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CHARACTERIZATION OF THE TRIMETHYLSILYL DERIVATIVES OF SUGAR PHOSPHATES AND RELATED COMPOUNDS BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

D. J. HARVEY AND M. G. HORNING
Institute for Lipid Research, Baylor College of Medicine, Houston, Texas 77025 (U.S.A.)
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SUMMARY

The gas chromatographic and mass spectrometric properties of the trimethylsilyl derivatives of sugar phospates and related compounds were investigated because of the importance of these phosphate esters in intermediary carbohydrate metabolism. Aldose-1-, -4-, -5-, and -6-phosphates and ketose diphosphates were chromatographed as the trimethylsilyl derivatives of the intact phosphate esters; the trimethylsilyl derivatives of the aldose diphosphates were found to be too unstable for chromatography. Substituted oxime derivatives were prepared from several of the sugar phosphates in an attempt to reduce the number of gas chromatographic peaks (due to α- and β-furanose and pyranose structures) and to increase the stability. The trimethylsilyl derivatives of these oximes gave single peaks in many cases, but the separation of isomeric hexoses was poor.

The methylene unit values were determined for forty-three sugar phosphates and related compounds on a polar (OV-17) and a non-polar (SE-30) phase. The structures of all derivatives were confirmed by mass spectrometry.

INTRODUCTION

Previous work has demonstrated that sugar phosphates and related compounds (phosphoric acid, glycerophosphates, hexitol phosphates, nucleotides and phosphatidyl inositols) can be converted to volatile derivatives which are suitable for analysis by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). A volatile trimethylsilyl (TMS) derivative of orthophosphoric acid was first reported by SAUER[1] in 1944, and this compound, *tris*-trimethylsilylphosphate, was subsequently analyzed by GC in 1967[2], and by GC–MS in 1969–1971[3–5]. The GC analyses of the TMS derivatives of several sugar phosphates and nucleotides have been described, but in the earlier work the derivatives were not characterized by MS[6–11]. In more recent papers, the mass spectral fragmentations of the TMS derivatives of sugar[12], inositol[13], ethylene glycol[14] and glycerophosphates[15], phosphatidyl inositols[16,17] and nucleotides[18–21] have been discussed extensively.

Relatively little difficulty has been encountered in preparing the TMS deriv-

atives of erythrose-4-, pentose-5-, or hexose-6-phosphates. However, the TMS derivatives of the aldose-1-phosphates are less stable and have not previously been characterized as the intact TMS phosphate esters[9]. Fructose-1-phosphate, on the other hand, forms a more stable derivative, and has been characterized by these methods[12].

By the use of suitable silylating reagents, we have been able to prepare derivatives of aldose-1-phosphates, ketose-diphosphates, phosphoenolpyruvate and various polyol phosphates not previously reported, and to characterize the intact phosphate esters by GC and GC–MS. The methylene unit (MU) values of various derivatives of 43 sugar and polyol phosphates, on both a polar and a non-polar phase, are reported in this paper.

EXPERIMENTAL

Materials and methods

Reference compounds were purchased, usually as the metal or cyclohexylammonium salts, from Sigma Chemical Co. and from Calbiochem. The free acids were isolated from the salts, where necessary, by DEAE-Sephadex chromatography[22]. Glycoaldehyde and glyceraldehyde phosphates were purchased as the salts (cyclohexylammonium and barium, respectively) of their diethylacetal derivatives; dihydroxyacetone and hydroxypyruvic acid phosphates were obtained as the cyclohexylammonium salts of their dimethylketal derivatives. The free aldo or keto acids were isolated from these derivatives according to the directions supplied by the manufacturer.

Preparation of derivatives

TMS-derivatives. TMS derivatives of all the phosphates except the pentose- and hexose-1-phosphates were prepared by reacting either 1 mg of the free phosphate, or its metal or cyclohexylammonium salt, with 0.1 ml of trimethylsilyltrifluoroacetamide (BSTFA) and 0.05 ml of trimethylchlorosilane (TMCS) in 0.1 ml of acetonitrile. The mixture was heated at 80° for about 10 min to ensure reaction of the metal salts.

