A Thesis entitled

STUDIES ON THE RESIDUE CHEMISTRY OF SOME ORGANOPHOSPHORUS PESTICIDES

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by

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RESULTS AND DISCUSSION

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SUMMARY

STUDIES ON THE RESIDUE CHEMISTRY OF SOME ORGANOPHOSPHORUS PESTICIDES

VERA MARIA SOARES

The plan of working has involved synthetic organic chemistry and analytical chemistry related to specific studies on two organophosphorus pesticides, viz. O,O-diethyl S-(N-ethoxycarbonyl-N-methylcarbamoylmethyl) phosphorodithioate (common name mecarbam) and O,O-diethyl S-chloromethyl phosphorodithioate (common name chlormephos).

The preparation of a full range of possible metabolites and their methylated derivatives was carried out and the compounds were characterised by mass spectrometry, nuclear magnetic resonance (^{31}P, ^{13}C, and ^{1}H), and infrared spectroscopy. The spectroscopic data were discussed in terms of structure.

The compounds have also been studied by both electron impact and fast atom bmbardment (FAB) mass spectrometry. In general the FAB spectra gave parent ions stronger than from the electron impact technique. The FAB technique, which has not previously been applied to these types of compounds, also presented additional modes pf fragmentation which are discussed.

Separation of the pesticides and their possible metabolites by gas chromatography has been investigated and the combined technique gc-ms (gas chromatography-mass spectromety) has also shown it is possible to identify certain components by this method.

Hydrolytic studies have shown that mecarbam at 70°C in 50% ethanol (buffer pH 7.0) has a half-life of approximately 3 hours. Chlormephos is considerably more stable. In the presence of alkali, hydrolysis took place by attack either at phosphorus to give O,O-diethyl phosphorothioate or at the carbamoyl carbonyl group to give diethoxyphosphinothioylthioacetic acid. Under similar conditions chlormephos also gave O,O-diethyl phosphorothioate and a number of other products resulting from reaction of the O,O-diethyl phosphorothioate and phosphorodithioate anions with a further molecule of chlormephos.

Degradation in the presence of lamb liver homogenate was shown to be rapid for both compounds. Any metabolites formed were too rapidly degraded for detection to be possible.

HISTORICAL

INTRODUCTION

Pesticides are substances or mixtures of substances that can prevent, destroy or repel different kinds of pests. The ideal pseticide would be the one that once having achieved its insecticidal, fungicidal, or herbicidal action, etc., degraded in the environment giving non-toxic residues. The organophosphorus pesticides usually undergo hydrolytic reactions under natural conditions. Chemical degradation by oxidation and isomerisation are also quite common processes. While the products of hydrolysis normally are non-toxic, the products of oxidation may be very poisonous. In addition, the products of hydrolysis are more soluble in water, more polar and therefore more easily eliminated from the organism. An investigation into the environmental degradation and hydrolysis of organophosphorus pesticides is thus essential.

The present investigations are concerned with the Murphy Chemical products mecarbam (1) [1], chlormephos (2) [2],

$(EtO)_2P(S)SCH_2CONMeCO_2Et$ $(EtO)_2P(S)SCH_2Cl$

1 2

and their metabolites.

Mecarbam is an organophosphorus insecticide and acaricide with slight systemic properties. Although it has been known since 1961, little has been published on its

detailed modes of degradation. Most of the work on mecarbam has been concerned with its toxicity in agricultural use, and with analytical methods for its determination and identification. In the chemical literature, mecarbam has been included in a comparative study of hydrolysis rates of some organophosphorus pesticides [3], in a study on the hydrolysis of organic phosphorus and carbamate pesticides in aquatic environments [4] and in studies on simple analytical methods for traces of pesticides in water and their acute toxicity to fish [5].

The standard analytical procedure for mecarbam is by gas chromatography using a flame ionization or thermionic detector and capillary gas chromatography has also been used [6].

A colorimetric method for the determination of organophosphorus pesticides that was also aplicable to mecarbam, was presented by Syoyma in 1977 [7], who also gave a classification procedure for the rapid identification of insecticides, including mecarbam by thin-layer and gas chromatography [8].

The only reported study on the degradation of mecarbam was published in 1981, giving its major degradation products in water and in crops [9]. The same authors, together with other co-workers, have also reported the electron impact mass spectrometry of mecarbam and of some related O,O-dialkyl S-(N-ethoxy-carbonyl-N-methyl-carbamoylmethyl)

and S-methyl carbamoylmethyl phosphorodithioates and phosphothioates [10].

Chlormephos is a contact insecticide for soil application. There are no reported studies on its mode of degradation but the chemical literature contains many papers on its activity and toxicity in crops. Methods for the determination and identification of chlormephos have been given by several workers. Dougherty and collegues investigated the chloride attachment negative chemical ionization mass spectra of organophosphate pesticides including chlormephos [11].

Gas chromatographic residue analysis of pesticides and their metabolites may not give sufficient information to identify the components of the residue. The complementary spectroscopic methods of infra-red and ultra-violet spectrocopy can be used to solve the structural identity of the residue. Mass spectrometry was first used to give the structural identity of pesticide metabolites in 1962 by Gunther [12]. Since then it has become an invaluable aid in pesticide analysis. The role of mass spectrometry in pesticide residue analysis has been discussed in textbooks such as: Analysis of Pesticide Residues [13], Mass Spectrometry: Technique and Application [14] and in several reviews e.g. The Mass Spectra of some Organophosphorus Pesticide Compounds [15], and Mass Spectra of Organophosphorus Esters and Alteration Products [16].

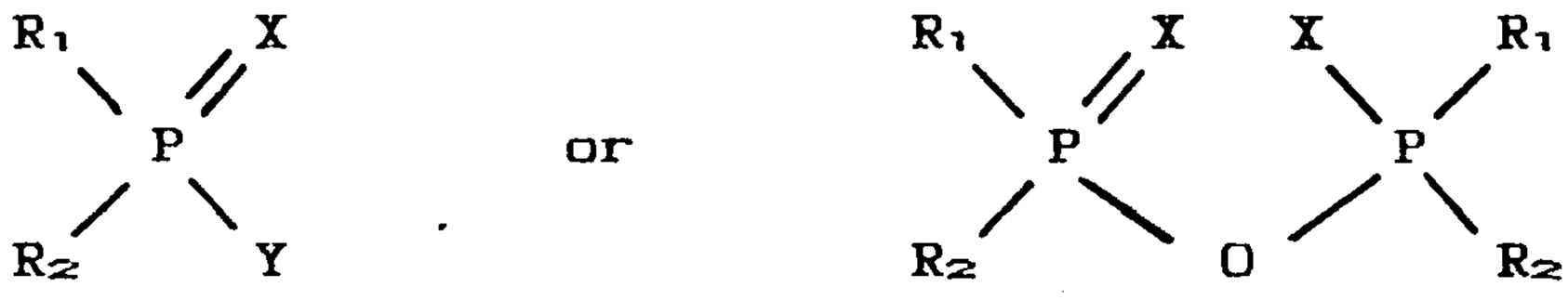
Considering the low sensitivity of complementary techniques such as nuclear magnetic resonance spectrometry, mass spectrometry is still the best approach to solve the identification of pesticide metabolites.

Gas chromatography coupled with mass spectrometry is in fact the best approach because of advantages such as rapid analysis, elimination of the necessity for isolating pure samples, and certainty in the identification of the molecule. Although an expensive technique for use in routine analysis, it is very useful in research because it obviates the necessity of using two or more techniques in obtaining definitive structural information. Diverse techniques such as electron impact, and field and chemical ionization have been used in pesticide mass spectrometry but eletron impact mass spectrometry is used mainly. Damico *et al* [17], compared field ionization with electron impact. Field ionozation gives more details about structural information while electron impact leaves in many cases doubts with reference to the identity of the molecular ion and its mode of decomposition. Fales *et al* [18], reported similar differences between chemical ionization and electron impact technique. Other reviews, such as Recent Applications of Mass Spectrometry and Combined Gas Chromatography-Mass Spectrometry to Residue Analysis [19], and Pesticide Mass Spectrometry [20], demonstrate that chemical ionization and low resolution electron impact mass spectrometry are complementary techniques in structure determination.

FAB (fast atom bombardment) is the most recent ionization technique for use in mass spectromety [21]. No reports on the use of FAB mass spectrometry for organophosphorus pesticides have been found in chemical literature so far. Such a technique could be useful for non-volitile or thermally unstable residues. The present studies have therefore included an investigation of the behaviour of the organophosphorus pesticides mecarbam and chlormephos and some other representative types under the conditions of FAB mass spectrometry.

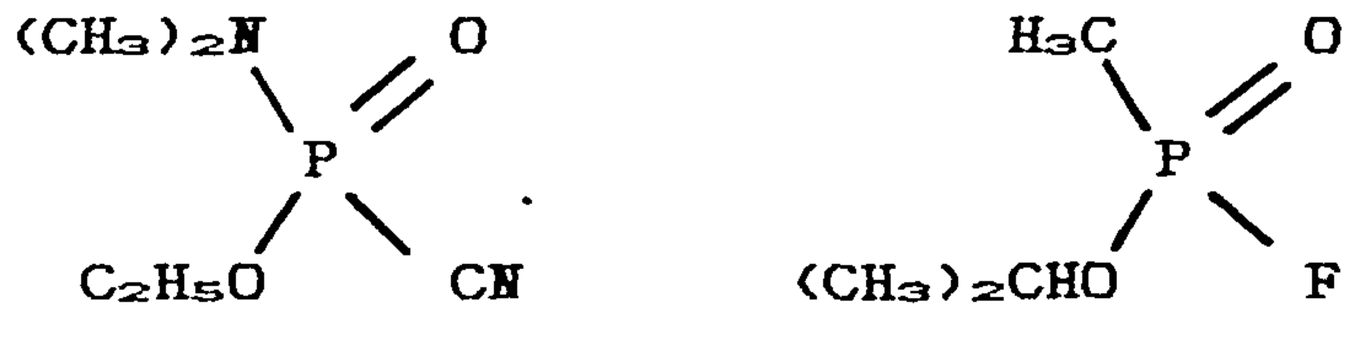
THE FIRST ORGANOPHOSPHORUS INSECTICIDES

The organophosphorus compounds showing insecticidal activity are hydrocarbon derivatives with one or two phosphorus atoms presenting the general structure:



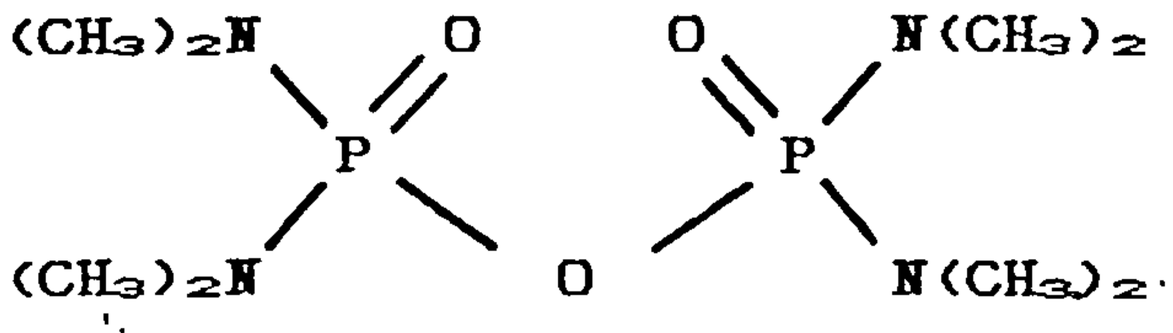
R_1 and R_2 are generally small alkyl, alkoxy, alkylthio or substituted amino groups ; X is oxygen or sulphur; Y is a leaving group or can be easily converted into one.

The discovery of insecticidal action in organophosphorus compounds was made during the Second World War, when the toxic nerve gases tabun (1), and sarin (2) prepared for use in warfare, were found to be effective insecticides by Gerhard Schrader and his colleagues in Germany.



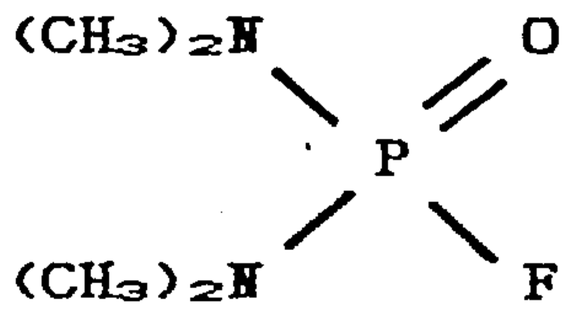
(1) (2)

The first systemic organophosphorus insecticide put on the market in 1941, was synthesised by Schrader, and was known as schradan or pestox(3).



(3)

At the same time, scientists under the direction of Saunders [22] in England, studied dimefox, (4), which is still used as a systemic insecticide for the control of aphids and red spider mites on crops by soil application.



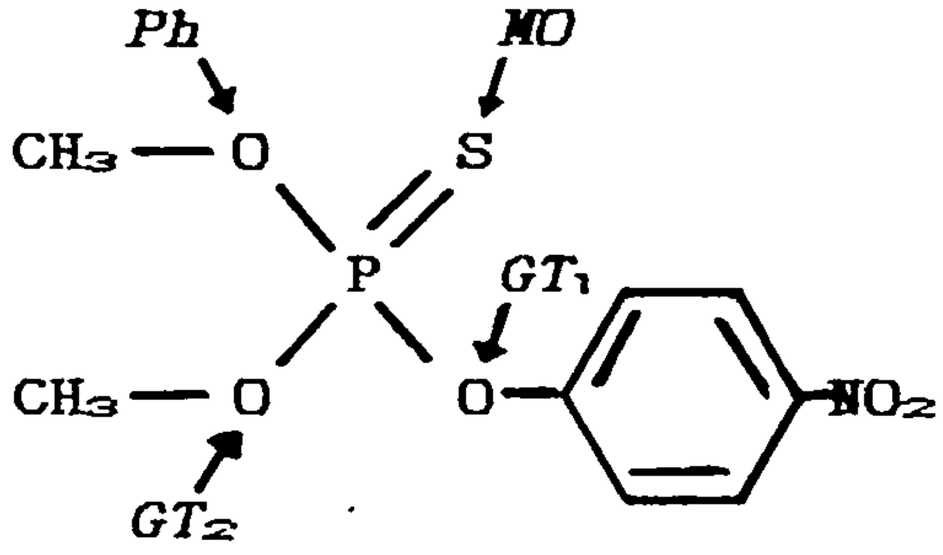
(4)

There are over 100 organophosphorus insecticides known and about 100 000 organophosphorus compounds have been named for their insecticidal action. The various members of this class of insecticides may differ greatly in their physicochemical properties, such as solubility in water, vapour pressure at room temperature, and chemical stability. Because of this some can be used as fumigants, and others as poisons or systemic compounds. To avoid mutiple application those that are very persistant, that is, have great chemical stability, must be used at the start of the season, and those that are not persistant should be used just before the harvest of the crops. They also show widely different toxicities to mammals, which makes some of them useful in animal hygiene.

DEGRADATION OF ORGANOPHOSPHORUS INSECTICIDES

The organophosphorus insecticides suffer decomposition in soil, plants, pests and mammals, and mechanisms by which this occurs depend on the physicochemical properties of the compounds themselves and on the environment. The degradation in soils, is a consequence of microbial activity, promoted principally by bacteria. In sterile soils, decomposition is catalyzed by clay surfaces, metal oxides, metal ions, and organic matter. The physicochemical properties of the soil such as humidity, temperature, alkalinity, acidity and the characteristics of the pesticides have a strong influence on the rates of degradation. Factors such as structure of the compound, solubility in water, its molecular size and shape are also important for the biochemical decomposition of the pesticide. In soil and water the degradation is limited to their surface since the ultra- violet light has little power of penetration.

In plants, pests and mammals, specific enzymes are responsible for degradation of the insecticide. Due to the structural variability of the organophosphorus compounds there is a considerable number of mechanisms by which they can be attacked by enzymes. The enzymes can attack various linkages in organophosphorus pesticides. For instance [23],

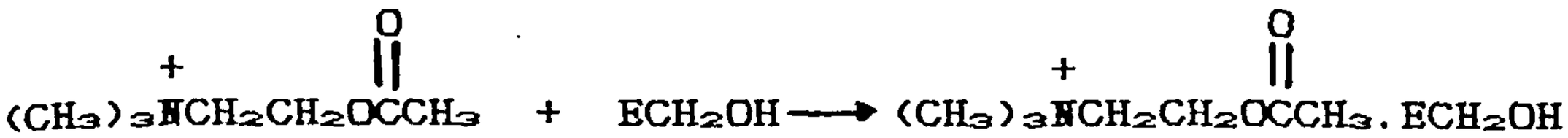


Ph, phosphatase/type A esterase; *MO*, microsomal mono-oxygenase; GT_1, glutathione-S-aryltransferase; GT_2, glutathione-S-alkyltransferase.

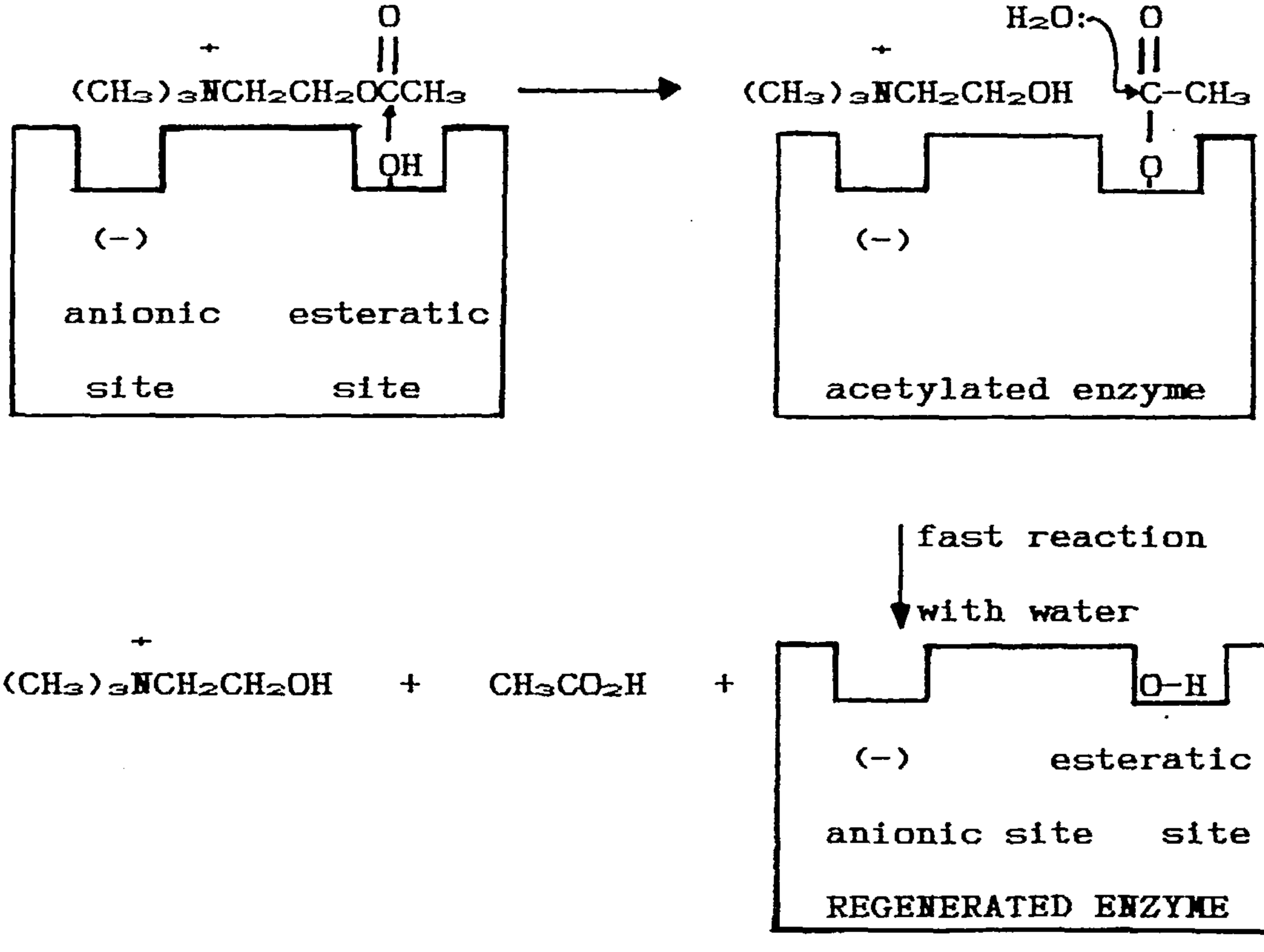
PRINCIPAL ENZYMES: ACETYLCHOLINESTERASE, MFO (MIXED FUNCTION OXIDASES), etc.

Acetylcholinesterase

The substance acetylcholine is responsible for the transmission of nervous impulses, between neurons and neurons and soft muscle. These are separated from each other by small gaps called synapses. When a nervous impulse arrives at the pre-synaptic cell the neurotransmitter (acetylcholine) is released, and passes through the synapse to the postsynaptic cell where it joins the receptor site. The acetylcholine is then hydrolysed by the enzyme acetylcholinesterase as shown.

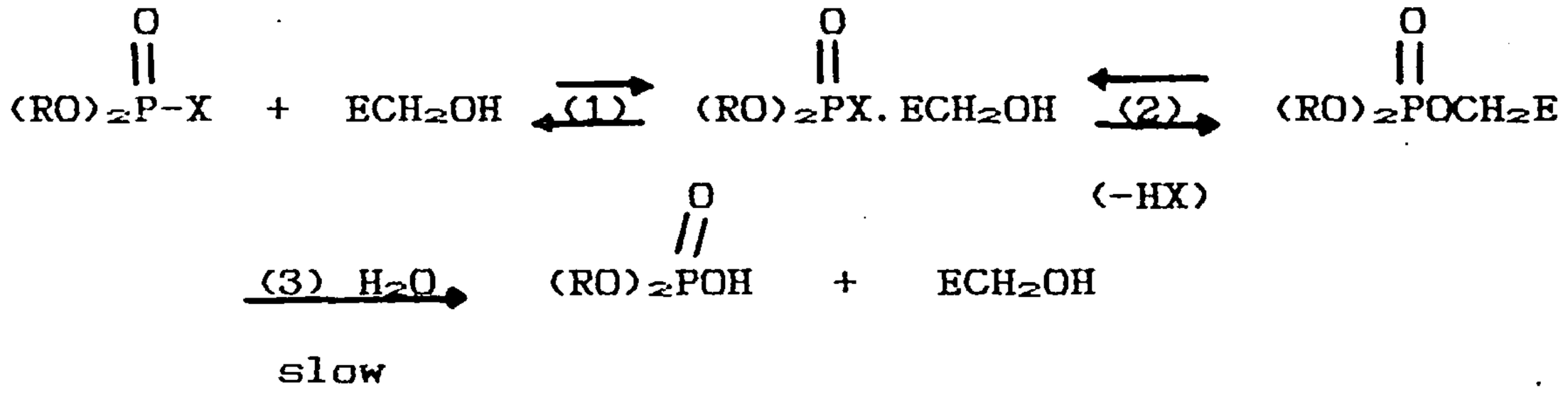


The active centre of the acetylcholinesterase has two reactive sites: the anionic site and the esteratic site, as shown.



In the presence of organophosphorus compounds that have the ability to phosphorylate the enzyme acetylcholinesterase, the acetylcholine remains in the synapses. The organophosphorus compounds mimic acetylcholine by joining to the esteratic site of the enzyme. The P-O bond of the phosphorylated enzyme is stronger than the C-O bond in the acetylated enzyme. This makes the hydrolysis of the phosphorylated enzyme very slow resulting in a continuous transmission of the nervous impulses. There is loss of muscular coordination, convulsions, and finally death.

The similarity between the two reactions can be appreciated below:



First a complex is formed between the enzyme and the phosphate. In a second stage the complex gives the phosphorylated enzyme, and in a third final stage a slow hydrolysis reaction takes place releasing the free enzyme. So the organophosphate effectively poisons the enzyme by phosphorylation and thus blocks efficient hydrolysis of acetylcholine into choline.

Mixed Function Oxidases (MFO)

There are a great variety of enzymes involved in the process of degradation of insecticides. Some of the most important are the mixed functions oxidases (MFO), also known as microsomal oxidase or mono-oxygenase, which occur in liver and fat tissues of animal, fish, and insect. They are responsible for oxidation of several lipophilic substrates such as steroids, lipids, and organophosphorus compounds. The oxidative reaction of MFO takes place in the presence of molecular oxygen and reduced nicotinamide adenine dinucleotide phosphate or reduced nicotinamide adenine dinucleotide. MFO are capable of inserting one of the oxygen atoms from an oxygen molecule into the substrate (RH) while the other is reduced to water:

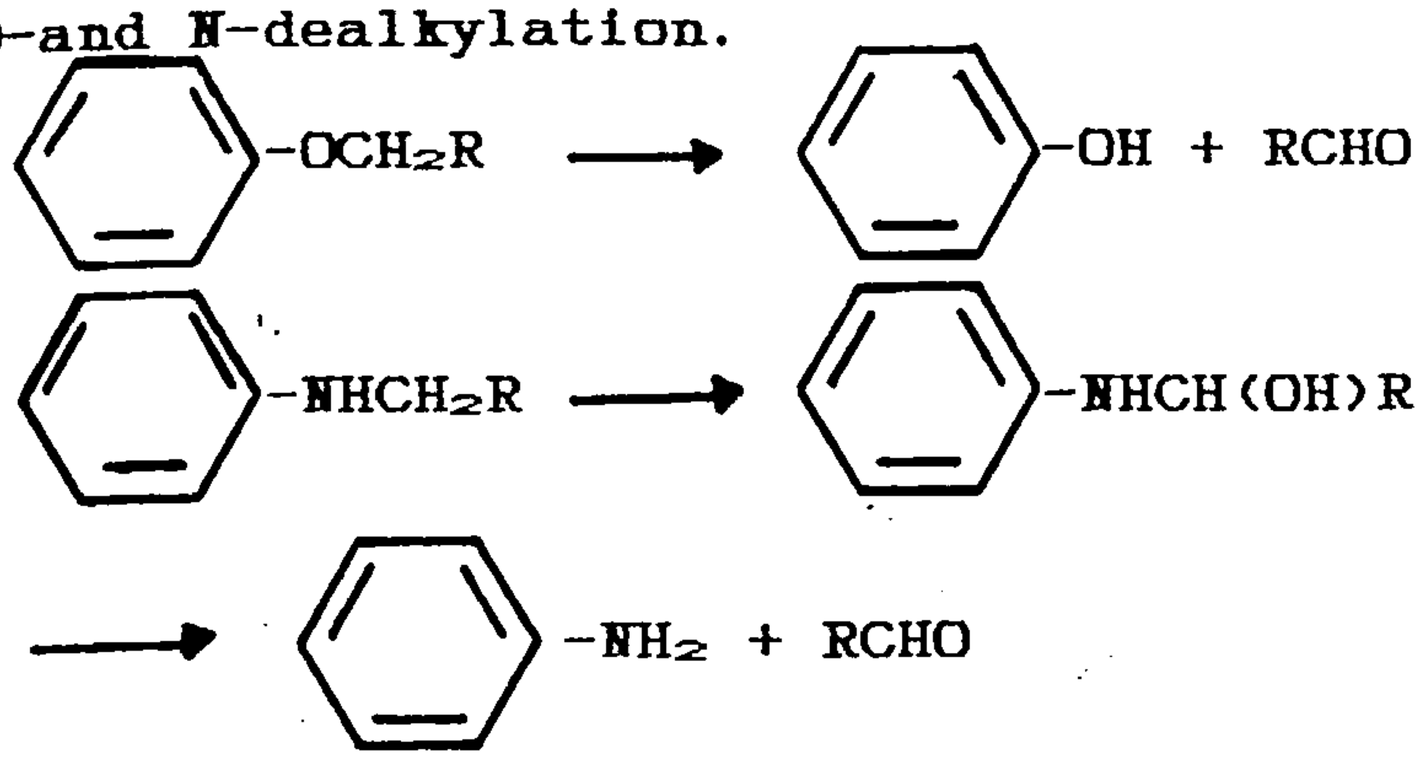
$$RH + NADPH + H^{+} + O_2 \xrightarrow{MFO} ROH + NADP^{+} + H_2O$$

Multifunction oxidases are involved in the following reactions [24]:

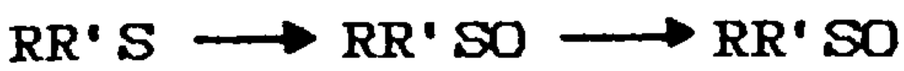
a) Hydroxylation.

$RH \longrightarrow ROH$

b) O-and N-dealkylation.

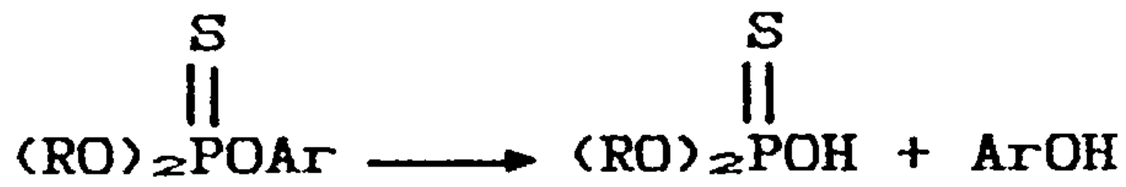


c) Oxidation of sulphides.

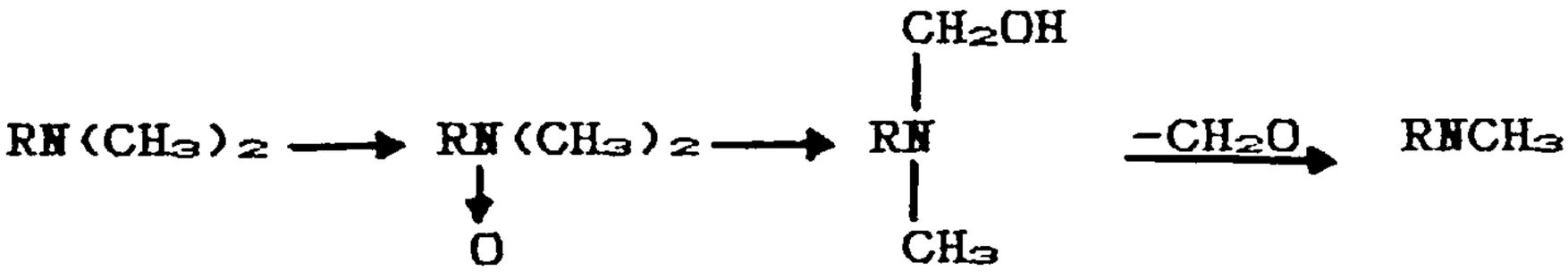


d) Oxidative desulphuration.

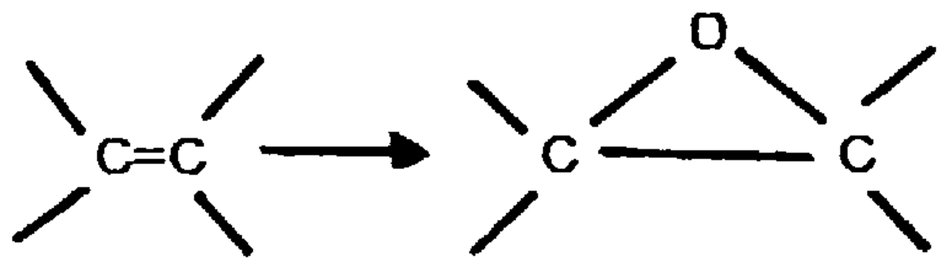
e) Deesterification.



f) Oxidation of tertiary amines.



g) Epoxidation.



Other Enzymes

Other enzymes of importance in the metabolism of organophosphorus pesticides are the hydrolases. The hydrolytic enzymes attack ester, amide or phosphate linkages and are named according to the substrate specificity; phosphatases attack R-O-P bonds, carboxylesterases attack R-COOR' bonds, carboxylamidases attack R-CONHR' linkages, etc. Esterases [25] can be classified into three groups (A,B,C) with respect to the organophosphates; A-type esterases hydrolyse organophosphates, B-type esterases are inhibited by organophosphates. C-type esterases may act on esters of acetic acid; they do not degrade organophosphates neither are they inhibited by them. The difference of behaviour between A and B esterases is a consequence of the relative magnitudes of the rate constants of the reactions in which the organophosphates bind to the enzyme and the following dephosphorylation process.

Hydrolysable linkages are not necessarily the locus of initial metabolic attack and these linkages are not only attacked by hydrolysis.

Some other enzymes of interest for the detoxification of the organophosphorus compounds are the glutathione-S-aryltransferases, glutathione-S-alkyltransferases, etc. These transferases [26] are present in the soluble cell fraction of mammalian liver and have molecular weights of the order of 45 000. These enzymes

undergo conjugation reactions, which are by definition, metabolic processees whereby foreign compounds and their metabolites, containing certain functional groups are linked to endogenous substrates giving more polar metabolites and are therefore less toxic.

THE PREDICTION OF ACTIVITY AND SELECTIVITY

The majority of the organophosphorus compounds that have capacity of phosphorylating show insecticidal activity. However, to determine the effectiveness of such compounds as insecticides it is necessary to synthesize a large number for biological screening. The most active compound is identified by trial and error methods.

