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DETERMINATION OF BIFUNCTIONAL COMPOUNDS

VII*. ETHYLPHOSPHONOTHIOIC DICHLORIDE AS A SELECTIVE REAGENT FOR THE TRACE ANALYSIS OF BIFUNCTIONAL COMPOUNDS BY GAS CHROMATOGRAPHY WITH PHOSPHORUS-SPECIFIC DETECTION

C. F. POOLE, S. SINGHAWANGCHA, L-E. CHEN HU and A. ZLATKIS
Department of Chemistry, University of Houston, Houston, Texas 77004 (U.S.A.)
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SUMMARY

Ethylphosphonothioic dichloride reacts selectively with bifunctional compounds containing OH, NH_2 and COOH groups to form derivatives which are stable to gas chromatography. These derivatives can be determined at the low picogram level with the nitrogen–phosphorus detector or with the flame photometric detector. The cyclic ethylphosphonothioic derivatives produce characteristic mass spectra with prominent molecular ions. The derivatives are suitable for identification purposes by gas chromatography–mass spectrometry and the prominent ion $[M - C_2H_5S]^+$ should be useful for trace analysis by single ion monitoring.

INTRODUCTION

From the advent of gas chromatography (GC), derivatives have been used to improve the thermal stability and chromatographic properties of polar molecules. In many cases, this was the only way these substances could be separated by GC. In recent years the development of sensitive and specific GC detectors has added an extra dimension to the general technique of derivatization —namely a method of introducing a label into a molecule which enables that substance to be determined selectively in a mixture with great sensitivity. If the derivative reaction can be made selective as well as the detection system used, then a simple form of analysis can be devised for selected substances in complex mixtures which does not rely entirely on complete chromatographic resolution. For biological and environmental samples, complete component resolution is rarely achieved with even the most advanced high-performance chromatographic systems.

Largely because of historical precedent, most derivatizing reagents commercially available are broad spectrum in application, reflecting the search for a single reagent which could be used with all functional groups. These reagents can obviously

* For Part VI see ref. 6.

be applied to bifunctional compounds but in this case no discrimination is achieved among the different types of functionalized molecules. The number of selective reagents which can be used for the analysis of bifunctional compounds is few. The most common reagents are the boronic acids, diacetoxydimethylsilanes and dichlorodimethylsilanes, ketones and *o*-phenylenediamine (for a review see ref. 1). With a few exceptions, these reagents are not suitable for use with selective GC detectors, so full advantage could not be taken of their use for trace analysis in complex mixtures. Recently, we have described the properties of a new series of substituted boronic acids for use with the electron-capture detector[2–6] for this purpose.

Although largely unevaluated in terms of analytical potential, many of the non-metal elements of the first three rows of the periodic table (*e.g.* sulfur, selenium, phosphorus, antimony, arsenic, germanium, silicon etc.) are known to form cyclic derivatives with bifunctional compounds. In the case of phosphorus and sulfur, specific element selective detectors are available commercially and widely available in many laboratories. It was for this reason that phosphorus reagents were initially selected for study and are reported here. Jaeger and co-workers[7–12] have prepared phosphorus derivatives of monofunctional compounds with a series of dimethylphosphinic and dimethylthiophosphinic reagents and shown these to have good GC and mass spectrometric (MS) properties. Diethylphosphate derivatives of alcohols and phenols[13,14], Δ^9-tetrahydrocannabinol[15] and amino acid methyl esters[16] are suitable for their determination by GC with selective phosphorus detection. Dimethylthiophosphoryl derivatives were used to analyse eight phenols obtained from the hydrolysis of carbamate pesticides[17]. Chlorophosphorolones have been used for the determination of small amounts of alcohols with the thermionic detector[18].

EXPERIMENTAL

Ethyl dichlorothiophosphate (Aldrich, Milwaukee, Wisc., U.S.A.) and ethylphosphonothioic dichloride (EPTD) (Ethyl Corporation, Ferndale, Mich., U.S.A.) were obtained commercially.

For GC, a Perkin-Elmer Sigma 2 gas chromatograph with flame-ionization (FID), flame photometric (FPD), nitrogen–phosphorus rubidium bead (NPD) and electron-capture (ECD) detectors was used. For the determination of retention index values a 90 cm × 0.2 cm I.D. nickel column packed with 1% OV-17 on Gas-Chrom Q (100–120 mesh) and a nitrogen flow-rate of 30 ml min^{-1} was used. For studies of detector sensitivity a 90 cm × 0.4 cm I.D. glass column packed with the above column material and operated with a nitrogen flow-rate of 30 ml min^{-1} was used.

