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DERIVATIZATION METHOD FOR THE HIGH-SENSITIVE DETERMINATION OF AMINES AND AMINO ACIDS AS DIMETHYLTHIOPHOSPHINIC AMIDES WITH THE ALKALI FLAME-IONIZATION DETECTOR

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SUMMARY

A derivatization method for the high-sensitive gas chromatographic determination of primary amines with the alkali flame-ionization detector is presented. Amino-containing compounds easily react with dimethylthiophosphinic chloride in the presence of triethylamine between −20 and + 20°. These derivatives show good gas chromatographic properties. The detection limit of N-dimethylthiophosphinylaniline was 500 fg.

The mass spectra of these amine derivatives were studied. The fragmentation pathways were elucidated by means of precise mass measurements and decoupled metastable transition determinations.

INTRODUCTION

Gas chromatography (GC) is widely used for the determination of organic amines. They can be separated on strongly basic stationary phases in their free form with a detection limit in the lower nanogram range[1]. For more sensitive quantitation at the picogram level, fluorine-containing derivatives have been used by several workers[2,3]. Another approach using the alkali flame ionization detector (AFID) has been described by Ertingshausen *et al.*[4]. In this method, the amino groups of various amino acids were derivatized to the corresponding N-diethylphosphorylamino acid methyl esters.

We have shown that numerous hydroxyl-containing compounds of biological interest can easily be converted into the corresponding esters by reaction with dimethylthiophosphinic chloride[5–8]. These derivatives show very good gas chromatographic properties, the lowest detectable amount being 100 fg.

The encouraging results on hydroxylated compounds prompted us to apply this derivatization procedure to primary amines and amino acids. The results obtained so far with these derivatives are presented in this paper.

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EXPERIMENTAL

Reagents and materials

All reagents and solvents were of analytical-reagent grade, with the exception of dimethylthiophosphinic chloride, which was obtained from Riedel-de Haën (Seelze, G.F.R.) and used after destillation as a 1 *M* solution in carbon tetrachloride or diethyl ether. Triethylamine was used as a 1 *M* solution in the same solvents.

Apparatus

A Model 1440 gas chromatograph (Varian, Palo Alto, Calif., U.S.A.), injector temperature 250°, with a 25 m × 0.35 mm I.D. SE-30 or OV-17 glass capillary column (LKB Producter, Bromma, Sweden), was used, with temperature programming from 90 to 280° at 6°/min, carrier gas helium at a flow-rate of 3 ml/min at 150° and a splitting ratio of 10:1. The temperature of the FID and AFID (rubidium sulphate) was 270°.

Mass spectra were obtained with a Varian-MAT 311 A double-focusing mass spectrometer using an SS 166 data system (Varian-MAT, Bremen, G.F.R.). The ionization energy was 70 eV and the source temperature was maintained at 200°. Sample introduction was performed with either a solid inlet probe for high-resolution measurements (R = 10,000) or a Varian Model 1440 gas chromatograph equipped with a 25-m SE-30 capillary column for low-resolution measurements (R = 1000). The column was coupled to the ion source of the mass spectrometer with an open split-type connection[9], which consisted of a platinum capillary (50 cm × 0.15 mm I.D.). The GC conditions were as described above. Decoupled metastable transitions were recorded by the direct analysis of daughter ions (DADI) technique[10] and the ion-defocusing technique[11]. These transitions are indicated by asterisks in the fragmentation scheme.

Method

The amine (1 μmole) dissolved in 100 μl of carbon tetrachloride was treated with 20 μl of dimethylthiophosphinic chloride solution and 50 μl of triethylamine solution for 5–60 min between −20 and + 20°. With amine hydrochlorides, 100 μl of triethylamine solution were used. After removing the volatiles with nitrogen, 100 μl of methanol and 30 mg of sodium hydrogen carbonate were added. The methanolysis was carried out for 30 min at 50° as described earlier[6,8]. The reaction products were dissolved in acetone and injected into the gas chromatograph.

Derivatization on the preparative scale was performed in the manner described above using a 1.5-fold excess of dimethylthiophosphinic chloride and the tertiary base.

