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DERIVATIVES FOR THE CHARACTERIZATION OF ALKYL- AND AMINOALKYLPHOSPHONATES BY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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SUMMARY

Trimethylsilyl derivatives of the alkylphosphonates and of certain aminoalkylphosphonates were found to be satisfactory for the characterization of these phosphorus compounds by gas chromatography and gas chromatography–mass spectrometry. The trimethylsilyl derivatives of the 1-aminoalkylphosphonates were, however, not sufficiently stable for this purpose. In order to analyze these compounds satisfactorily, the amino group was converted into the N-acetyl derivative, acetone Schiff base, or isothiocyanate; trimethylsilyl ester formation was retained for the phosphonate system. These derivatives, particularly the isothiocyanates, were stable and had good gas chromatographic and mass spectrometric properties.

INTRODUCTION

The isolation in 1959 of 2-aminoethylphosphonic acid from the ciliate of sheep rumen[1] has initiated extensive research into the biochemistry of compounds containing a C–P bond[2]. Several additional aminoalkylphosphonates and aminophosphonocarboxylic acids have subsequently been identified in various species. A number of methods have been used to isolate and identify these compounds, but there has been little application of gas chromatography (GC) or combined gas chromatography and mass spectrometry (GC–MS) to this field. KARLSSON[3], however, has recently emphasized the utility of these techniques in differentiating between closely related compounds such as 2-aminoethylphosphonic acid and O-phosphorylethanolamine.

The low volatility of these compounds necessitates their conversion to suitable derivatives prior to analysis by gas phase methods. We have recently examined the GC–MS characteristics of the trimethylsilyl (TMS) derivatives of a series of alkylphosphonates and aminoalkylphosphonates containing a primary amino group, and have found that although these adducts are suitable for the characterization of many of these compounds, they are not satisfactory in all cases. Consequently, we have investigated other derivatives which are more stable and therefore applicable to a wider range of aminoalkylphosphonates.

EXPERIMENTAL

Materials

All the compounds discussed in this paper were obtained from commercial suppliers.

Preparation of derivatives

Per-TMS derivatives. The phosphonate (1 mg) in acetonitrile (0.1 ml) was treated with bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.1 ml) and trimethylchlorosilane (TMCS, 0.05 ml). Formation of the TMS derivatives of the alkylphosphonates was complete after a few minutes at room temperature. The aminoalkylphosphonates were heated with the reagents at 80° until the compounds had dissolved (15 min–2 h). Aliquots of these solutions were then analyzed directly.

TMS esters of the aminoalkylphosphonates (–NH_2 *group not derivatized*). The TMS esters of the aminoalkylphosphonates were prepared as follows:

Method 1: The aminoalkylphosphonate (1 mg), BSTFA (0.1 ml) and TMCS (0.05 ml) were heated at 80° until the aminoalkylphosphonate had dissolved (15 min–4 h). Aliquots of these solutions were analyzed directly.

Method 2: The aminoalkylphosphonate (1 mg), dimethylsulfoxide (0.1 ml), hexamethyldisilazane (HMDS, 0.1 ml) and dioxane (0.4 ml)[4] were heated at 80° until the aminoalkylphosphonate had dissolved (1–100 h). This reaction produced fewer N-TMS byproducts, but often took an inconveniently long time.

d_9-TMS derivatives. These were synthesized by the above methods using d_{18}-BSA and d_9-TMCS in place of the BSTFA and TMCS respectively.

N-Acetyl/TMS derivatives. The N-acetyl TMS derivatives were prepared as follows:

Method 1: The aminoalkylphosphonate (1 mg) was heated at 80° with a 10 % solution of acetic anhydride in pyridine (0.2 ml). When the aminoalkylphosphonate had dissolved, the reagent and solvents were removed (N_2 stream) and the residue was redissolved by heating at 80° for 10 min with acetonitrile (0.1 ml), BSTFA (0.1 ml) and TMCS (0.05 ml). This method was not satisfactory for all compounds because several of them were insoluble in the reagents.