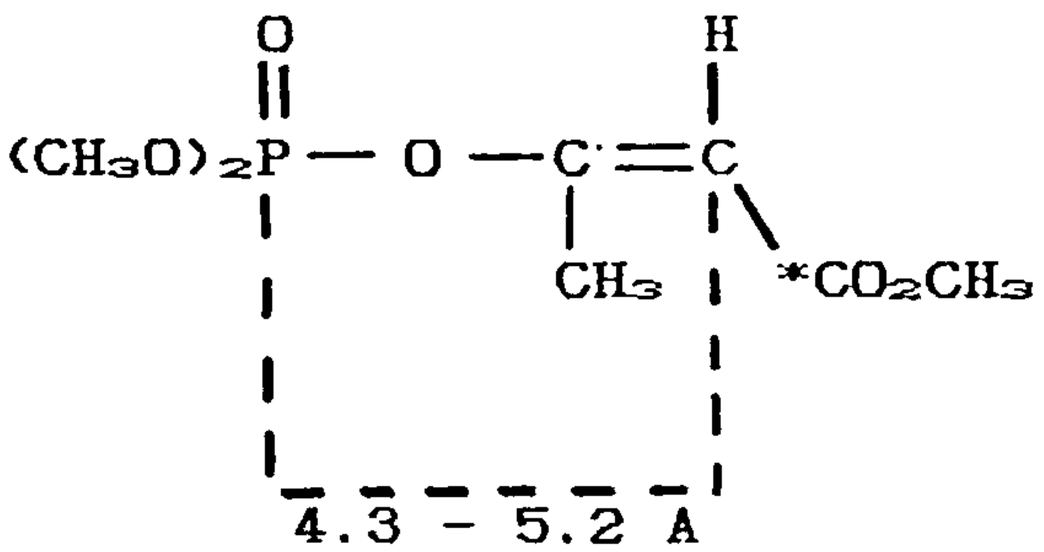
It is found that very similar compounds may have different types and degrees of activity. For example, 2,4-dichlorophenyl and 2,4,5-trichlorophenyl phosphorothioates are nematicides, acaricides, or soil insecticides [27]. An example of different degrees of activity is given by the 3-methyl and 3-chloro derivative of parathion that have their mammalian toxicities reduced compared to parathion: parathion LD_{50} (oral) to rats 6.4 mg/Kg; the 3-chloro derivative, chlorthion LD_{50} (oral) to rats 400 mg/kg, the 3-methyl derivative, sumithion LD_{50} (oral) to rats 500 mg/kg. It is impossible to predict these effects since the

substituents do not alter very much the physicochemical properties [28].

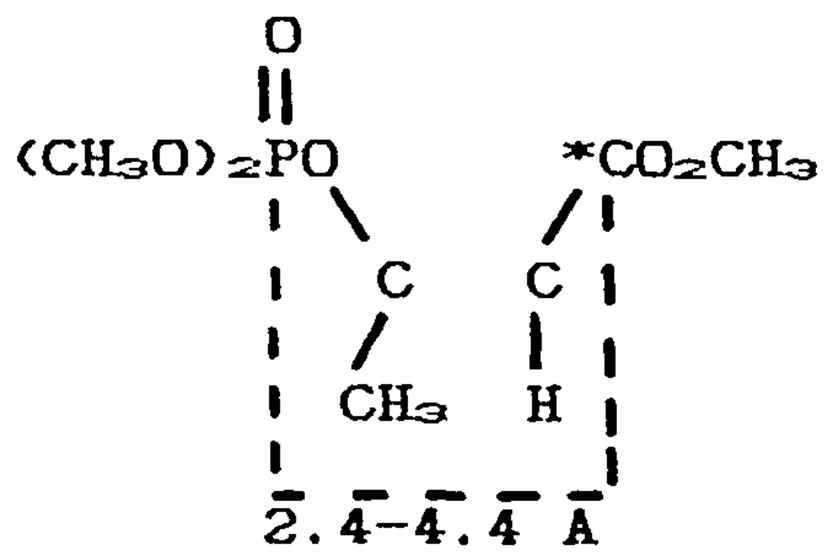
SOME FACTORS RELATED TO ACTIVITY

Steric Hindrance

Several enol phosphates which contain carboxylate groups exhibit enhanced insecticidal activity by protonation which occurs due to interaction between the carboxylic group and the esteratic site of the acetylcholinesterase. This interaction is affected by steric factors, thus *cis*-mevinphos is a more powerful cholinesterase inhibitor than its *trans*-isomer. In *trans*-mevinphos, the interaction between the carboxylic group and the enzyme is sterically hindered by the presence of the *cis*-dimethoxyphosphoryl moiety.



Cis-mevinphos

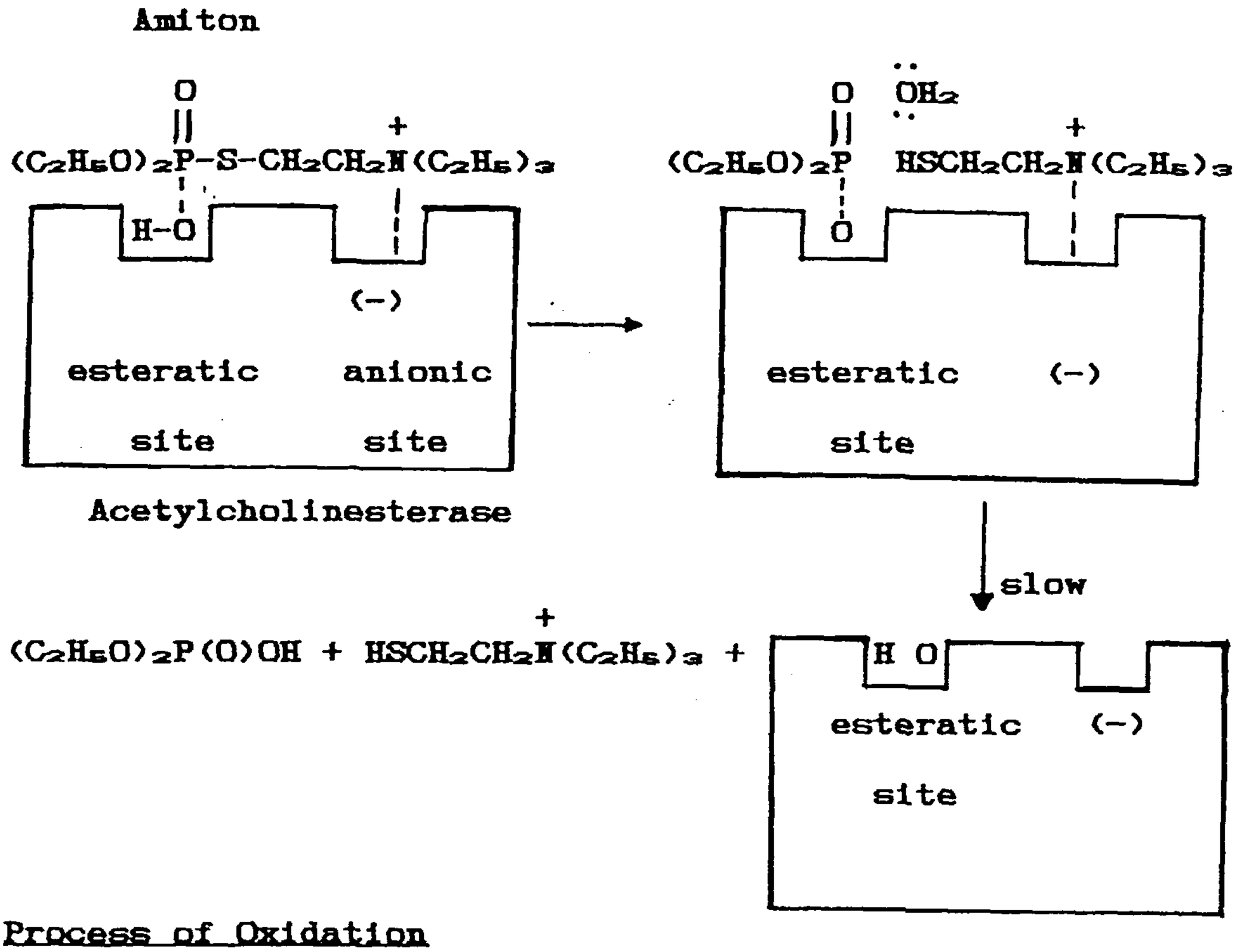


Trans-mevinphos

Suitable Distance between the Active Centres.

In addition, the good insecticidal activity of *cis*-mevinphos is possible because the active centres of acetylcholinesterase, the anionic and esteratic sites are approximately the same distance apart (4.5 - 5.9 A) as the phosphorus and carbonyl carbon atoms (4.3 - 5.5 A). The distance in the *trans*-isomer is 2.2 - 4.4 A which does not fit well with the active sites of the acetylcholinesterase.

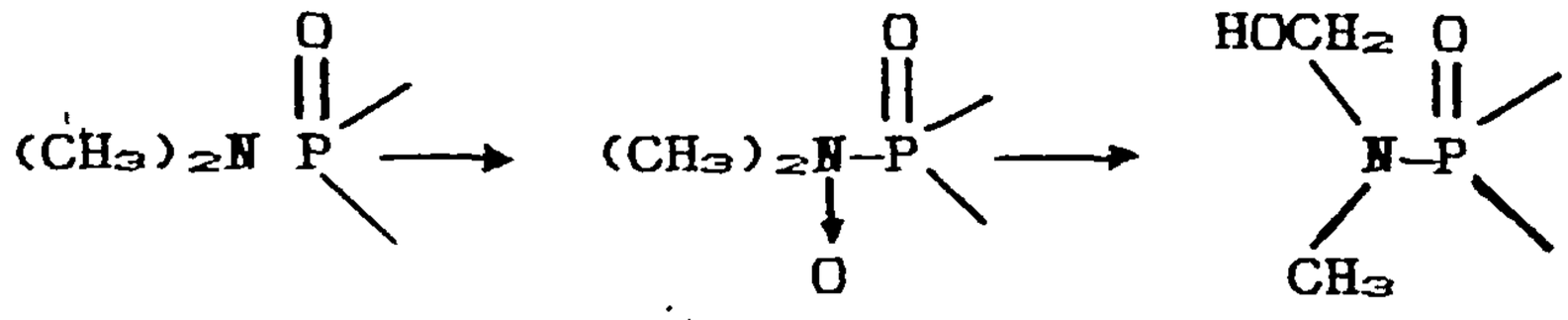
Another example of the significance of suitable distance between the active centres in order to obtain a good fit of the toxicant molecule to the acetylcholinesterase is given by the insecticide amiton, LD_{50} (oral) to rats 3 mg/Kg. The quaternary nitrogen atom of this insecticide has an extraordinary affinity for the anionic site of the enzyme, and is suitably placed to bring the phosphoryl group into close proximity with the esteratic site.



Process of Oxidation

In vivo oxidation by MFO (mixed function oxidases) is responsible for the insecticidal activity of some organophosphorus compouonds for instance, the thiophosphoryl (P=S) group may be activated to phosphoryl (P=O). The oxygen atom being more electronegative than sulphur pulls electrons from the P atom in the P=O bond, making phosphorylation possible. Also a strong electron donor group e.g. $(CH_3)_2N$ may be converted into an electron-withdrawing group [28]:

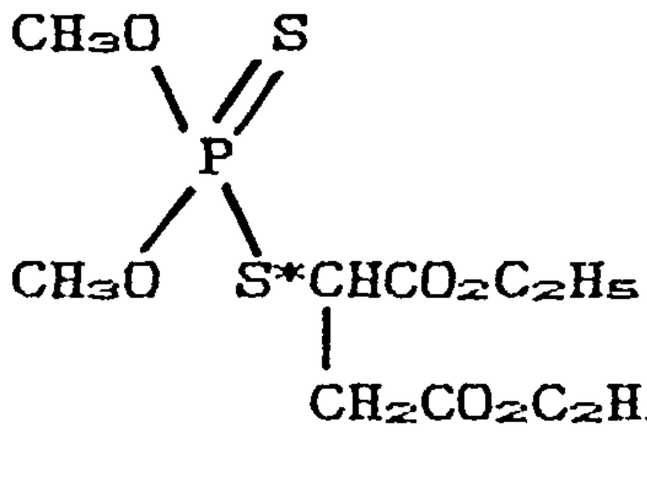
An example of activation process is the enzymic oxidation of schradan ,to the N-oxide or methylol:



The positive charge on the nitrogen increases the electrophilicity of the phosphorus atom converting the compound into a strong phosphorylating agent.

Asymmetry

Asymmetry in an insecticidal molecule may influence its activity and this is to be expected since the enzymes are themselves asymmetric molecules. In the case of malathion, the dextro form is the more toxic and effective inhibitor of acetylcholinesterase agent and liver carboxyesterase [24], and it has greater insecticidal potency.



*C asymmetric carbon atom

Malathion

Differences in the Nervous Systems of Insects and Mammals

These differences may be responsible for specific toxic effects of some organophosphorus compounds. For instance, amiton is an analogue of acetylcholine but *in vivo* it ionizes and its ionic form is selectively toxic to mammals. The nerve junctions of insects have a lipid sheath that repels the ionic form of amiton while the mammalian nervous system does not present such a barrier [24].

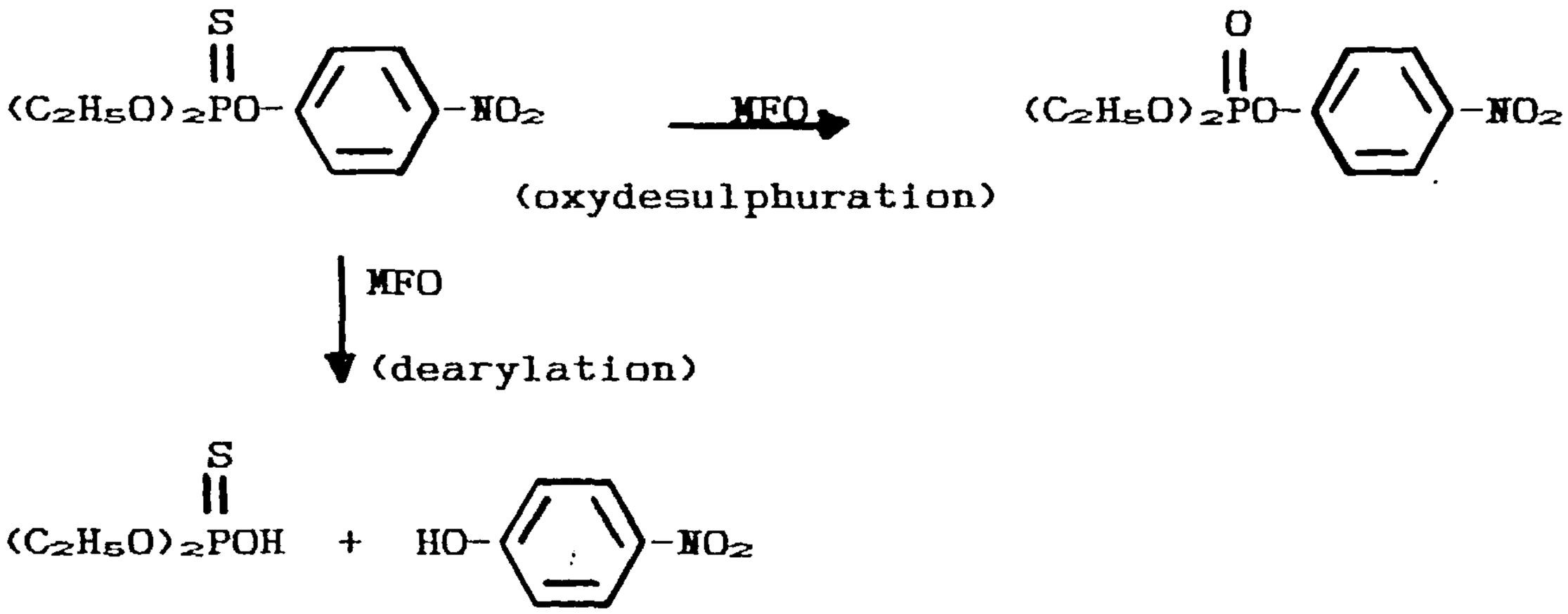
Transport of the Toxicant Molecule to the Site of Action

The importance of this is exemplified by the low solubility of the insecticide tetra-chlorvinphos in water and organic solvents which makes its transport and penetration to the active site of the cholinesterase difficult. In consequence, the insecticide has low mammalian toxicity.

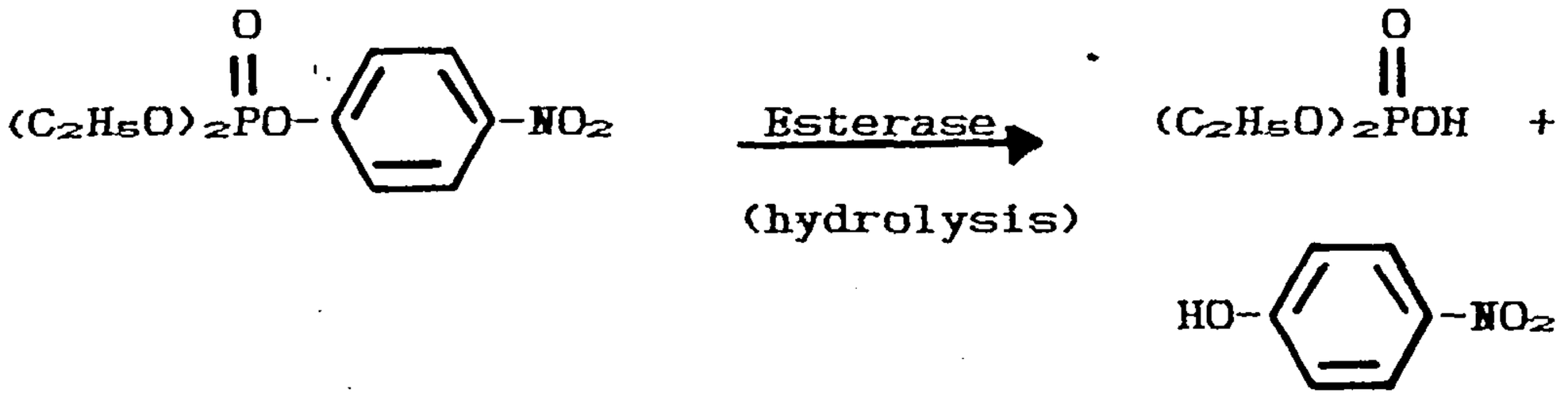
BIOLOGICAL DEGRADATION OF SOME ORGANOPHOSPHORUS COMPOUNDS

The study of the metabolism of selected types of pesticides in plants, animals, and insects is an important aspect of the investigation of the mode of action of these compounds. Such studies may make it possible to predict probable metabolites for similar compounds and to understand their selective toxicity. The metabolism of some organophosphorus insecticides is shown below.

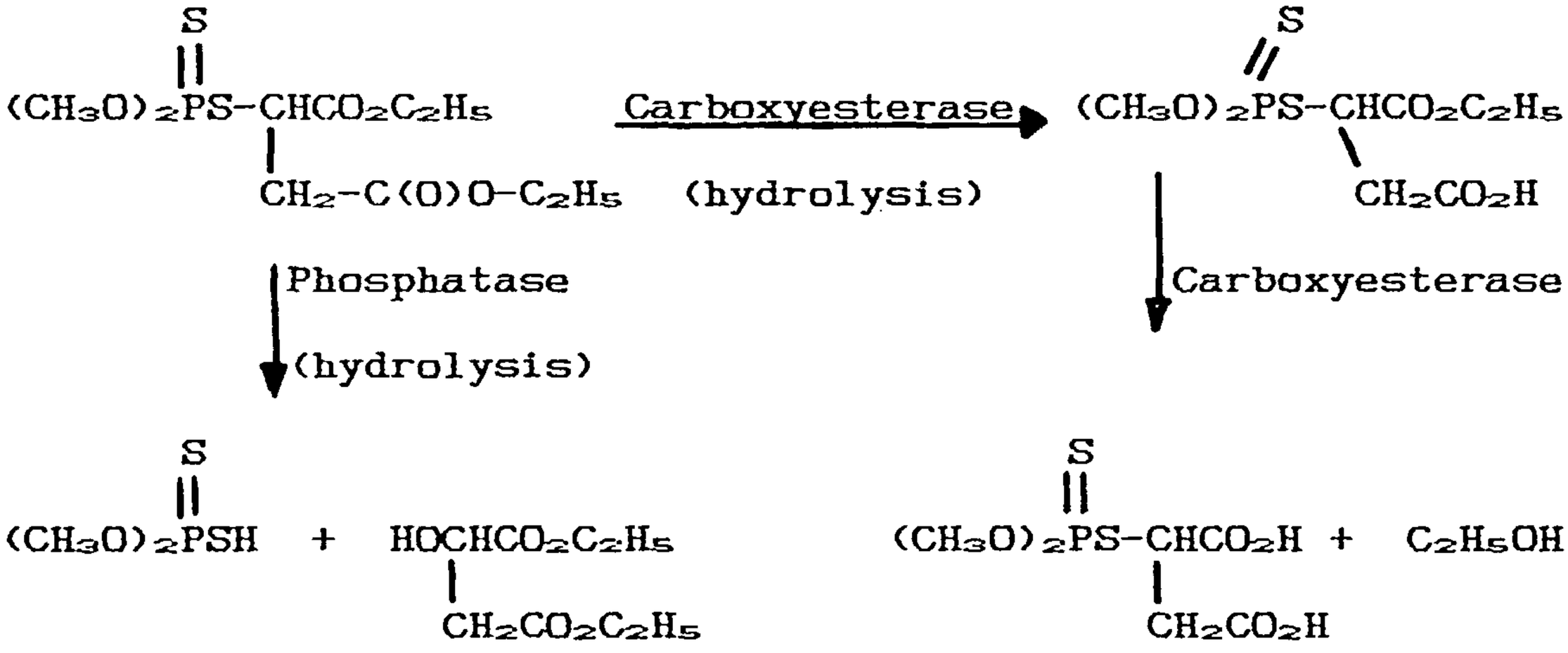
Parathion LD_{50} (oral) to rats 6.4 mg/Kg is a systemic insecticide, that is, it penetrates the plant tissues and consequently is translocated within the plant vascular system. Phosphorothioates like parathion are poor inhibitors of acetylcholinesterase while the oxo analogues are very active. Parathion is converted to paraoxon by the MFO and it can also be deactivated by the MFO in a hydrolysis reaction which results in dearylation. In plants the oxidases are responsible for its detoxification [29].



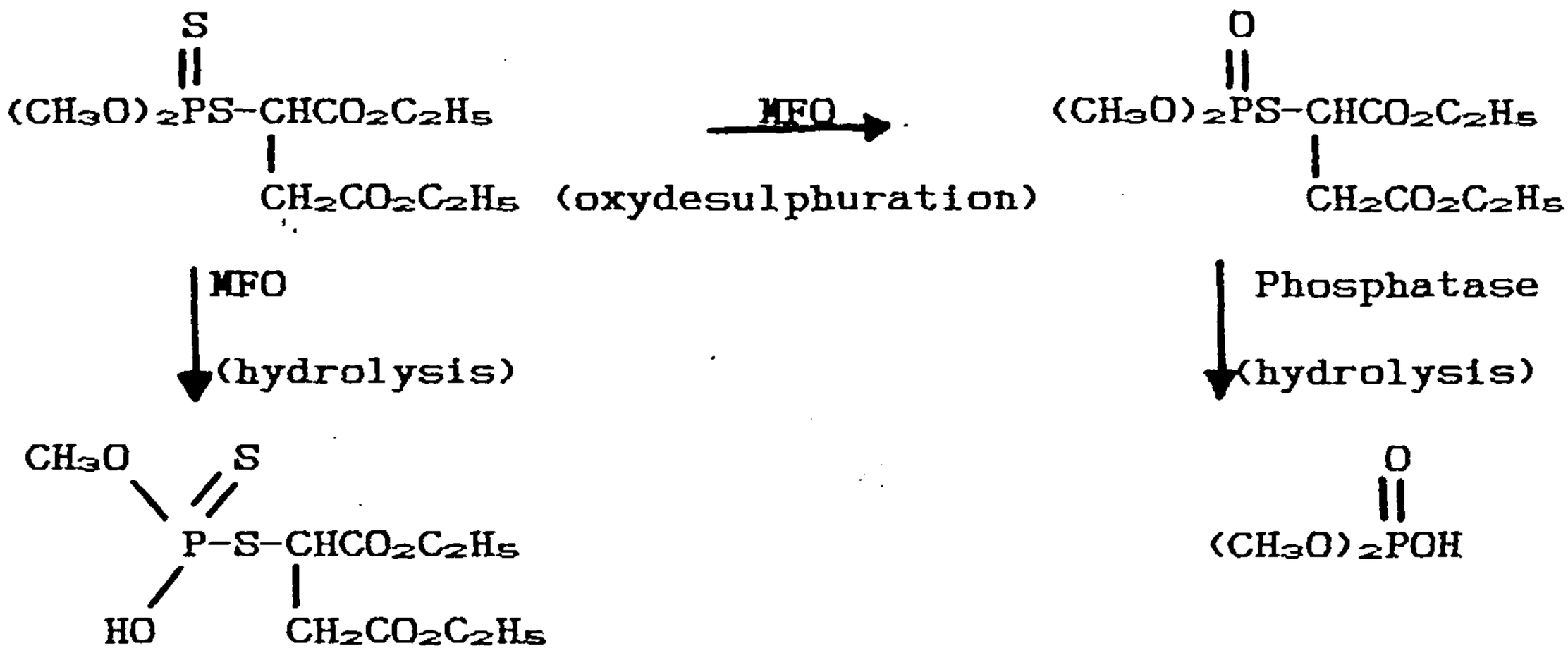
Paraoxon, LD_{50} 3.0 mg/Kg, has moderate persistency. It is detoxified by esterases as follows:



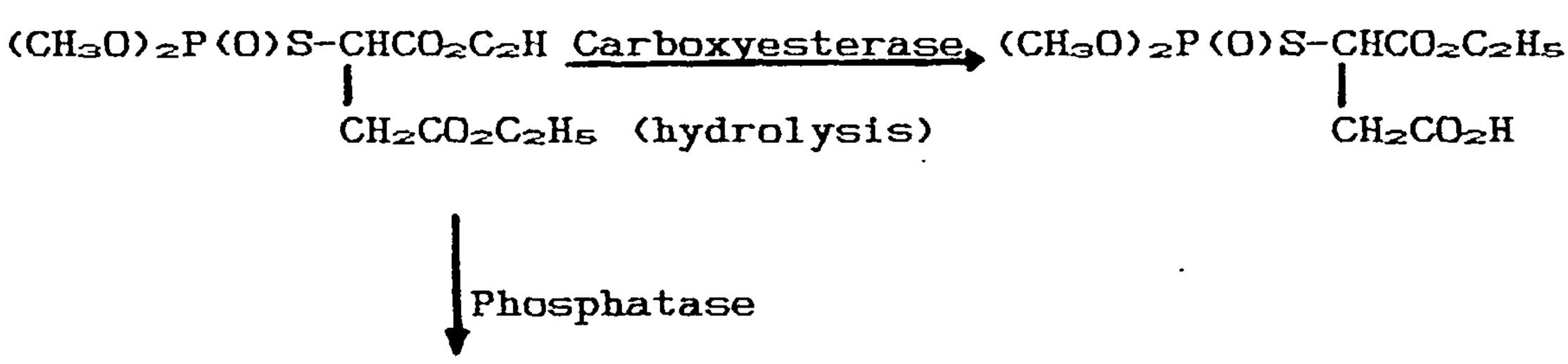
Malathion LD_{50} 1300 mg/Kg, is an effective contact insecticide and also used as an acaricide. It is detoxified principally by carboxyesterase in vertebrates. In insects, especially with houseflies, the phosphatase hydrolyses the P-S-C linkage. The reactions are shown in the following:



Malathion is also oxydesulphurated to malaoxon by the multifunction oxidases in mammals and insects or undergoes hydrolysis of one of the CH_3O-P bonds. Deactivation may also occur by hydrolysis of the P-S linkage.

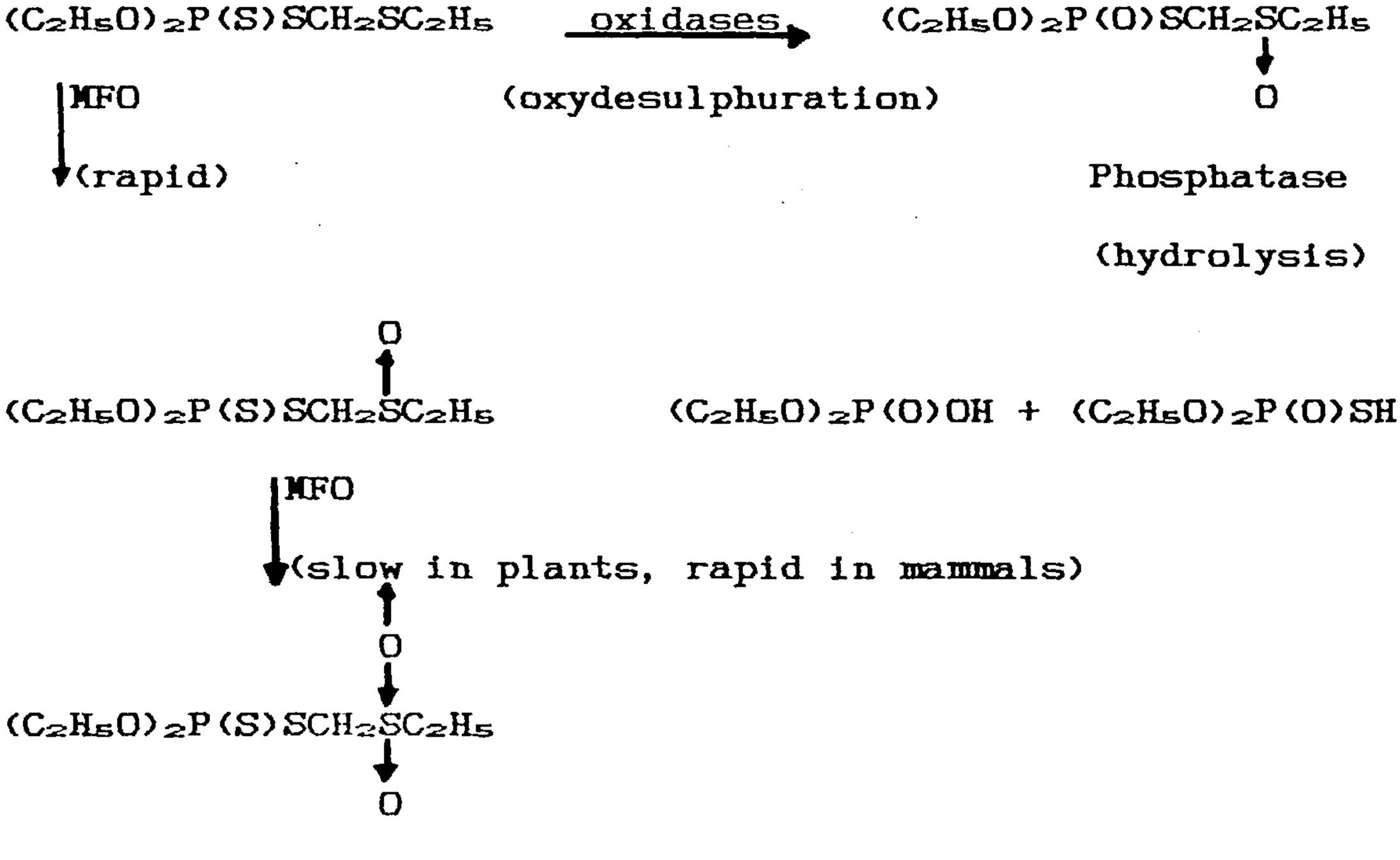


Malaoxon, LD_{50} 88 mg/Kg, is a highly active anticholinesterase agent. It is deactivated by phosphatase or carboxyesterase which hydrolyses one or both carboethoxy groups. Mammals have stronger carboxyesterase activity[24].