RESULTS AND DISCUSSION

Primary aliphatic, aromatic and heterocyclic amines react with dimethylthiophosphinic chloride in the presence of excess of triethylamine to give the corresponding N-dimethylthiophosphinic amides:

$$R\text{–}NH_2 + ClP(S)Me_2 \xrightarrow{NEt_3} R\text{–}NH\text{–}P(S)Me_2 + HCl$$

TABLE I

PHOSPHINYLATED COMPOUNDS INVESTIGATED

Methylamine	Cyclohexylamine	2-Aminopyridine
Ethylamine	Aniline	3-Aminopyridine
Isopropylamine	*o*-Toluidine	Glycine methyl ester
Allylamine	*p*-Toluidine	Alanine methyl ester
sec.-Butylamine	*m*-Xylidine	Valine methyl ester
n-Butylamine	Anisidine	Leucine methyl ester
Ethylenediamine	*o*-Phenylenediamine	Phenylalanine methyl ester
Putrescine	*p*-Phenylenediamine	γ-Aminobutyric acid methyl ester
Benzylamine	α-Naphthylamine	

This reaction is well known for the synthesis of such derivatives on the preparative scale[12–14].

The derivatives (Table I) are easily formed between −20 and +20°. By treating the reaction solution with methanol–sodium hydrogen carbonate the excess of dimethylthiophosphinic chloride can be removed. For high-sensitive determination an additional purification by thin-layer chromatography is necessary.

With amino acids prior to the phosphinylation step, the carboxylic group is selectively methylated with methanol–hydrochloric acid at room temperature. During the phosphinylation of some α-amino acids we observed the formation of dipeptides with one phosphinic group as by-products. Therefore, the reaction conditions for these compounds have to be optimized so as to achieve uniform reaction products.

Dimethylthiophosphinic amides show excellent stability against hydrolysis. Thus the derivatives of aniline, cyclohexylamine and ethylenediamine were not affected by a 3-h treatment with 20% aqueous methanol (50°) or 20% aqueous dioxane

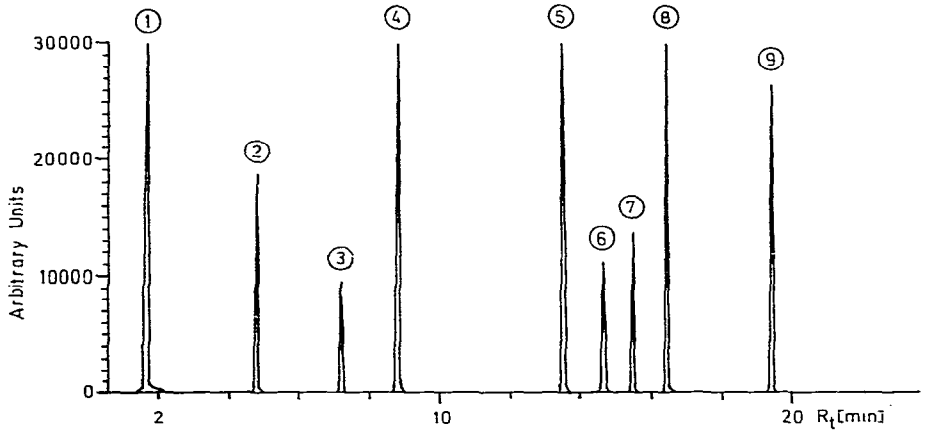

Fig. 1. Mass chromatogram (*m/e* 93) of a mixture of N-phosphinylated primary amines after gas chromatographic separation on a 25 m × 0.35 mm I.D. SE-30 glass capillary column (90–230°; 6°/min). Peaks: 1 = dimethylthiophosphinic acid methyl ester, 2 = N-dimethylthiophosphinylethylamine; 3 = N-dimethylthiophosphinyl-*sec.*-butylamine; 4 = N-dimethylthiophosphinyl-*n*-butylamine; 5 = N-dimethylthiophosphinylcyclohexylamine; 6 = N-dimethylthiophosphinylaniline; 7 = N-dimethylthiophosphinyl-*o*-toluidine; 8 = N-dimethylthiophosphinylbenzylamine; 9 = N-dimethylthiophosphinylanisidine.