Method 2: TMS esters of the aminoalkylphosphonate (free NH_2 group) were prepared by one of the above methods and the reagents and solvents were removed (N_2 stream). These TMS esters were treated with a 10 % solution of acetic anhydride in pyridine (0.2 ml) and heated at 80° for 30 min. The reagents were again removed (N_2 stream) and the product was dissolved in acetonitrile (0.1 ml), BSTFA (0.1 ml) and TMCS (0.05 ml) by heating for 10 min at 80° to ensure complete reaction of the phosphonate group.

Acetone Schiff base/TMS derivatives. The TMS esters of the aminoalkylphosphonates were prepared as described above, blown to dryness (N_2 stream), and heated with acetone (0.1 ml) for 30 min at 80°. After removal of the acetone (N_2 stream) the compound was treated with acetonitrile (0.1 ml), BSTFA (0.1 ml) and TMCS (0.05 ml) and heated at 80° for 10 min.

Isothiocyanate/TMS derivatives. TMS esters of the aminoalkylphosphonic acids were prepared as described above and the reagents were removed (N_2 stream). The residue was treated with carbon disulfide (0.25 ml) and the mixture was allowed

to stand at room temperature overnight. Aliquots of this solution were analyzed directly.

Gas chromatography

GC analyses were made with a Barber Coleman Series 5000 gas chromatograph fitted with a flame ionization detector. The columns were 12 ft. × 4 mm glass "W" columns packed with 1 % SE-30, or 1 % OV-17 on acid-washed and silanized Gas-Chrom P (100–120 mesh). Nitrogen was used as the carrier gas and the flow-rate was adjusted to 40 ml/min at 200°. The flash heater was maintained at 250–300° and the detector bath at 310°.

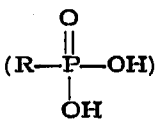
Mass spectrometry

An LKB 9000 mass spectrometer was used to record the low resolution spectra of the derivatives. Sample introduction was via the GC inlet; a 9 ft. × 4 mm glass coil packed with 1 % SE-30 on 80–100 mesh acid-washed and silanized Gas-Chrom P was used. The column temperature was adjusted to give a retention time of about 10 min for each compound. Mass spectra were recorded at 70 eV, the source temperature was 250°, and the accelerating voltage, 3.5 kV.

High-resolution spectra of selected compounds were recorded with a CEC21-110B mass spectrometer.

RESULTS AND DISCUSSION

The trimethylsilyl derivatives of the alkylphosphonic acids



gave single peaks when examined by both a polar (OV-17) and a non-polar (SE-30) GC phase. Mass spectrometric analysis of these compounds confirmed the presence of two TMS groups on the phosphonate system. Formation of these derivatives was rapid at room temperature and once formed, the derivatives could be stored for several weeks at 0° without extensive decomposition. Similar results were obtained for the TMS derivatives of the phosphonoalkylcarboxylic acids and methylenediphosphonic acid. The methylene unit (MU) values of these derivatives are given in Table I.

The per-trimethylsilyl derivatives of 2-aminoethyl and 3-aminopropylphosphonic acid were also satisfactory for GC analysis. As reported by KARLSSON, two TMS groups are introduced on to the nitrogen atom by the derivatization method employed. Slight decomposition of these derivatives on the GC column was observed, similar to the behavior of the TMS derivatives of organic phosphates discussed by SHERMAN *et al.*[5]. The phosphonate TMS esters appeared more stable than the corresponding TMS derivatives of the phosphates, however. This was clearly seen when the relative peak heights of the TMS derivatives of 2-aminoethylphosphonic acid and O-phosphorylethanolamine were compared at different column temperatures. Reduction of peak height with increased retention time was more apparent with the

TABLE I

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE TMS DERIVATIVES OF THE PHOSPHONIC ACIDS

Compound	*MU*			*Mass spectrometric data*			
	1% SE-30	*1% OV-17*	*Temperature*	M^+		$[M-15]^+$	
				m/e	*% of base peak*	*m/e*	*% of base peak*
Methylphosphonic acid	11.35	12.40	90	240	12	225	100
Ethylphosphonic acid	12.05	13.10	90	254	21	239	100
Phenylphosphonic acid	15.30	17.15	140	302	20	287	100
1-Phenylmethylphosphonic acid	16.50	18.20	160	316	86	301	100
Methylene diphosphonic acid	18.00	18.95	170	464	24	449	100
Phosphonoacetic acid	15.40	16.50	160	356	21	341	100
Phosphonopropionic acid	16.55	17.65	160	370	7	355	100