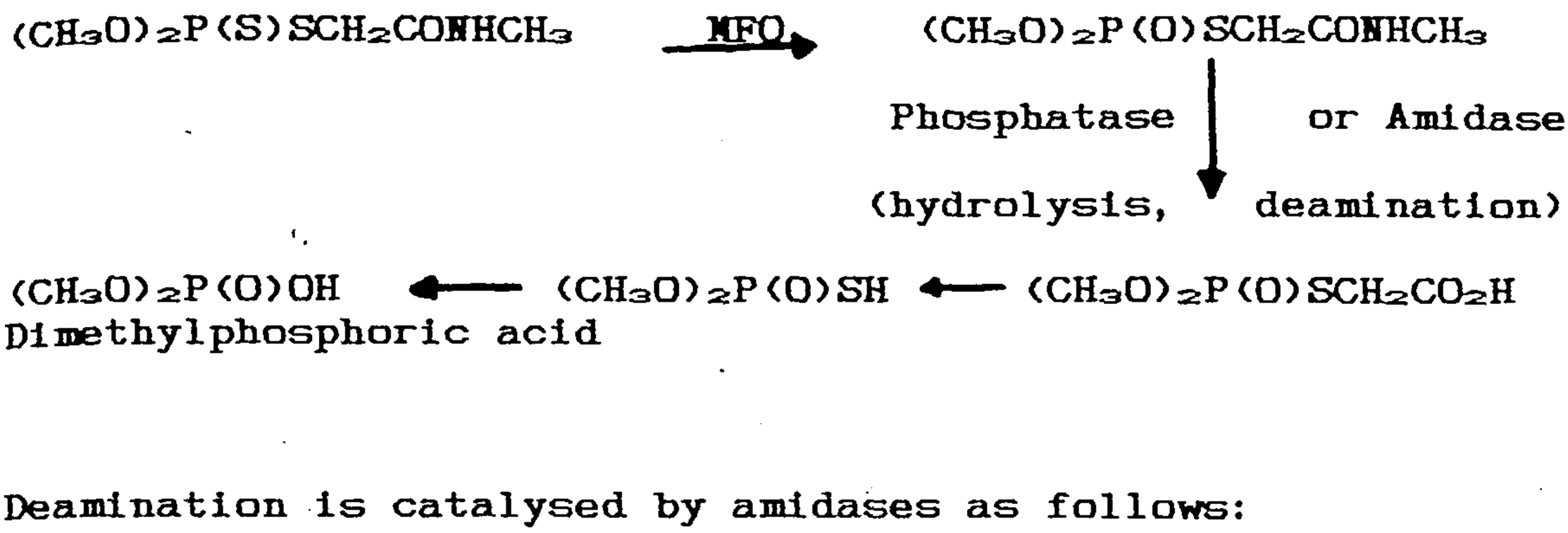
Phorate or thimet, LD_{50} 2 mg/Kg, has both systemic and contact insecticidal action. In plants, animals, and insects the sulphide group is oxidized to the corresponding sulphoxide and then to sulphone. Oxydesulphuration occurs in plants but not in insects by oxidases.[24] The phosphatase carries out hydrolysis with cleavage of the P-S bond in

insects. The various metabolites are shown in the following scheme[30,31].

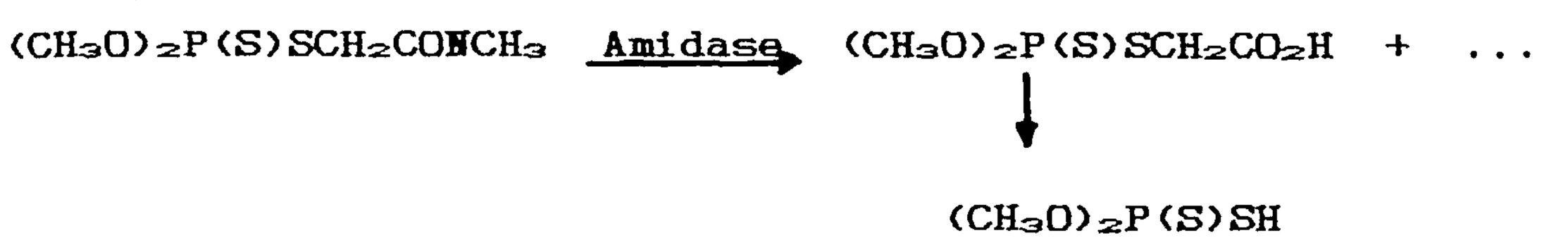


Rogor or dimethoate, LD_{50} 230 mg/Kg, is a systemic insecticide and acaricide and it is not absorbed by the lipid phase. In consequence it gives low quantity of residues.

The metabolism is similar in plants, insects and vertebrates but it is more rapidly degraded in mammals [27, 30, 31]. The multifunction oxidases cause O- and N-dealkylation and desulphuration, the phosphatases hydrolyse the P-O and P-S bonds, and the amidases are responsible for deamination. Desulphuration occurs in olive fruit fly, the oxo compound being then degraded to dimethylphosphoric acid.

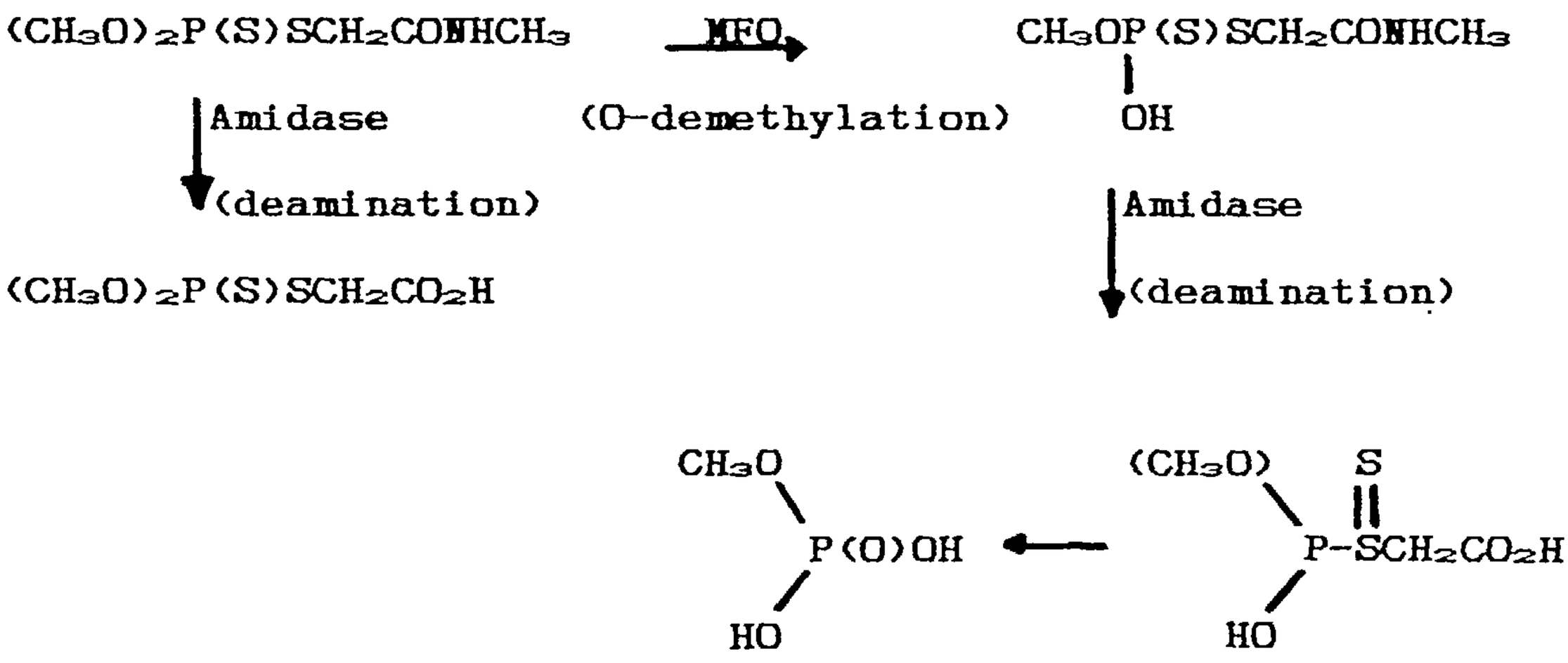


Deamination is catalysed by amidases as follows:



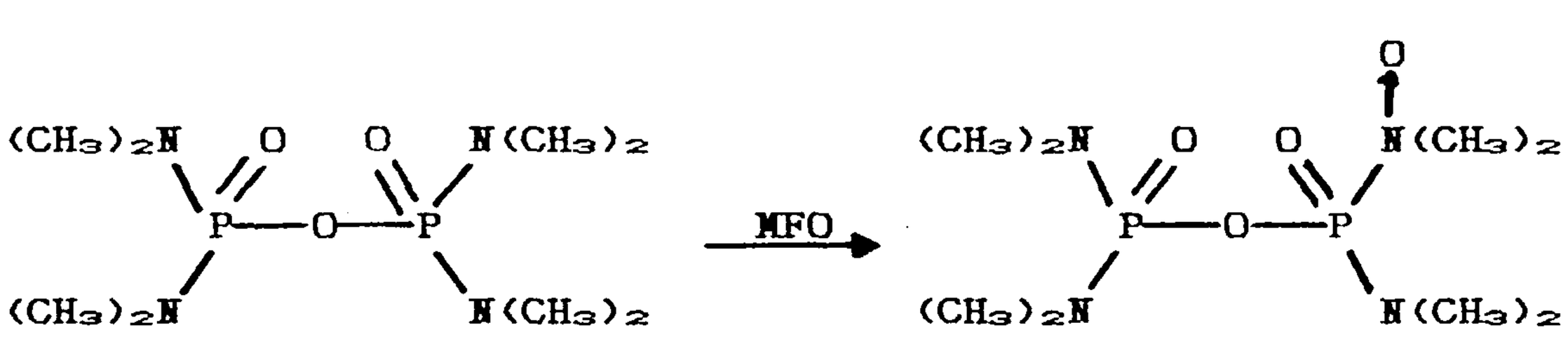
In mammals, the amidase action takes place in the liver and the products of degradation are eliminated in the urine. The first degradation in vertebrates is caused by the amidase attack [31].

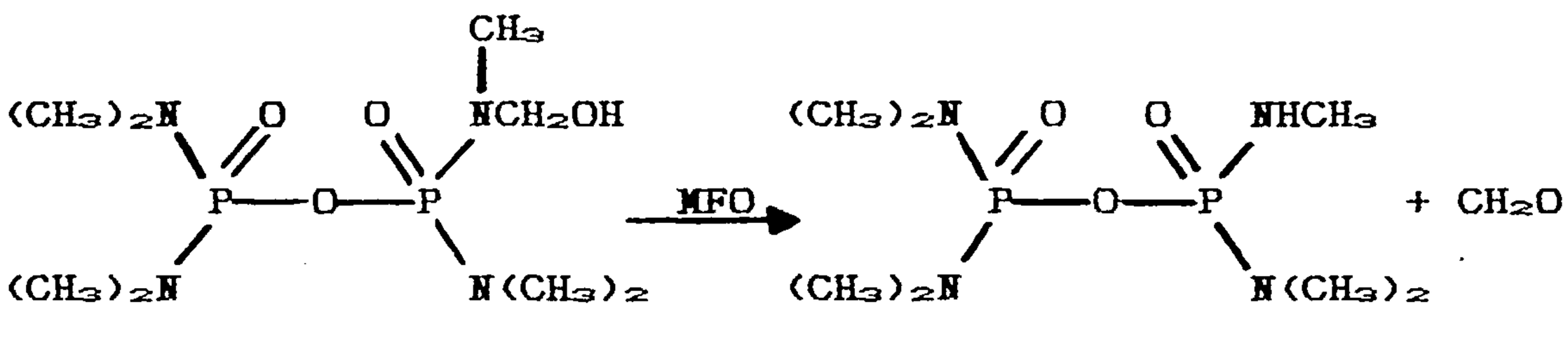
In plants, the hydrolysis is mainly caused by phosphatases rather than carboxyesterases or amidases while multifunction oxidases realize O-demethylation.



The toxicity of Rogor (dimethoate) for insects is due to its conversion into the oxo analogue, its rapid penetration in the fly, and the fact that the fly acetylcholinesterase is very susceptible to phosphorylation.

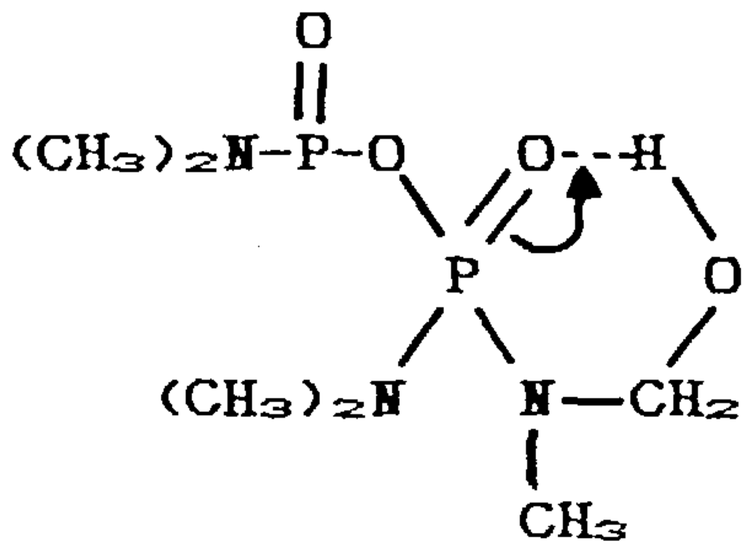
Schradan, LD_{50} 8 mg/Kg, is a systemic insecticide whose activity is due to oxidative activation of the tertiary amino group. It does not itself have an anticholinesterase effect. In mammals and in insects, the MFO oxidises the tertiary amino group of schradan (A), to give the N-oxide (B), which rearreanges to the N-methylol derivative (C). This subsequently decomposes to formaldehyde and heptamethylpyrophosphoramide (D).



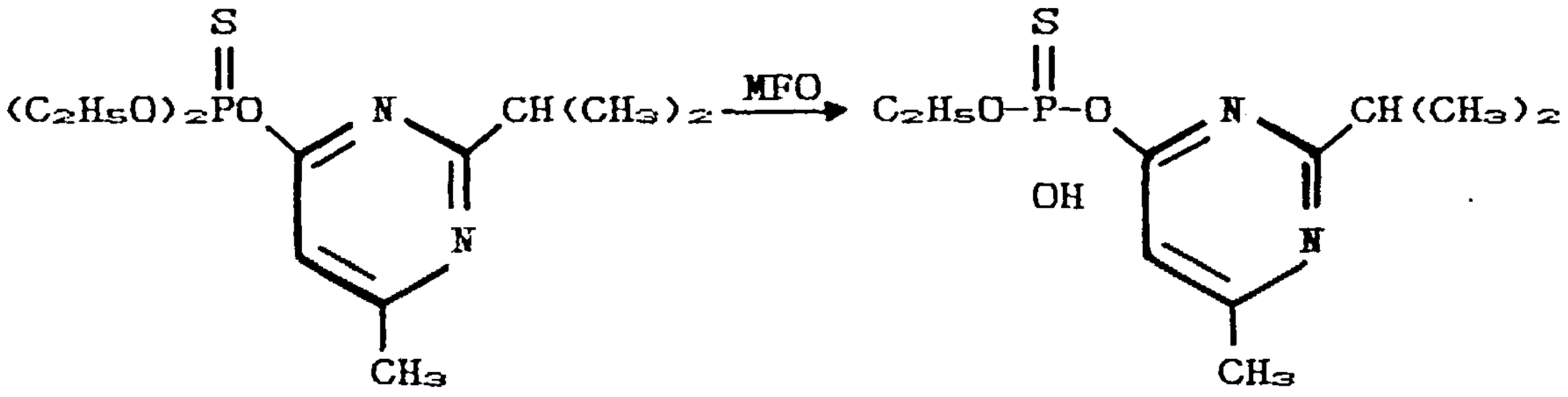


First it was supposed that the N-oxide (B), was the most active metabolite of schradan because charge on the nitrogen atom increases the electrophilicity of phosphorus.

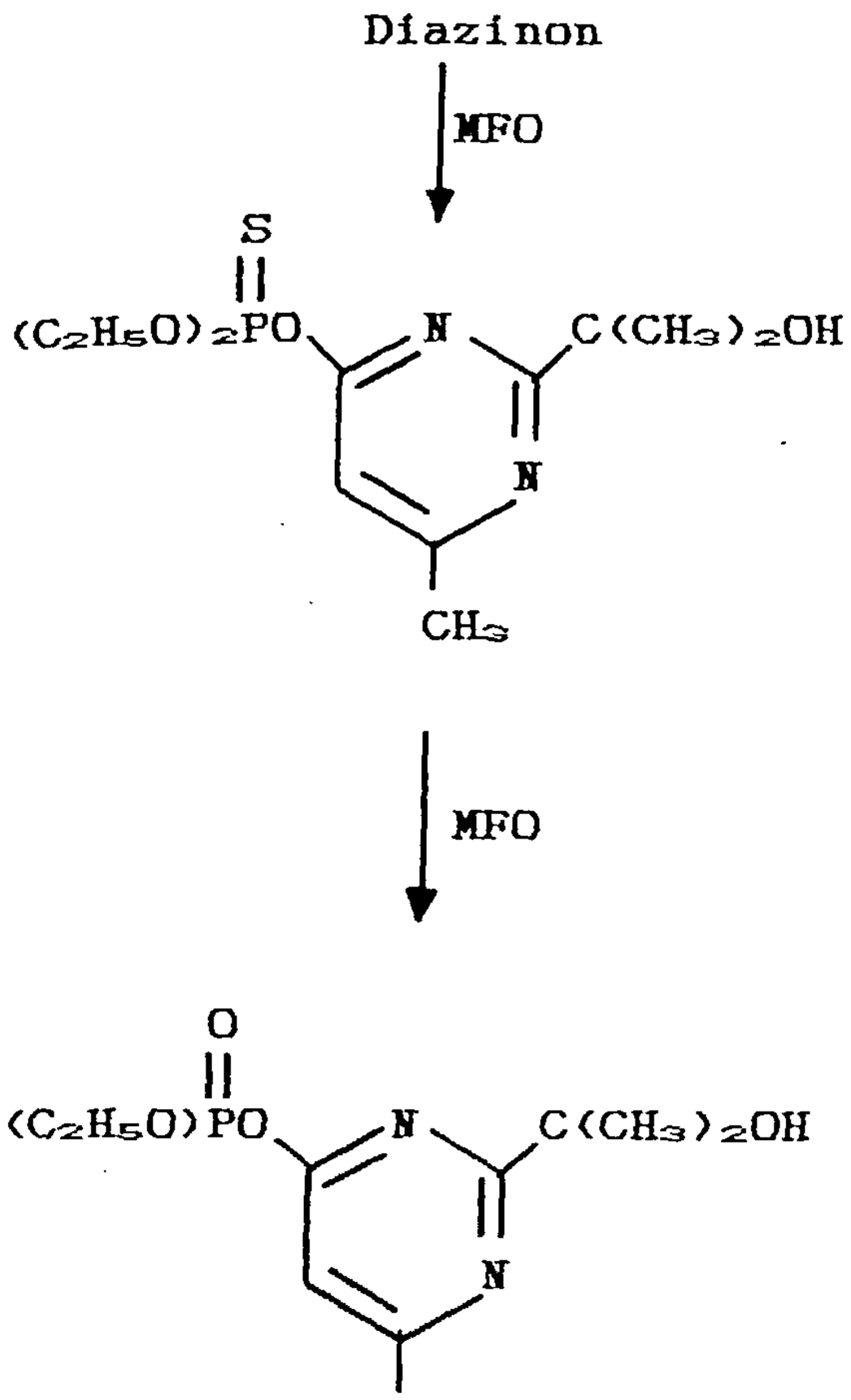
Further studies have however shown that the most active metabolite is methylol derivative (C). The hydrogen bonding provides an increase of electron drift from the phosphorus atom which enhances the phosphorylation ability of the compound.

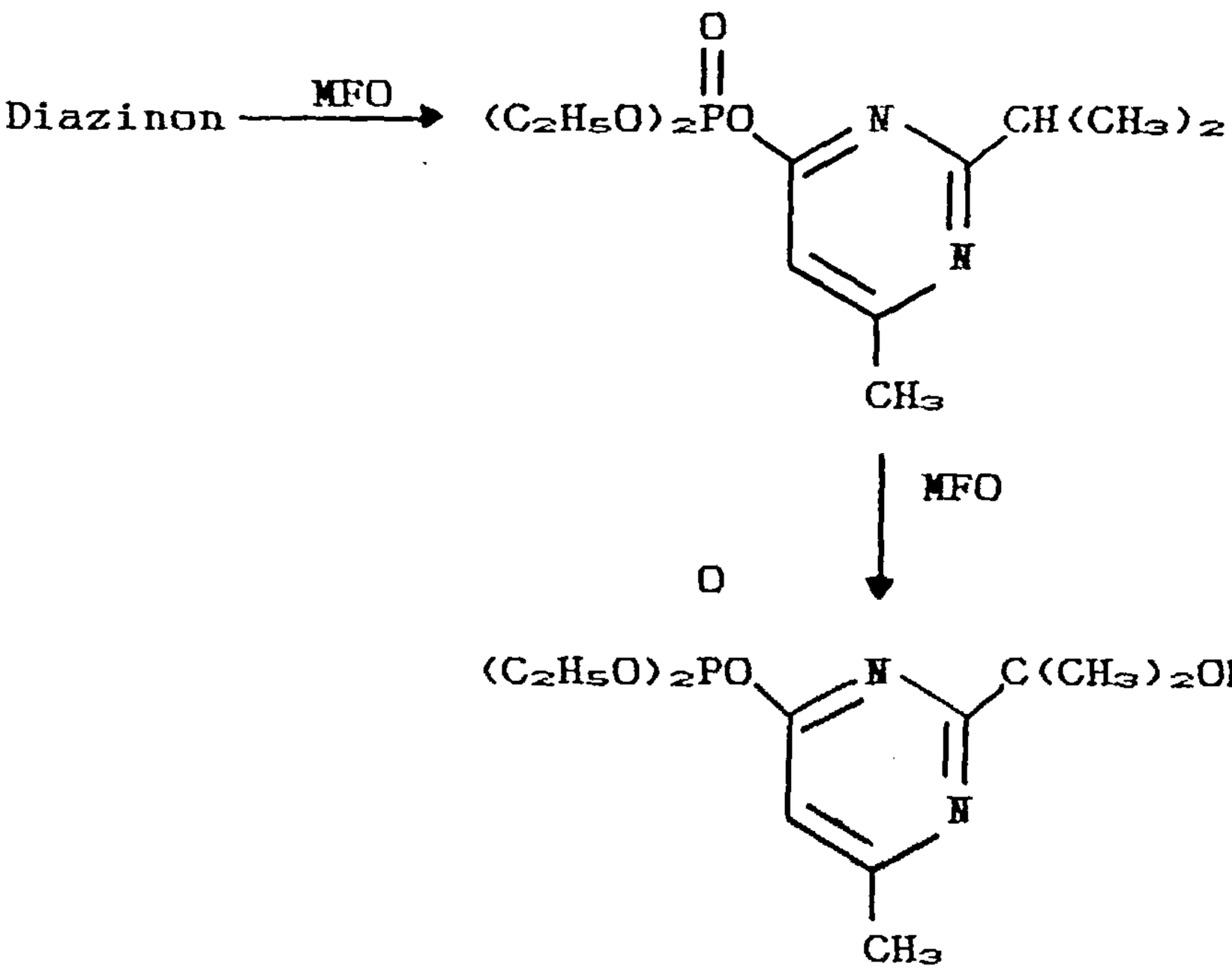


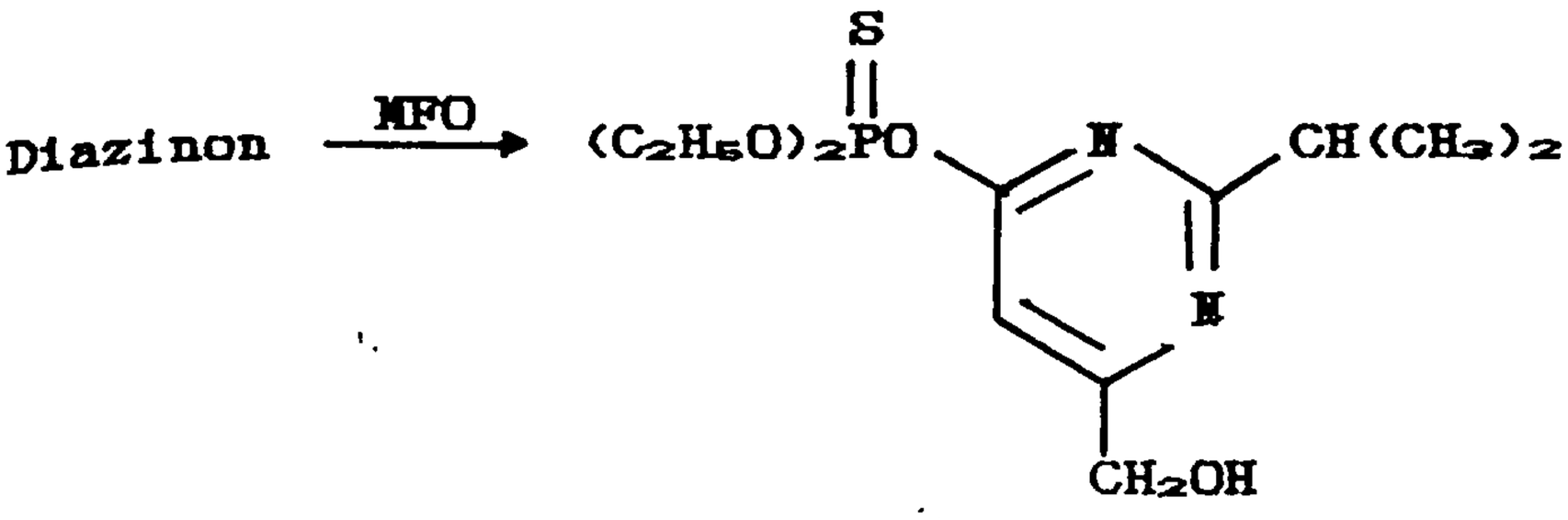
Diazinon, LD_{50} 150 mg/Kg, is an example of an organophosphorus insecticide that has a heterocyclic ring in its molecule. In mammals, the MFO oxidises diazinon giving a small proportion of its oxo analogue but it gives mostly active metabolites by hydroxylation of the methyl or isopropyl groups.



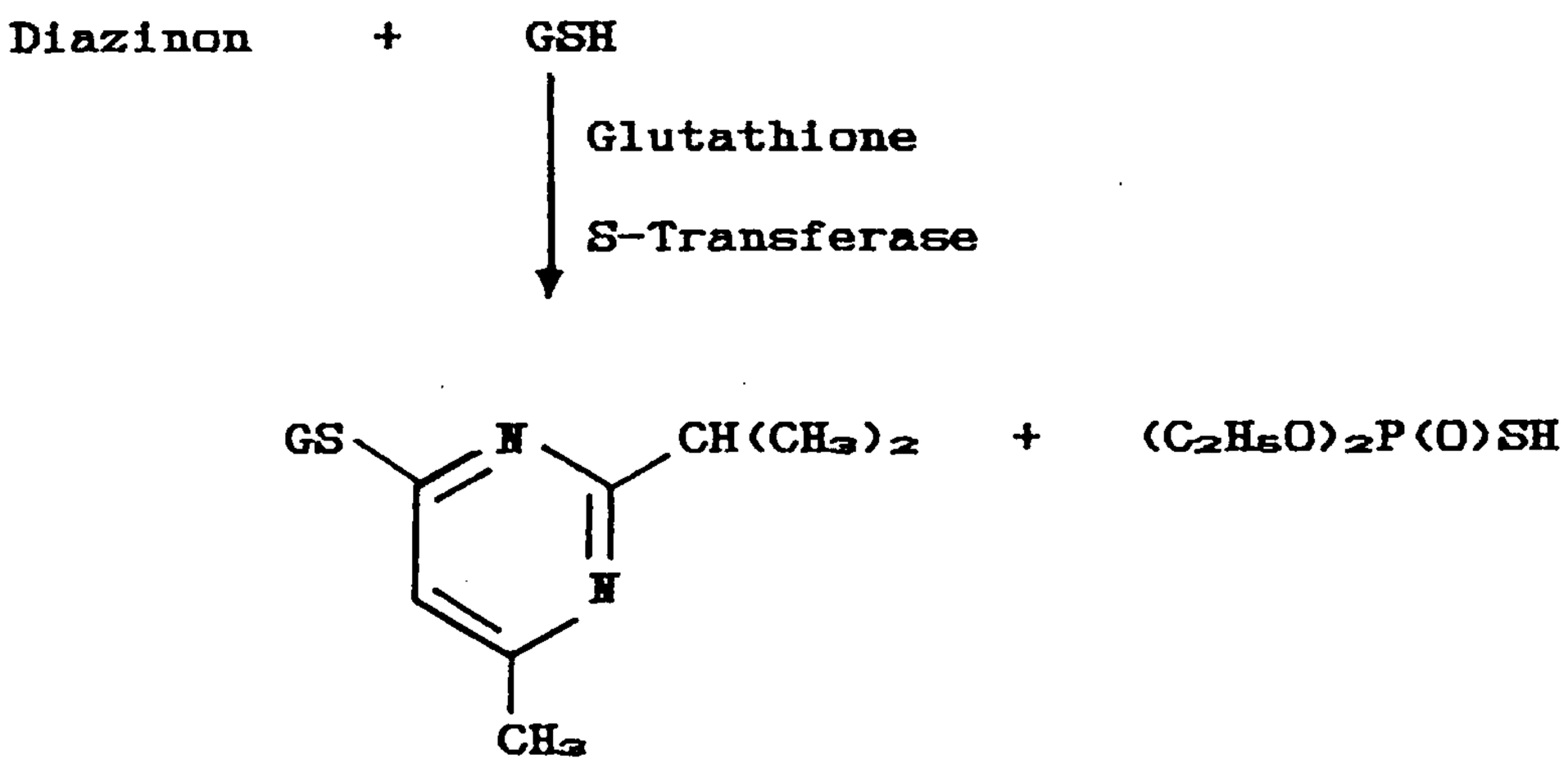
Diazinon


Diazinon
MFO
S
(C2H5O)2PO
N
C(CH3)2OH
N
CH3
MFO
O
(C2H5O)PO
N
C(CH3)2OH
N
CH3


Diazinon
MFO
O
(C2H5O)2PO
N
CH(CH3)2
N
CH3
MFO
O
(C2H5O)2PO
N
C(CH3)2OH
N
CH3



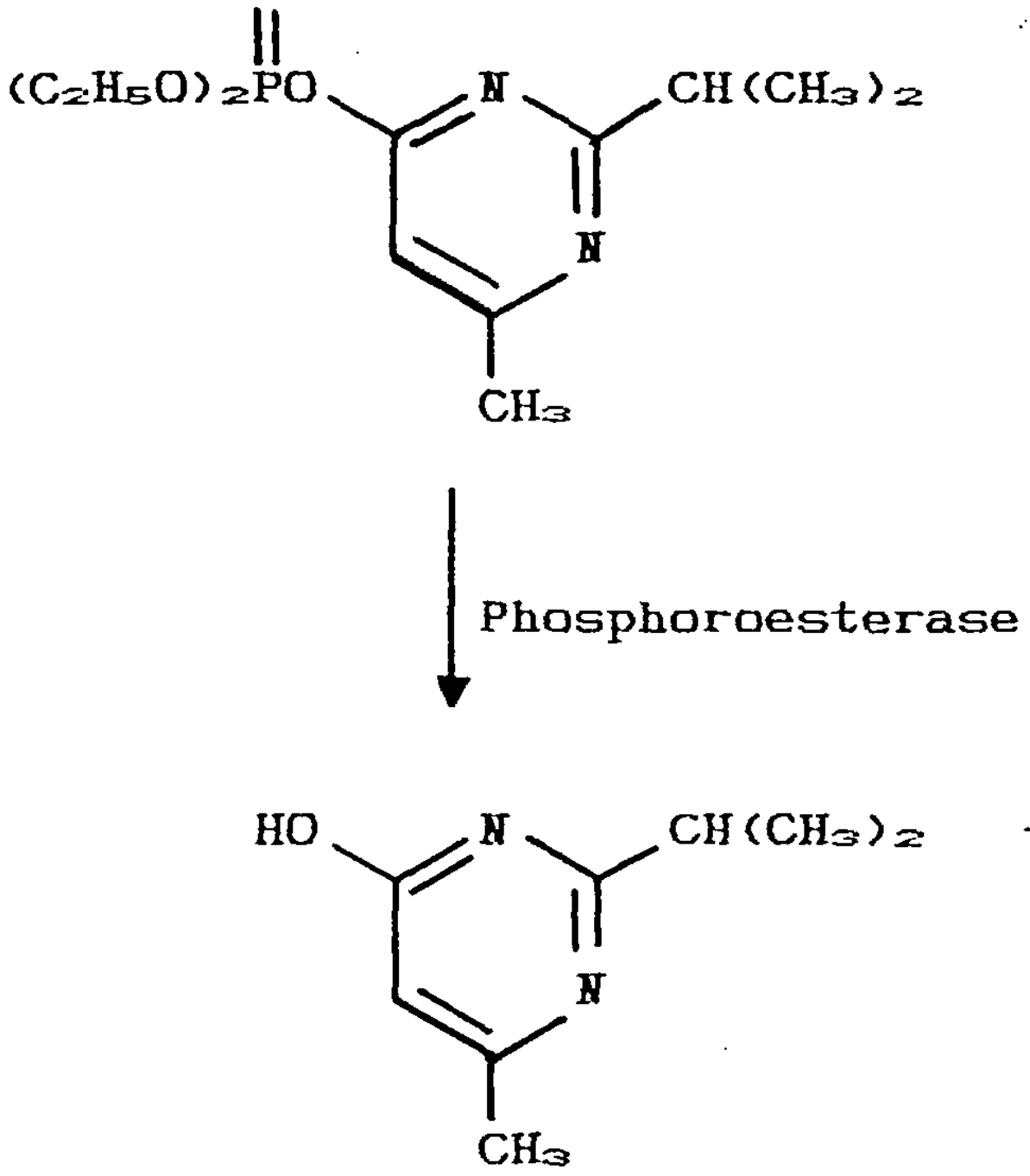
The reaction of cleavage of the P-O-aryl bonds is catalyzed by the enzyme glutathione S-transferase in the presence of glutathione, principally in rat liver and cockroaches to give pyrimidinyl glutathione and diethyl phosphorothioic acid [30].