For GC–MS a Hewlett-Packard 5992A mass spectrometer equipped with a single-stage glass-jet separator and a 6 ft. × 0.4 cm I.D. glass column packed with 3% OV-1 on Gas-Chrom Q (100–120 mesh) and operated with a nitrogen flow-rate of 30 ml min^{-1} was used. Electron-impact mass spectra were recorded at an ionization potential of 70 eV.

For the preparation of derivatives for GC, 15 μl of EPTD and 33 μl of triethylamine were added to a 10 mmole solution of the bifunctional compound in 1.0 ml of acetonitrile. The mixture was heated for 30 to 45 min at 80 °C in a nitrogen atmosphere, cooled to room temperature and a few drops of water added with a Pasteur pipette (sufficient to dissolve all the salt formed). Finally, 1.0 ml of diethyl ether was

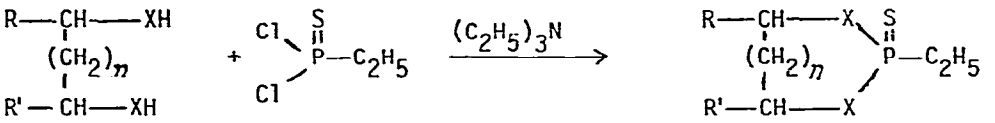
added to the mixture and the solution shaken for 1.0 min. For analysis, a 1.0-μl aliquot was removed from the upper layer and injected into the gas chromatograph with an injection port temperature of 250 °C.

For studies of reaction rate and of derivative stability, 2,7-dimethylnaphthalene was added as an internal standard.

The cyclic ethylphosphonothioic derivative of pinacol was prepared on the preparative scale, essentially by the above method and the fraction boiling between 90–95 °C at 0.4 mmHg collected.

RESULTS AND DISCUSSION

EPTD reacts with bifunctional compounds containing OH, NH_2 and COOH groups in the presence of triethylamine to form cyclic ethylphosphonothioic derivatives according to the following equation:



$n = 0, 1$
XH = OH, NH_2, COOH

EPTD also reacts with *ortho*-substituted bifunctional aromatic compounds as well as with compounds containing enolizable ketone groups (*e.g.* 2,4-pentanedione) to form derivatives stable to GC. Chromatographically stable derivatives were not obtained with dicarboxylic acids (*e.g.* succinic acid), α-hydroxy acids (*e.g.* lactic acid, mandelic acid) or with diols and diamines in which $n \geqslant 2$. Retention index data for a representative series of bifunctional compounds forming EPTD derivatives are summarized in Table I. The structures of all derivatives were confirmed by MS. All derivatives gave symmetrical peaks on the silicone phases used in this study and could be stored for at least 24 h in the extraction solution without appreciable decomposition.

TABLE I

RETENTION INDEX DATA FOR THE CYCLIC ETHYLPHOSPHONOTHIOIC DERIVATIVES

Cyclic ethylphosphonothioic derivative	*Retention index (I_{OV-17})*		*Column temperature (°C)*
	1st Peak	*2nd Peak*	
Ethylene glycol	1563	—	110
1,3-Propanediol	1568	—	110
Pinacol	1639	—	110
Catechol	1733	—	130
3-Amino-1-propanol	1788	—	130
1,3-Propanediamine	1968	—	150
cis-1,2-Cyclohexanediol	1961	2020	150
o-Aminophenol	2042	—	150
Phenyl-1,2-ethanediol	2185	2221	170
o-Phenylenediamine	2385	—	170