TMS derivatives of the sugar-1-phosphates were prepared according to the following procedure: 1 mg of the sugar phosphate, either as its cyclohexylammonium salt or as the free phosphate isolated by DEAE-Sephadex chromatography, was treated with 0.1 ml of trimethylsilylimidazole (TMSI) in 0.1 ml of acetonitrile and allowed to stand at room temperature for about 5 min. An aliquot, usually 1 μl, was analyzed directly. This method could also be used for the free phosphates and cyclohexylammonium salts of all the other phosphates but not for the metal salts.

d_9-TMS analogs of these compounds were synthesized by substituting the appropriate deuterated silylating reagent for the unlabelled reagents in the above preparations.

Methyloxime-TMS derivatives. A solution of 1 mg of the sugar phosphate and 1 mg of methoxylamine hydrochloride in 0.2 ml of pyridine was kept at room temperature for 1 h. The resulting methoximes were converted into their TMS derivatives by the addition of 0.1 ml of either BSTFA or TMSI. After heating the mixture at 60° for 5 min, the derivative was analyzed by GC.

Ethyl- and TMS-oximes. These derivatives were prepared by substituting ethoxylamine hydrochloride, or hydroxylamine hydrochloride in the methoxylamine procedure.

GC analysis

GC analyses were carried out using a Barber Colman Series 5000 gas chromatograph fitted with two 12 ft. × 4 mm I.D. glass W columns and flame ionization detectors. The column packings used were 1 % or 5 % SE-30 and 1 % OV-17 on acid washed and silanized Gas-Chrom P (100–200 mesh). The flow-rate of carrier gas (nitrogen) was 40 ml/min at 200°. The temperature of the flash heater was 250–300° and the detector bath temperature was 310°. Separations were usually carried out by temperature programming at 2°/min.

GC–MS analyses

Mass spectra were recorded at 70 eV with an LKB 9000 mass spectrometer. The source temperature was 270°, accelerating voltage 3.5 eV, and the ionising current 60 μA. Sample introduction was via the GC inlet using a 9 ft. × 4 mm I.D. glass coil packed with 1 % SE-30 on acid washed and silanized Gas-Chrom P (100–120 mesh). The column temperature was adjusted to give a retention time of 8–10 min for each sample.

RESULTS AND DISCUSSION

The structures of all the derivatives were confirmed by MS using a combined gas chromatograph–mass spectrometer, demonstrating that the phosphate esters could be analyzed by these techniques as the intact compounds.

Although the *tris*-TMS ester of orthophosphoric acid was quite stable and satisfactory for characterization purposes, the corresponding derivative of pyrophosphoric acid was less stable and decomposed slowly on the GC column to give the *tris*-TMS ester of orthophosphoric acid. The TMS derivative of tripolyphosphoric acid was very unstable and the only peaks observed in its gas chromatogram were produced by the TMS derivatives of ortho- and pyrophosphoric acid formed by decomposition. As can be seen from Table I, good separation of the TMS derivatives of ortho- and pyrophosphoric acid was achieved on both a non-polar (SE-30) and a polar (OV-17) phase, permitting ready identification of pyrophosphate in the presence of orthophosphate.

The TMS derivatives of a series of C_2 and C_3 phosphate esters gave single peaks on both phases. The compounds studied and their MU values[23] are listed in Table I; it is apparent that most of these derivatives can be separated on either phase. A separation of the TMS derivatives of L-α- and β-glycerophosphate and 2- and 3-phosphoglyceric acid is shown in Fig. 1. Although these derivatives were satisfactory for purposes of identification, they were not sufficiently stable for quantitative analysis because of loss of the labile carboxylic or phosphate ester silyl groups on the GC columns. Partial decomposition of the analogous derivatives of sugar phosphates has been discussed by SHERMAN *et al.*[11]. The derivatives could, however, be stored at −14° (freezer) for several weeks without extensive decomposition.