[GSH = glutathionel.

Insects generally have poor transferase activity, although a diazinon-resistant strain of houseflies owed their tolerance to the presence of glutathione S-transferase[30].

Dephosphorylation takes place in liver of rats under the influence of phosphoesterase, thus deactivating the molecule to give 6-pyrimidinol and diethylphosphoric acid [27,30].



EXPERIMENTAL

STARTING MATERIALS

The following chemicals were used in the course of this work.

Ethanol - G.P.R., BDH Chemicals Ltd.

Phosphorus pentasulfide - G.P.R., BDH Chemicals Ltd.

Potassium hydroxide pellets - AnalaR, BDH Chemicals Ltd

Toluene - AnalaR, BDH Chemicals Ltd.

Acetone - AnalaR, BDH Chemical Ltd.

Acetone - nanograde for pesticide residue analysis, BDH Chemicals Ltd.

Chloroacetic acid - G.P.R., Hopkin & Williams Ltd.

Methyl bromoacetate - b.p. 51-52°C/15 mmHg, Aldrich Chemical Co. Ltd.

Iodomethane - G.P.R., BDH Chemicals Ltd.

Sulphur - flowers G.P.R., Hopkin & Williams Ltd.

Diethyl phosphite - G.P.R., BDH Chemicals Ltd.

1,2-dichloroethane - G.P.R., BDH Chemicals Ltd.

Ammonia - gas cylinder, BOC. Special Gases

Diethyl ether anyhydrous - May & Baker ltd.

Anhydrous sodium sulphate - ROSE Chemical Ltd.

Calcium chloride - G.P.R., BDH Chemicals Ltd.

Ethyl chloroformate - SynchemicA Reagents for Organic sythesis (ethyl chlorocarbonate).

Methylamine - gas cylinder, BDH Chemicals Ltd.

Chloroacetyl chloride - G.P.R., BDH Chemicals Ltd.

Petroleum spirit b.p. 60-80°C and 40-60°C - G.P.R., BDH Chemicals Ltd.

Charcoal - Chemical for Science and Industry. Hopkin & Williams Ltd.

Soda lime - Chemical for Science and Industry. Hopkin & Williams Ltd.

Sodium chloride - AnalaR, BDH Chemicals ltd.

Potassium carbonate, anhydrous - G.P.R. BDH Chemicals Ltd.

Florisil - about 30-60 U.S. mesh for chromatographic analysis. BDH laboratory reagents.

Hexane - nanograde (for pesticide analysis). BDH Chemicals Ltd.

Paraformaldehyde - Chemical for Science and Industry. Hopkin & Williams Ltd.

Anhydrous hydrogen chloride - gas cylinder, BOC. Special Gases.

Hydrogen peroxide solution - G.P.R. (100 volumes) BDH Chemicals Ltd.

Dichloromethane - 99.5% ROSE Chemicals Ltd.

Diazald - 99% Aldrich Chemical Co Ltd.

Dipotassium hydrogen orthophosphate - G.P.R., Hopkin & Williams Ltd.

Hydrochloric acid - G.P.R. BDH Chemicals Ltd.

Chlormephos - Murphy Chemical Ltd.

Mecarbam - Cheminova.

GENERAL APPARATUS

Homogeniser - A Silverson Machines Ltd. Heavy duty laboratory mixer emulsifier, Model L2 AIR was used for the extraction of samples.

Griffin Flask Shaker - This was used for the extraction of pesticides and their metabolites in the hydrolysis studies.

Rotatory film Evaporator - BüCHI 011, made in Switzerland.

Refractometer - Bellingham & Stanley Ltd.

Water Bater - Thermostatically controlled, Compenstat, England.

SPECTROSCOPIC METHODS

Perkin-Elmer 781 and Perkin-Elmer 1310 Infrared Spectrophotometers, were used for obtaining infrared spectra with samples as liquid films, Nujol mulls or KBr discs.

Routine ^{1}Hnmr spectra were obtained with a Perkin Elmer R12B 60 MHz, whilst a Bruker WP-80 MHz instrument was used for better resolved proton spectra (80 MHz), for carbon-13 spectra (20.12 MHz), and for phosphorus-31 spectra (32.4 MHz). A few ^{31}P spectra were also recorded on a Bruker 400 operating at 162 MHz (Service provided by the

manufacturers at the X International Conference on Phosphorus Chemistry, Bonn, September 1986). Chemical shifts are relative to TMS (^{1}H or ^{13}C spectra) or to 85% phosphoric acid (^{31}P spectra), downfield positive.

Electron-impact mass spectra were obtained using an MSS-AEI/MS9 Double Focussing Mass Spectrometer operating at 70 eV and at the lowest temperature necessary for volatilisation of the sample.

FAB mass spectra were obtained through the SERC service at PCMU (Physico-Chemical Measurement Unit, Harwell), with a VG Analytical ZAB-1F spectrometer. A primary beam of xenon atoms was produced from an ion gun (Ion-Tech Ltd.) operating at 8 Kv.

GAS CHROMATOGRAPHIC METHODS

Gas chromatography was carried out with a Varian Model 3700 Gas Chromatograph equipped with flame-ionization (FID) and thermionic (TSD) detectors, and on a Varian Model 3300 instruments fitted with FID detection system. The carrier gas was nitrogen.

GAS-CHROMATOGRAPHY-MASS SPECTROMETRY

GC-MS was carried out by the SERC Mass Spectrometry Centre, University College, Swansea, and at the School of Pharmacy, University of London, on a Hewlett Packard instrument, model 5890 A, VG Masslab 12-250 quadrupole Mass Spectrometer.

PREPARATIONS OF COMPOUNDS

Preparation of potassium O,O-diethyl phosphorodithioate, $(EtO)_2P(S)SK$

$$4C_2H_5OH + P_2S_5 \longrightarrow 2(C_2H_5O)_2PS_2H + H_2S$$

$$(C_2H_5O)_2\ PS_2H + KOH \longrightarrow (C_2H_5O)_2PS_2K + H_2O$$

To a 250 cm^3 three-necked round bottom flask equipped with a magnetic stirrer, phosphorus pentasulfide (22.2 g, 0.100 mol) and toluene (30cm^3) were added. The flask was heated to 70°C, and ethanol (18.7 g, 0.407 mol) was added dropwise. The mixture was then refluxed at 100°C for 2 hours, after which time toluene (20 cm^3) was added and the flask was cooled to room temperature. KOH pellets (13.2 g of 85% KOH, 0.2 mol) were ground into a powder and then added to the flask and a white precipitate was formed. Further toluene (20 cm^3) was added and the mixture was left stirring overnight. The precipitate was filtered, washed with toluene (2 x 10 cm^3), and dried in a vacuum for 0.5 hour. The product was recrystallised by dissolving it in acetone (2 x 100 cm^3), filtering off the impurities, concentration at room temperature, and the addition of diethyl ether (150 cm^3), to give potassium O,O diethyl phosphorodithioate (17.6 g, 33.3%), m.p.198°C, (Pianka [32],

m.p. 200-201°C). The filtrate was evaporated to dryness, under vaccum at room temperature, to give a further quantity of the potassium salt (1.0 g, 1.9%).

Preparation of diethoxyphosphinothioylthioacetic acid, $(EtO)_2PS_2CH_2CO_2H$

$$(EtO)_2PS_2K + ClCH_2CO_2H \longrightarrow (EtO)_2PS_2CH_2CO_2H + KCl$$

Chloroacetic acid (1.1 g, 11.6 mmol) was dissolved in AnalaR acetone (32 cm^3) and added to potassium O,O-diethylphosphorodithioate (2.5 g, 11.2 mmol). The mixture was refluxed for 0.5 hour at a temperature of 80°C. The product was cooled and left to settle for one day, then filtered under vacuum. The filtrate was evaporated under vacuum to a third of its volume to leave a viscous liquid residue of diethoxyphosphinothioylthioacetic acid (0.92 g, 87.6 %), n_D^{20} 1.5213. δ_P 92 (t quintet, J_{POCH} 8.3 Hz, J_{PSCH} 16.6 Hz); δ_H 1.33 (CH_3, t, J_{HCCH} 8.0 Hz), 3.66 (SCH_2, d, J_{PSCH} 17.5 Hz), 4.16 (CH_2O, d of quartets, J_{HCCH} 7.0 Hz, J_{POCH} 10.5 Hz), 11.80 (OH, s); δ_C 15.8 (CH_3, d, J_{POCC} 8.0 Hz), 34.9 (SCH_2, d, J_{PSC} 3.1 Hz), 64.5 (CH_2O, d, J_{PCO} 5.5 Hz), 175.0 (C=O, d, J_{PSCC} 3.0 Hz). FAB: m/z 245 (MH^+, 100%). The electron impact mass spectrum does not show a molecular ion; the base peak was at m/z 62.

Preparation of methyl diethoxyphosphinothioylthioacetate, $(EtO)_2PS_2CH_2CO_2Me$.

$$(EtO)_2S_2K + BrCH_2CO_2Me \longrightarrow (EtO)_2PS_2CH_2CO_2Me + KBr$$

Methyl bromoacetate (1.4 g, 9.15 mmol) was added to AnalaR acetone (25 cm^3) and transferred to a round-bottom flask containing potassium O,O-diethyl phosphorodithioate (2.0 g, 8.93 mmol). This mixture was refluxed for 3 hours at a temperature of 80°C and then filtered. The solvent was evaporated at room temperature and the liquid residue was distilled to give methyl diethoxyphosphinothioacetate a viscous, colourless liquid, b.p. 100°C at 0.07 mmHg, (Lui *et al* [33] b.p. 99.6-100.5°C/0.07 mmHg), n_D^{19} 1.5059, δ_P 92.3 (t quintet, J_{POCH} 8.6 Hz, J_{PSCH} 17.2 Hz); δ_H 1.40 (CH_3, t, J_{HCCH} 6.0 Hz), 3.60 (SCH_3, d, J_{HCCH} 12.0 Hz), 3.74 (OCH_3, s), 4.15 (CH_2O, d of quartets, J_{HCCH} 6.0 Hz), J_{POCH}, 10.0 Hz) ; δ_C 15.7 (CH_3, d, J_{POCC} 8.5 Hz), 34.8 (SCH_2, d, J_{PSC} 3.7 Hz), 52.8 (OCH_3, s), 64.2 (CH_2O, d, J_{POC} 6.1 Hz), 169.1 (C=O, d, J_{PSCC} 3.7 Hz). E.I. mass spectrum m/z 258(M^+, 74%) and a base peak at m/z 97.

Preparation of O,O-diethyl S-methyl phosphorodithioate, $(EtO)_2PS_2CH_3$.

$$(EtO)_2PS_2K + CH_3I \longrightarrow (EtO)_2PS_2CH_3 + KI$$

Potassium O,O-diethyl phosphorodithioate (1.5 g, 6.70 mmol) was dissolved in AnalaR acetone (10 cm^3) and iodomethane (1.0 g) was added. The precipitation of KI was left to be completed overnight at room temperature. Then it was filtered off and washed with acetone. The filtrate and washings showed a slightly yellow colour. The solvent was removed at room temperature under vacuum to leave a reddish liquid and a white solid. The supernatant liquid was removed, left for 0.5 hour under vacuum, and was then distilled to give O,O-diethyl S-methyl phosphororodithioate as a pale yellow liquid, b.p. 75°C at 0.5 mmHg. δ_P 95.0 ppm, (m); δ_H 1.14 (CH_3, t, J_{HCCH} 7.0 Hz), 2.05 (SCH_2, d of quartets, J_{PSCH} 15.0 Hz), 3.94 (CH_2O, d of quartets, J_{HCCH} 5.0 Hz, J_{POCH} 7.5 Hz); δ_C 14.9 (SCH_3, d, J_{PSC} 4.3 Hz), 15.9 (CH_3, d, J_{POCC} 7.9 Hz), 63.9 (CH_2O, d, J_{PSC} 6.1 Hz). E.I. mass spectrum: m/z 200 (M^+, 100%)

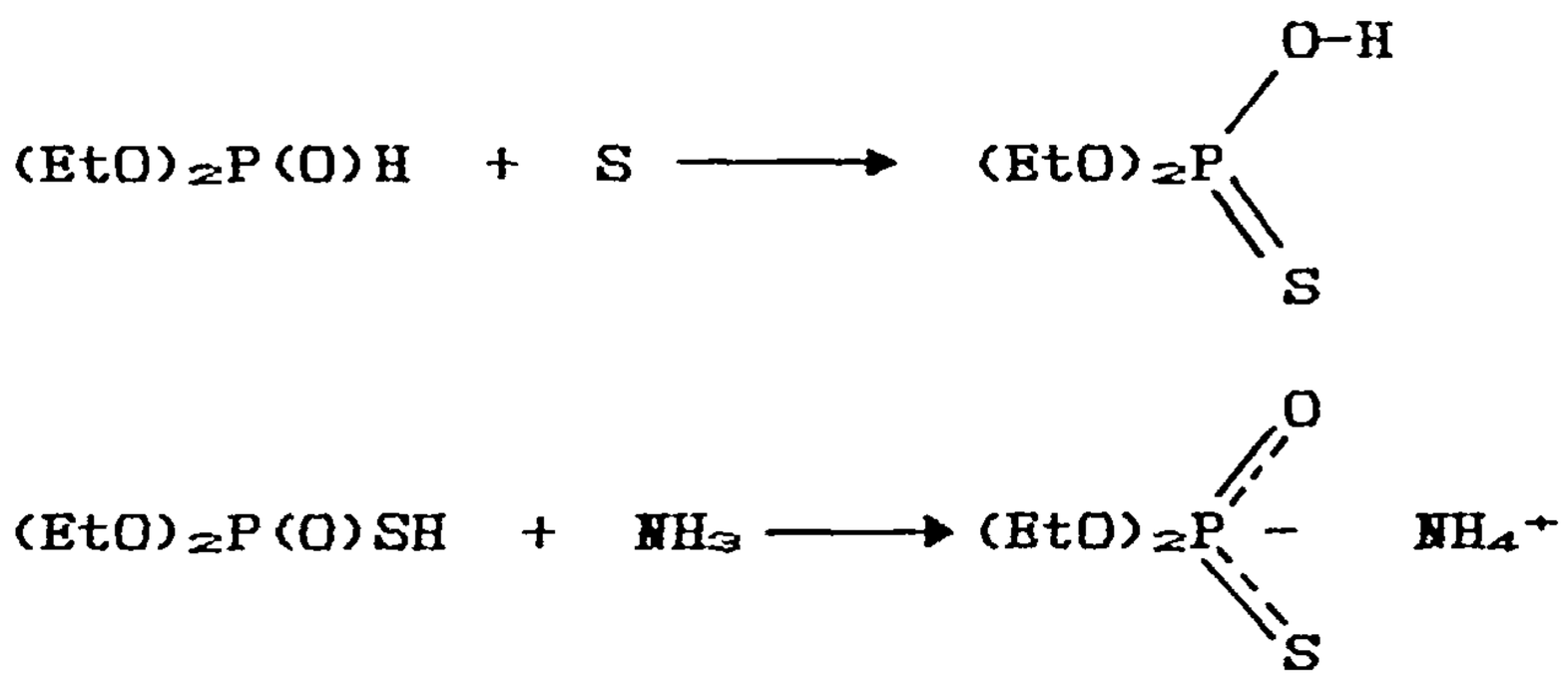
Preparation of O,O-diethyl dithiophosphoric acid, $(EtO)_2PS_2H$.

$$(EtO)_2PS_2K + HCl \longrightarrow (EtO)_2PS_2H + KCl$$

Potassium O,O-diethyl phosphorodithioate (4.0 g, 17.9 mmmol) was dissolved in a minimum of water and concentrated HCl (2.0 cm^3) was added. O,O-Diethyldithiophosphoric acid was extracted thrice with an

equal volume of diethyl ether and the ether layers were combined. Anhydrous Na_2SO_4 was added to remove water. The solution was filtered under vacuum, and the filtrate was evaporated on a water bath under vacuum to give a slightly green liquid residue which was distilled to give O,O-diethyldithiophosphoric acid as a colourless liquid, b.p. 52°C at 0.05 mmHg, n_D^{20} 1.5130. δ_P 85 (quintet J_{POCH} 10.2 Hz), (Jancke [34], δ_H 84.3, J_{POCH} 10.2 Hz); δ_H 1.35 (CH_3, t, J_{HCCH} 6.0 Hz), 3.40 (SH, s), 4.25 (CH_2O, d of quartets, J_{HCCH} 6.0 Hz, J_{POCH} 10.0 Hz); δ_C 15.7 (CH_3, d, J_{POCC} 8.5 Hz), 64.2 (CH_2O, d, J_{POC} 5.5 Hz). FAB: m/z 187 (MH^+, 12.5%). E.I. mass spectrum: m/z 186 (M^+, 16%).

Preparation of ammonium O,O-diethyl phosphorothioate, $(EtO)_2P(O)S^-NH_4^+$.



Sulphur (5.3 g, 0.166 g atom) and dry 1,2-dichloroethane (100 cm^3) were placed in a 250 cm^3 three-necked flask equipped with a magnetic stirrer. To the centre

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quartets, J_{HCCH} 5.0 Hz, J_{POCH} 7.0 Hz). FAB: m/z 188 (MH^+, 43.3%).

Preparation of O,O-diethoxyphosphinylthioacetic acid, $(EtO)_2P(O)SCH_2CO_2H$.

$$(EtO)_2P(O)SNH_4 + ClCH_2CO_2H \longrightarrow (EtO)_2P(O)SCH_2CO_2H + NH_4Cl$$

Ammonium O,O-diethyl phosphorothioate (1.0 g, 5.3 mmol) was dissolved in AnalaR acetone (11.0 cm^3) and chloroacetic acid (0.5 g, 5.3 mmol) was added. The system was refluxed for 2.0 hours at 80°C, and then filtered under vacuum to remove ammonium chloride. Solvent was removed from the filtrate under vacuum (0.5 hour) to leave O,O-diethoxyphosphinylthioacetic acid, as a yellow viscous oil. FAB: m/z 229 (MH^+ 3.9%). E.I. mass spectrum: m/z (M^+1%).

Preparation of methyl diethoxyphosphinylthioacetate, $(EtO)_2P(O)SCH_2CO_2Me$.

$$(EtO)_2P(O)SNH_4 + BrCH_2CO_2Me \longrightarrow (EtO)_2P(O)SCH_2CO_2Me + NH_4Br$$

Ammonium O,O diethyl phosphorothioate (1.2 g, 6.4 mmol) was dissolved in AnalaR acetone (10 cm^3) and methyl bromoacetate (1.0 g, mmol) was added. A white precipitate of

ammonium bromide was formed and the system was stirred for 4.0 hours. The solid was then filtered off under vaccum and washed with acetone. The combined filtrate and washings were evaporated under reduced pressure and liquid residue then distilled to give a pale yellow liquid, b.p. 84°C at 0.5 mmHg, δ_P 24.8 (t quintet, J_{POCH} 7.9 Hz, J_{PSCH} 15.8 Hz); δ_H 1.32 (CH_3, t, J_{HCCH} 6.0 Hz), 3.50 (SCH_2, d, J_{PSCH} 15.0 Hz), 3.65 (OCH_3, s), 4.00 (CH_2O, d of quartets, J_{HCCH} 9.0 Hz J_{POCH} 10.5 Hz), [Baboulene [36], δ_H 1.41 (Ha, t), 3.62 (Hc, d with J(P-Hc)= 15.0 Hz, 3.78 (Hd, s), 4.22 (Hb, q with J(P-Hb)= J(Ha-Hb)= 7.42 Hz)]; δ_C 15.8 (CH_3, d, J_{POCC} 7.3 Hz), 32.1 (SCH_2, d, J_{PSC} 3.6 Hz), 52.7 (OCH_3, s), 63.9 (CH_2O, d, J_{POC} 5.5 Hz). E.I. mass spectrum: m/z 242 (M^+, 18%).

Preparation of O,O-diethyl S-methyl phosphorothioate, $(EtO)_2P(O)SCH_3$.

$$(EtO)_2P(O)SNH_4 + CH_3I \longrightarrow (EtO)_2P(O)SCH_3 + NH_4I$$

Ammonium phosphorothioate (3.0 g, 16.3 mmol) was dissolved in AnalaR acetone (30 cm^3). Iodomethane (2.3 g, 16.2 mmol) was added and the reacton mixture was left heated under reflux at 50°C for 1 hour. The liquid was evaporated under reduced pressure and the solid residue formed from which was extracted with dry ether thrice, the crude $(EtO)_2P(O)CH_3$. The yellowish liquid was distilled,

b.p. at 20 mmHg 65°C, to give O,O-diethyl S-methyl phosphorothioate as a colourless liquid. δ_P 28.5 ppm, (m); δ_H 1.35 (CH_3, t, J_{HCCH} 6.5 Hz), 2.75 (SCH_3, d, J_{PSCH} 14.5 Hz), 4.25 (CH_2O, d of quartets, J_{HCCH} 6.5 Hz, J_{POCH} 9.0 Hz); δ_C 12.3 (SCH_3, d, J_{PSC} 4.8 Hz), 16.1 (CH_3, d, J_{POCC} 6.7 Hz), 63.6 (CH_2O, d, J_{POC} 6.1 Hz).

Preparation of O,O-diethyl hydrogen phosphorothioate, $(EtO)_2P(O)SH$.

$$(EtO)_2P(O)SNH_4 + HCl \longrightarrow (EtO)P(O)SH + NH_4Cl$$

Ammonium phosphorothioate (5.0 g, 26.7 mmol) was dissolved in a minimum of water, and concentrated HCl was added until the solution was acid. The product was extracted thrice with an equal volume of diethyl ether, and the ether layers were combined. Anhydrous Na_2SO_4 was added to remove water. The solution was then filtered and the filtrate evaporated under high vacuum to give O,O-diethyl hydrogen phosphorothioate as a colourless liquid . δ_P 60.6 (quintet, J_{POCH} 9.1 Hz); δ_H 1.40 (CH_3, t, J_{HCCH} 6.5 Hz), 4.25 (CH_2O, d of quartets J_{HCCH} 6.0 Hz, J_{POCH} 10.5 Hz), 7.00 (SH, s); δ_C 15.9 (CH_3, d, J_{POCC} 7.3 Hz), 64.5 (CH_2O, d, J_{POC} 5.5 Hz).

Preparation of ethyl N-methylcarbamate, EtOCONHMe

$$EtOCOCl + 2MeNH_2 \xrightarrow{toluene} EtOCONHMe + MeNH_3Cl$$

Toluene (100 cm^3) was added to ethyl chloroformate (32.8 g, mol). The flask was cooled in an ice bath, and methylamine was bubbled for 20 minutes giving a white precipitate of methylammmonium chloride, which was filtered off and washed with toluene (20 cm^3). The filtrate was concentrated to constant weight and then distilled to give ethyl N-methylcarbamate as colourless liquid, (15.6 g, 50.2%), b.p. 70°C at 0.05 mmHg, n_D^{20} 1.4155.

Preparation of ethyl N-chloroacetyl-N-methylcarbamate, $ClCH_2CONMeCO_2Et$

$$EtOCONHMe + ClCH_2COCl \longrightarrow ClCH_2CONMeCO_2Et + HCl$$

Ethyl N-methylcarbamate (18.8 g, 0.18 mol) and chloroacetyl chloride (20.6 g, 0.18 mol) were allowed to react under reflux for 3 hours at 130°C. The system was left to cool to room temperature and then cooled in ice bath. After 10 minutes, the crystals that appeared were filtered off and recrystallised from petroleum ether, b.p. 30-40°C, to give a first bath of product. Further crystals from the

filtrate were recrystallised from petroleum ether, b.p. 60-80°C, with charcoal treatment. The two batches were then combined, washed with cold petroleum ether, and dried to give N-chloroacetyl-N-methylcarbamate, m.p. 32°C. (Pianka [37], m.p. 34-36°). ν_{max} 1740(sh), 1720(m) (COOEt), 1700(w), 1680(sh) (CONMe), 780(s), 720(sh), 700(s) (C-Cl). δ_H 1.35 (CH_3, t, J_{HCCH} 6.0 Hz), 3.20 (NCH_3, s), 4.25 (CH_2O, d, J_{HCCH} 7.0 Hz), 4.70 ($ClCH_2$, s); δ_C 14.2 (CH_2CH_3, s), 31.8 (NCH_3, s), 46.5 ($ClCH_2$, s), 63.6 (OCH_2CH_3, s), 154.4 (COC, s), 163.7 (COO, s).

Preparation of S-(N-Ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorodithioate, $(EtO)_2PS_2CH_2CONMeCO_2Et$.

$$ClCH_2CONMeCO_2Et + (EtO)_2PS_2K \longrightarrow (EtO)_2PS_2CH_2CONMeCO_2Et + KCl$$

N-chloroacetyl-N-methylcarbamate (7.2 g, 40 mmol) in acetone (40 cm^3) was added dropwise to potassium O,O-diethyl phosphorodithioate (8.32 g, 37 mmol) in acetone (100 cm^3) at 35°C. The mixture was heated under reflux for 1 hour, allowed to stand at room temperature for 20 hours and then filtered to yield potassium chloride (2.8 g, 100%) and a filtrate from which acetone was removed under reduced pressure at 35°C. The residue was dissolved in diethyl ether (100 cm^3) and the resulting solution was washed with saturated aqueous sodium chloride containing potassium

carbonate (1.0%), (3 x 20 cm^3). The ether layer was then dried with potassium carbonate and the ether removed under reduced pressure at 30°C. The residue was a viscous liquid which was purified on a column (34 x 2.5 cm) of florisil, using a mixture of 50% hexane-dried ether as eluent (4 x 50 cm^3). The first fraction was passed a second time through the florisil column to give a colourless liquid residue of S-(N-ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorodithioate (7.1 g, 58.2%). δ_P 93.5 ppm, (m); δ_H 1.37 (CH_3, t, J_{HCCH} 7.1 Hz, 3.22 (NCH_3, s), 4.20 (CH_2, m), 4.27 (SCH_2, d, J_{PSCH} 15.1 Hz); δ_C 14.2 (CH_3, s), 15.8 ($\underline{C}H_3CH_2O$, d, J_{POCC} 7.5 Hz), 31.6 (NCH_2, s), 39.9(SCH_2, d, J_{PSC} 2.4 Hz), 63.5 ($CH_3\underline{C}H_2OCO$, s) 64.2($CH_3\underline{C}H_2OP$, d, J_{POC} 6.1 Hz). FAB: m/z 330(MH^+, 28.3%), base peak at m/z 227.

Preparation of S-(N-Ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorothioate, $(EtO)_2P(O)SCH_2CONMeCO_2Et$.

$$ClCH_2CONMeCO_2Et + (EtO)_2P(O)SNH_4 \longrightarrow (EtO)_2P(O)SCH_2CONMeCO_2Et + NH_4Cl$$

Ammonium O,O-diethyl phosphorothioate (6.28 g, 33 mmol) was dissolved in acetone (75 cm^3) and N-chloroacetyl-N-methylcarbamate (5.4 g, 30 mmol) in acetone (30 cm^3) was added dropwise with stirring. The reaction mixture was heated under reflux for 1 hour at 65°C. Then it

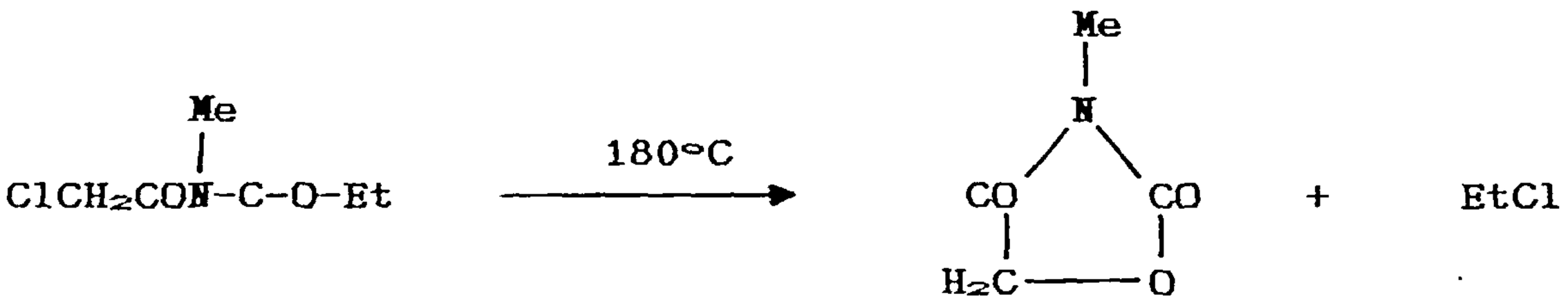
was left to reach room temperature and filtered. The solid ammonium chloride was dried for 3 hours to give a yield of 1.10 g, (61.5%). The filtrate was evaporated under reduced pressure at 40°C to remove acetone, and dry diethyl ether (100 cm^3) was added producing a precipitate that was rejected. The supernatant liquid was washed with a saturated aqueous sodium chloride solution containing potassium carbonate (1%), (3 x 20 cm^3), dried over anhydrous K_2CO_3 (1.0 g) and left to dry for 1 hour, and the ether was removed under high vacuum. The product was passed through a column (1.8 x 30 cm^3) of florisil (7.0 g), using petrolem ether b.p. 80°C (3 x 50 cm^3) as eluent, to give S-(N-ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorothioate as a slightly yellow liquid (6.0 g, 57.1%), δ_P 270 ppm (m); δ_P 26.3 ppm; δ_H 1.35 (CH_3, t, J_{HCCH} 6.0 Hz), 3.20 (NCH_3, s), 4.25 (SCH_2, J_{PSCH} 13.9 Hz), 4.26 (CH_2, m); δ_C 14.2 (CH_3, s), 16.0 ($\underline{C}H_3CH_2O$, d, J_{POCC} 7.3 Hz), 31.7 (NCH_3, s) 37.6 (SCH_2, d, J_{PSC} 2.4 Hz), 63.6 ($CH_3\underline{C}H_2OCO$, s); 63.9($CH_3\underline{C}H_2OP$, d, J_{POC} 6.1 Hz), 154.4 ($N\underline{C}O_2Et$, s), 170.6 ($SCH_2\underline{C}O$, d, J_{PSCC} 2.0 Hz). The gc-ms gives M^+ 313(1%).

Preparation of O,O-diethyl S-(hydroxymethyl) phosphorodithioate, $(EtO)_2PS_2CH_2OH$.

$$(EtO)_2PS_2H + (CH_2O)_n \xrightarrow[HCl]{\underline{dried\ ether}} (EtO)_2PS_2CH_2OH$$

O,O-Diethyl phosphorodithioate (4.5 g, 24 mmol) was dissolved in 50 cm^3 of anhydrous diethyl ether. Then paraformaldehyde (0.73 g, 24.3 mmol) and anhydrous diethyl ether (.50 cm^3) were added. The mixture was cooled to about 0°C and anhydrous hydrogen chloride was bubbled through continuously for 2.5 hours. Then diethyl ether was removed, under reduced pressure to leave O,O-diethyl S-hydroxymethyl phosphorodithioate as a colourless oil. δ_P 92.6 (t quintet, J_{POCH} 9.0 Hz, J_{PSCH} 18.0 Hz); δ_H 1.38 (CH_3, t, J_{HCCH} 7.1 Hz), 3.64(OH, s) 4.18 (CH_2O, d, of quartets J_{HCCH} 6.6 Hz, J_{POCH} 10.0 Hz), 5.13 (SCH_2, d, J_{PSC} 22.6 Hz; δ_C 15.8 (CH_3, d, J_{POCC} 7.9 Hz, 64.4 (CH_2O, J_{POC} 6.7 Hz, 68.1 (SCH_2, d, J_{PSC} 3.7 Hz). FAB: m/z 217 (MH^+, 5.7%), base peak at m/z 199. E.I. mass spectrum does not showed a molecular ion and the base peak was at m/z 97.

Preparation of 3-methyl-oxazolid-2,4-dione



N-chloroacetyl-N-methylcarbamate (0.7 g, 3.9 mmol) was heated at 180°C for 110 minutes, then cooled to room temperature. The product was obtained as a solid that after

removal of traces of ethyl chloride had m.p. 124°C, (Spielman [38], 128°C, Iwaya [39], 133°C). ν_{MAX} 1830 (C=O), 1755, 1735, 1720 (sh) (C=O), [Pianka [37], 1826 (2-CO), 1748, 1732 (4-CO)] cm^{-1}. δ_H 1.35(CH_3, t, J_{HCCH} 6.0 Hz), 3.20 (NCH_3, s), 4.25 (CH_2, d, J_{HCCH} 7.0 Hz), 4.70 ($ClCH_2$, s); δ_C 25.9(NMe, s), 68.2 (CH_2, s), 156.6 ($\underline{C}OCH_2$, s), 170.6 ($\underline{C}O$, s).