TMS derivative of O-phosphorylethanolamine. Decomposition by the loss of the TMS ester of phosphoric acid is thought to contribute to the instability of the latter compound. Good separation of the TMS derivatives of 2-aminoethylphosphonate and O-phosphorylethanolamine was achieved on both phases as is evident from Fig. 1, which shows a separation of these two compounds and the TMS derivative of 3-aminopropylphosphonic acid on SE-30. MU values are listed in Table II. Single GC peaks were also obtained for the TMS derivatives of 3-aminophosphonopropionic and 4-aminophosphonobutyric acids.

Per-TMS derivatives were not satisfactory for the characterization of the 1-aminoalkylphosphonic acids.

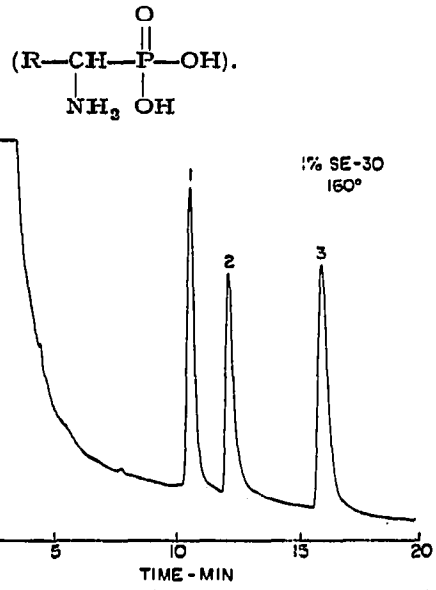


Fig. 1. GC separation of the TMS derivatives of 2-aminoethylphosphonic acid (peak 1), O-phosphorylethanolamine (peak 2) and 3-aminopropylphosphonic acid (peak 3) on a 12-ft. 1% SE-30 column at 160°.

TABLE II

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE TMS DERIVATIVES OF THE AMINOPHOSPHONIC ACIDS

Phosphonic acid	*No. of TMS groups*	*MU*			*Mass spectrometric data*				
		1% SE-30	*1% OV-17*	*Temperature*	*M+*		*[M-15]+*		
					m/e	*% of base peak*	*m/e*	*% of base peak*	*Base peak*
Aminomethyl	2	13.10	14.60	120	255	16	240	16	211
	3	14.55	15.25	120	327	1.5	312	10	102
1-Aminoethyl	2	13.25	14.55	120	269	5	254	2	44
	3	14.75	15.30	120	341	1.3	326	2	116
1-Aminopropyl	2	13.95	15.20	120	283	2.2	268	1.7	58
	3	15.30	15.80	120	355	1.3	340	2.2	130
1-Aminobutyl	2	14.65	15.85	130	297	1.3	282	0.8	72
	3	15.85	16.30	130	369	1.6	354	1.8	144
1-Aminopentyl	2	15.55	16.70	140	311	1.6	296	1	86
	3	16.55	17.00	140	383	1.5	368	1.7	158
1-Amino-2-methylpropyl	2	14.50	15.55	140	297	1.2	282	1.8	72
	3	—[a]	16.05	140	369	1.1	354	2.4	144
1-Amino-2(4-hydroxyphenyl)ethyl	3		24.25[b]	200	433	0.5	418	5.5	254
		22.55							
	4		23.65[b]	200	505	0.5	490	3.1	254
2-Aminoethyl	3	—[a]	15.55	140	341	0	326	2.5	211
	4	17.60	18.00	140	413	0.3	398	100	398
3-Aminopropyl	3	14.85	16.55	150	355	0	340	5	225
	4	18.75	19.10	150	427	0.5	412	100	412

[a] Satisfactory peak could not be obtained.
[b] Poor separation. The N-TMS derivative decomposed on the column to give the free amino compound.