TABLE I

MU VALUES FOR THE DERIVATIVES OF ORTHO- AND PYROPHOSPHORIC ACID AND THE C_2 AND C_3 PHOSPHATE ESTERS

Compound	*Derivative*	*1% SE-30*	*1% OV-17*
Orthophosphoric acid	TMS	12.70	13.45
Pyrophosphoric acid	TMS	16.60	18.00
Glycoaldehyde phosphate	Di-Et acetal, TMS	16.20	17.60
	Me-oxime, TMS	14.80	16.25
	Et-oxime, TMS	Not recorded	16.90 (17.00)[a]
L-α-Glycerophosphate	TMS	18.05	18.65
β-Glycerophosphate	TMS	17.65	18.25
2-Phosphoglyceric acid	TMS	18.15	19.20
3-Phosphoglyceric acid	TMS	18.45	19.45
Glycerol-1,2-diphosphate	TMS	22.25	23.35
Glycerol-1,3-diphosphate	TMS	22.55	23.75
2,3-Diphosphoglyceric acid	TMS	22.40	23.85
2-Phosphoenolpyruvic acid	TMS	15.95	17.35
Hydroxypyruvic acid phosphate	Di-Me ketal, TMS	18.25	19.95
DL-Glyceraldehyde phosphate	Di-Me acetal, TMS	18.70	19.70
	Me-oxime, TMS	17.55 (17.40)	18.70 (18.50)
	Et-oxime, TMS	18.10	19.30 (19.05)
	TMS-oxime, TMS	18.85 (18.50)	19.65 (19.20)
Dihydroxyacetone phosphate	Di-Me ketal, TMS	17.75	18.90
	Me-oxime, TMS	17.75 (17.85)	19.00 (19.15)
	Et-oxime, TMS	18.25	19.50 (19.60)
	TMS-oxime, TMS	18.65	19.55 (19.65)

[a] Values in parentheses represent minor peaks.

The TMS derivatives of the keto and aldehyde phosphates were less stable, and it was necessary to convert the carbonyl groups into suitable derivatives before silylation in order to prepare adducts which could be chromatographed successfully. Methoxime-, ethoxime-, and TMS-oxime-TMS derivatives were prepared from glycoaldehyde, DL-glyceraldehyde and dihydroxyacetone phosphate and were found to be more stable than the TMS derivatives of the free keto compounds. The gas chromatograms of DL-glyceraldehyde and dihydroxyacetone phosphates, derivatized in this manner, exhibited two peaks presumably produced by the *syn* and *anti* isomers of the oximes. These could be separated on both SE-30 and OV-17 columns. The mass spectra of these isomeric compounds showed similar fragmentation patterns, although the relative intensities of the ions varied considerably. TMS derivatives of the diethylacetals of glycoaldehyde and DL-glyceraldehyde phosphates and of the dimethylketals of dihydroxyacetone and hydroxypyruvic acid phosphates were also found to be more stable than corresponding TMS derivatives of the compounds containing free carbonyl groups. All of these compounds gave single GC peaks on both phases. The MU values for these derivatives are listed in Table I. No single set of derivatives was found which would separate all the compounds listed in Table I on a single phase, but it was possible to separate all the pairs of isomeric compounds (for example, L-α-glycerophosphate and β-glycerophosphate). By using two GC phases, or by preparing two sets of derivatives, all the parent compounds listed in Table I could eventually be separated from each other.

Hydroxypyruvic acid phosphate could not be satisfactorily chromatographed

either as the TMS derivative (free keto group) or as the oxime-TMS derivative. The dimethylketal-TMS derivative was, however, more stable and gave a single GC peak on both phases. The *tris*-TMS ester of 2-phosphoenolpyruvic acid was reasonably stable although some decomposition was noted during chromatography.