Oxidation of O,O-Diethylphosphorodithioic acid to the Disulfide, $[(EtO)_2PS_2]_2$

$$2(EtO)_2PS_2H + H_2O_2 \longrightarrow [(EtO)_2PS_2]_2 + 2H_2O$$

The acid (0.23 g, 12.4 mmol) was treated with 0.1 cm^3 of hydrogen peroxide (30%) and 2.3 cm^3 H_2O (iced water). After allowing to stand overnight, the product was extracted with methylene chloride, dried with sodium sulphate, and the solvent evaporated to give the disulfide, as colourless liquid. δ_P 84.8 (quintet, J_{POCH} 10.1 Hz), (Lippman [40], δ_P 80.4 ppm; δ_H 1.40 (CH_3, t, J_{HCCH} 6.3 Hz), 4.25 (CH_2, m); δ_C 15.8 (CH_3, d, J_{POCC} 8.5 Hz), 64.9 (CH_2O, d, J_{POCH} 5.5 Hz). FAB: m/z 371 (MH^+, 100%). E.I. mass spectrum: m/z 370 (M^+, 25%).

HYDROLYSIS STUDIES

Rate measurements using gas-chromatography

Mecarbam and benzophenone (as internal standard) were dissolved in a solvent system consisting of:

(a) 50% EtOH/50% buffer, pH 6.6 or 6.8;

(b) 50% dioxane/50% buffer, pH 7.0.

The solution was heated at 70°C in a thermostatted bath for several hours, during which time aliquots were taken at half hour intervals, and cooled for 5 min in a refrigerator, before analysis.

Gas Chromatographic Conditions

A Varian model 3700 gas chromatograph was used fitted with FID (Flame Ionization Detector) and a glass column packed with 4% SE 30 on Chromosorb W AW DMCS, of 6' in length, 1/4" O.D. (outside diameter), and 2 mm I.D. (internal diameter).

The analysis was carried out by temperature program starting at 151°C for 2 min, and heating at 20°C/min to 253°C. The temperature of the injector was 167°C, and the temperature of the detector was 399°C. The initial flow-rate

in the column was 30 cm^3/min (24 psig), with H_2 at 3 cm^3/min, Air at 300 cm^3/min in the detector.

First-order rate constants for the disappearance of mecarbam in the presence of excess of solvent were calculated from the equation:

$$k_1 = \frac{1}{t} \ln \frac{a}{a-x}$$

where: a= initial concentration of mecarbam

x= concentration at time t

A plot of ln(a/a-x) versus t thus gave a straight line of slope equal to k_1. The half-life for the reaction was given by:

$$t_{1/2} = \frac{0.693}{k_1}$$

Identification of hydrolysis products by gc-ms

Mecarbam

Two experiments for the study of hydrolysis of mecarbam were carried out, considering two different levels of degradation, partially (sample 2) and more nearly complete degraded (sample 1).

Experiment one: To 0.1529 g of mecarbam was added 5 ml of water and one pellet of KOH and 3 ml of hexane. After 1 hour shaking was added HCl until the solution is acid.

Experiment two: To 0.2076 g of mecarbam was added 5 ml of water and one pellet of KOH and 3 ml of hexane. After 2 hours shaking was added HCl until the solution was acid.

The hexane layers were methylated using diazomethane as described below and gas chromatographic analysis carried out on the Varian model 3700 fitted with a FID (flame ionization detector) and a glass column packed with 4% SE 30 on chromosorb W AW DMCS, of 6' in length, 1/4'' O.D. (outside diameter)and 2 mm I.D.(internal diameter). These samples were also analysed by g.c.-m.s., and the results are discussed in the section " g.c.-m.s. analysis for hydrolysis studies".

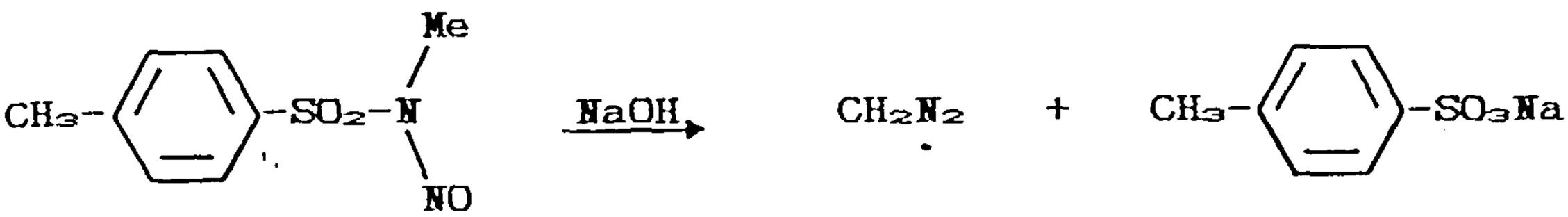
Chlormephos

The experiment for the study of hydrolysis of chlormephos was carried out in the conditions below.

To 0.2230 g of chlormephos was added 3 ml of water and one pellet of KOH and 5 ml of hexane. After 1 hour shaking was added HCl until the solution was acid.

The hexane layer was methylated and analysed by g.c., and further by g.c.-m.s.. See section "results and discussion" for analysis of the results.

Methylation [41]



Diazald

Nitrogen was passed through a system of four subsequent tubes. The first tube contained 20 cm³ diethyl ether. The second tube contained 7.5 cm³ diethyl ether, 0.5 g of diazald (N-methyl-N-nitroso-p-toluenesulfonamide), and 2.5 cm³ of alcoholic KOH. The third tube contained an aliquot of the sample. The fourth tube contained 15 cm³ of a mixture(1:1) of glacial acetic acid and diethyl ether.

The contents in tube 2 were immersed in a large volume of acetic acid to neutralize alkali and remove diazomethane. Nitrogen was passed through at two or three bubbles *per sec* until the solution turned yellow and then for a further five minutes.

MECARBAM AND CHLORMEPHOS DEGRADATION STUDIES IN LIVER HOMOGENATE

Mecarbam

The experiments (A and B) for the metabolic studies of mecarbam in lamb liver homogenate were carried out using the following procedure.

Experiment A: The mecarbam solution (4 ml) (5014 µg/ml) was added to 5 g of lamb liver, corresponding to 4011.2 ppm, and the mixture homogenised in 125 ml of water.

A control was made by homogenising 5g of liver in 125 ml of water.

Aliquots of the sample and of the control (25 ml) were taken immediately after homogenisation (taken as zero time) at room temperature.

To slow down the reaction the homogenates were placed in a cooling bath at -10°C and further aliquots of 25 ml from the sample and the control were taken after 1/6, 1/3 and 1/2 hour.

Extraction of the pesticide and its metabolites was carried out using the following organic solvents successively: hexane, dichloromethane, and diethyl ether. Dipotassium hydrogen phosphite was added to avoid formation of emulsion.

Each organic layer was collected in a round-bottomed flask and evaporated down to approximately 1 ml on a rotatory evaporator. A N_2 stream was then used until almost dry. Exactly 1 ml of hexane was added and the solution was analysed by gas chromatography. The sample was then methylated by diazomethane (as described) and further analysed by g.c. or gc-ms for the identificaton of metabolites.

Experiment B: The mecarbam solution (4 ml) (5014 µg/ml) was added to 1.5 g of lamb liver, corresponding to 13370.6 ppm and homogenised in 25 ml of water. Aliquots (10 ml) were taken 2 minutes after homogenisation at room temperature. The extraction of the pesticide and its metabolites was carried out with hexane (10 ml). The rest of the procedure was carried out as for experiment A.

Chlormephos

The experiments for the degradation studies of chlormephos were carried out using the the same procedure as used for mecarbam. However due to the high stability of this compound a much smaller concentration of the pesticide has been used.

Experiment A: Thus 1 ml of solution of chlormephos (484 µg/ml) was added to 50 g of lamb liver which corresponds to 9.68 ppm and was homogenised in 250 ml of water.

The control contained 50 g of liver in 250 ml of water.

Aliquots of the sample and control (25 ml) were taken immediately after homgenisation, (taken as zero hour time) at room temperature.

The sample and control were placed in a water bath at 41°C, and further aliquots were taken at one, two, three, four, five and six hours.

The rest of the procedure was carried out as for mecarbam.

Experiment B: The chlormephos solution (0.5 ml) (484 µg/ml) was added to 1.5 g of lamb liver, corresponding to 161.3 ppm and and homogenised in 25 ml of water. Aliquots were taken after 5 minutes at room temperature. The extracts of the pesticide and its metabolites was carried out in hexane. The rest of the procedure was carried out as for experiment A.

1 µl of the pesticide samples (mecarbam and chlormephos) were injected into the g.c. under the conditions below and the results are discussed in the section "g.c.-m.s. analysis for degradation studies".

Gas Chromatographic Conditions

A Varian model 3700 chromatograph was used fitted with a TSD (thermionic detector) and a glass column packed with 8% silicone fluid MS 200/12500 on chromosorb W-HP (4 mm x 2 m), T_{max} 220, of 2 m in length, 1/4" outside diameter and 4 mm internal diameter.

The parameters for analysis of mecarbam were: temperature program starting at 151°C for 5 minutes, and heating at 20°C/min to 210°C. The initial flow-rate in the column was 30 cm^3 (30 psig), with H_2 at 3 cm^3/min, and air at 300 cm^3/min in the detector.

The temperature program for the analysis of chlormephos differed in the final temperature, which was 180°C. The injection temperature, T_i was 250°C, and the detector temperature, T_d, 300°C.

RESULTS AND DISCUSSION

PREPARATION AND CHARACTERISATON BY NMR OF POSSIBLE METABOLITES

A range of phosphorus compounds related to mecarbam and to chlormephos have been prepared with the aim of investigating their possible formation as metabolites of these pesticides. The starting materials for this synthetic work were potassium O,O-diethyl phosphorodithioate (1) and ammonium O,O-diethyl phosphorothioate (2) which were prepared as follows [37].

$$4\ EtOH + P_2S_5 \longrightarrow 2(EtO)_2P(S)SH + H_2O$$

(3)

$$(EtO)_2P(S)SH + KOH \longrightarrow (EtO)_2P(S)SK + H_2O$$

(1)

$$(EtO)_2P(O)H + S + NH_3 \longrightarrow (EtO)_2P(O)SNH_4$$

(2)

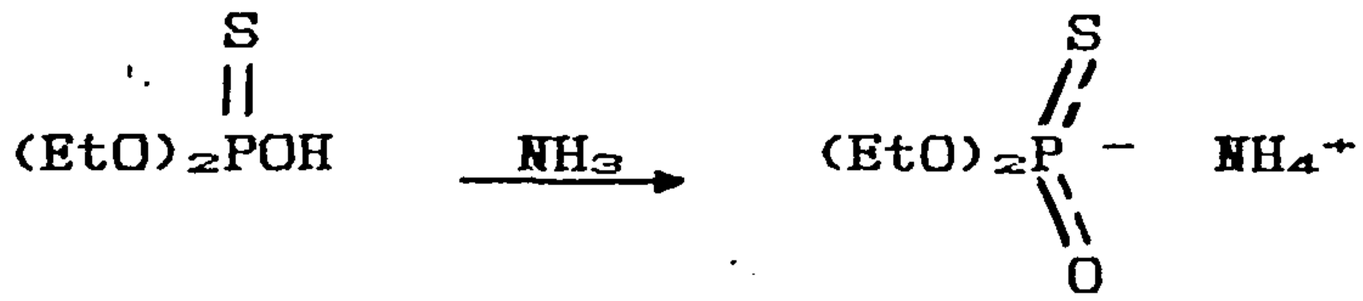
This second reaction is thought to involve direct addition of sulphur to the trivalent form of diethyl phosphite.

$$(EtO)_2PH \longrightarrow (EtO)_2POH \xrightarrow{S} (EtO)_2POH$$

(4) (5)

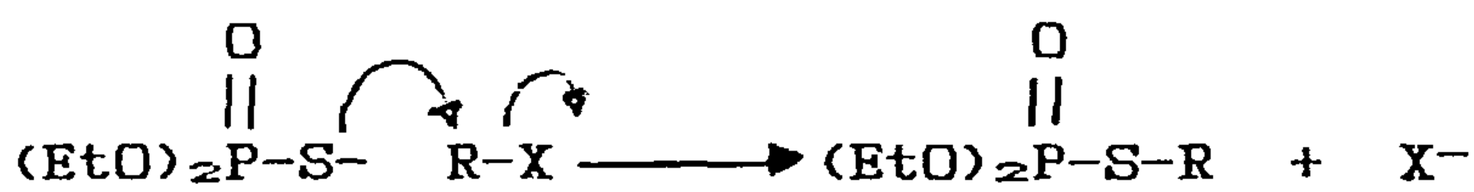
Although the acid (5) has the thiono structure as shown, the ammonium salt has a mesomeric anion in which

either the S or the O atom can act as a nucleophile. For reactions

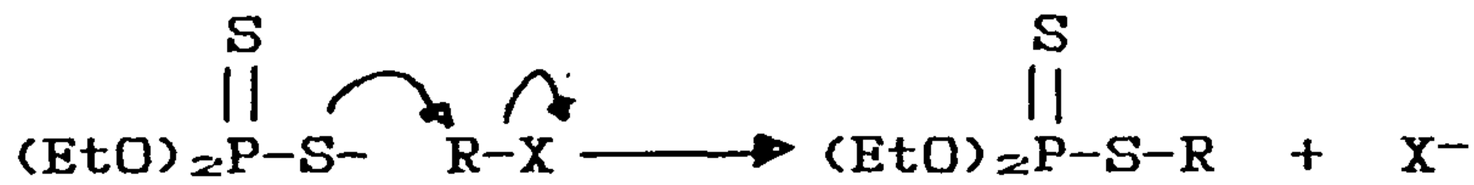


(2)

with halogeno compounds it is the softer S atom which reacts giving product containing a P=O rather than a P=S bond. P=O analogues of the pesticides and their metabolites can therefore be prepared.



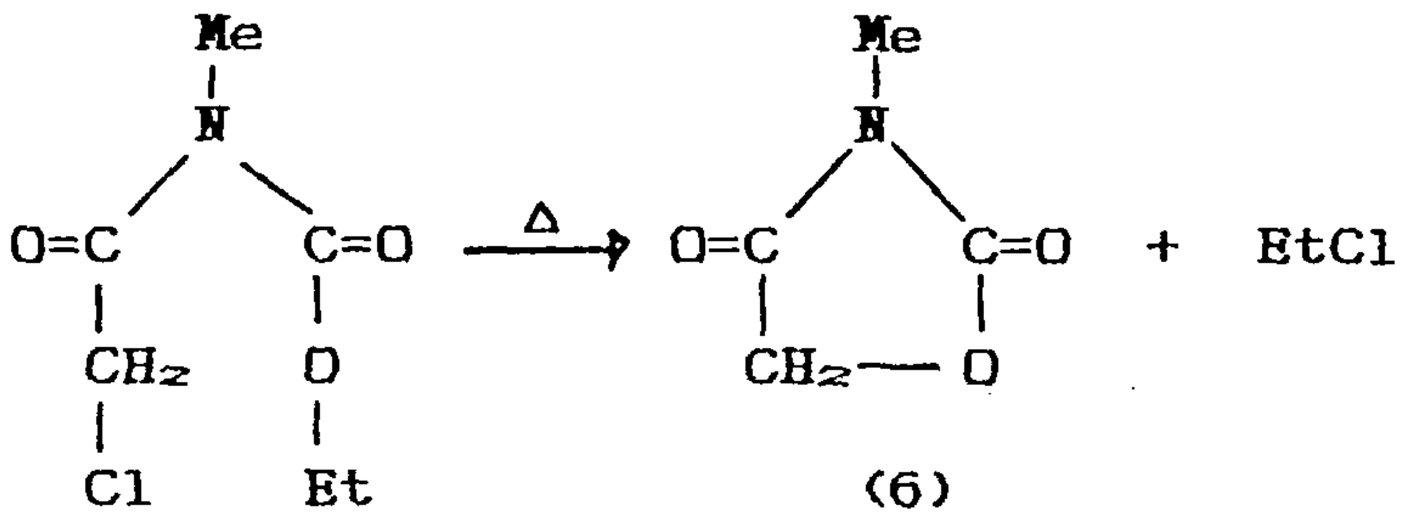
Using this procedure and also the potassium dithioate for the dithio derivatives, the compounds were prepared.



The chloro compound required for the preparation of mecarbam and its oxygen analogue was prepared by the following stages from ethyl chloroformate.

$$EtOCONHMe + ClCH_2COCl \xrightarrow{Et_3N} EtOCON(Me)COCH_2Cl + Et_3NHCl$$

It is known that this product can undergo elimination of ethyl chloride to form a cyclodione [37,43] and this was also prepared as it was considered to be a further possible degradation product of mecarbam



(6)

O,O-Diethyl S-hydroxymethyl phosphorodithioate (7) was prepared by a method involving the addition of formaldehyde to O,O-diethyl dithiophosphoric acid (3).

$$(EtO)_2PSH + CH_2O \longrightarrow (EtO)_2PS^{-}CH_2OH$$

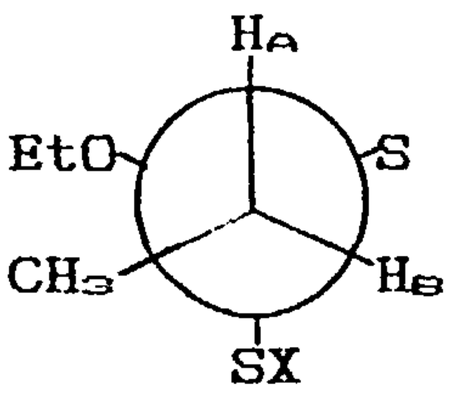
(7)

This is a somewhat unstable compound but has been described as an intermediate for the preparation of chlormephos by subsequent chlorination [44].

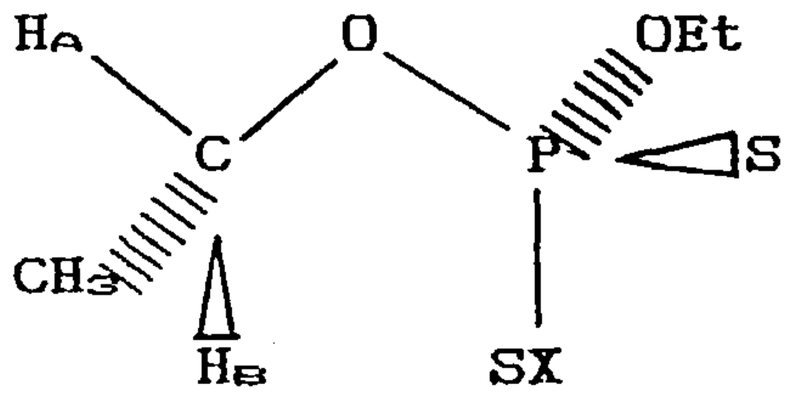
The compounds were identified mainly by n.m.r. spectroscopy. The ^{31}P n.m.r. chemical shifts (δ_P) confirmed the environment of the phosphorus atoms, while ^{1}H n.m.r. and ^{13}C n.m.r. spectra showed the characteristic patterns, chemical shifts (δ_H, δ_C) and coupling constants (J) for the

ethyl groups (CH_3CH_2) and other protons and carbon atoms in the molecule (Tables 1-3).

Further examination of the n.m.r. spectra of compounds of the type $(EtO)_2P(S)SX$ (when X = H, Me, ... CH_2OH, etc) showed that the non-equivalence of the α-methylene protons is due to intrinsic asymetry at the P atom 45.



or



When the nearer atom to the α-C of an ethyl group is a CH_3 group, and the atom at the rear is P (linked to C_α via O), this gives rise to an AB pattern for the CH_2 protons instead of a simple signal. The CH_2 protons are also coupled to the CH_3 group and to the P atom giving a total of 32 lines. ^{13}C n.m.r. showed the two ethyl groups to be equivalent.

^1H n.m.r. of $(EtO)_2P(S)SCH_2Cl$ at 80 MH_z (Bruker WP 80 NMR Spectrometer), gave a spectrum that is very complex, probably because of AB coupling between the two CH_2 protons. Higher resolution will be necessary to interpret the spectrum fully. The CH_2 protons of the ethyl group nevertheless showed a signal approximating to 4 overlapping quartets (see Table 2). Another interesting feature at 80 MHz is that P coupling to the CH_3 group of the ethoxy group

is revealed, showing a doublet of triplets with J_{POCC} 0.98 Hz. The CH_2 attached to the S (SCH_2) appeared at δ 4.91 ppm, as a doublet with J_{PSCH} 21.5 Hz. Wang [46] found for $(EtO)_2PS_2CH_2Cl$ δ_H 4.90 ppm and J_{PSCH} 21.0 Hz.

Table 1. ^{31}P n.m.r. Data ($CDCl_3$ or CD_3COCD_3), δ (ppm), for Mecarbam and Chlormephos Derivatives [a].

$(EtO)_2PS_2CH_2CO_2H$	92.0 (t of quintet, J_{POCH} 8.3 Hz, J_{PSCH} 16.6 Hz)
$(EtO)_2PS_2CH_2CO_2Me$	92.3 (t of quintet, J_{POCH} 8.6 Hz J_{PSCH} 17.2 Hz)
$(EtO)_2PS_2CH_3$	95.0 (mutiplet)
$(EtO)_2PS_2H$	85.0 (quintet, J_{POCH} 10.2 Hz)
$[(EtO)_2PS_2]_2$	84.8 (quintet, J_{POCH} 10.1 Hz)
$(EtO)_2PS_2CH_2CONMeCO_2Et$	93.5 (mutiplet)[b]
	92.4 (mutiplet)
$(EtO)_2PS_2CH_2Cl$	91.6 (3 overlapping quintets, J_{POCH} 10.3 Hz, J_{PSCH} 20.6 Hz)[b]
	91.2 (3 overlapping quintets, J_{POCH} 10.3 Hz, J_{PSCH} 20.6 Hz)
$(EtO)_2PS_2CH_2OH$	92.6 (t of quintet, J_{POCH} 9.0 Hz, J_{PSCH} 18.0 Hz)[b]
	95.4 (s), [^1H][bb]
$(EtO)_2P(O)SNH_4$	56.2 (mutiplet)
$(EtO)_2P(O)SCH_2CO_2Me$	24.8 (t of quintet, J_{POCH} 7.9 Hz, J_{PSCH} 15.8 Hz)
$(EtO)_2P(O)SCH_3$	28.5 (multiplet)
$(EtO)_2P(O)SH$	60.6 (quintet, J_{POCH} 9.1 Hz)
$(EtO)_2P(O)SCH_2CONMeCO_2Et$	27.0 (mutiplet)[b]
	26.3 (mutiplet)

[a] Determined on Bruker WP-80 Spectrometer unless otherwise indicated. [b] Determined on Bruker 400 instrument.

^{31}P n.m.r. data are given in Table 1 and were mainly recorded on the WP-80 spectrometer. For better interpretation of the coupling constants the spectra of mecarbam, its oxygen analogue, cholormephos and O,O-diethyl S-hydroxymethyl phosphorodithioate were also scanned at 400 MHz (Bruker spectrometer), but surprisingly no improvement was obtained. These spectra are discussed below.

Assuming the coupling of four CH_2O protons and of two SCH_2 protons to phosphorus, a 15 line spectrum (3 quintets) may be expected.

In most cases in which a $PSCH_2$ is present, the coupled spectrum showed a symmetrical pattern of equally spared lines. Seven were usually quite distinct and nine in total were sometimes recorded, corresponding to the overlap of three quintets for which J_{PSCH} is equal to twice J_{POCH}.

The spectrum of mecarbam showed only 7 lines on the 400 MHz instrument, although on the WP-80 a total of 11 were clearly seen. The spacings between the corresponding lines were similar but were unequal, and a clear interpretation was not possible. By comparison with other similar compounds a value for J_{POCH} of 9-10 Hz seems likely. The chemical shifts (δ) were at 93.5 and 92.4 ppm respectively.

Line-spacings for ^{31}P n.m.r. signal of mecarbam (Hz).

32.4 MHz	162.0 MHz
9.85	–
9.84	9.85
4.92	4.92
4.92	] 9.85
4.92	
5.91	] 9.84
3.94	
5.91	5.91

The spectrum of the oxygen analogue of mecarbam also showed 7 lines on both instruments but only 4 were sufficiently resolved on the 400 MHz spectrometer for their positions to be recorded, although the spaces between them were equal. The coupling constant value J_{POCH} was approximately 6-7 Hz, whilst the chemical shift were δ 27.0 and 26.3 ppm.

The spectrum of chlormephos appeared clearly as a nonet on both intruments. The overlapping of the the three quintets results in 9 lines in the spectrum with J_{POCH} = 10.3 Hz and J_{PSCH} = 20.6 Hz. The chemical shifts (δ) were 91.6 and 91.2 ppm respectively and the average value for the coupling constants was 10.3 Hz.

Table 2. ^1H n.m.r. Data ($CDCl_3$ or CD_3COCD_3), δ (ppm), for Mecarbam and Chlormephos Derivatives [a]

$(EtO)_2PS_2H$	1.35(CH_3, t, J_{HCCH} 6.0 Hz), 3.40 (SH, s), 4.25 (CH_2, d of quartets, J_{HCCH} 6.0 Hz, J_{POCH} 10.0 Hz).
$(EtO)_2P(O)SH$	1.40(CH_3, t, J_{HCCH} 6.5 Hz), 4.25 (CH_2, d of quartets J_{HCCH} 6.0 Hz, J_{HCCH} 10.5 Hz), 7.0 (SH, s).
$(EtO)_2PS_2CH_3$	1.14(CH_3, t, J_{HCCH} 7.0 Hz), 2.05 (SCH_2, d, J_{PSCH} 15.0 Hz), 3.94 (CH_2, d of quartets J_{HCCH} 5.0 Hz, J_{POCH} 7.5 Hz).
$(EtO)_2P(O)SCH_3$	1.35(CH_3, t, J_{HCCH} 6.5 Hz), 4.25 (CH_2, d of quartets J_{HCCH} 6.5 Hz, J_{POCH} 9.0 Hz), 2.75 (SCH_2, d, J_{PSCH} 14.5 Hz).
$(EtO)_2PS_2CH_2CO_2H$	1.33(CH_3, t, J_{HCCH} 8.0 Hz), 3.66 (SCH_2, d, J_{PSCH} 17.5 Hz), 4.16 (CH_2, d of quartets, J_{HCCH} 7.0 Hz, J_{POCH} 10.5 Hz), 11.80 (OH, s).
$(EtO)_2PS_2CH_2CO_2Me$	1.40(CH_3, t, J_{HCCH} 6.0 Hz), 3.60 (SCH_2, d, J_{HCCH} 12.0 Hz), 3.74 OCH_3, s), 4.15 (CH_2, d of quartets, J_{HCCH} 6.0 Hz, J_{POCH} 10.0 Hz).
$(EtO)_2P(O)SCH_2CO_2Me$	1.32 (CH_3,t, J_{HCCH} 6.0 Hz), 3.50 SCH_2, d, J_{PSCH} 15.0 Hz), 3.65 (OCH_3, s), 4.00 (CH_2, d of quartets, J_{HCCH} 9.0 Hz, J_{POCH} 10.5 Hz).
$(EtO)_2PS_2CH_2OH$	1.38(CH_3, t, J_{HCCH} 7.1 Hz), 3.64 (OH, s), 4.18 (CH_2, d of quartets, J_{HCCH} 6.6 Hz, J_{POCH} 10.0 Hz), 5.13 (SCH_2, d, J_{PSCH} 22.6 Hz).
$[(EtO)_2PS_2]_2P$	1.40 (CH_3, t, J_{HCCH} 6.3 Hz), 4.23 (CH_2, m of 13 lines centred at 4.25, between 4.0-4.5).

continuation of Table 2

$(EtO)_2PS_2CH_2CONMeCO_2Et$[b]	1.37(CH_3, t, J_{HCCH} 7.1 Hz), 3.22 (NMe, s), 4.20 (CH_2, m), 4,27 (SCH_2, d, J_{PSCH} 15.1 Hz).
$(EtO)_2P(O)SCH_2CONMeCO_2Et$[b]	1.35 (CH_3, t, J_{HCCH} 6.0 Hz), 3.20 (NCH_3, s), 4.25 (SCH_2, J_{PSCH} 13.9 Hz), 4.26 (CH_2, m).
$(EtO)_2PS_2CH_2Cl$ [b]	1.38 (CH_3, d of triplet, J_{HCCH} 7.1 Hz, J_{POCH} 0.98 Hz), 4.20 (CH_2, 4 overlapping quartets, J_{PCCH} 7.4 Hz, J_{POCH} 7.1 Hz, Δ AB 1.9 Hz), 4.91 (SCH_2, d, J_{PSCH} 21.5 Hz).
$(EtO)_2P(O)SNH_4$	1.18 (CH_3, t, J_{HCCH} 6.0 Hz), 3.80 (CH_2, d of quartets, J_{HCCH} 5.0 Hz, J_{POCH} 7.0 Hz).
$ClCH_2CONMeCOOEt$	1.35 (CH_3, t, J_{HCCH} 6.0 Hz), 3.20 (NCH_3, s), 4.25 (CH_2, d, J_{HCCH} 7.0 Hz), 4.70 ($ClCH_2$, s).

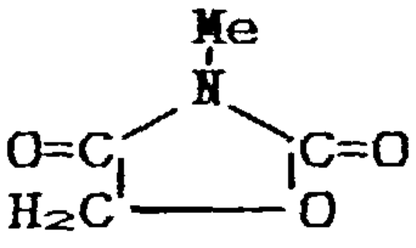
a Determined on a Perkin Elmer R 12B-60 MHz unless otherwise indicated.

b Determined on a Bruker WP-80 Spectrometer.

Table 3. ^{13}C n.m.r. Data ($CDCl_3$ or $CDCOCD_3$, δ (ppm), for Mercabam and Chlormephos Derivatives

$(EtO)_2PS_2H$	15.7(CH_3, d, J_{POCC} 8.5 Hz), 64.2 (CH_2, d, J_{POC} 5.5 Hz).
$(EtO)_2P(O)SH$	15.9(CH_3, d, J_{POCC} 7.3 Hz), 64.5 (CH_2, d, J_{POC} 5.5 Hz).
$(EtO)_2PS_2CH_3$	14.9(SCH_3, d, J_{PSC} 4.3 Hz), 15.9 (CH_3, d, J_{POCC} 7.9 Hz), 63.9 (CH_2, d, J_{PSC} 6.1 Hz).
$(EtO)_2P(O)SCH_3$	12.3(SCH_3, d, J_{PSC} 4.8 Hz), 16.1 (CH_3, d, J_{POCC} 6.7 Hz), 63.6 (CH_2, d, J_{POC} 6.1 Hz).
$(EtO)_2PS_2CH_2CO_2H$	15.8(CH_3, d, J_{POCC} 8.0 Hz), 34.9 (SCH_2, d, J_{PSC} 3.1 Hz), 64.5 (CH_2, d, J_{POC} 5.5 Hz), 175.0 (C=O, d, J_{PSCC} 3.0 Hz).
$(EtO)_2PS_2CH_2CO_2Me$	15.7(CH_3, d, J_{POCC} 8.5 Hz), 34.8 (SCH_2, d, J_{PSC} 3.7 Hz), 52.8 (OCH_3, s), 64.2 (CH_2, d, J_{POC} 6.1 Hz), 169.1 (C=O, d, J_{PSCC} 3.7 Hz).
$(EtO)_2P(O)SCH_2CO_2Me$	15.8(CH_3, d, J_{POCC} 7.3 Hz), 32.1 (SCH_2, d, J_{PSC} 3.6 Hz), 52.7 (OCH_3, s), 63.9 (CH_2, d, J_{POC} 5.5 Hz).
$(EtO)_2PS_2CH_2OH$	15.8(CH_3, d, J_{POCC} 7.9 Hz), 64.4 (CH_2, d, J_{POC} 6.7 Hz), 68.1(SCH_2, d, J_{PSC} 3.7 Hz).
$[(EtO)_2PS_2]_2$	15.8 (CH_3, d, J_{POCC} 8.5 Hz), 64.9 (CH_2, d, J_{POC} 5.5 Hz).
$(EtO)_2PS_2CH_2CONMeCO_2Et$	14.2(CH_3 ,s), 15.8($\underline{CH_3}CH_2O$, d, J_{POCC} 7.5 Hz), 31.6 (NCH_3, s), 39.9 (SCH_2, d, J_{PSC} 2.4 Hz), 63.5 ($CH_3\underline{CH_2}OCO$, s), 64.2 ($CH_3\underline{CH_2}OP$, d, J_{POC} 6.1 Hz).

continuation of table 3.

$(EtO)_2P(O)SCH_2CONMeCO_2Et$	14.2(CH_3, s), 16.0($\underline{C}H_3CH_2O$, d, J_{POCC} 7.3 Hz), 31.7(NCH_3, s), 37.6 (SCH_2, d, J_{PSC} 2.4 Hz), 63.6 ($CH_3\underline{C}H_2OCO$, s), 63.9 ($CH_3\underline{C}H_2OP$, d, J_{POC} 6.1 Hz),154.4 ($N\underline{C}O_2Et$, s), 170.6 ($SCH_2\underline{C}O$, d, J_{PSCC} 2.0 Hz).
$(EtO)_2PS_2CH_2Cl$	15.8(CH_3, t, J_{HCCH} 8.5 Hz), 47.6 (SCH_2, d, J_{PSC} 4.9 Hz), 64.7 (CH_2, d, J_{POC} 7.0 Hz).
$ClCH_2CONMeCO_2Et$	14.2 ($CH_2\underline{C}H_3$, s) 31.8 (NMe, s), 46.5 ($ClCH_2$, s), 63.6($O\underline{C}H_2CH_3$,s), 154.4 ($\underline{C}OC$, s), 163.7 ($\underline{C}OO$, s).
	25.9 (NMe, s), 68.2 (CH_2, s), 156.2 ($\underline{C}OCH_2$, s), 170.6 ($\underline{C}OO$, s).