Two peaks of varying height were obtained on both GC phases for most of the compounds studied. Analysis by mass spectrometry showed that the second compound eluted contained one TMS group on the nitrogen atom and that this appeared to decompose by loss of the N-TMS moiety to give the compound producing the first peak. This latter compound had the same MU value and mass spectrum as the derivative containing a free NH_2 group prepared by the reaction of HMDS on the aminoalkylphosphonate. The derivatives (free NH_2 group) prepared by this latter method were found to be more satisfactory for characterization of the 1-aminoalkylphosphonic acids than the N-TMS analogs as only one peak was obtained in most cases. Unfortunately, two poorly resolved peaks were produced by the compounds containing aromatic substituents; these corresponded to the $-NH_2$ and –NHTMS derivatives. Multiple peaks were also produced by the di- and tri-TMS derivatives of 2-aminoethyl- and 3-aminopropyl-phosphonic acids.

In view of the unstable nature of many of these N-TMS compounds, several other derivatives of the amino group were investigated in the hope of finding a set of derivatives suitable for all compounds. The phosphonic acid moiety was converted into its bis-TMS ester as before. The zwitterionic character of these aminoalkylphosphonic acids renders them very insoluble and unreactive in most organic systems. Trimethylsilyl ester formation was found to be the most satisfactory method of

rendering these compounds soluble and consequently the bis-TMS esters were prepared prior to derivatization of the amino function. In some cases, however, heating for several days at 80° was necessary for complete reaction. This was particularly evident for the reaction with HMDS to produce derivatives containing the free NH_2 group. Decomposition of the parent aminoalkylphosphonates under these conditions, however, seemed to be minimal. After formation of the bis-TMS ester of the phosphonate was complete, various derivatives of the $-NH_2$ group were prepared.

TABLE III

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE N-ACETATE/TMS DERIVATIVES OF THE AMINOPHOSPHONIC ACIDS

Phosphonic acid	*MU*			*Mass spectrometric data*				
	1% SE-30	*1% OV-17*	*Temperature*	*M+*		*[M-15]+*		
				m/e	*% of base peak*	*m/e*	*% of base peak*	*Base peak*
Aminomethyl	15.95	18.20	160	297	47	282	57	211
1-Aminoethyl	15.90	17.90	160	311	8.5	296	16	211
1-Aminopropyl	16.40	18.30	160	325	9.5	310	25	211
1-Aminobutyl	17.00	18.90	160	339	12	324	30	226
1-Aminopentyl	17.75	19.70	170	353	9	338	20	86
1-Amino-2-methylpropyl	16.65	18.35	160	339	3.5	324	23	211
1-Amino-2-phenylethyl	20.95	23.90	230	387	26	372	36	120
1-Amino-2(4-hydroxyphenyl)ethyl[a]	24.35[b]	25.55[b]	240	445	11	430	25	177
1-Amino-2(4-hydroxyphenyl)ethyl[c]	24.15	26.60	230	475	0.5	460	12	294
2-Aminoethyl	16.85[b]	19.20[b]	180	311	18.5	296	23	211
3-Aminopropyl	18.30[b]	20.95[b]	200	325	6	310	21	240

[a] O-Acetyl derivative.
[b] Peak tailed badly.
[c] O-TMS derivative.

TABLE IV

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE ACETONE SCHIFF BASE/TMS DERIVATIVES OF THE AMINOPHOSPHONIC ACIDS

Phosphonic acid	*MU*			*Mass spectrometric data*				
	1% SE-30	*1% OV-17*	*Temperature*	*M+*		*[M-15]+*		
				m/e	*% of base peak*	*m/e*	*% of base peak*	*Base peak*
Aminomethyl	14.45	15.95	120	295	8.7	280	9.3	70
1-Aminoethyl	14.25	15.50	120	309	1.1	294	2.0	84
1-Aminopropyl	14.60	15.85	120	322	0.2	308	3.4	98
1-Aminobutyl	15.35	16.45	130	337	0.1	322	1.6	112
1-Aminopentyl	16.00	17.15	150	351	0.1	336	1.6	126
1-Amino-2-methylpropyl	14.95	15.95	130	337	0.1	322	1.0	112
1-Amino-2-phenylethyl	19.05	20.95	180	385	1.0	370	4.5	160
1-Amino-2(4-hydroxyphenyl)ethyl	22.45	24.00	220	473	0.5	458	8.0	294
2-Aminoethyl	15.75	17.30	150	309	6.0	294	15	84
3-Aminopropyl	16.50	18.15	160	323	6.5	308	24	84