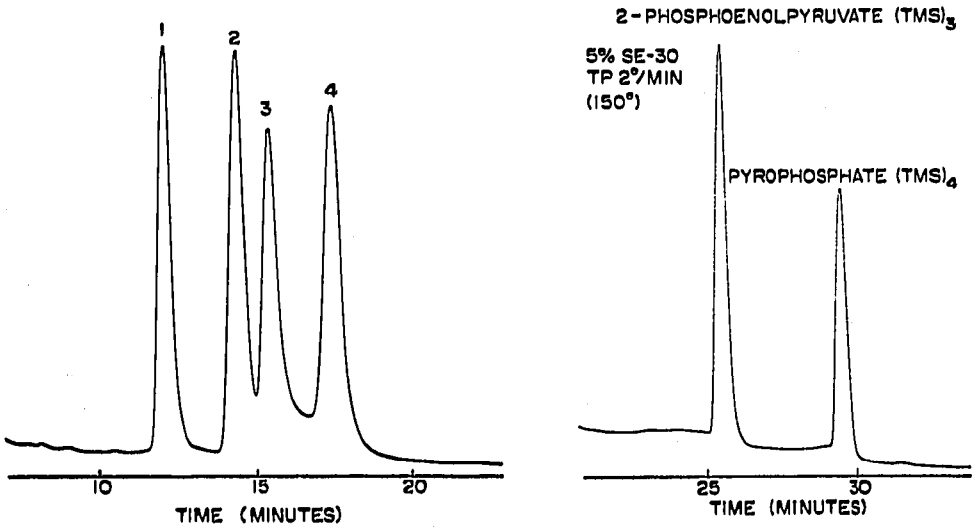


Fig. 1. GC separation of the TMS derivatives of β-glycerophosphate (peak 1), L-α-glycerophosphate (peak 2), 2-phosphoglyceric acid (peak 3) and 3-phosphoglyceric acid (peak 4) on 1% SE-30 (12 ft.) at 160°.

Fig. 2. GC separation of the TMS derivatives of 2-phosphoenolpyruvate and pyrophosphate on 5% SE-30 (12 ft.). The column was programmed from 150° at 2°/min. Decomposition of these unstable derivatives can be clearly seen.

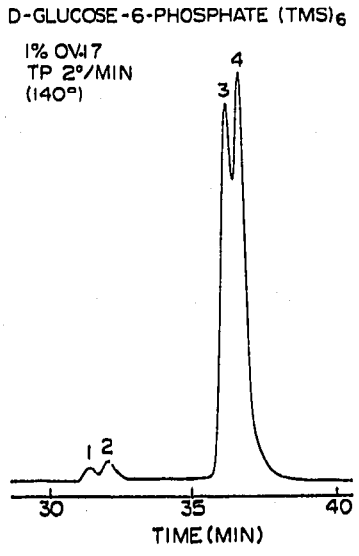


Fig. 3. GC trace of the TMS derivative of D-glucose-6-phosphate on 1% OV-17 (12 ft.) programmed from 140° at 2°/min. Peaks 1 and 2 are produced by the α- and β-furanose forms of the sugars and the larger peaks 3 and 4 represent the α- and β-pyranose structures.

Fig. 2 illustrates one of the problems associated with GC analysis of the TMS derivatives of the more unstable phosphates. From the GC tracing, it is apparent that the TMS derivatives of both pyrophosphate and 2-phosphoenolpyruvate decompose slowly on the column.

The MU values of the TMS derivatives of the C_4 to C_7 sugar phosphates are listed in Table II. Multiple peaks were obtained for the aldose sugars, pentose-5-, hexose-6- and heptose-7-phosphates, on an SE-30 column and usually on an OV-17 column. This is illustrated in Fig. 3 which shows a GC tracing of the TMS derivative of D-glucose-6-phosphate. These multiple peaks are produced by the TMS derivatives of the α- and β-furanose and -pyranose forms of the sugars. It is generally assumed, by analogy with the unphosphorylated sugars, that the first peak of each pair is produced by the α isomer and the second peak by the β isomer. In contrast to the

TABLE II

MU VALUES FOR THE TMS DERIVATIVES OF THE C_4–C_7 SUGAR PHOSPHATES AND RELATED COMPOUNDS

Compound	*1% SE-30*			*1% OV-17*		
D-Erythrose-4-phosphate	18.95			19.70		
2-Deoxy-D-ribose-5-phosphate	20.00			21.00		
D-Xylose-1-phosphate	22.00			22.50		
D-Xylulose-5-phosphate	21.70			22.40		
D-Ribose-1-phosphate	20.60			22.00		
D-Ribose-5-phosphate	21.65	(21.75)[a,b]		22.20		
D-Ribulose-5-phosphate	21.75			22.35		
D-Ribulose-1,5-diphosphate	[c]			26.90		
2-Deoxy-D-glucose-6-phosphate	22.75	(23.15)		23.35	23.70	
2-Deoxy-6-phosphogluconic acid	23.50			23.65		
α-D-Glucose-1-phosphate	23.45	23.80		23.85	24.45	
D-Glucose-6-phosphate (furanose)	(23.50)	(23.70)		(23.70)	(23.85)	
D-Glucose-6-phosphate (pyranose)	24.55	24.85		24.80	24.95	
1-Phosphoglucuronic acid	23.75			25.00	(24.90)	
D-Glucosamine-6-phosphate	24.15			25.00		
6-Phosphogluconic acid	25.10			24.75		
α-D-Mannose-1-phosphate	22.60	23.35		23.25		
D-Mannose-6-phosphate	23.60	(24.40)		23.70	24.35	
α-D-Galactose-1-phosphate	23.30	22.90[d]	23.80[d]	22.78	23.55[d]	24.35[d]
D-Galactose-6-phosphate (furanose)	(23.24)[e]	(23.62)[e]		(23.10)[e]	(23.30)	(23.45)[e]
D-Galactose-6-phosphate (pyranose)	24.00	24.35		24.00	24.35	
1-Phosphogalacturonic acid	24.55	(24.00)		26.10	(25.25)	
D-Fructose-1-phosphate	23.05	23.35	23.60	22.85	22.30	23.50
D-Fructose-6-phosphate	23.35			23.25		
D-Fructose-1,6-diphosphate	27.30	27.60		27.65	28.05	
Sedoheptulose-7-phosphate	25.30	25.45		24.75	24.95	
D-Mannitol-1-phosphate	24.45			23.75		
D-Sorbitol-6-phosphate	24.55			23.60		
Myoinositol-2-phosphate	25.55	(24.50)		25.05	(24.35)	
(—)-Inositol-3-phosphate	23.65	(24.10)[a]		22.70	(23.25)	
D-Glycerol-1-(L-myoinositol)-1-phosphate	29.10			28.00		