CHARACTERISATION OF ORGANOPHOSPHORUS COMPOUNDS BY INFRARED SPECTROSCOPY

Organophosphorus compounds containing sub-groups within the molecule such as P-H, P-OH, P=O, P=S, P-OR, P-Cl etc. give not only characteristic keys for identification of their functional groups but also yield information about the nature of the groups attached to the P=O, P=S, P-OH groups etc. The small frequency variations of a given functional group tend to be systematic, so that this frequency variation can give relevant information about the nature of the chemical groups adjacent to the phosphorus group in question [47].

The P=O vibration always produces a strong band from 1150-1310 cm^{-1}, however it may occur that occasional compounds give an wider range from 1080-1380 cm^{-1} [47].

The P=S stretching vibration is more variable in intensity than the P=O vibration and is found in the range of 550-760 cm^{-1}. It is calculated to be at 675 cm^{-1}.It is intense and very useful when a chlorine or sulfur atom is substitued directly on the phosphorus. It is weak when all the substituents are carbon, nitrogen or oxygen. There is strong coupling between the P=S stretching and the single bond stretching of the substituents, so in order to obtain a

better interpretation of the spectrum, correlation for substituent structures must be considered [47].

Structure of the type P-O-R gives strong absorption bands. The structure is best considered as having two principal vibrations, the C-O and P-O, which are coupled and may cause complex bands corresponding to asymmetric and symmetric stretching [47]. The P-O-C (alkyl) is in the range of 1050-995 cm^{-1}, the P-O-CH_3 gives a medium to weak well defined band at about 1190 cm^{-1} assigned to methyl rock, and P-O-CH_2CH_3 gives similar bands at 1160 and 1100 cm^{-1}, as well as a characteristic C-C band near 960 cm^{-1}.

The -OH group of P-OH gives a broad, weak-to-medium intensity absorption, from 3100-2100 cm^{-1}, on which broad maxima may be superimposed. The P-OH may not appear greatly different from other strongly hydrogen bonded hydroxyl groups. However, where there is one OH on a P=O, distinctive bonding occurs which lowers the P=O frequency and gives a broad OH band around 1660-1700 cm^{-1} [47].

Infrared data for DETA prepared in the present work are shown below.

Abbreviations (s), (sh), (m), (w) correspond to strong, shoulder, medium and weak respectively.

INFRARED DATA

$(EtO)_2PS_2H$		$\sqrt{}$ (cm^{-1})		
3000(s)	2940(s)	2920(s)	2880(s)	2780(w)
2720(w)	2540(sh)	2460(s)	2360(sh)	1800(w)
1740(w)	1700(w)	1620(s)	1540(w)	1520(sh)
1480(s)	1440(s)	1420(sh)	1390(s)	1290(m)
1160(s)	1100(s)	1040(sh)	1015(s)	965(s)
845(m)	835(sh)	785(s)	660(s)	

The P=S stretching vibration for DETA was found at 660 cm^{-1} as a very intense absorption and has been reported to occur near 650 cm^{1} [48].

Chen Wen and collegues [49] studied the infrared and Raman spectra of Q,Q-diethyl phosphorodithioic acid at different temperatures and found that it presents four bands at 668, 658, 646 and 636 cm^{-1} as the temperature of the sample decreases. At room temperature the bands at 658 and 646 cm^{-1} overlap to give a single band at 654 cm^{-1}.

Strong aborptions for the $P(-O)_2$ stretching vibration were at 845, and 780 cm^{-1} and a shoulder at 835 cm^{-1}.

The $P(-O)_2$ stretching vibrations have also been reported to occur in the range 860-760 cm^{-1} [48,50].

Cheng recorded two strong bands at 850 and 775 cm^{-1}. The first results from the antisymmetric stretching vibration of $P(-O)_2$ and the second from symmetric stretching.

It was not possible to see the P-S stretching vibration on the instrument used, since it occurs in the range of 540-500 cm^{-1} [48,50].

A strong absorption was seen at 2460 cm^{-1} and shoulders at 2540 and 2360 cm^{-1}, and are assigned to the S-H stretching vibration.

Cheng [49] observed two S-H stretching vibrations at 2460 and 2360 cm^{-1}; the band at 2460 cm^{-1} increases in intensity as temperature decreases.

Shagidullin [51] also observed for DETA at 20°C a broad band of irregular shape with a maximum at 2460 cm^{-1} and shoulders at 2540 and 2578 cm^{-1} which correspond to associated and free SH vibrations [52,53]..

Shagidullin [51] supported by the works of other researchers [54] postulated that open and cyclic dimers of DETA can exist in the liquid state. This explains the complex form of the SH bands of the associated molecules.

The appearance of free SH vibrations may be due either to the presence of monomeric dithiophosphate molecules existing in the form of at least two rotational isomers [55,56] or terminal SH groups of linear self-associates.

Infrared data for the organophosphorus compounds prepared are presented in the following tables.

INFRARED DATA

$(EtO)_2PS_2CH_3$		$\sqrt{}$ (cm^{-1})		
3960(w)	2990(s)	2950(m)	2900(m)	2875(sh)
2840(sh)	2760(w)	2730(w)	2400(w)	2300(w)
2200(w)	1830(s)	1760(w)	1720(w)	1700(w)
1630(w)	1460(m)	1445(s)	1395(s)	1325(s)
1295(s)	1265(w)	1170(s)	1105(m)	1045(sh)
1020(w)	960(m)	835(w)	800(w)	660(s)

INFRARED DATA

$(EtO)_2PS_2CH_2CO_2H$		$\sqrt{}$ (cm^{-1})		
3180(s)	3120(sh)	3100(sh)	2884(w)	2760(w)
1715(s)	1680(sh)	1515(w)	1475(m)	1440(m)
1420(w)	1395(s)	1295(s)	1205(m)	1160(s)
1100(s)	1040(sh)	1015(s)	970(s)	890(sh)
830(m)	800(m)	660(s)		

INFRARED DATA

$(EtO)_2PS_2CH_2CO_2Me$		ν (cm^{-1})		
3480(w)	2990(s)	2960(w)	2900(s)	2880(sh)
2840(sh)	1750(s)	1620(m)	1520(w)	1480(w)
1440(s)	1420(sh)	1390(s)	1305(s)	1280(sh)
1200(m)	1160(s)	1105(m)	1040(s)	1015(s)
970(s)	895(m)	835(s)	800(s)	665(s)

INFRARED DATA

$[(EtO)_2PS_2]_2$		ν (cm^{-1})		
2990(s)	2950(m)	2900(sh)	2875(sh)	1820(w)
1720(m)	1475(m)	1440(s)	1400(w)	1395(s)
1370(sh)	1295(s)	1265(sh)	1195(s)	1125(s)
1020(s)	980(s)	830(s)	810(s)	645(s)

INFRARED DATA

$(EtO)_2PS_2CH_2CONMeCO_2Et$	ν (cm^{-1})			
2995(s)	2950(sh)	2910(sh)	1730(s)	1695(w)
1680(sh)	1540(w)	1515(w)	1480(w)	1470(w)
1445(w)	1425(w)	1375(m)	1325(s)	1310(sh)
1240(w)	1220(w)	1180(m)	1160(w)	1110(w)
1100(w)	1075(w)	1020(sh)	1015(s)	960(s)
900(w)	870(w)	830(m)	800(w)	775(m)
700(m)	655(s)			

INFRARED DATA

$(EtO)_2P(O)SH$	ν (cm^{-1})			
3000(m)	2960(w)	2920(w)	2410(w)	2330(w)
1850(w)	1630(w)	1480(s)	1450(s)	1400(s)
1300(s)	1220(w)	1110(w)	1030(w)	970(w)
820(sh)	790(s)	650(w)	620(s)	

INFRARED DATA

$(EtO)_2P(O)SCH_3$	$\int$ (cm^{-1})			
3700(s)	3005(m)	2880(sh)	2870(sh)	2800(sh)
2730(sh)	2200(w)	1785(sh)	1740(s)	1710(sh)
1690(sh)	1650(sh)	1580(w)	1520(sh)	1485(w)
1450(sh)	1440(s)	1380(w)	1310(w)	1260(w)
1210(w)	1170(w)	1100(w)	1020(w)	980(w)
810(w)	800(w)	770(w)	710(s)	

INFRARED DATA

$(EtO)_2P(O)SCH_2CO_2Me$	$\int$ (cm^{-1})			
3460(s)	2980(m)	2960(sh)	2900(sh)	2880(sh)
2760(sh)	2720(sh)	2640(sh)	2280(w)	2200(sh)
1760(sh)	1740(s)	1720(sh)	1680(sh)	1640(sh)
1550(sh)	1530(sh)	1510(sh)	1480(m)	1440(m)
1410(sh)	1380(s)	1370(w)	1300(sh)	1260(m)
1195(w)	1160(w)	1100(m)	1015(w)	980(w)
900(sh)	880(sh)	795(sh)	760(w)	695(s)

INFRARED DATA

$(EtO)_2P(O)SCHCONMeCO_2Et$		$\int$ (cm^{-1})		
3450(s)	3000(s)	2980(sh)	2965(sh)	2950(s)
1740(w)	1710(w)	1690(sh)	1660(sh)	1560(w)
1540(w)	1530(w)	1540(w)	1480(w)	1460(w)
1440(w)	1400(w)	1380(w)	1330(w)	1310(w)
1260(s)	1190(w)	1170(w)	1120(w)	1110(w)
1080(w)	1030(m)	980(s)	900(s)	880(s)
810(sh)	790(s)	710(s)		

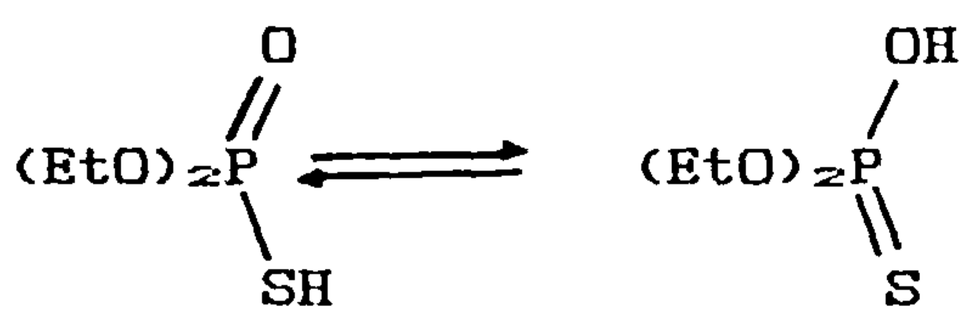
INFRARED DATA

$(EtO)_2PS_2CH_2Cl$		$\int$ (cm^{-1})		
2990(s)	2940(w)	2900(m)	2860(w)	1520(w)
1480(m)	1470(sh)	1445(s)	1395(s)	1295(s)
1240(s)	1170(s)	1105(m)	1020(sh)	1015(s)
970(s)	850(w)	830(m)	805(m)	730(s)
655(s)				

INFRARED DATA

$(EtO)_2PS_2CH_2OH$		$\int$ (cm^{-1})		
3420(s)	2980(s)	2920(m)	2900(m)	2880(sh)
2760(sh)	2720(sh)	2600(w)	2440(w)	2320(w)
2240(sh)	2060(m)	1920(w)	1760(w)	1730(w)
1700(w)	1610(s)	1540(sh)	1510(sh)	1480(s)
1470(sh)	1440(s)	1420(sh)	1380(sh)	1360(sh)
1340(sh)	1310(s)	1260(m)	1220(m)	1990(m)
1160(s)	1100(w)	1035(sh)	1015(s)	960(s)
910(sh)	820(sh)	790(s)	665(sh)	640(s)

For all the compounds presented above the P=S bond usually is in the range 650-665 cm^{-1}. Only $(EtO)_2(O)SH$ showed a strong absorption at 620 and a shoulder at 650 cm^{-1}. This could be caused by tautomerism.



The P=O strectching vibration was observed between 1310-1160 cm^{-1}. With $(EtO)_2P(O)SH$ the P=O absorption was seen at 1300 and 1220 cm^{-1} while for $(EtO)_2P(O)SCH_3$ was at 1310, 1260, 1210 and 1170 cm^{-1}. For the oxygen-analogue of

mecarbam the P=O vibration was assigned at 1310, 1260, 1190 and 1170 cm^{-1}. $(EtO)_2P(O)SCH_2Me$ showed the P=O vibration at 1300, 1260, 1195 and 1160 cm^{-1}.

The $P(-O)_2$ stretching vibration was present in all the compounds in the range of 770 to 870 cm^{-1}. These absorptions are assigned to be at the positions shown below.

	ν (cm^{-1})
$(EtO)_2PS_2Me$	835-800
$(EtO)_2PS_2CH_2CO_2H$	830-800
$(EtO)_2PS_2CH_2CO_2Me$	835-800
$[(EtO)_2PS_2]$	830-810
$(EtO)_2PS_2CH_2CONMeCO_2Et$	870, 830, 800, 775
$(EtO)_2P(O)SH$	820-790
$(EtO)_2P(O)SMe$	800-770
$(EtO)_2P(O)SCH_2Me$	795,760
$(EtO)_2P(O)SCH_2CONMeCO_2Et$	790-810
$(EtO)_2PS_2CH_2Cl$	850, 830, 805
$(EtO)_2PS_2CH_2OH$	820,790

The C=O ketone vibration is expected at 1710 cm^{-1}. For $(EtO)P_2CH_2CO_2H$ the C=O frequency occurs as a strong vibration at 1715 cm^{-1} and for $(EtO)_2PS_2CH_2CO_2Me$ also as a strong absorption at 1750 cm^{-1}. For $(EtO)_2P(O)SCH_2CO_2Me$ the C=O absorption was assigned as a strong band at 1740 and two shoulders at 1760 at 1720 cm^{-1}. Mecarbam presented a strong band at 1730 cm^{-1} due to C=O absorption from the ester

(COOEt) group and two bands at 1695 (weak) and 1680 cm^{-1}(shoulder), due to C=O frequency from the amide (CONMe-) group. For the oxygen analogue of mecarbam the ester C=O frequency occurs at 1740 and 1710 as weak bands and the C=O vibration from the amide group at 1690 (shoulder) and 1660 cm^{-1} (shoulder).

For both compounds $(EtO)_2PS_2CH_2CO_2H$ and $(EtO)_2PS_2CH_2OH$ the OH frequency is a strong band at 3180 and 3420 cm^{-1} respectively.

The halogen bond C-Cl usually appears in the range 800-600 cm^{-1} and in the case of chlormephos was assigned to the strong band at 730 cm^{-1}.

CHARACTERISATION OF ORGANOPHOSPHORUS COMPOUNDS BY FAB MASS SPECTROMETRY

The programme has involved a study of some typical organophosphorus compounds by electron-impact mass spectrometry and by the modern technique, FAB, fast atom bombardment mass spectrometry. FAB is new soft ionization method to obtain mass spectra, particularly for non-volatile or thermally unstable compouds. No previous work using the FAB technique has been reported in the study of organophosphorus pesticides. The chemical literature presents, however, FAB mass spectra for other diverse types of compound. The FAB technique for solids was found to be applicable to a wide range of organic, organo-metallic and metallic compounds. For example, the positive ion fast atom bombardment mass spectrum of methionyllsylbradykinin has been reported. In this work was described the mass spectral study in the solid state of molecules which were previously difficult or impossible to study by other ionization methods. The technique uses the phenomenon of ion sputtering and employs a beam of fast neutral atoms, typically of argon or xenon at 2-8 Kev, as the primary particles. The mass spectra obtained have high pseudomolecular ion selectivity, usually showing $[M+H]^+$ and $[M-H]^-$ ions in positive and negative ion spectra respectively [57].

The FAB technique has also been used by Joel to obtain the spectra of NADP tetra-sodium salt and streptomycin sulphate [21]. FAB allows ionization and the generation of fragment ions, and often gives a peak for $[M+H]^+$ even with compounds of low volatility, thermally labile compounds and salts. Fragment ions are obtainable along with positive ions and negative ions of almost the same intensity. Ions may be generated for a long time, approximately 1 hour with some compounds. Also a stable ion beam is obtainable because FAB hardly gives little rise to the charge-up phenomenon which takes place when the specimen is bombarded with charged particles such as ions [21].

Fast atom bombardment mass spectra have been obtained of salts of analogues of inorganic pyrophosphoric acid, e.g. substituted methylene bisphosphonates, and positive ion fast atom bombardment mass spectrometry has been proved to be more succesful than negative ion for analysis of these salts [58].

The use of FAB for the study of thick layers of organic compounds is discussed by Surnam and co-workers [59]. The same authors have also investigated the use of fast atom bombardment quadrupole mass spectrometry for the study of structures of non-volatile organic compounds. The technique is illustrated using the aminoacids L-histidine and L-arginine [60].

FAB mass spectrometry has also been applied to a range of polar organic molecules, such as organic salts,

polar antibiotics, and nucleoside phosphates [61]. In these classes of compound, molecular weights in the range 300-2000 daltons have been routinely determined, operating in both positive and negative ion modes. Molecular weights of peptides were readily obtained on less than 1 nmol of material, and sequence information was conveniently deduced from sample sizes in the range 2-50 nmol.

Finally it is important to report the characterisation of amino- and guanidino-phosphonic acids by FAB mass spectrometry [62], these results being particularly relevant to the possible analysis of glyphosate and related zwitterionic herbicides and their metabolites by this method. The production of an intense $[M+H]^+$ peak in addition to a relatively simple fragmentation pattern, shows that the FAB technique is a invaluable tool for the characterisation of compounds of these types.

FAB MASS SPECTROMETRY OF PHOSPHORODITHIOATES AND PHOSPHOROTHIOATES.

In the present investigation, the FAB spectra were recorded at the Physicochemical Measurements Unit (PCMU), Harwell, and were obtained using a glycerol matrix on a VG Analytical ZAB-1F Spectrometer. The sample in glycerol was

bombarded with fast xenon atoms that were produced from an ion gun (Ion-tech Ltd) operating at 8 Kev.

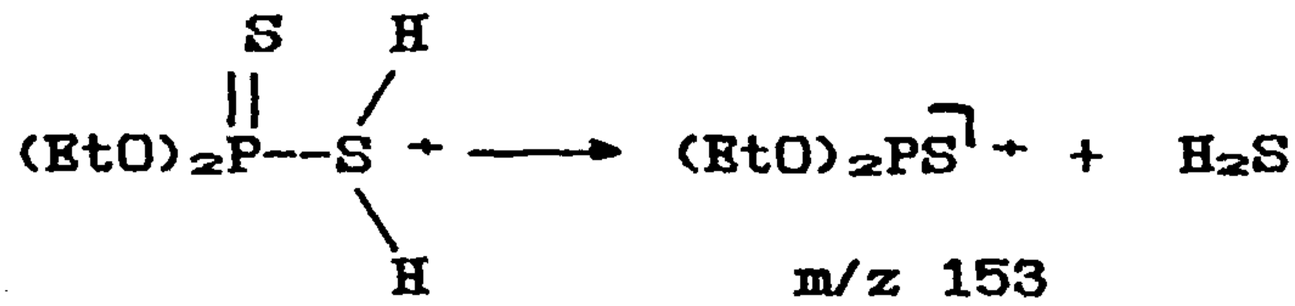
Results are given in the following tables (1-8) and possible fragmentation pathways are discussed.

Table 1a. FAB mass spectral data for O,O-diethyl dithiophosphoric acid, $(EtO)_2PS_2H$ (DETA).

Mwt 186

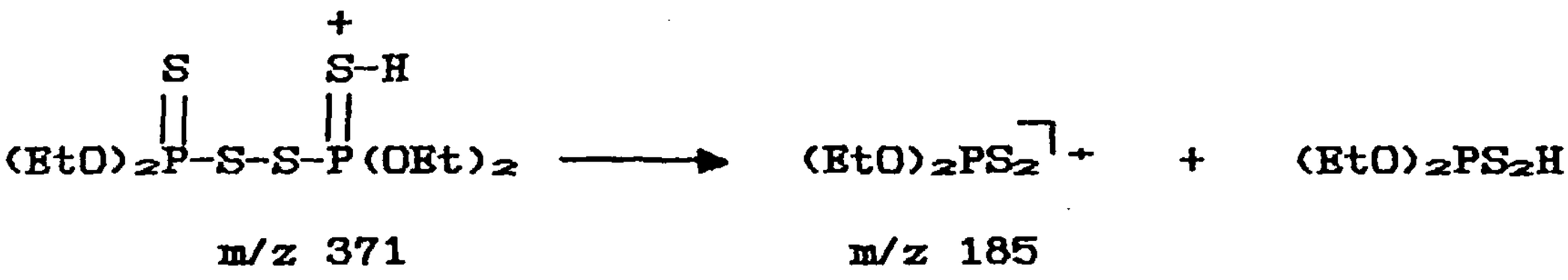
m/z	Relative intensity (%)	Assignment
371	31.3	$[(EtO)_2P(S)S]_2H^+$
187	12.5	$[M+H]^+$
186	8.0	$(EtO)_2P_2H^+$
185	94.9	$(EtO)_2PS_2^+$
157	28.7	$(EtO)(OH)PS_2^+$
153	69.2	$(EtO)_2PS^+$
125	53.1	$(EtO)(OH)PS^+$
121	100.0	$(EtO)_2P^+$
93	42.9	$(EtO)(OH)P^+$

Diethyl dithiophosphoric acid shows a peak at $[M+H]^+$, of relative intensity 12.5%, which might possibly give rise to the peak at m/z 153 (69.2%) by loss of H_2S (cf. loss of H_2O from analogous ions in certain later examples).

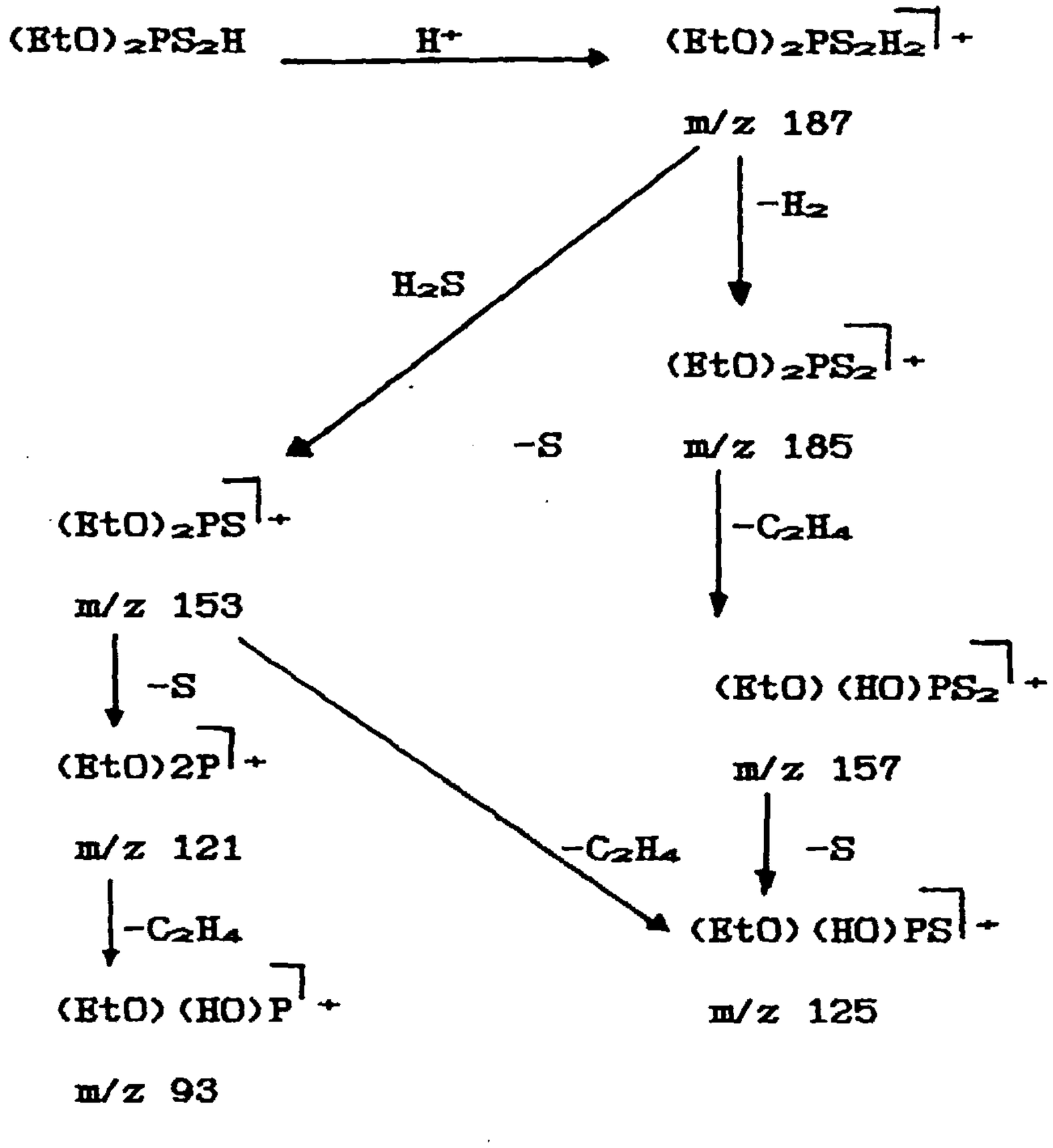


This process occurs by heterolytic fission in which the pair of electrons of the P-S bond is retained by the S atom and the positive charge remains with the fragment ion $(EtO)_2PS^+$.

The overall fragmentation is however confused by the presence of ions which may possibly arise from the oxidation product of DETA, $(EtO)_2P(S)SSP(S)(OEt)_2$. The presence of this is shown clearly by the corresponding molecular ion at m/z 371 which as shown later may give rise to the ion at m/z 185 by S-S cleavage.



In addition, the characteristic peaks at m/z 157, 153, 125, 121, and 93 may be formed by further fragmentation e.g. as shown in Scheme 1.



Scheme 1

A further pure sample of DETA was therefore prepared. The data are presented in table 1b.

Table 1b.

m/z	371	341	295	263	187	185	171	153	143	125	121	115	97
%		10	10	15	20	20	20	100	5	10	3	4	12	8

The fragmentation patterns was not entirely reproducible, probably due to the fact that DETA is not a very stable compound.

The B/E linked scan spectrum for the ion, m/z 187 of O,O-diethyldithiophosphoric acid suggested that ions at m/z 186, 185, 171, 159, 153, 143, 133 and 129 all originate from the ion m/z 187, although not all of these have been assigned.

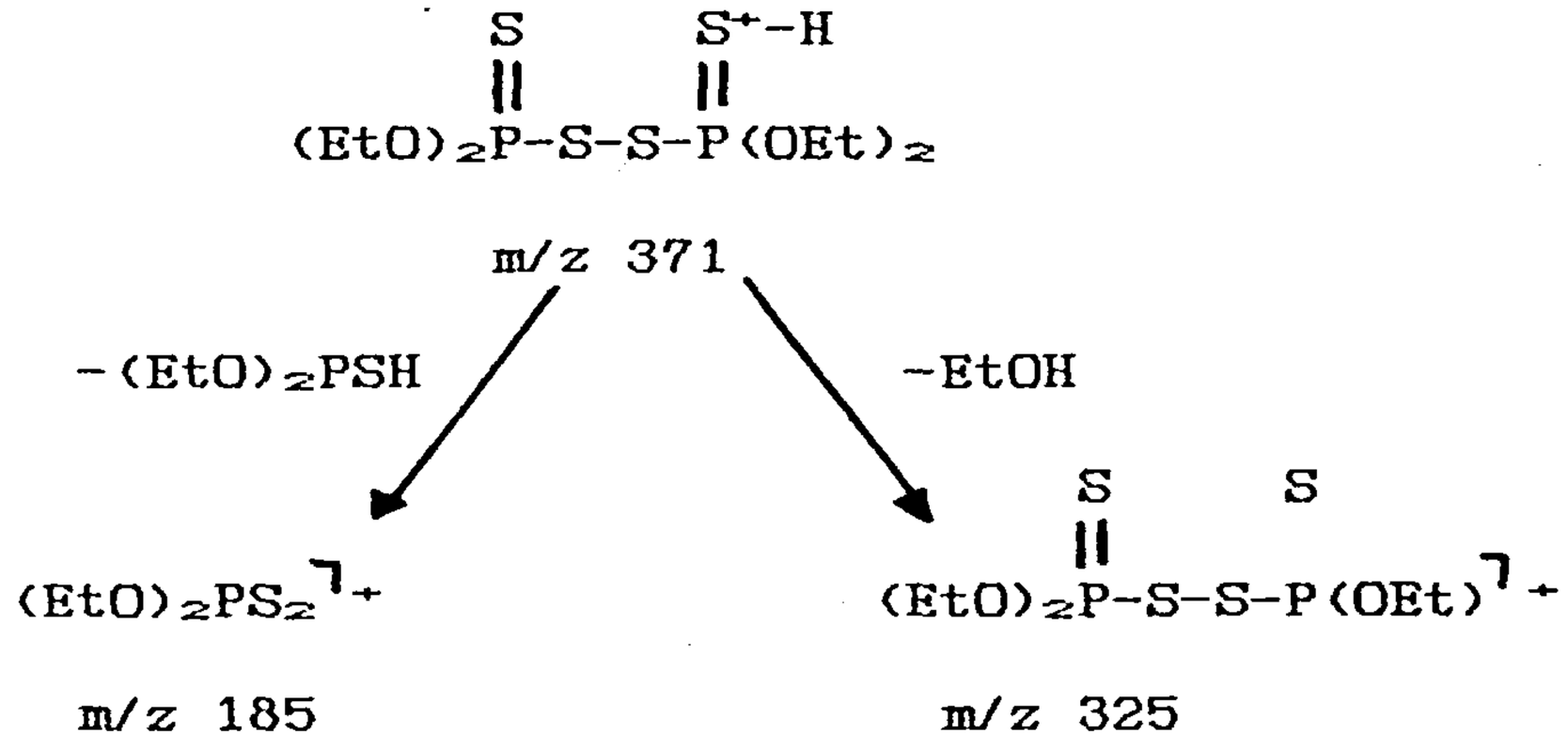
In order to investigate the fragmentation pattern for the oxidation product of DETA further the compound itself was prepared and studied by FAB mass spectrometry which gave the result in Table 2.

Table 2. FAB mass spectral data for the product of oxidation of O,O-diethyldithiophosphoric acid, the disulfide, $(EtO)_2P(S)SSP(S)P(OEt)_2$.

m/z	587	555	523	491	399	371	370	338	325	309	293	277	261	249
%	5	15	20	15	18	100	55	10	35	8	15	5	15	10
m/z	217	199	185	157	153	129	125	121	97	93	65			
%	12	5	80	17	40	11	25	65	25	21	0			

In order to confirm the fragmentation pathways for the molecular ion of $[(EtO)_2PS_2]_2$, the B/E linked scan was also obtined. This spectrum showed the ions at m/z 325, 306,

231 and 185 to originate from the parent ion, while the ion at m/z 185 gives rise only to the peak at m/z 157. The main initial pathaways are shown in Scheme 2.



Scheme 2

Further stepwise loss of ethylene from m/z 185 gives ions at m/z 157 and m/z 129, whilst P-S cleavage accounts for ions at m/z 153 and m/z 121 as shown also in the fragmentation of other phosphorodithioates.

Table 3. FAB mass spectral data for diethoxyphosphinothioylthioacetic acid, $(EtO)_2P(S)SCH_2CO_2H$.