TABLE II

MASS SPECTRA OF ALIPHATIC N-DIMETHYLTHIOPHOSPHINYLAMINES (TEN MOST INTENSE IONS)

Compound	*m/e (%)*
Methylamine	123[M]$^+$ (100), 108 (68), 94 (86), 93 (56), 79 (73), 76 (20), 65 (25), 63 (18), 62 (22), 60 (36)
Ethylamine	137[M]$^+$ (100), 122 (10), 104 (7), 94 (98), 93 (51), 79 (38), 76 (15), 65 (20), 63 (17), 61 (19)
Allylamine	149[M]$^+$ (17), 116 (6), 94 (100), 93 (21), 79 (19), 78 (6), 65 (12), 63 (7), 61 (6), 56 (97)
Isopropylamine	151[M]$^+$ (8), 118 (2), 94 (16), 93 (12), 79 (4), 76 (3), 65 (4), 62 (3), 59 (2), 58 (100)
sec.-Butylamine	165[M]$^+$ (20), 136 (5), 132 (12), 94 (54), 93 (38), 76 (7), 72 (100), 65 (6), 56 (6), 55 (8)
n-Butylamine	165[M]$^+$ (62), 132 (11), 122 (6), 94 (93), 93 (46), 79 (10), 76 (10), 72 (100), 65 (8), 56 (4)
Benzylamine	199[M]$^+$ (56), 166 (48), 107 (52), 106 (100), 94 (60), 93 (53), 91 (59), 79 (58), 77 (35), 65 (46)
Ethylenediamine	244[M]$^+$ (14), 151 (5), 135 (52), 122 (13), 110 (24), 95 (13), 94 (63), 93 (100), 65 (24), 63 (19)

(100°). The amides can be well separated on glass capillary columns (Fig. 1). The slight tailing on the non-polar SE-30 phase could be reduced by using OV-17 columns. The detection limit for N-dimethylthiophosphinylaniline was 500 fg with a two-fold signal-to-noise ratio.

For these derivatives, the AFID applied showed a linear range of 1:1000.

These derivatives are also of interest in combined GC–mass spectrometry, as they show intensive molecular ions. Therefore, their mass spectrometric behaviour was investigated in detail.

Mass spectrometry

The dominating ions in the mass spectra of the aliphatic and cycloaliphatic dimethylthiophosphinic amides are presented in Table II. All spectra exhibit abundant

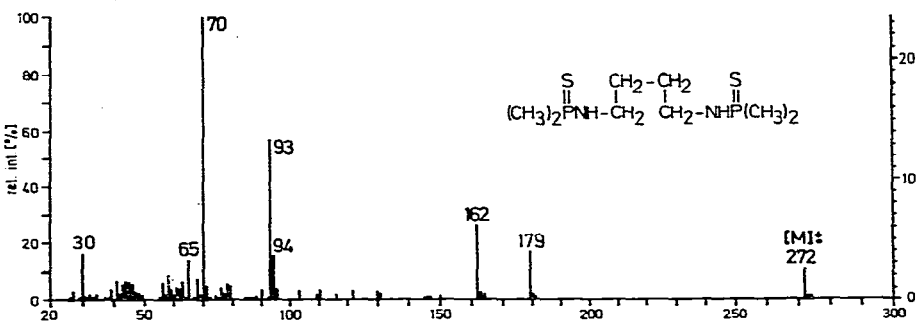

Fig. 2. Mass spectrum (70 eV) of N,N'-bis(dimethylthiophosphinyl)-1,4-diaminobutane.

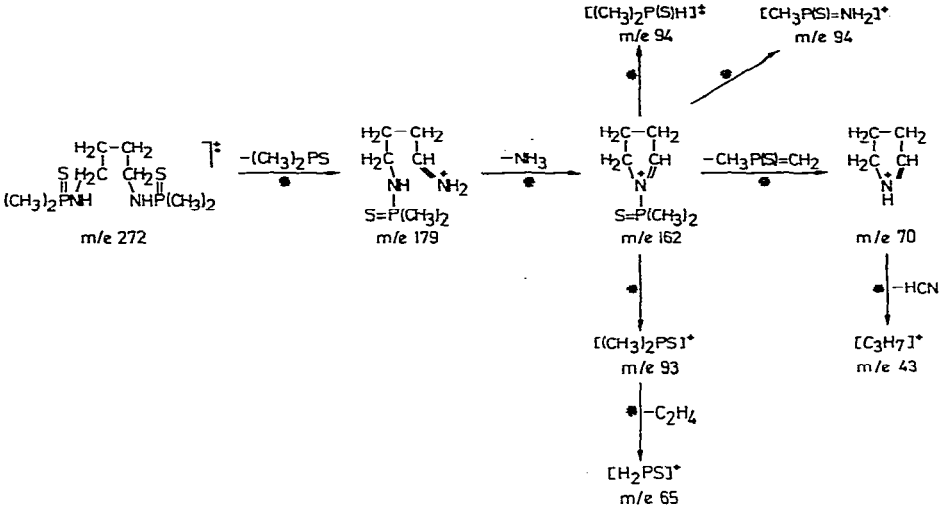

Fig. 3. Fragmentation scheme of N,N'-bis(dimethylthiophosphinyl)-1,4-diaminobutane.