[a] Shoulder.
[b] Values in parentheses are minor peaks.
[c] Satisfactory peak not available.
[d] Does not contain phosphorus.
[e] Probably an impurity.

aldose phosphates, the TMS derivatives of the ketose phosphates gave single peaks on both phases. The TMS derivatives of the aldonic acids, 2-deoxy-6-phosphogluconic acid and 6-phosphogluconic acid, also gave single peaks and were comparable in stability to the phosphoglyceric acids. Glucosamine-6-phosphate, the only amino sugar phosphate studied, formed an N-silyl derivative with BSTFA, which gave a single peak on both OV-17 and SE-30.

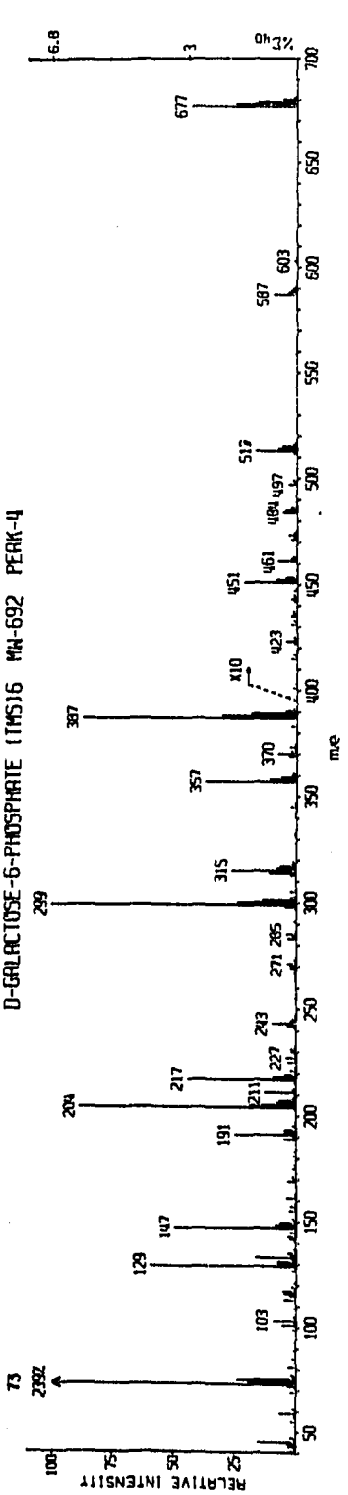
In order to prepare TMS derivatives of the aldose-1-phosphates and 1-phosphoalduronic acids, it was necessary to modify the procedure for derivative formation to reduce loss of the phosphate group. The catalyst, TMCS, was omitted and TMSI was employed as the silylating agent. Derivative formation was rapid and apparently complete within 5 min at room temperature for the seven compounds listed in Table II. Xylose-1-phosphate and ribose-1-phosphate gave single peaks on both phases, but the other compounds usually gave multiple peaks on at least one phase. The mass spectra of the compounds producing the additional peaks observed for glucose-1-phosphate and galactose-1-phosphate showed no ions characteristic of the phosphate system indicating that partial decomposition had occurred during sample preparation or chromatography.