The N-acetyl/TMS derivatives of the aminoalkylphosphonates were prepared by treating the TMS esters, synthesized as above, with acetic anhydride in pyridine. These derivatives generally gave single peaks on both GC phases. Peak shapes were not ideal and some tailing was noted, particularly with the 2-aminoethyl and 3-aminopropyl compounds, but separation of most of the aminoalkylphosphonates was achieved. The N-acetyl derivatives had somewhat longer retention times than the N-TMS analogs; methylene unit values are listed in Table III. These derivatives tended to decompose when stored and additional peaks appeared in their gas chromatograms after a few days. It was possible with the lower molecular weight compounds to introduce the N-acetyl group before the phosphonate moiety was silylated, but this procedure could not be applied to all compounds.

Acetone Schiff bases were also prepared from the TMS esters (free NH_2 group) of these compounds. These derivatives gave better peak shapes than the N-acetyl derivatives but were generally less stable and it was more difficult to obtain single peaks. Separation characteristics were similar to those of the other derivatives (Table IV).

The most satisfactory derivatives studied were those in which the amino function was converted into the isothiocyanate group. This was conveniently achieved by the reaction of the TMS ester with carbon disulfide overnight. Analogous derivatives of the amino acids[6,7] and phenylethylamines[8,9] have recently been investigated and found to be satisfactory for the purpose of characterizing these amino compounds by GC–MS. Simple, well-shaped peaks were obtained for the NCS/TMS derivatives of all the aminoalkylphosphonic acids studied. The stability of these compounds was also better than that of the other derivatives. Consequently, good

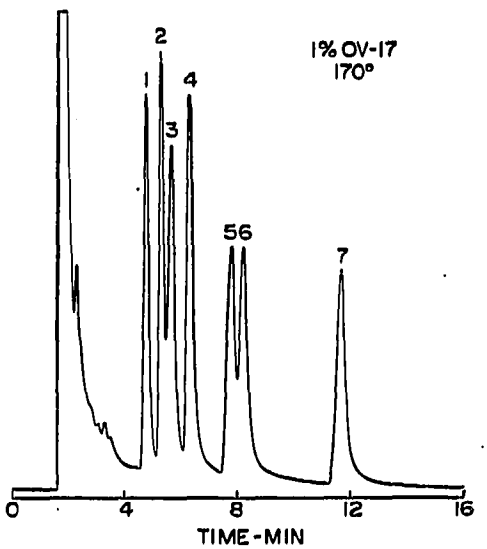


Fig. 2. GC separation of the isocyanate-TMS derivatives of 1-aminoethylphosphonic acid (peak 1), 1-aminopropylphosphonic acid (peak 2), 1-amino-2-methylpropylphosphonic acid (peak 3), 1-aminobutylphosphonic acid (peak 4), 2-aminoethylphosphonic acid (peak 5), 1-aminopentylphosphonic acid (peak 6) and 3-aminopropylphosphonic acid (peak 7). The column was 1% OV-17 (12 ft.) at 170°.

TABLE V

GAS CHROMATOGRAPHIC AND MASS SPECTROMETRIC DATA FOR THE ISOTHIOCYANATE/TMS DERIVATIVES OF THE AMINOPHOSPHONIC ACIDS

Compound	*MU*[a]		*Mass spectrometric data*				
	1% SE-30	*1% OV-17*	*M*+		*[M-15]*+		
			m/e	*% of base peak*	*m/e*	*% of base peak*	*Base peak*
Aminoethylphosphonic acid	15.25	17.20	297	46	282	100	282
1-Aminoethylphosphonic acid	15.35	17.10	311	29	296	29	225
1-Aminopropylphosphonic acid	15.90	17.50	325	21	310	27	225
1-Aminobutylphosphonic acid	16.65	18.20	339	9	324	28	225
1-Aminopentylphosphonic acid	17.50	19.00	353	15	338	33	225
1-Amino-2-methylpropylphosphonic acid	16.35	17.80	339	22	324	34	225
1-Amino-2-phenylethylphosphonic acid	20.80	23.35	387	2.5	372	17	329
1-Amino-2(4-hydroxyphenyl)ethylphosphonic acid	24.35	26.25	475	0.0	460	13	417
2-Aminoethylphosphonic acid	16.75	18.85	311	41	296	42	268
3-Aminopropylphosphonic acid	17.70	19.95	325	33.5	310	63	225