TABLE III

CHARACTERISTIC FRAGMENTS IN THE HIGH-RESOLUTION MASS SPECTRUM OF N,N'-BIS(DIMETHYLTHIOPHOSPHINYL)-1,4-DIAMINOBUTANE

Empirical formula	*Calculated*	*Observed*
$C_8H_{22}N_2P_2S_2$	272.0698	272.0709
$C_6H_{16}N_2PS$	179.0771	179.0774
$C_6H_{13}NPS$	162.0505	162.0498
C_2H_7PS	94.0005(a)*	94.0001
CH_5NPS	93.9879(b)*	93.9876
C_2H_6PS	92.9927	92.9922
C_4H_8N	70.0657	70.0658
H_2PS	64.9614	64.9615

* Doublet: (a) = 10%, (b) = 90% relative intensity.

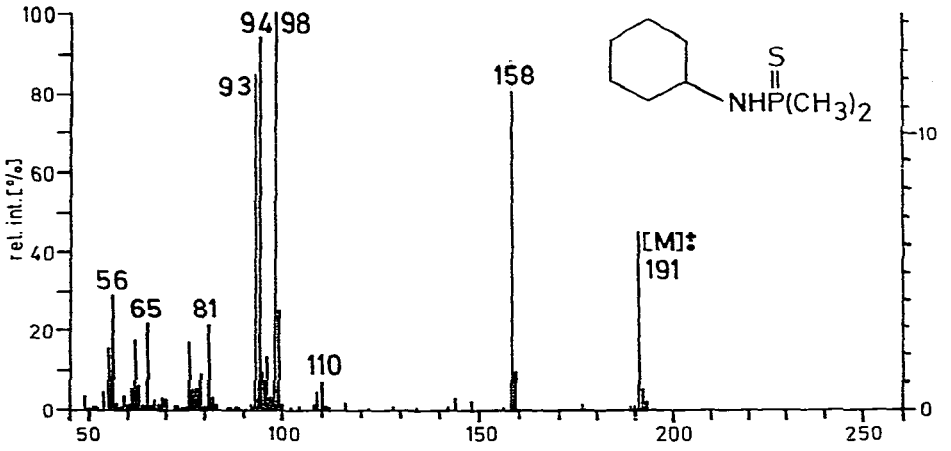

Fig. 4. Mass spectrum (70 eV) of N-dimethylthiophosphinylcyclohexylamine.

TABLE IV

CHARACTERISTIC FRAGMENTS IN THE HIGH-RESOLUTION MASS SPECTRUM OF N-DIMETHYLTHIOPHOSPHINYLCYCLOHEXYLAMINE

Empirical formula	*Calculated*	*Observed*	*Origin**
$C_8H_{18}NPS$	191.0897	191.0905	M^+
$C_8H_{17}NP$	158.1097	158.1097	M−SH
$C_6H_{12}N$	98.0969	98.0965	$M-(CH_3)_2PS$; *m/e* 158 $-CH_3P(CH_2)$
C_2H_7PS	94.0005(a)**	94.0015	—
CH_5NPS	93.9879(b)**	93.9888	*m/e* 109
C_2H_6PS	92.9927	92.9939	*m/e* 110
C_6H_9	81.0704	81.0703	*m/e* 98 $-NH_3$

* Determined by metastable transition measurements.
** Doublet: (a) = 30%, (b) = 70% relative intensity.

molecular ions. For the elucidation of their fragmentation pathways, we studied the spectrum of the putrescine derivative (Fig. 2). The main fragmentations (Fig. 3) are derived from precise mass measurements (Table III) and metastable transition determinations. Contrary to the saturated steroidal dimethylthiophosphinates[15], the expected loss of the phosphinic group by a McLafferty rearrangement was not observed. However, the most important cleavage reaction of the phosphinic group is the elimination of $(CH_3)_2PS$, similar to the fragmentation behaviour of the phenolic dimethylthiophosphinates[16]. Intense P-containing ions are formed by $[(CH_3)_2P(S)H]^+$ (*m/e* 94), $[CH_3P(S)NH_2]^+$ (*m/e* 94), $[(CH_3)_2PS]^+$ (*m/e* 93) and $[H_2PS]^+$ (*m/e* 65).