In addition to the glycero diphosphates (Table I), the ketose diphosphates, ribulose-1,5-diphosphate and fructose-1,6-diphosphate formed reasonably stable TMS derivatives suitable for GC analysis. It was not possible, however, to prepare TMS derivatives of the aldose diphosphates (glucose-1,6-diphosphate and ribose-1-pyrophosphate-5-phosphate) that could be analyzed as the intact diphosphate esters.

Methoxime-TMS derivatives[24] were prepared from the aldose-4-, -5-, -6-, and -7-phosphates and the ketose phosphates in an attempt to reduce the number of peaks produced by the α and β anomers. When these derivatives were chromatographed on a 1 % SE-30 column, a single peak was usually obtained, but the sample usually gave two peaks when analyzed on OV-17. This is again thought to be due to the separation of the *syn* and *anti* isomers of the methoximes, although the occurrence of cyclic–acylic structures analogous to those reported[25] for the TMS oximes of unphosphorylated sugars, is also possible. Although fewer peaks are obtained, these derivatives are of limited use for identification because the isomeric sugars (glucose, mannose and galactose, for example) have very similar MU values (Table III). Attemps to improve the separation of the sugars by preparing ethoxime- and TMS-oxime-TMS derivatives were unsuccessful (Table III).

The TMS derivatives of the hexitol phosphates, D-mannitol-1- and D-sorbitol-6-phosphate, gave single peaks on OV-17 and SE-30 as did D-glycerol-1-(L-*myo*-inositol)-1-phosphate. The cyclic hexitol phosphates, *myo*-inositol-2-phosphate and (−)-inositol-3-phosphate, gave two peaks; the smaller peak is probably due to an impurity in the starting material.

The mass spectra of the TMS derivatives of the compounds discussed in this paper showed similar fragmentation patterns. Molecular ions were generally absent or of very low abundance, but ion-molecule reaction products were often observed as low intensity peaks at M + 1 and M + 73 (refs. 15, 26, 27). When the spectra were recorded at higher pressures, additional peaks produced by ion-molecule reactions became apparent. Previous work has shown that these ions were produced by interaction of the neutral molecule with abundant fragment ions containing a siliconium center, for example, M + 147, M + 299 (refs. 15, 27, 28). As the M + 1 and M + 73



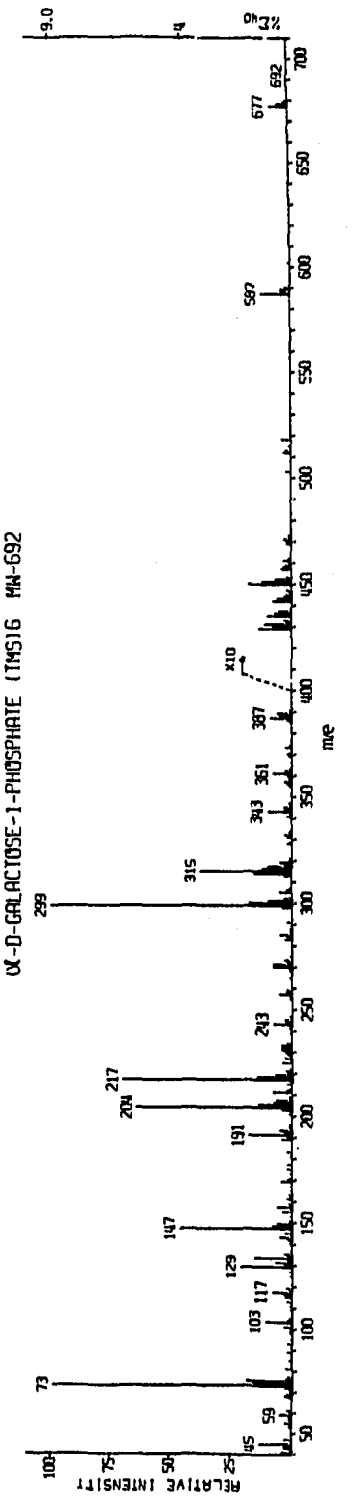


Fig. 4. Mass spectra of the TMS derivatives of D-galactose-6-phosphate (pyranose form) and α-D-galactose-1-phosphate recorded at 70 eV. Marked differences in the intensities of the peaks at *m/e* 357 and 387 are apparent and aid in distinguishing between the two isomers.