[a] Temperature programmed from 100° at 2°/min.

results were obtained in temperature programmed runs; the other derivatives gave better results under isothermal conditions where retention times were shorter. One of the difficulties encountered with the N-acetyl and Schiff base derivatives was the appearance of additional peaks produced by the N-TMS analogs. This did not occur with the isothiocyanate derivatives; displacement of the N-TMS moiety by the

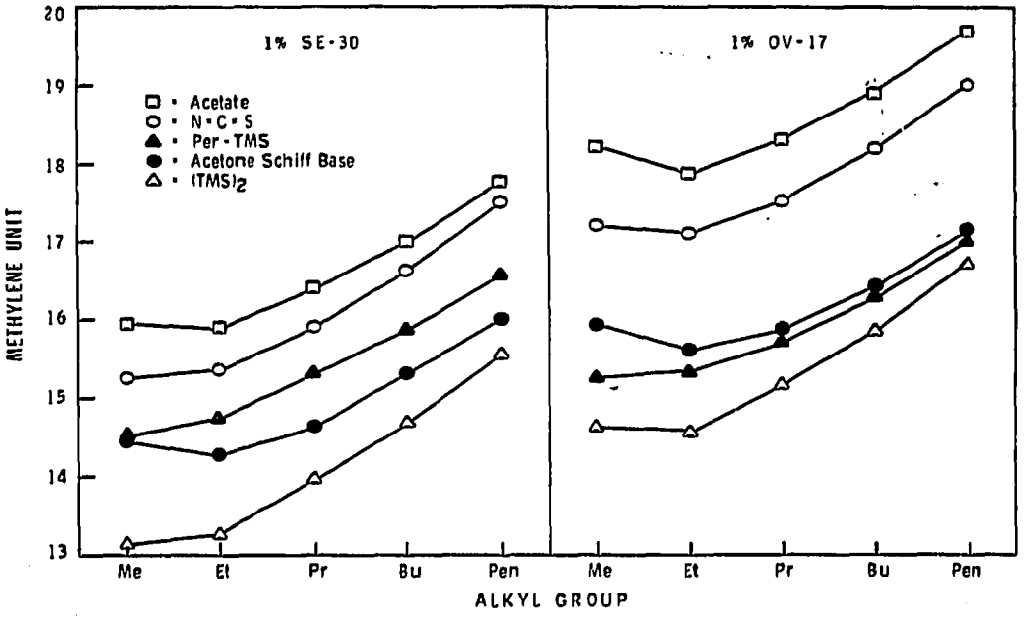


Fig. 3. Comparison of the methylene unit values for the various derivatives of the 1-aminoalkylphosphonic acids (Me-Pen) on two phases.

carbon disulfide over several hours resulted in single peaks for all compounds. It was not possible to achieve good separation of aminomethyl and 1-aminoethylphosphonic acids but all of the other compounds separated on both phases as can be seen from Fig. 2. MU values are listed in Table V. These derivatives could be stored for several months at 0° without appreciable decomposition.

Fig. 3 shows a comparison between the methylene unit values of the different derivatives of the 1-aminoalkylphosphonate on two phases.

MASS SPECTROMETRIC CHARACTERISTICS

The relative abundances of the molecular and $[M-15]^+$ ions are given in the Tables I–V. Full details of the fragmentation pathways and detailed mass spectral information will be published elsewhere.