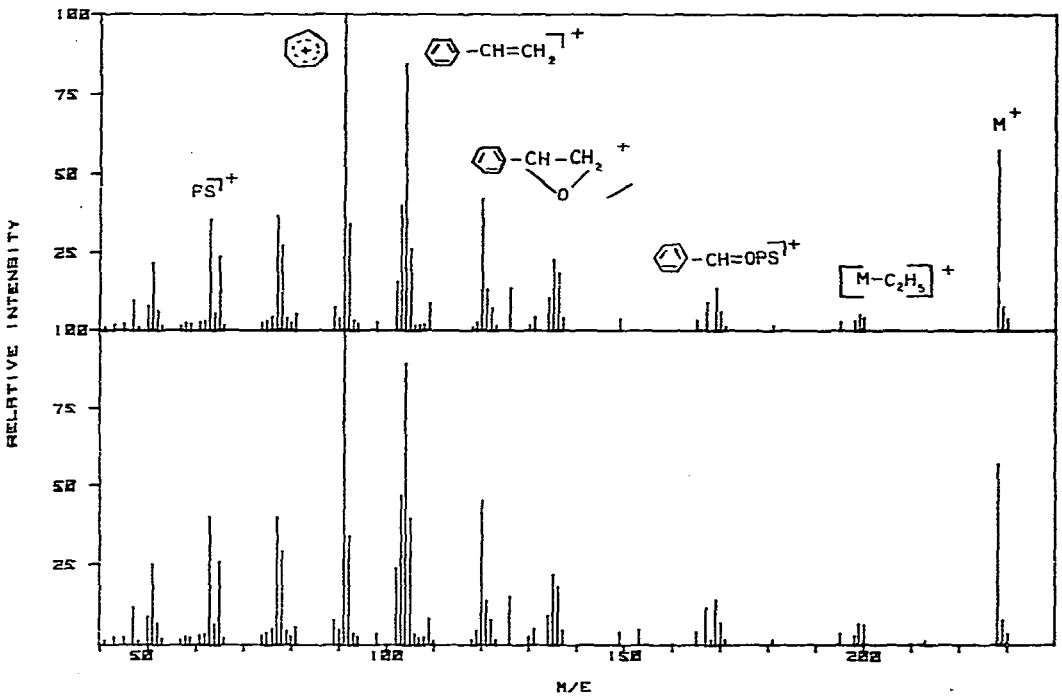
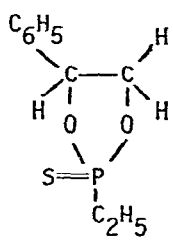
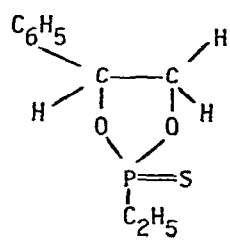


Fig. 1. The mass spectra of the diastereoisomers resulting from the reaction of ethylphosphonothionic dichloride with phenyl-1,2-ethanediol.

Some derivatives produced two peaks on gas chromatography (2nd peak in Table I) and the origins of these peaks were investigated by GC–MS. In the case of phenyl-1,2-ethanediol, two peaks of equal height were obtained which produced essentially identical mass spectra (Fig. 1). These peaks are most probably diastereoisomers (see below) and are not separated on short GC columns.





In the case of the *cis*-1,2-cyclohexanediol derivative, two peaks of approximately equal height were obtained on GC. The mass spectra had identical molecular ions and similar principles modes of fragmentation. The most likely explanation here is that under the conditions used for derivatization *cis–trans* isomerization occurred giving a mixture of the two geometrical isomers.

Although the reaction rate depends on the steric arrangement and the chemical nature of the bifunctional group (aromatic bifunctional compounds react slower than their aliphatic equivalent) the reactions of all compounds studied were complete

within 30–45 min at 80 °C. With the exceptions mentioned above, single peaks free of any significant byproducts were obtained in all cases.

In an exactly similar manner to that described for EPTD, ethyl dichlorothiophosphate was tested. This reagent did not form any derivatives stable to GC, perhaps due to the greater lability of the phosphorus–ethoxy bond compared to the phosphorus–ethyl bond under the conditions necessary for derivative formation.

A derivative of pinacol was prepared to assess the possibility of using different selective detectors for the sensitive determination of the cyclic ethylphosphonothioic derivatives. Phosphorus can be determined with the NPD and the FPD, sulfur can be determined with the FPD and also the thiophosphono group can be determined with the ECD[19]. Optimum conditions of operation were established for each detector and the minimum detectable quantity (MDQ) for the pinacol derivative determined. The results are summarized in Table II. Hetero-element detection limits are in agreement with the manufacturers specifications for the instrument with the most favorable detection limit being obtained with the NPD. This detector also responds to nitrogen in the NP mode so that the FPD would be preferrable for some applications when a high degree of selectivity is required. To optimize the ECD a study of the effect of detector temperature on detector response (Fig. 2) was made[20]. This indicated a dissociative mechanism of electron-capture with the greatest response being obtained at high detector temperatures. However, with this detector at 385 °C, detection limit is not sufficient for general use.