TABLE III

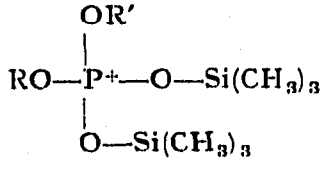
MU VALUES FOR THE OXIME-TMS DERIVATIVES OF THE C_4–C_7 SUGAR PHOSPHATES

Compound	*Oxime*	*1% SE-30*	*1% OV-17*
D-Erythrose-4-phosphate	Me	19.65	20.40 (20.70)
2-Deoxy-D-ribose-5-phosphate	Me	20.75	21.55
D-Ribose-5-phosphate	Me	21.85	21.95 (21.40)
D-Ribulose-5-phosphate	Me	21.70 (21.40) (21.85)	21.95 22.25 (21.60)
D-Xylulose-5-phosphate	Me	21.80 (21.70)	22.30 (22.05)
2-Deoxy-D-glucose-6-phosphate	Me	22.85	23.25
D-Glucose-6-phosphate	Me	24.05	24.00
	Et	24.45 (24.75)	24.40 (24.55)
	TMS	24.90	24.45 (24.55)
D-Galactose-6-phosphate	Me	23.90	23.95 (23.00) (24.05)
	Et	24.35 (24.70)	24.15 (24.50)
	TMS	24.90 (25.25)	24.40 (24.65)
D-Mannose-6-phosphate	Me	24.00	23.75 (23.25)
	Et	24.25	23.95
	TMS	24.85	24.25
D-Fructose-6-phosphate	Me	23.95	23.80 (23.05)
	Et	24.25	24.10
	TMS	24.55 (23.20)	23.85 (23.00)
Sedoheptulose-7-phosphate	Me	26.45	26.05

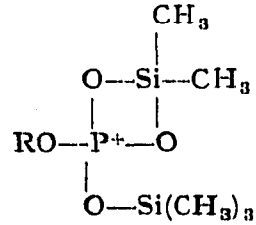
ions were observed when the spectra were recorded under the normal electron impact conditions, care must be taken to avoid erroneous assignments of the molecular ion in the spectra of the unknown sugars. These ion-molecule reaction products may be readily detected, however, because of the variation of their abundance with pressure.

The fragment ion of highest mass usually observed was $[M\text{-}15]^+$, formed by loss of a methyl radical from a TMS group. This was shown by its shift to $[M\text{-}18]^+$ in the spectra of the d_9-TMS derivatives[20]. Determination of the extent of hydroxylation of these compounds is greatly facilitated by observing the shift of the M^+ or $[M\text{-}15]^+$ ions in the spectra of the d_9-TMS analogs.

The spectra are dominated by abundant rearrangement ions containing a resonance stabilized phosphonium cation[15]. Ions a, b, and c are formed by migration of TMS groups and/or hydrogen atoms to the phosphate moiety. Ions d, e, and f



a, b, c



d, e

(a) $R = R' = Si(CH_3)_3$, $m/e = 387$
(b) $R = H$ and $R' = Si(CH_3)_3$, $m/e = 315$
(c) $R = R' = H$, $m/e = 243$
(d) $R = Si(CH_3)_3$, $m/e = 299$
(e) $R = H$, $m/e = 227$

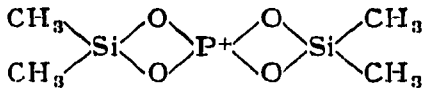
TABLE IV

PARTIAL MASS SPECTRA OF THE TMS DERIVATIVES OF SELECTED SUGAR PHOSPHATES SHOWING THE VARIATION IN THE RELATIVE ABUNDANCE OF THE MAIN REARRANGEMENT IONS