TABLE V

MASS SPECTRA OF AROMATIC AND HETEROCYCLIC N-DIMETHYLTHIOPHOSPHINYLAMINES (TEN MOST INTENSE IONS)

Compound	*m/e (%)*
2-Aminopyridine	186[M]+ (90), 171 (100), 153 (13), 137 (28), 123 (15), 94 (28), 93 (37), 78 (18), 67 (20), 65 (23)
3-Aminopyridine	186[M]+ (82), 171 (10), 156 (10), 153 (31), 139 (7), 137 (5), 94 (27), 93 (100), 78 (9), 65 (28)
o-Toluidine	199[M]+ (100), 184 (14), 166 (15), 136 (11), 107 (36), 106 (73), 94 (9), 93 (43), 77 (11), 65 (8)
p-Toluidine	199[M]+ (100), 184 (21), 150 (15), 136 (10), 107 (75), 106 (36), 94 (14), 93 (48), 77 (12), 65 (15)
m-Xylidine	213[M]+ (41), 198 (6), 180 (11), 121 (24), 120 (100), 106 (8), 93 (26), 91 (8), 77 (11), 65 (12)
Anisidine	215[M]+ (100), 200 (11), 168 (12), 123 (70), 122 (74), 121 (14), 108 (36), 95 (13), 93 (69), 65 (21)
α-Naphthylamine	235[M]+ (100), 220 (6), 202 (13), 172 (23), 144 (10), 143 (87), 142 (7), 115 (26), 93 (34), 65 (7)
o-Phenylenediamine	292[M]+ (100), 259 (18), 199 (25), 167 (79), 166 (60), 151 (68), 137 (45), 107 (29), 93 (96), 65 (29)
p-Phenylenediamine	292[M]+ (100), 277 (5), 260 (5), 200 (17), 199 (55), 167 (7), 108 (5), 107 (67), 93 (76), 65 (9)

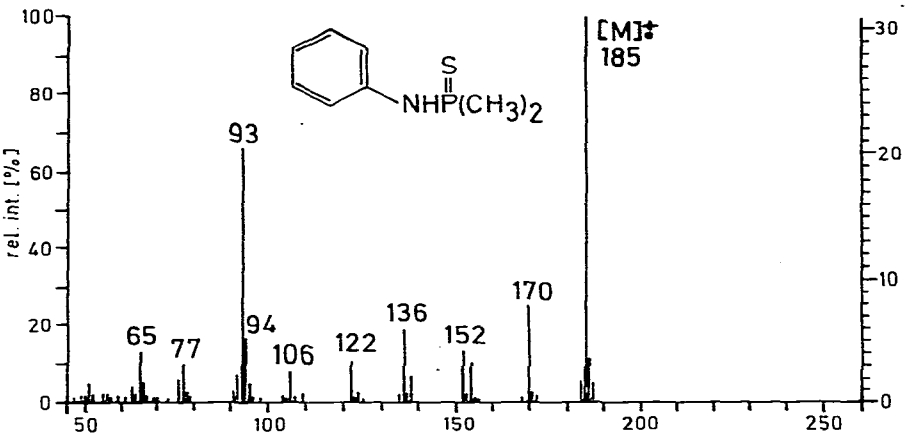

Fig. 5. Mass spectrum (70 eV) of N-dimethylthiophosphinylaniline.

TABLE VI

CHARACTERISTIC FRAGMENTS IN THE HIGH-RESOLUTION MASS SPECTRUM OF N-DIMETHYLTHIOPHOSPHINYLANILINE

Empirical formula	*Calculated*	*Observed*	*Origin**
$C_8H_{12}NPS$	185.0426	185.0421	M^+
C_7H_9NPS	170.0192	170.0203	$M-CH_3$
$C_8H_{11}NP$	152.0629	152.0613	$M-SH$
C_7H_7NP	136.0316	136.0319	*m/e* 170–H_2S
C_6H_5NP	122.0159	122.0155	—
CH_5NPS	93.9879	93.9862	M
C_6H_7N	93.0578(a)**	93.0580	$M-CH_3P(S)CH_2$
C_2H_6PS	92.9927(b)**	92.9944	M
C_6H_5	77.0391	77.0390	M; *m/e* 136; *m/e* 122

* Determined by metastable transition measurements.