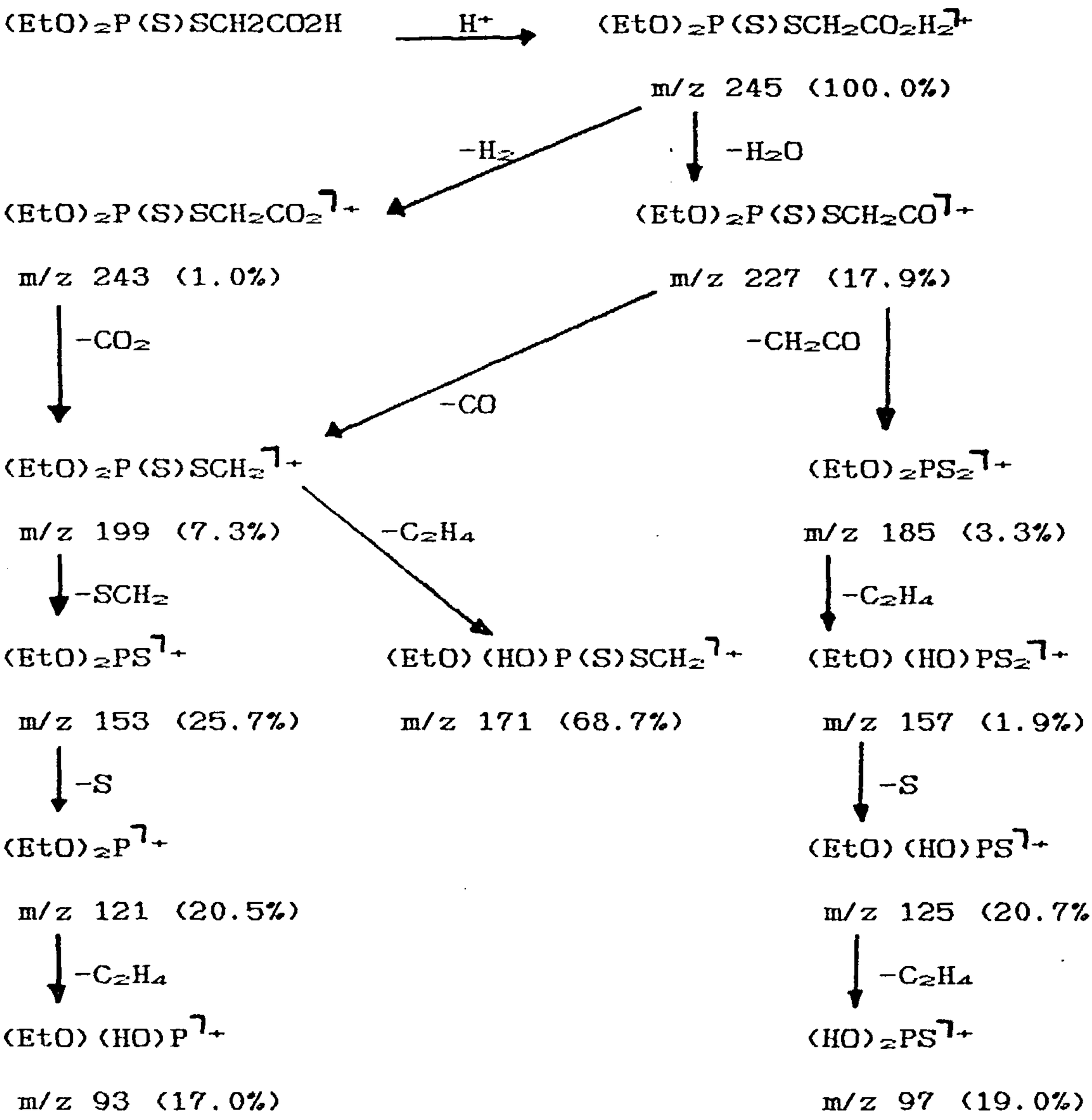
Mwt 244

m/z	Relative intensity (%)	Assignment
245	100.0	$(EtO)_2P(S)SCH_2CO_2H_2^+$
243	1.0	$(EtO)_2P(S)SCH_2CO_2^+$
227	17.9	$(EtO)_2P(S)SCH_2CO^+$
199	7.3	$(EtO)_2P(S)SCH_2^+$
185	3.3	$(EtO)_2P(S)S^+$
171	68.7	$(EtO)(HO)P(S)SCH_2^+$
157	1.9	$(EtO)(HO)P(S)S^+$
153	25.7	$(EtO)_2P(S)^+$
125	20.7	$(EtO)(HO)PS^+$
121	20.5	$(EtO)_2P^+$
97	19.0	$(HO)_2PS^+$
93	17.0	$(EtO)(HO)P^+$

The main fragmentations for diethoxyphosphinothioylthioacetic acid are thought to occur as shown in Scheme 3.

The $[M+H]^+$ ion corresponds to the base peak which is very common in FAB mass spectrometry. The subsequent steps then appear to involve the loss of H_2O to give the medium intense m/z 227 ion, which expels CO to give a weak peak at m/z 199. This is followed by the loss of thioformaldehyde, SCH_2, to form the relatively intense m/z 153 ion which undergoes stepwise loss of sulphur and of ethylene by known types of process to give peaks at m/z 121 and m/z 93. An

alternative pathway from the peak at m/z 199 may be considered to give the m/z 171 by loss of ethylene. The formation of the peak at m/z 157 is satisfactorily explained by sequential loss of ketene, CH_2CO, and ethylene from the ion at m/z 227. Further fragmentation by loss of S and ethylene can then be presumed to occur.



Scheme 3

The possibility of the loss of H_2 by the molecular ion m/z 245 is indicated by a very weak peak at m/z 243, from which the loss of CO_2 could be an alternative route to the formation of the important ion at m/z 199.

A certain similarity between the FAB and electron impact fragmentation patterns is observed, however the intensities of the corresponding ions differ greatly. The m/z 153, m/z 127, m/z 97 ions observed in the FAB spectra are less intense than those originated by the electron impact technique. (See later for a discussion of the electron impact mass spectrometry of this compound and the

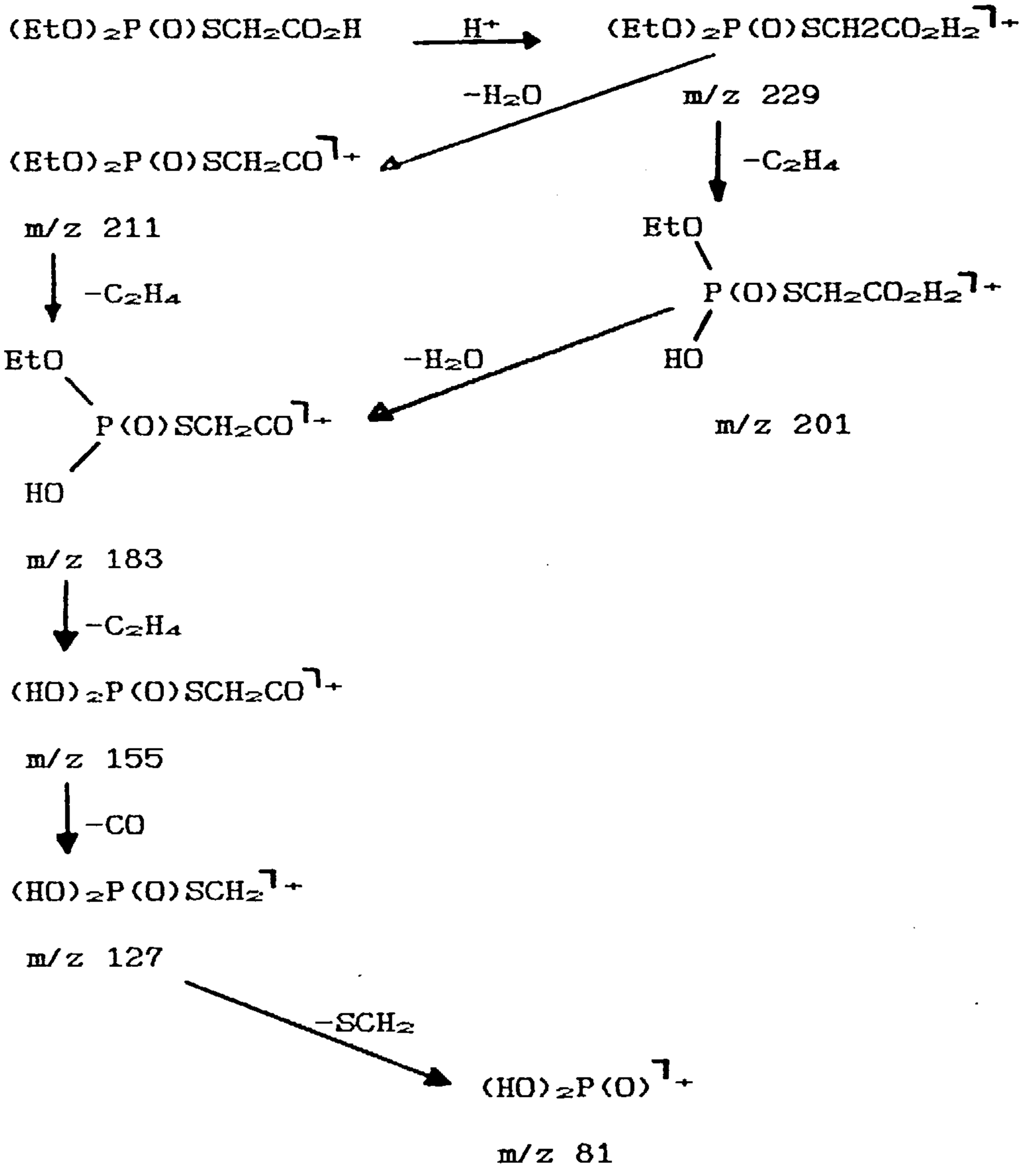
Table 4. FAB mass spectral data for diethoxyphosphinyl-thioacetic acid, $(EtO)_2P(O)SCH_2CO_2H$.

Mwt 228

m/z	Relative intensity (%)	Assignment
338	40.2	[M + glycerol + H_2O]$^+$
257	13.2	not identified
229	3.9	$(EtO)_2P(O)SCH_2CO_2H_2]^{+\cdot}$
211	2.9	$(EtO)_2P(O)SCH_2CO]^+$
201	22.2	$(EtO)(HO)P(O)SCH_2CO_2H_2]^+$
183	4.7	$(EtO)(HO)P(O)SCH_2CO]^+$
161	26.4	not identified
155	6.0	$(HO)_2P(O)SCH_2CO]^+$
127	3.3	$(HO)_2P(O)SCH_2]^+$
116	100.0	not identified
81	9.4	$(HO)_2P(O)]^+$

possibility of thermal decomposition).

The FAB mass spectrum for diethoxyphosphinyl-thioacetic acid is very complex compared to that for the dithio analogue above. Only a certain number of probable fragmentations can be identified and these are shown in Scheme 4.



Scheme 4

The peak at m/z 338 corresponds to the [M + glycerol + H_2O]$^+$ ion. The spectrum gives a base peak (m/z 116) whose structure was not determined. The molecular ion [M+H]$^+$ is very weak but appears to undergo loss of H_2O to give another weak peak at m/z 211. This by stepwise loss of ethylene, can give the m/z 183 and m/z 155 ions and the latter ion by cleavage of the P-S bond may form the ion at m/z 81.

An alternative mode of fragmentation may be by initial loss of ethylene from the [M+H]$^+$ ion, followed by loss of water, and subsequent loss of ethylene to give the m/z 155 ion.

Except for the m/z 116, 161, 201, 257, 338 ions, the relative intensities in this FAB spectrum are generally small.

In addition to the metabolites discussed above two salts of diethyl dithiophosphoric acid were also investigated by FAB. In case of the potassium salt the [M+H]$^+$ peak which would occur at m/z 225 does not appear but two successive eliminations of ethylene from this would account for the relatively abundant ion detected at m/z 169 (Scheme 5).

Finally this fragmentation route arrives at the m/z 137 ion by loss of S and direct hydrogen rearrangement to the P atom.

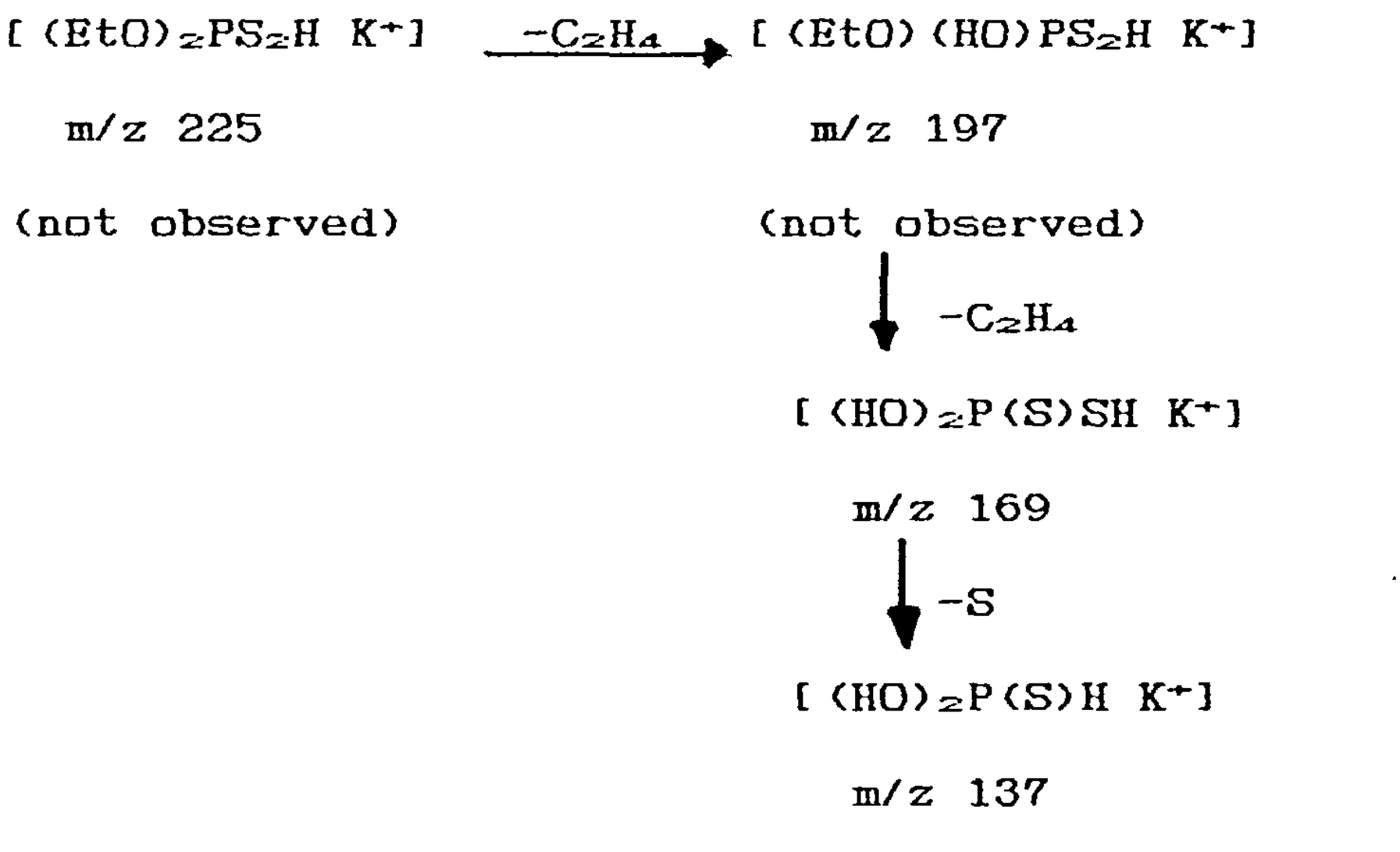
The base peak, m/z 263, may correspond to the aggregate containing two potassium ions which by stepwise loss of two ethylene molecules gives the weak ion at m/z

207, (Scheme 6). It is interesting that in neither fragmentation pathway is the ion observed which results from loss of the first ethylene molecule.

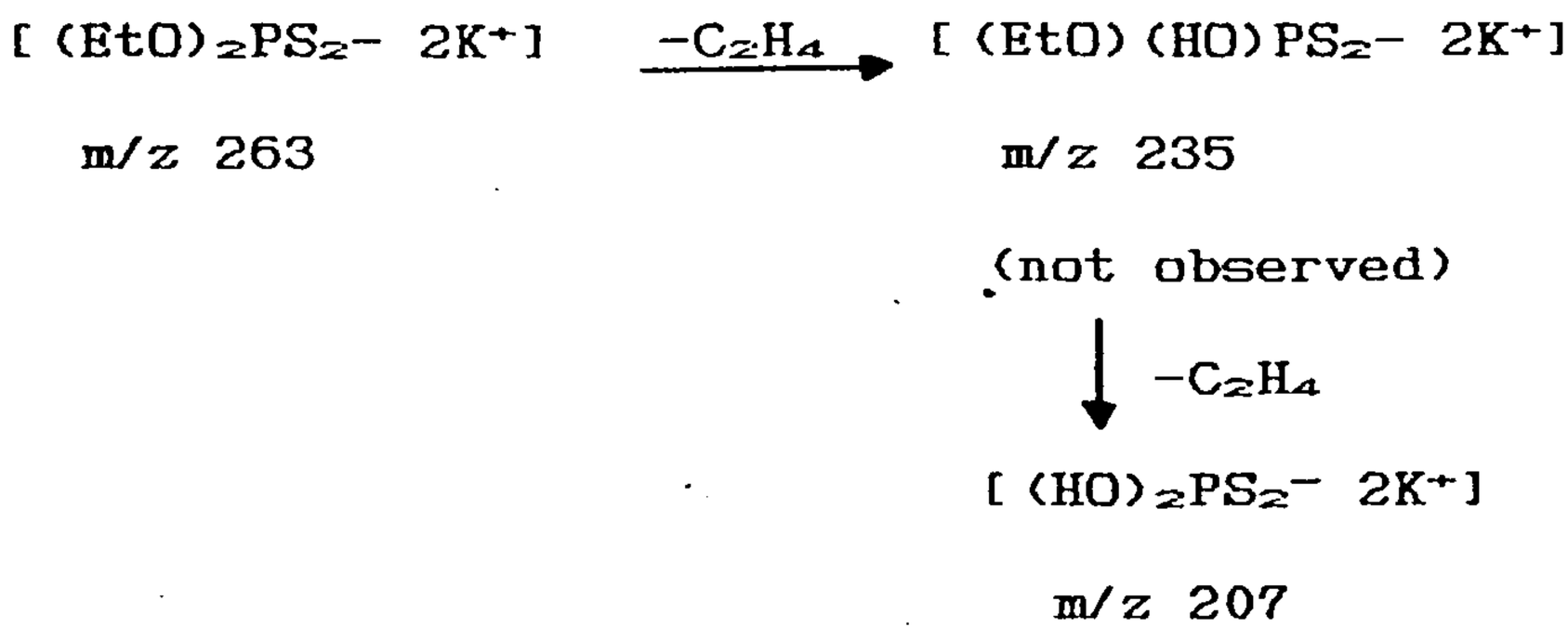
Table 5. FAB mass spectral data for potassium O,O-diethyl-phosphorodithioate, $(EtO)_2P(S)SK$.

Mwt 224

m/z	Relative intensity (%)	Assignment
273	28.8	not identidied
265	21.7	not identified
263	100.0	$(EtO)_2P(S)S^-\ 2K^{+}$
207	4.0	$(HO)_2P(S)S^-\ 2K^{+}$
169	24.8	$(HO)_2P(S)SH\ K^{+}$
137	4.5	$(HO)_2P(S)H\ K^{+}$
111	39.2	not identified



Scheme 5



Scheme 6

Table 5. FAB mass spectral data for ammonium O,O-diethylphophorothioate, $(EtO)_2P(O)SNH4$.

Mwt 187

m/z	Relative intensity (%)	Assignment
280	6.9	[M+H + glycerol]+
188	43.3	$(EtO)_2POSH\ NH_4]^+$
187	5.1	$(EtO)_2POS\ NH_4]^+$
171	100.0	$(EtO)_2POHSH]^+$
170	9.8	$(EtO)_2PS^-\ NH_3]^+$
143	20.3	$(EtO)(OH)POHSH]^+$
138	1.7	$(EtO)_2POH]^+$
121	0.5	$(EtO)_2P]^+$
115	25.0	$(OH)_2POHSH]^+$
110	62.8	$(EtO)(OH)POH]^+$

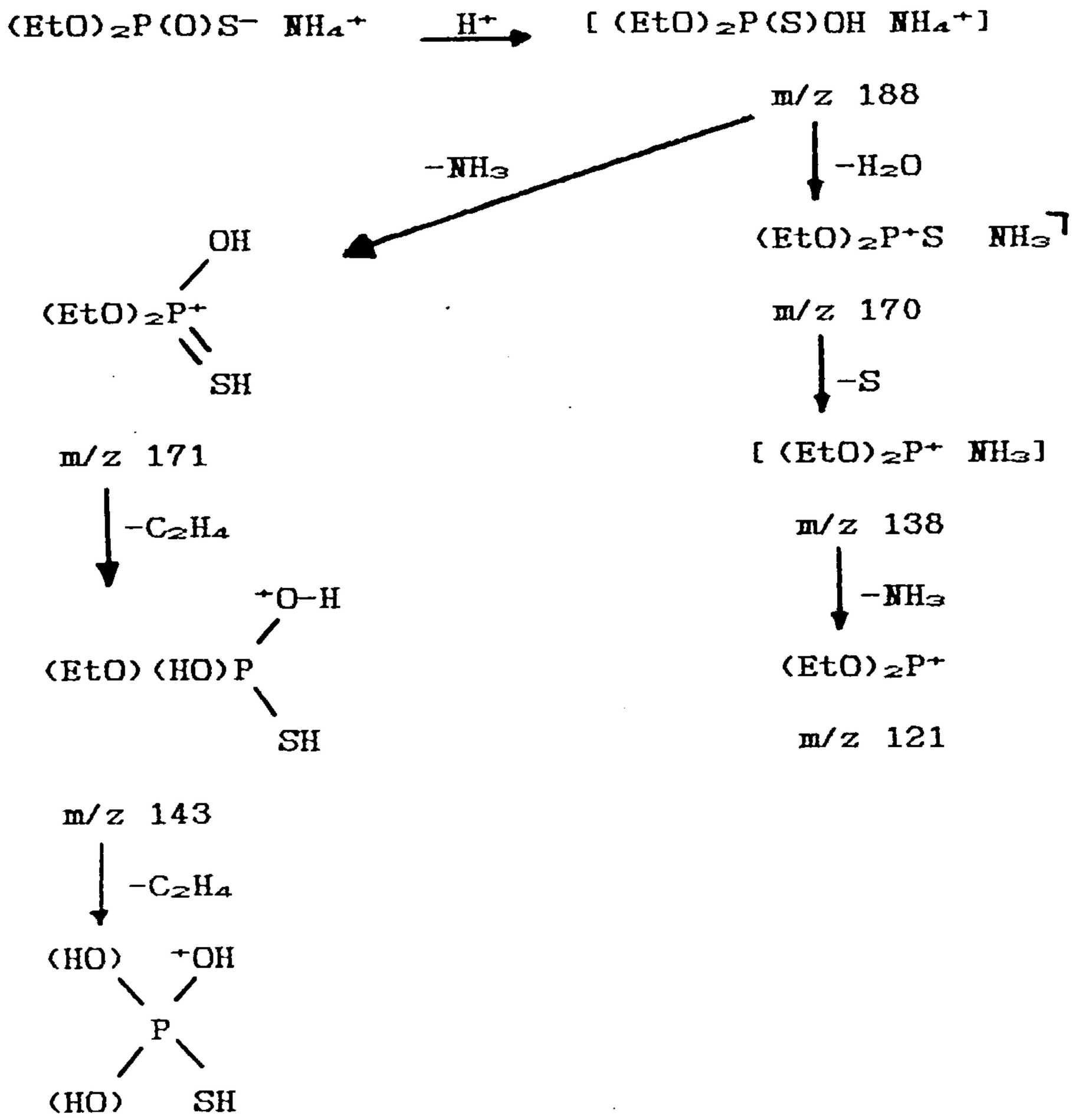
The mass spectrum of the ammonium salt is quite different from that of the potassium phosphorodithioate referred to above, the main fragmentation pathway being thought to occur as shown in Scheme 7.

The peak at m/z 280 corresponds to the protonated molecular ion together with the glycerol molecule. The protonated molecular ion is itself an abundant ion which results from protonation of the anion, probably at phosphoryl oxygen i.e. $[(EtO)_2P(S)OH\ NH_4^+]$. This ion may then lose water to give the ion at m/z 170, a process which would involve simultaneous loss of hydroxide ion from the phosphorus moiety and a proton from the ammonium cation.

The protonated ion (m/z 188), may also expel NH_3 to give the base peak, m/z 171. This ion by stepwise loss of ethylene gives the m/z 143 and 115 ions, which are relatively intense.

The very weak peaks at m/z 138 and m/z 121 could possibly arise by the sequential loss of sulphur and of ammonium from the m/z 170 ion. Their formation from the ion at m/z 171 by loss first of SH and then of OH seems less likely as the the first step would involve the formation of $(EtO)_2POH^+$ as an odd-electron species.

Although further loss of ethylene could account for the formation of the very strong ion observed at m/z 110, it is more likely that the latter corresponds to the ammonium ion plus glycerol, $[NH_4^+\ C_3H_8O_3]$, a combination that has been reported in the case of other ammonium salts [63].


(EtO)2P(O)S− NH4+
H+
[(EtO)2P(S)OH NH4+]
m/z 188
−NH3
−H2O
OH
(EtO)2P+
SH
(EtO)2P+S NH3
m/z 170
−S
m/z 171
−C2H4
+O−H
(EtO)(HO)P
SH
[(EtO)2P+ NH3]
m/z 138
−NH3
(EtO)2P+
m/z 121
m/z 143
−C2H4
(HO) +OH
P
(HO) SH
m/z 115

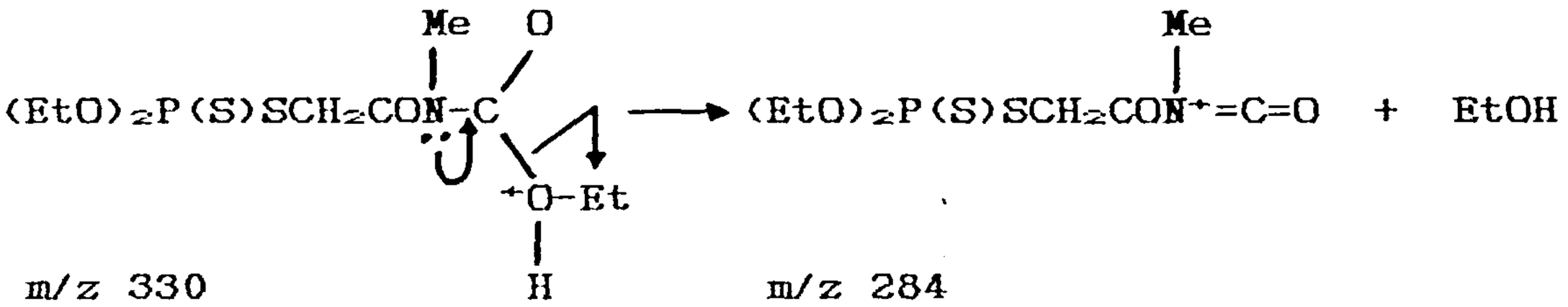
Scheme 7

Table 7. FAB mass spectral data for S-(N-ethoxycarbonyl-N-methylcarbamoylmethyl) O,O-diethyl phosphorodithioate, $(EtO)_2P(S)SCH_2CONMeCO_2Et$ (mecarbam).

Mwt 329

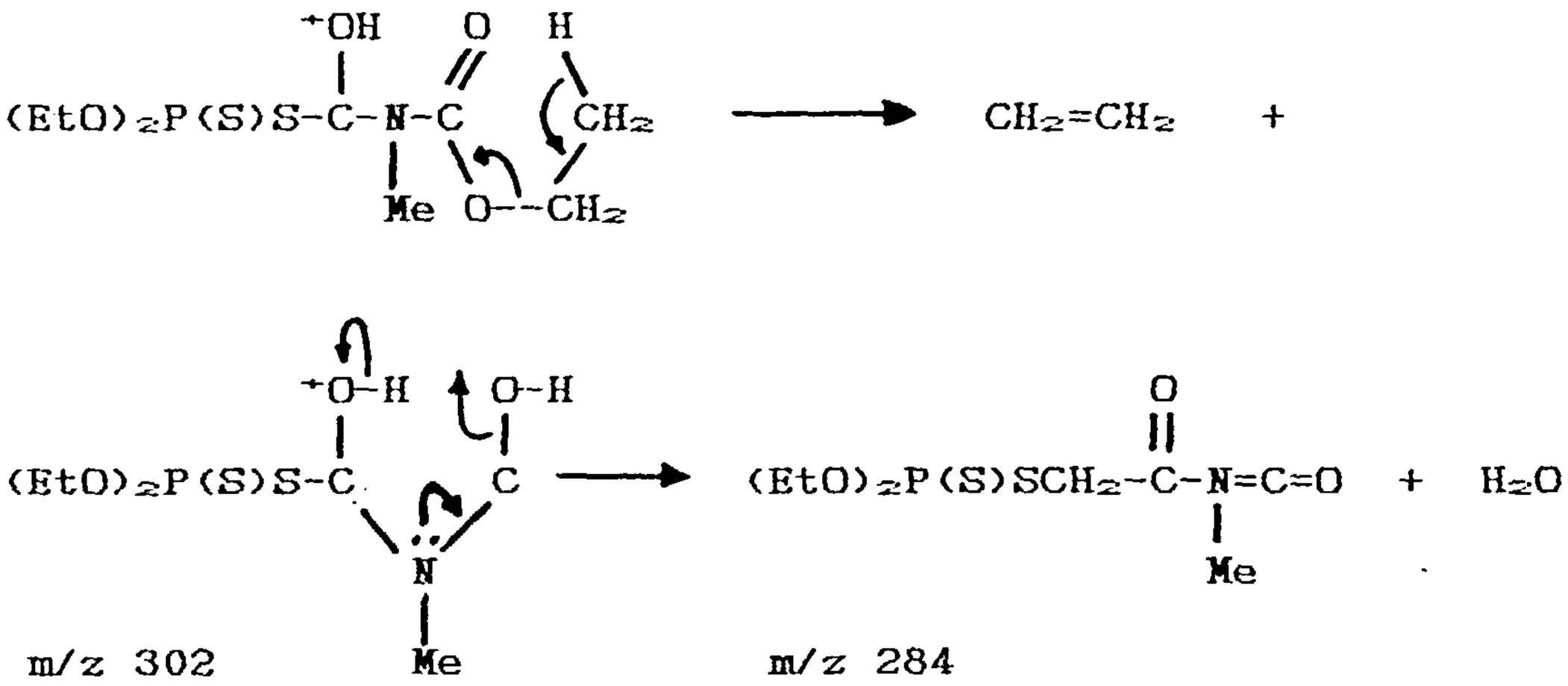
m/z	Relative intensity (%)	Assignment
330	28.3	$(EtO)_2P(S)SCH_2CONMeC(OH)(OEt)^+$
302	0.5	$(EtO)_2P(S)SCH_2CONMeC(OH)_2^+$
284	29.8	$(EtO)_2P(S)SCH_2CONMeCO^+$
256	1.7	$(EtO)_2P(S)SCH_2CONMe^+$
227	100.0	$(EtO)_2P(S)SCH_2CO^+$
226	2.9	$(EtO)_2P(S)SCHCO^+$
199	16.5	$(EtO)_2P(S)SCH2^+$
177	1.9	$SCH_2CONMeCOHOEt^+$
171	7.0	$(EtO)(HO)P(S)SCH_2^+$
160	15.3	$C_6H_9NO_2S^+$
159	10.7	$C_6H_{10}NO_2S^+$
153	34.0	$(EtO)_2PS^+$
149	0.4	$SCH_2CONMeC(OH)_2^+$
144	42.6	$(HO)_2P(S)SCH_3^+$
143	5.7	$(HO)_2P(S)SCH_2^+$
131	8.4	$SCH_2CONMeCO^+$
125	33.2	$(EtO)(HO)P(S)^+$
116	51.2	$CH_2CONMeCO_2H^+$
97	30.1	$(HO)_2P(S)^+$
93	23.2	$(EtO)(HO)P^+$

Mecarbam shows an $[M+H]^+$ peak that is relatively intense. The site of protonation cannot be stated with certainty although several are possible. Direct elimination of ethanol to give the peak at m/z 284 would be analogous to the loss of water from certain carboxylic acids or hydroxy compounds and suggests protonation of the carboxylate group (Scheme 8).



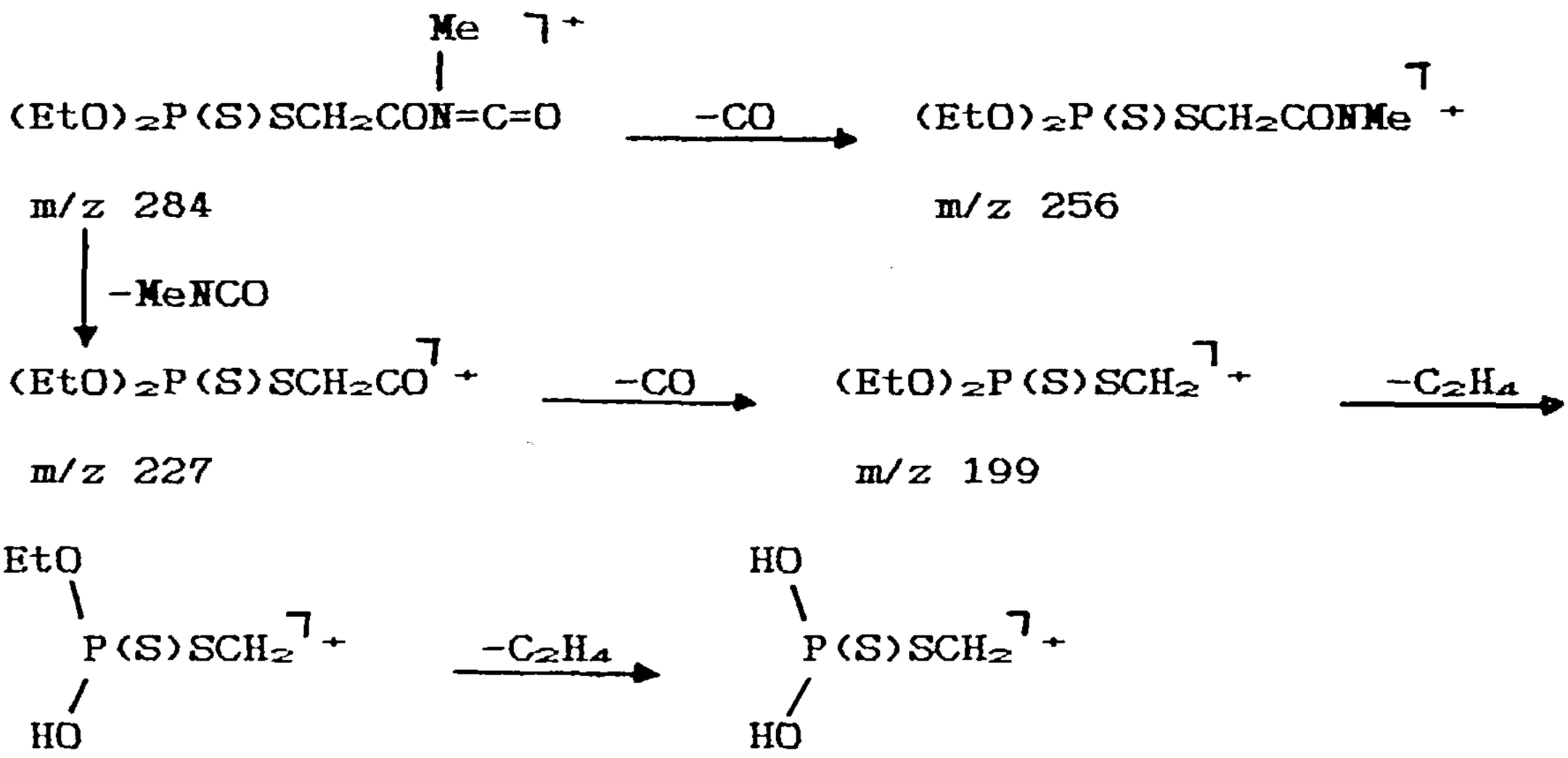
Scheme 8

Two stages could however be involved as the weak peak at m/z 302 suggests the initial loss of ethylene. In this case protonation of the carbamoyl oxygen may be involved (Scheme 9).