TABLE II

MINIMUM DETECTABLE QUANTITY (MDQ) OF THE EPTD DERIVATIVE OF PINACOL

Detector	*MDQ*		
	Pinacol ($\times 10^{-9}$)	*P* ($g\ sec^{-1}$, $\times 10^{-15}$)	*S* ($g\ sec^{-1}$, $\times 10^{-10}$)
Nitrogen–phosphorus detector	0.002	2.0	—
Flame photometric detector (P mode)	0.5	2000	—
Flame photometric detector (S mode)	1.0	—	2.0
Electron-capture detector	10.0	—	—

The principal phosphorus-containing ions in the mass spectra of the cyclic ethylphosphonothioic derivatives of the bifunctional compounds studied are summarized in Table III. These ions often constitute an appreciable quantity of the total ion current for the mass spectrum. A notable feature of the spectra is the prominent molecular ion observed in all cases. The loss of the fragments of ethyl sulfide from the molecular ion is a facile cleavage providing the base peak for many of the mass spectra. This plus the ions $[C_2H_5PS]^5$ (*m/e* 92] and $[PS]^+$ (*m/e* 63) serve to characterize the derivatives. As a general observation, those derivatives with an oxygen atom bonded directly to phosphorus tend to show a loss of ethene $[C_2H_4]$ from the molecular ion while those with phosphorus nitrogen bonds shows a preferential loss of ethane $[C_2H_5]$ (see Table III). The mass spectra of the EPTD derivatives of ethylene glycol

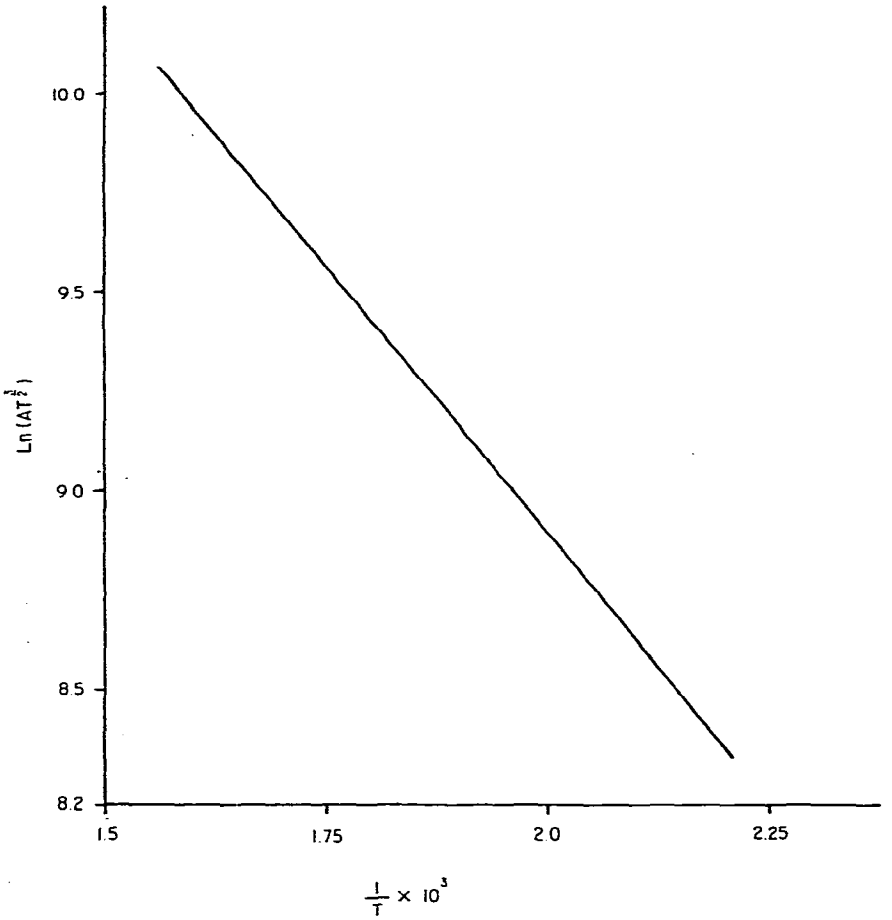


Fig. 2. The effect of detector temperature on detector response for the EPTD derivative of pinacol, plotted in the form of ln $AT^{3/2}$ *vs.* $1/T$ where A = peak area for a fixed amount of derivative and T = detector temperature in °K.