Compound	*Base*[b] *m/e*	M^+		*M-15*		*m/e* 387 (%)	*m/e* 315 (%)	*m/e* 299 (%)	*m/e* 243 (%)	*m/e* 227 (%)	*m/e* 211 (%)	*m/e* 357 (%)
		m/e	%	*m/e*	%							
L-α-Glycerophosphate	299	460	0	445	25	9.5	18	100	8	8	32	98
β-Glycerophosphate	243	460	0	445	11	1.5	15	48	100	9.5	25	3.5
2-Phosphoglyceric acid	299	474	1	459	90	47	78	100	4	13	39	6.5
3-Phosphoglyceric acid	147	474	0.5	459	26	34	45	80	3.5	85	51	70
Glycerol-1,2-diphosphate	299	612	0	597	12	10.5	49	100	19	10.5	35	5
Glycerol-1,3-diphosphate	299	612	0	597	12	7	25	100	8	9	29	63
D-Ribose-1-phosphate	217	590	0	575	1.5	3.5	42	70	14	6.5	12	1
D-Ribose-5-phosphate	315	590	0	575	5.5	2.5	100	51	8.5	11	16.5	2
α-D-Glucose-1-phosphate	299	692	0	677	0.5	16	42.5	100	15	4.5	12.5	5
α-D-Glucopyranose-6-phosphate[a]	204	692	0	677	3.5	99	28	52	7.5	7	12.5	27
β-D-Glucopyranose-6-phosphate	204	692	0	677	3.5	96	23	67	6.5	6	11	24
α-D-Galactose-1-phosphate	299	692	0	677	0.5	9	37	100	8	3	8	3
α-D-Galactopyranose-6-phosphate[a]	387	692	0	677	4	100	39	86.5	14	7	16	41
β-D-Galactopyranose-6-phosphate[a]	299	692	0	677	4	87	26	100	10	4.5	13	37
α-D-Mannopyranose-1-phosphate	315	692	0	677	0.5	7	100	64	15	5	11	4
α-D-Mannopyranose-6-phosphate[a]	387	692	0	677	7	100	27	73	8	6.5	12.5	29
β-D-Mannopyranose-6-phosphate[a]	204	692	0	677	5.5	96	23	80	7.5	5	12	25.5
D-Fructose-1-phosphate	217	692	0.3	677	4.5	3	12	54	3.5	2.5	5.5	5
D-Fructose-6-phosphate	315	692	0	677	5	16.5	100	56	4.5	12.5	14	2.5
D-Sorbitol-6-phosphate	299	766	0	751	1.5	82	79	100	12.5	6.5	9	33
D-Mannitol-1-phosphate	387	766	0	751	3.2	100	71	62	3.5	3	7	28

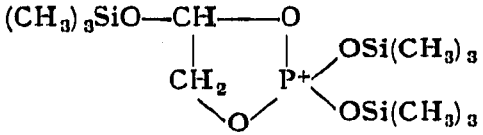
[a] Tentative assignment of α and β isomers.

[b] *m/e* 73 was disregarded when assigning the base peak because of its general irreproducibility.



f (m/e = 211)

are fragment ions formed from ions a, b, and c by mechanisms discussed previously[12,15,19]. The phosphorus-containing fragment ion, g, of mass m/e 357 contains an additional two carbon atoms from the carbon skeleton and is again characteristic of this type of derivative.



g (m/e 357)

Isomeric compounds can usually be differentiated by comparing the relative intensities of the characteristic ions a–g. For example, in Fig. 4, the mass spectrum of D-galactose-6-phosphate and α-D-galactose-1-phosphate are compared; the ions at m/e 387 and 357 are much more abundant in the spectrum of the TMS derivative of the 6-phosphate than of the 1-phosphate. Other examples are listed in Table IV. In all the examples studied, the intensity of m/e 387 was found to be considerably greater in the spectra of the hexopyranose-6-phosphates than in the corresponding derivatives of the 1-phosphates. The spectra of the α and β anomers of each hexose phosphate were generally similar as can be seen from Table IV.

The differences in relative abundance of the rearrangement ions a–e is undoubtedly a reflection of the stereochemistry of the molecule[30]. The abundance of the rearrangement ion m/e 147 has also been shown to be a function of stereochemistry in compounds of rigid structure such as sugars[31], cyclohexanediols[32], and steroids[33] suggesting a similar stereochemical dependence for the formation of ions a–e in the spectra reported here.

Certain other ions aid in defining the carbon skeleton of the phosphate esters. For example, m/e 318 is abundant in the spectra of the TMS derivatives of the inositols (cyclic hexitols) but is present in low abundance in the spectra of the linear hexitols. Ions characteristic of the TMS derivatives of furanose and pyranose sugars and sugar phosphates have been discussed previously[12,34].

A large number of other ions, characteristic of TMS derivatives of polyhydroxy compounds, are also present in these mass spectra. The structures of these ions have been discussed in the recent literature[34,35].

Complete MS data for the compounds discussed in this paper will be published in the *Archives of Mass Spectral Data*.

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