Molecular ions were present in moderate abundance in the spectra of the alkylphosphonic acids (no amino group) and in all cases the base peak was the $[M-15]^+$ ion. In the spectra of the TMS derivatives of the aminoalkylphosphonates, (both NH_2 and NHTMS analogs), the molecular ions were of very low abundance or completely absent. The N,N-bis-TMS derivatives of 2-aminoethyl- and 3-aminopropylphosphonic acid TMS esters exhibited no molecular ions, but small peaks at $[M-1]^+$ were present. An interesting feature of the spectra of the latter compounds and those of the TMS derivatives of the alkylphosphonates was the appearance of peaks at $M+73$, representing the addition of a trimethylsilyl cation to the neutral molecules in an ion molecule reaction. Reactions of this type have also been noted in the mass spectra of other TMS derivatives[10,11] and have been investigated in more detail[12]. These ions were present at about 1 % relative intensity when the spectra were recorded under normal electron impact conditions. Consequently, in view of the low abundance or absence of molecular ions, care must be taken in assigning molecular weights to unknown phosphonates. The variations in abundance of the $M+73$ ions with ion source pressure (sample concentration) permits their ready identification.

Molecular ions of very low abundance were also observed for the acetone Schiff base derivatives; in these spectra, the dominant ions are produced by charge localization on the nitrogen atoms and subsequent α-cleavage. There was little additional fragmentation. Molecular ions of higher abundance were observed for the N-acetyl and isothiocyanate derivatives (Fig. 4). This is particularly valuable in the case of the isothiocyanates because these derivatives also exhibited the most satisfactory GC properties.

Fragment ions characteristic of the TMS-phosphonate system were present in all spectra at *m/e* 121, 195, 211, and 225–227. Their compositions and structures were determined by means of high resolution mass measurements and deuterium labelling (d_9-TMS derivatives). The ion at *m/e* 225 contains the TMS-phosphonate moiety and is produced by simple cleavage. Ions at *m/e* 226 and 227 are produced by 1 and 2 hydrogen migration to the phosphonate moiety, respectively. The predominant ions in the *m/e* 225–227 range were found to be a function of hydrogen mobility. Abundant ions produced by extensive rearrangement were present in the spectra of most derivatives, especially the N-acetates and isothiocyanates; details of these fragmentations will be published elsewhere.

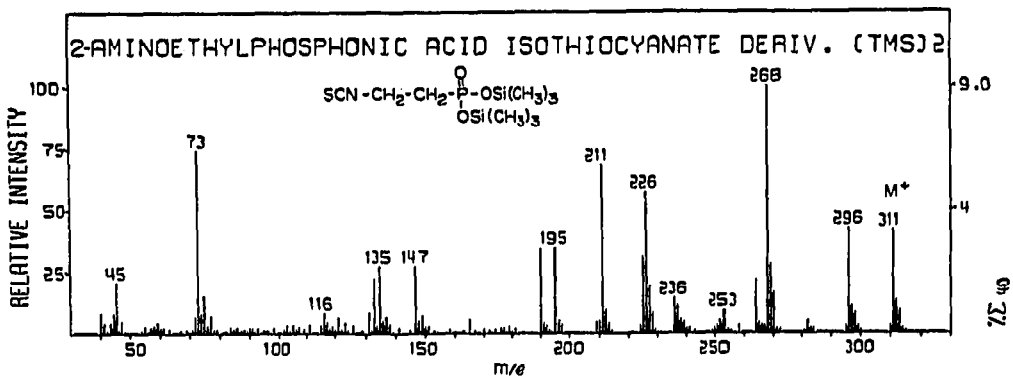


Fig. 4. Mass spectrum (70 eV) of the isocyanate-TMS derivative of 2-aminoethylphosphonic acid. An abundant molecular ion (*m/e* 311) is present. Ions at *m/e* 226, 211 and 195 are characteristic of the TMS-phosphonate moiety. The base peak (*m/e* 268) results from loss of ethylene from the M-15 ion (*m/e* 296).

CONCLUSIONS

Trimethylsilyl derivatives were satisfactory for the characterization of the alkylphosphonates, 2-aminoethyl- and 3-aminopropylphosphonic acids. The 1-aminophosphonates were best characterized as the TMS esters of the isothiocyanate derivative; this derivative was also satisfactory for the 2- and 3-amino compounds. Ions characteristic of the phosphonate system were present in the mass spectra of all the derivatives permitting rapid identification of these compounds. The isothiocyanate derivatives were the most satisfactory of the derivatives studied as they gave good GC characteristics, and their mass spectra were structurally informative.

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