TABLE III

PRINCIPAL IONS IN THE MASS SPECTRA OF THE EPTD DERIVATIVES OF SOME BIFUNCTIONAL COMPOUNDS

Compound	*Relative abundance*					
	M^+	$[M-C_2H_4]$	$[M-C_2H_5]$	$[M-C_2H_5S]$	C_2H_5PS (*m/e* 92)	*PS* (*m/e* 63)
Ethylene glycol	51.2	31.4	4.5	100	7.8	14.0
Phenyl-1,2-ethanediol	56.6	5.7	5.9	11.4	34.3	40.2
Pinacol	39.6	0.2	0.2	7.5	59.9	9.5
cis-1,2-Cyclohexanediol	29.6	1.5	—	28.5	3.9	14.2
1,3-Propanediol	48.5	24.5	2.7	100	2.5	12.8
2,4-Pentanedione	100	7.9	4.9	38.5	2.3	21.8
Catechol	33.8	11.7	3.2	100	12.4	28.1
o-Phenylenediamine	47.1	4.0	39.7	100	7.3	8.9
o-Aminophenol	30.0	4.8	9.6	100	1.6	—
Salicylic acid	41.0	8.1	1.9	100	53.9	30.1
Anthranilic acid	13.1	8.1	100	21.9	20.5	
1,3-Propanediamine	85.4	11.6	93.7	100	18.9	55.1
3-Aminopropanol	54.3	7.1	8.2	100	3.9	21.3

(Fig. 3), *o*-phenylenediamine (Fig. 4) and anthranilic acid (Fig. 5) can be considered to be typical of the spectra studied here.

Compared to the monofunctional dimethylthiophosphinic derivatives studied by Jacob, Vogt and co-workers[9–12] no evidence for partial isomerization of the P–O, P–S bonds was observed and thus the mode of fragmentation for the bifunctional derivatives is different to that of the monofunctional derivatives described by these workers.

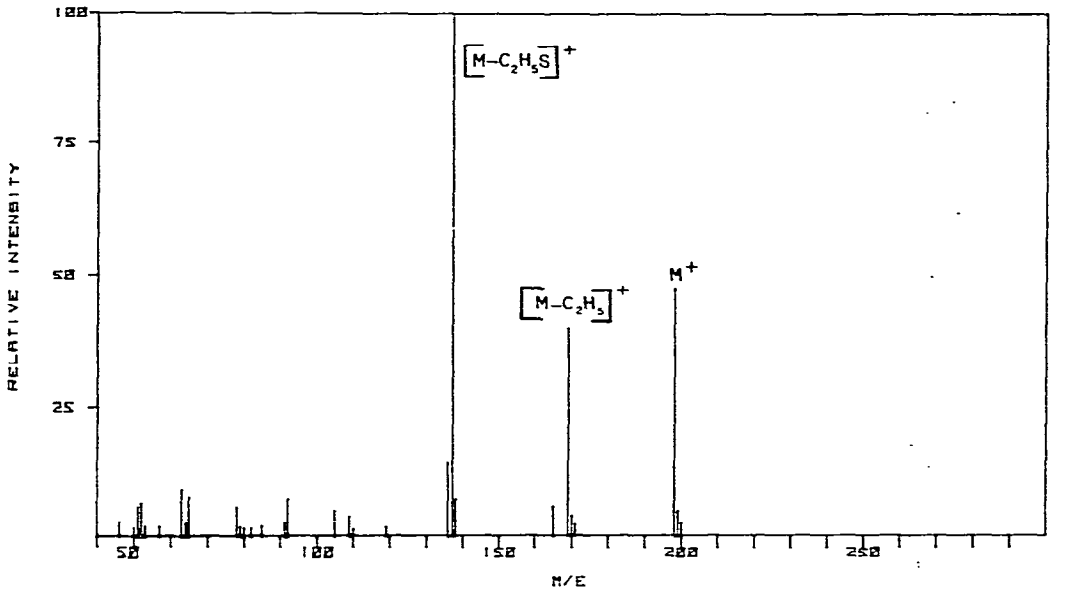


Fig. 3. Mass spectrum of the EPTD derivative of ethylene glycol.