** Doublet: (a) = 50%, (b) = 50% relative intensity.

TABLE VII

MASS SPECTRA OF N-DIMETHYLTHIOPHOSPHINYLAMINO ACID METHYL ESTERS (TEN MOST INTENSE IONS)

Compound	*m/e (%)*
Glycine	181[M]+ (51), 149 (16), 122 (19), 109 (22), 94 (74), 93 (100), 88 (61), 79 (17), 65 (23), 63 (14)
Alanine	195[M]+ (80), 136 (76), 109 (22), 103 (20), 102 (100), 94 (76), 93 (72), 79 (21), 70 (15), 65 (35)
Valine	223[M]+ (13), 164 (17), 130 (81), 109 (34), 94 (82), 93 (100), 72 (33), 70 (45), 65 (33), 55 (16)
Leucine	237[M]+ (11), 178 (12), 149 (17), 144 (81), 102 (16), 94 (89), 93 (100), 88 (88), 86 (20), 65 (31)
Phenylalanine	271[M]+ (17), 222 (16), 180 (24), 178 (72), 120 (15), 109 (70), 94 (53), 93 (100), 91 (23), 65 (14)

The fragmentation routes in the spectrum of the cyclohexylamine derivative (Fig. 4) occur with only slight modifications. The results of accurate mass measurements are given in Table IV.

Characteristic fragments of the aromatic and heterocyclic dimethylthiophosphinic amides are given in Table V. The spectra are dominated by highly intense molecular ions, even forming the base peak in many instances. The mass spectrum of the aniline derivative is presented in Fig. 5. The elemental compositions of essential fragments and their origins were determined by high-resolution and metastable transition measurements (Table VI).

The fragmentation patterns of the mass spectra of the α-amino acid derivatives (Table VII) are nearly identical. They contain abundant amine fragments $[M - COOCH_3]^+$. The phosphinic groups are exclusively eliminated by the expulsion of a $(CH_3)_2PS$ radical. As expected, an intense ester fragment is observed only in the spectrum of the phenylalanine derivative at *m/e* 180.

A different fragmentation behaviour is exhibited in the spectrum of the γ-aminobutyric acid derivative (Fig. 6). From the molecular ion are lost CH_3OH (*m/e* 177) and $(CH_3)_2PS$ (*m/e* 116). Both ions lead to the key fragment at *m/e* 84, which is a well documented cleavage product in the spectra of glutamic acid derivatives[17].

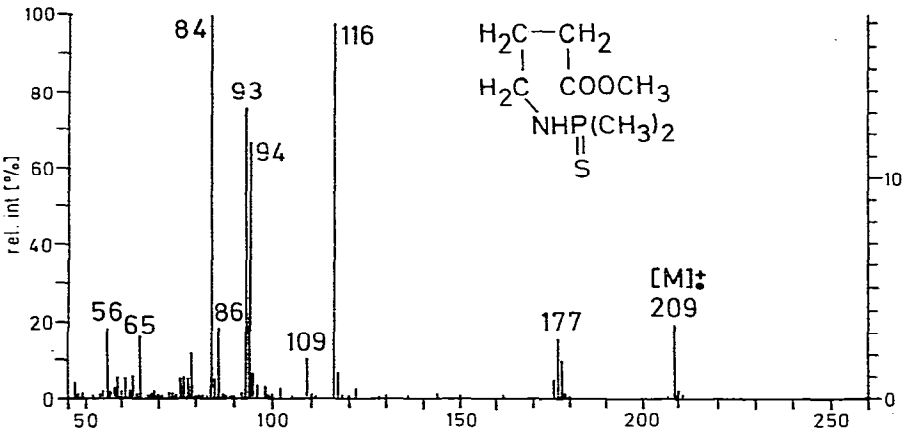

Fig. 6. Mass spectrum (70 eV) of N-dimethylthiophosphinyl-γ-aminobutyric acid methyl ester.

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