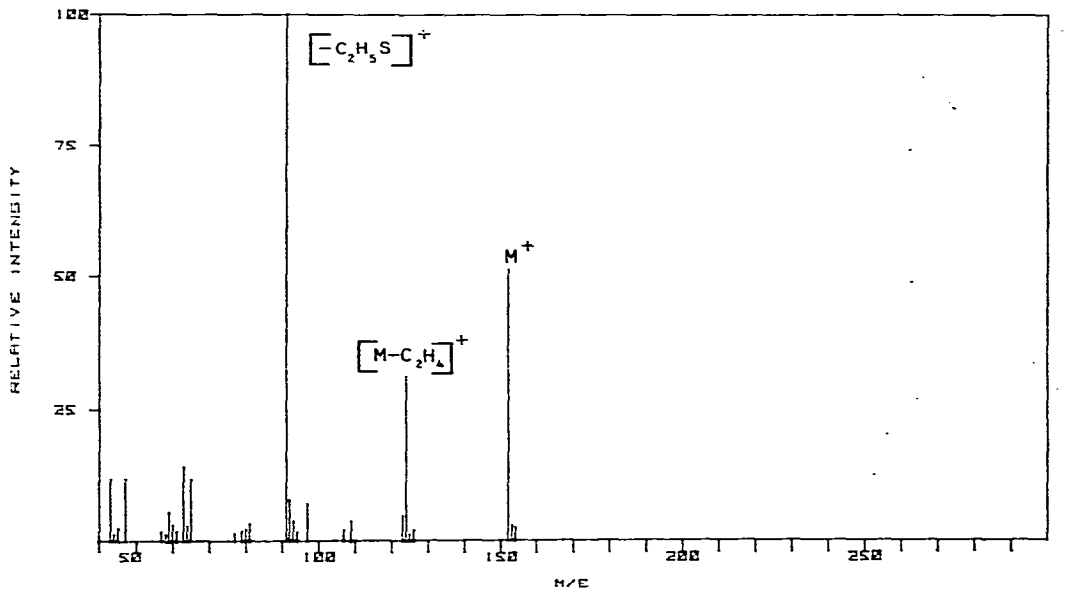


Fig. 4. Mass spectrum of the EPTD derivative of *o*-phenylenediamine.

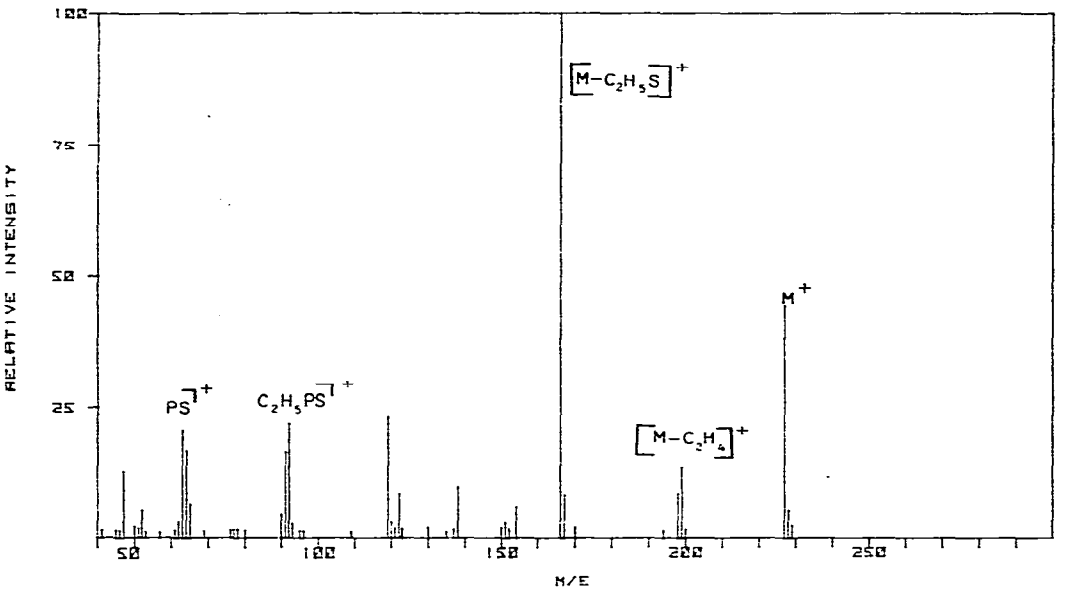


Fig. 5. Mass spectrum of the EPTD derivative of anthranilic acid.

An analysis of the mass spectra of the diol derivatives indicated that the four ions, *m/e* 65, 97, 109 and 126 (Table IV) were characteristic of aliphatic dihydroxy derivatives (phenolic OH groups do not produce these fragments). These ions were either absent or not generally prominent in the spectra of the diamines or aromatic carboxylic acid derivatives.

TABLE IV

CHARACTERISTIC IONS IN THE MASS SPECTRA OF EPTD DIOL DERIVATIVES

Compound	*Relative abundance*			
	$P(OH)_2^+$*, *m/e 65*	$P(OH_2S)^+$, *m/e 97*	$C_2H_5PS(OH)^+$, *m/e 109*	$C_2H_5PS(OH)_2^+$, *m/e 126*
Ethylene glycol	11.6	7.0	3.7	2.0
Phenyl-1,2-ethanediol	25.7	0.7	7.9	14.9
Pinacol	0.9	—	20.7	10.4
cis-1,2-Cyclohexanediol	9.1	11.2	11.2	2.2
2,4-Pentanedione	27.9	28.8	13.0	6.4
Propane-1,3-diol	21.4	3.9	7.7	3.5

* An alternative assignment could be $[H_2PS]^+$ (ref. 9).

CONCLUSIONS

The cyclic ethylphosphonothioic derivatives of bifunctional compounds are thermally and hydrolytically stable. They can be determined at the low picogram level using the NPD or the FPD. The derivatives have characteristic mass spectra with prominent molecular ions suitable for identification and analysis.

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REFERENCES

1 A. Darbre, in K. Blau and G. S. King (Editors), *Handbook of Derivatives for Chromatography*, Heyden, London, 1977, pp. 262–316.
2 C. F. Poole, S. Singhawangcha and A. Zlatkis, *Chromatographia*, 11 (1978) 347.
3 C. F. Poole, S. Singhawangcha and A. Zlatkis, *J. Chromatogr.*, 158 (1978) 33.
4 C. F. Poole, S. Singhawangcha and A. Zlatkis, *Analyst (London)*, 104 (1979) 82.
5 S. Singhawangcha, C. F. Poole and A. Zlatkis, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 77.
6 C. F. Poole, S. Singhawangcha and A. Zlatkis, *J. Chromatogr.*, (1979) in press.
7 W. Vogt, K. Jacob and M. Knedel, *J. Chromatogr. Sci.*, 12 (1974) 658.
8 K. Jacob, W. Vogt, I. Fischer and M. Knedel, *Tetrahedron Lett.*, (1975) 1927.
9 K. Jacob, W. Vogt, M. Knedel and W. Scanfer, *Biomed. Mass Spectrom.*, 3 (1976) 64.
10 K. Jacob, W. Vogt, G. Schwertfeger, E. Maier, A. Ohnesorge and M. Knedel, in M. Eggstein and H. M. Liebich (Editors), *Mass Spectrometry and Combined Techniques in Medicine, Clinical Chemistry and Clinical Biochemistry*, Tübingen, 1977, p. 246.
11 K. Jacob and W. Vogt, *J. Chromatogr.*, 150 (1978) 339.
12 K. Jacob, C. Falkner and W. Vogt, *J. Chromatogr.*, 167 (1978) 67.
13 P. G. Deo and P. H. Howard, *J. Ass. Offic. Anal. Chem.*, 61 (1978) 210.
14 W. A. Aue, *Advan. Chem. Ser.*, 104 (1971) 53.
15 N. K. McCallym, *J. Chromatogr. Sci.*, 11 (1073) 508.
16 G. Ertingshausen, C. W. Gehrke and W. A. Aue, *Separ. Sci.*, 2 (1967) 681.
17 M. C. Bowman and M. Beroza, *J. Ass. Offic. Anal. Chem.*, 50 (1967) 926.
18 R. Vilceanu and P. Schulz, *J. Chromatogr.*, 82 (1973) 279.
19 C. E. Cook, C. W. Stanley and J. E. Barney, *Anal. Chem.*, 36 (1964) 2354.
20 C. F. Poole, *J. Chromatogr.*, 118 (1976) 280.