Scheme 9

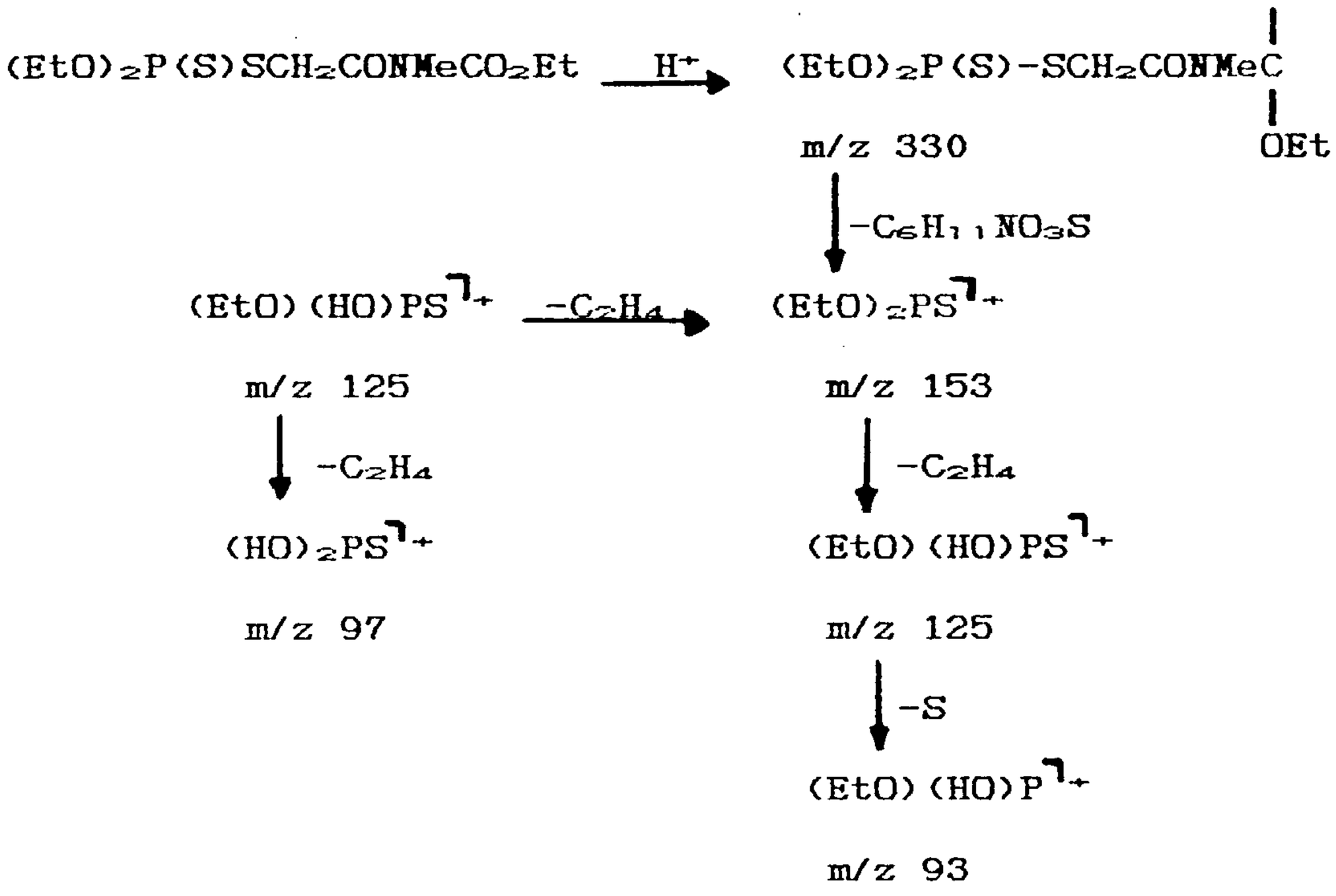
The expulsion of CO by the m/z 284 ion could then account for the weak peak at m/z 256. The cleavage of the CO-N bond with loss of methyl isocyanate would give the base peak, m/z 227, which then expels CO to form the ion at m/z 199. Finally via stepwise loss of ethylene the m/z 171 and m/z 143 ions are formed (Scheme 10).



Scheme 10

In the fragmentation route shown in Scheme 11, the cleavage of the bond between phosphorus and the thiolo-sulphur atom gives the m/z 153 ion which is also a characteristic peak in the electron impact mass spectra for the phosphorodithioates [64]. This ion, by loss of ethylene, gives the m/z 125 peak and subsequent loss of S gives the m/z 93 ion. The m/z 153 ion may also fragment by stepwise loss of ethylene to give the ions at m/z 125 and 97.

The m/z 97 ion, assigned $(HO)_2PS^+$ is more abundant in the electron impact mass spectrum [10], and is considered to be the most characteristic feature in the fragmentations of diethyl phosphorodithioates [65].

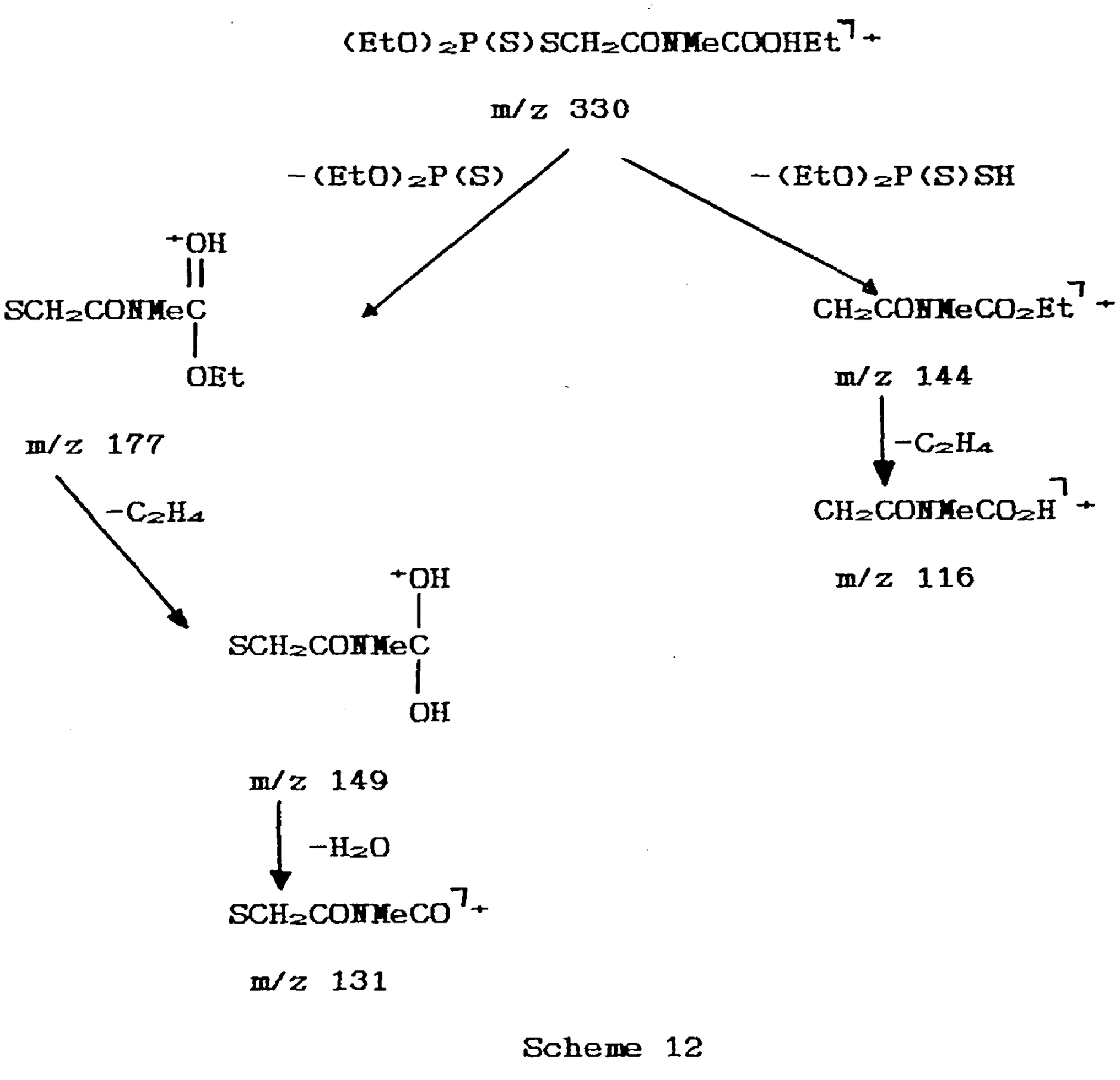


Scheme 11

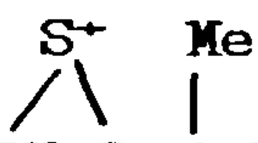
Other possible routes for fragmentation of $[M+H]^+$ are shown in Scheme 12, the cleavage of the thiolo sulphur atom from carbon, with rearrangement of hydrogen, giving the abundant m/z 144 ion, which by loss of ethylene gives the important peak m/z 116.

The $[M+H]^+$ ion may also give the weak m/z 177 peak by cleavage of the P-S bond. This ion, via loss of ethylene, may give the m/z 149 peak and subsequent loss of water will

give the weak m/z 131 ion, assigned to be $SCH_2CONMeCO^+$. (It should be noted that the formulations given for a number of the ions discussed here are for simplicity shown as resulting from simple bond cleavage. The structures of these ions may be different).



Scheme 12

The m/z 131 ion  in the electron impact mass spectrum [10] is thought to be formed from the m/z 159 ion  , which comes from the molecular ion (m/z 329) by fission of the P-S bond and hydroxyl transfer. Hydroxyl transfer has been reported for organometallic derivatives [66]. The m/z 159 ion loses ethylene giving the m/z 131 ion (base peak). The formation of m/z 131 from m/z 159 is supported by the metastable ion at m/z 108.

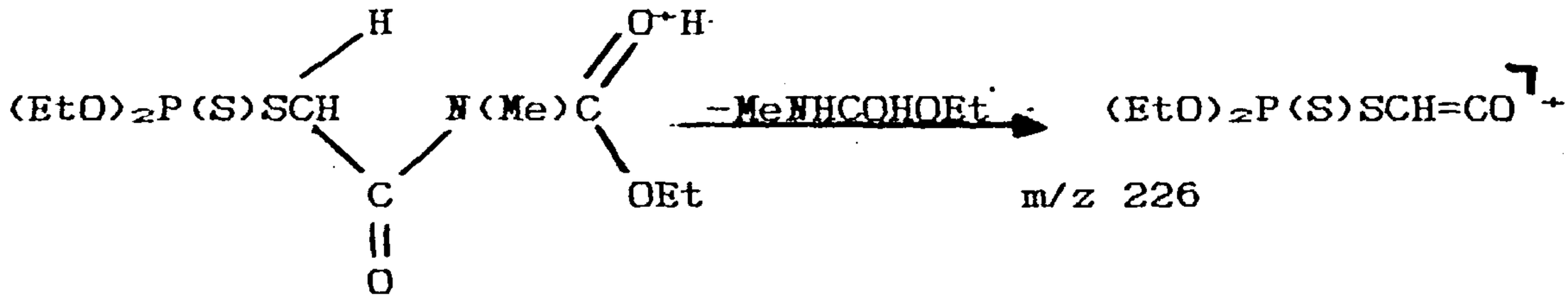
In addittion to the ion at m/z 159, the electron impact mass spectrum shows a peak at m/z 160 ($C_6H_{10}NO_2S$) which it is suggested is formed by cleavage of the P-S bond and oxygen transfer to the phosphorus in the keto-form of the parent ion.

The peaks at m/z 159 and 160 are observed in the FAB mass spectrum showing intensities of 10.7% and of 15.3% respectively.

In Scheme 13 a possible route is shown for the formation of the m/z 226 ion from the parent ion by fission of the CO-N bond with hydrogen transfer from the phosphorus containing moiety.

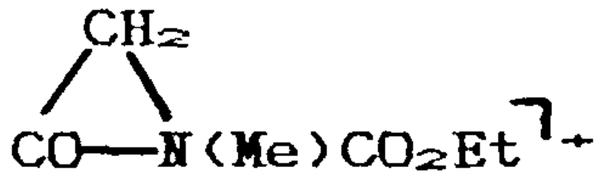
The origin of the m/z 226 from the molecular ion was confirmed in its electron impact mass spectrum [10] by accelerating voltage scanning. This ion was also proved to be formed from an unidentified ion at m/z 327, however not detectable.

The m/z 227 ion which is shown to be the base peak, could similarly be formed in FAB m.s. by fission of CO-N without hydrogen transfer or possibly as in Scheme 10 above.



Scheme 13

The electron impact mass spectrum of mercabam [19] showed that ions resulting from primary fission of bonds in the carbamoyl chain are of minor significance. Exceptions are the phosphorus-containing ion m/z 199 (68%) and the carbamate moiety m/z 144 (14%), resulting from S-CH_2 bond cleavage. It is suggested that the m/z 144 ion is stabilized by ring-closure with involvement of the nitrogen atom to give the following structure:



The fragmentation of this ion via loss of ethylene was confirmed by the metastable peak, m* 93.5.

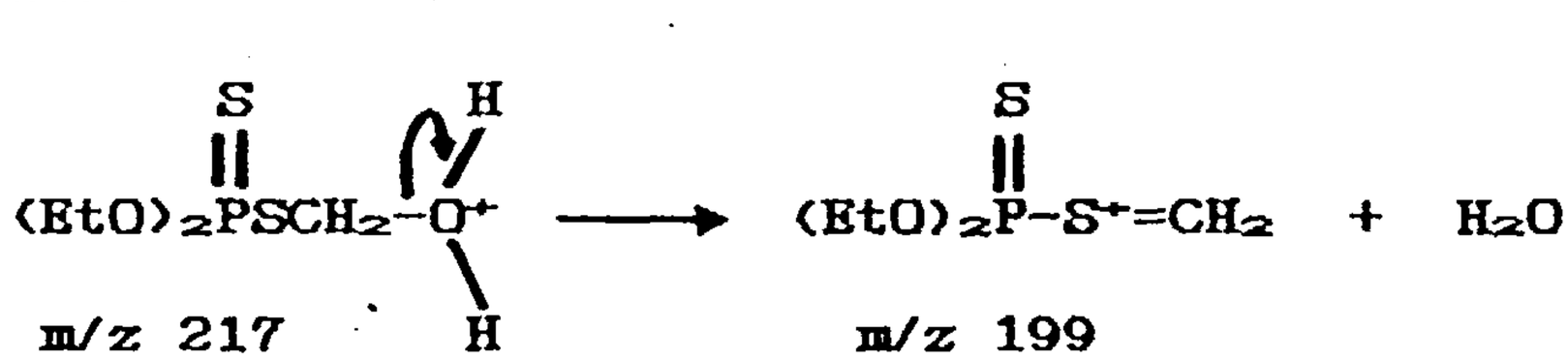
Such fragmentations are similar in the FAB mass spectrum except that the intensities differ, that is m/z 144 is more abundant for the FAB m.s. (Scheme 12) while m/z 199 is more abundant for the e.i. mass spectrum.

Table 8. FAB mass spectral data for O,O-diethyl S-hydroxymethyl phosphorodithioate, $(EtO)_2P(S)SCH_2OH$.

Mwt 216

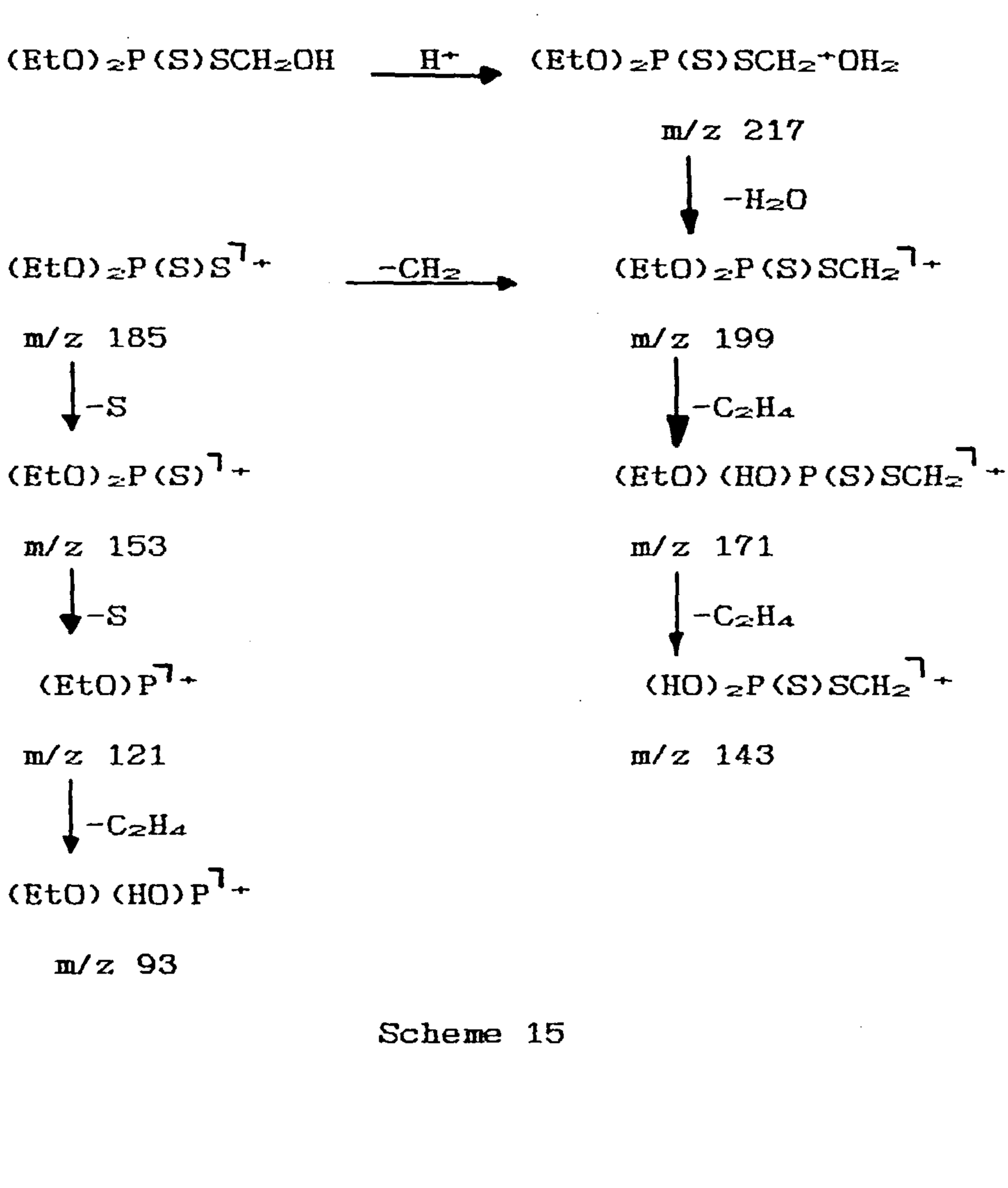
m/z	Relative intensity (%)	Assignment
309	1.0	$[M+H + glycerol]^+$
217	5:7	$(EtO)_2P(S)SCH_2OH]^+$
199	100.0	$(EtO)_2P(S)SCH_2]^+$
186	2.9	$(EtO)_2P(S)SH]^+$
185	14.1	$(EtO)_2P(S)S]^+$
171	14.8	$(EtO)(HO)P(S)SCH_2]^+$
153	53.4	$(EtO)(HO)P(S)SCH_2]^+$
143	8.8	$(HO)_2P(S)SCH_2]^+$
121	42.8	$(EtO)_2P]^+$
93	24.0	$(EtO)(HO)P]^+$

The most likely site of protonation in the $[M+H]^+$ ion is the oxygen atom as shown, as oxygen is more basic than sulphur. This structure then readily accounts for the loss of H_2O to give the base peak at m/z 199 which is presumbly stablished in the sulphonium structure (Scheme 14).



Scheme 14

Following the initial loss of H_2O (Scheme 15) fragmentation occurs mainly by the stepwise loss of ethylene and of sulphur. However, the peak at m/z 185 appears to require the unusual loss of methylene, CH_2, as the neutral fragment from m/z 199.



Scheme 15

The most important initial fragment in both FAB and electron impact mass spectra (see section on e.i. spectra) is that at m/z 199. Although its subsequent fragmentation in both cases is similar, it is of interest to note that the

peak (m/z 186) arising from the loss of formaldehyde is much more intense (100%) in the case of electron impact.

The fragmentation patterns obtained by FAB seem less complicated than those from electron impact.

ELECTRON IMPACT MASS SPECTROMETRY OF PHOSPHORODITHIOATES AND PHOSPHOROTHIOATES.

The existing literature on electron impact mass spectra of phosphorodithioates and phosphorothioates is very extensive [10,11,14,16,17,19,20,64,66]. However, only a few of the compounds presented in this work have been studied before. The mass spectrometry of O,O-diethyl dithiophosphoric acid, was reported by Lynch [67] while the spectra of mecarbam and its oxygen analogue were discussed in chapter III of this work [67], and have been extensively studied by Charalambous *et al* [10]. The present research was carried out with the purpose of comparing the results obtained with those already recorded and studying the modes of fragmentation for the other posssible metabolites of mercabam and chlormephos.

The electron impact mass spectra of the compounds studied were recorded on an AEI MS9 Spectrometer at an electron impact energy of 70 ev and a source temperature that was variable with the nature of the compounds.

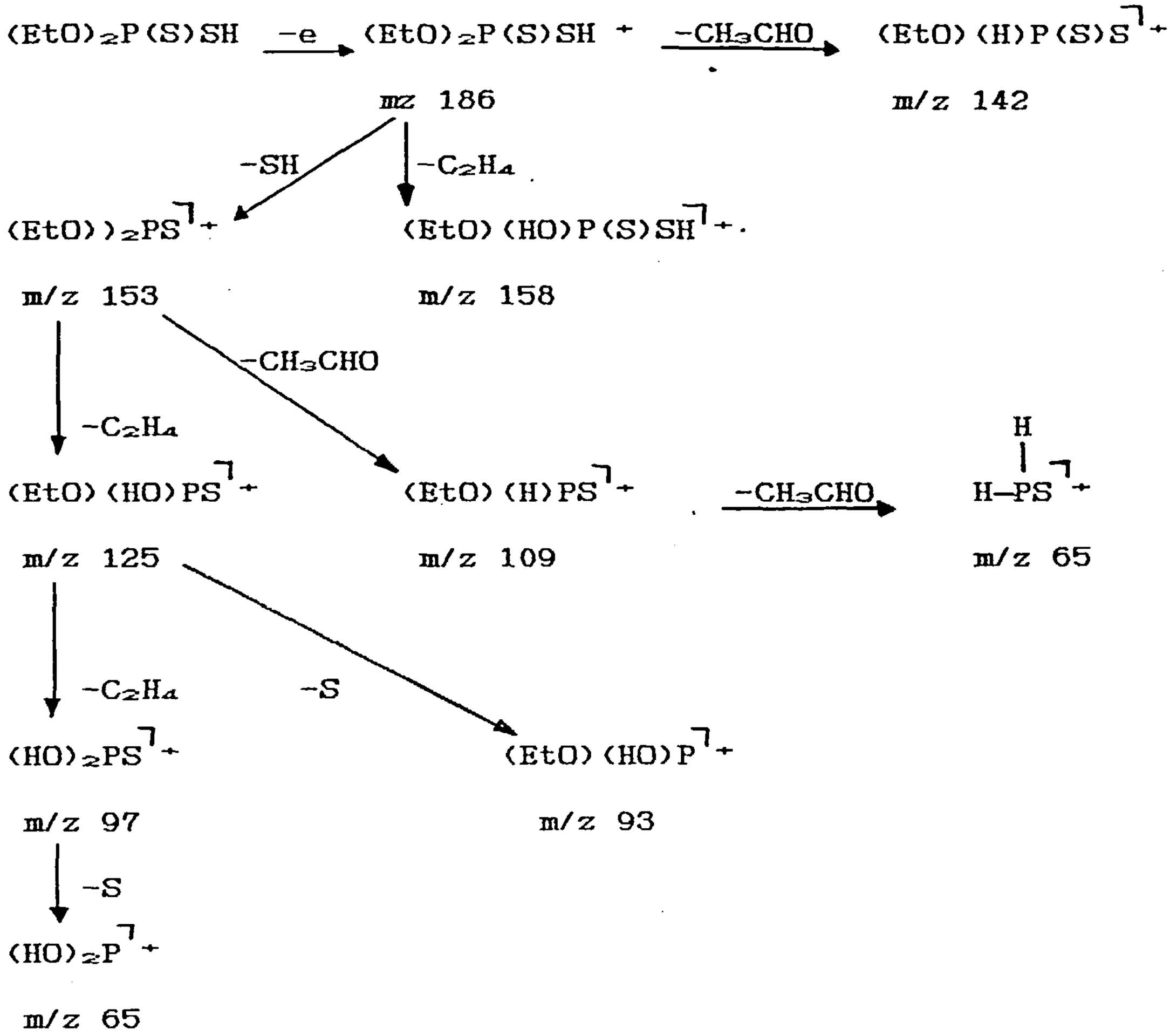
Table 9a. Mass spectral data for $(EtO)_2P(S)SH$ at 200°C.

m/z	272	244	236	227	226	199	198	195	187	186	185	181	172	171
%	23	10	6	8	3	7	8	11	3	16	3	5	6	77
m/z	170	169	167	159	158	157	155	154	153	143	142	139	131	130
%	14	9	5	5	17	8	9	10	21	26	9	5	6	8
m/z	127	126	125	121	120	119	115	114	113	112	111	109	106	105
%	10	8	50	49	11	6	28	10	6	5	6	13	6	5
m/z	99	97	95	94	93	92	91	88	85	83	81	80	79	78
%	6	100	7	6	43	12	6	7	8	12	13	5	5	5
m/z	77	76	75	74	73	71	70	69	67	65	64	63	61	60
%	6	12	12	17	7	16	8	19	5	49	13	6	10	13
m/z	59	58	56	55	54									
%	13	8	27	8	20									

Table 9b. Mass spectral data for $(EtO)_2P(S)SH$ at 350°C.

m/z	244	198	187	186	185	173	172	171	170	158	155	154	153	148
%	8	17	1	5	2	5	7	74	46	14	7	11	15	6
m/z	143	142	141	138	129	127	126	125	121	115	114	113	111	110
%	33	14	6	17	10	12	21	38	28	47	35	7	17	9
m/z	109	108	106	99	98	97	96	93	92	91	83	82	81	80
%	26	6	15	13	8	100	5	54	7	8	9	12	37	6
m/z	78	75	74	65	64	63	62	60	59	58	57	56		
%	8	9	20	59	6	6	97	26	15	12	13	8		

Principal fragmentations are as follows



Scheme 16

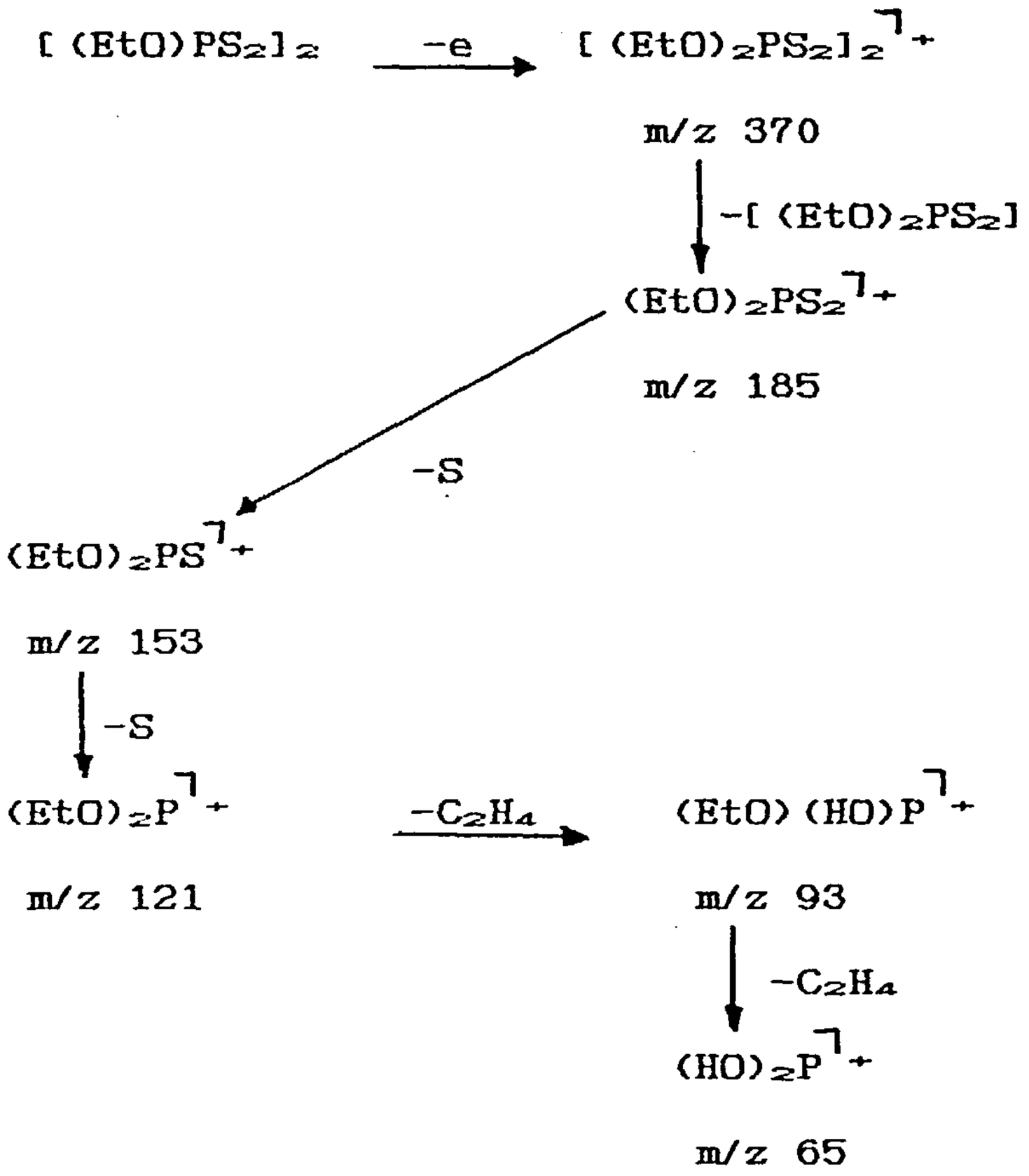
DETA shows a parent ion (m/z 186) of moderate intensity (16%), the most abundant peaks then resulting from cleavage of the P–SH bond and the stepwise loss of ethylene and S to give $(OH)(EtO)PS^{+}$, $(OH)_2PS^{+}$, and $(OH)_2P^{+}$. Direct loss of ethylene from the parent ion may also account for the peak at m/z 158, assgined to $(EtO)(OH)P(S)SH^{+\cdot}$.

Hydrogen rearrangement directly to the phosphorus atom has been already suggested by Damico [17]. The mass spectral data for $(EtO)_2P(S)SH$ referred to by Lynch [67] confirm this rearrangement and here it may explain the m/z 142, and m/z 109. Direct hydrogen rearrangement to the P atom is also a possibility to account for the structure of the abundant m/z 65 ion occurring in the spectrum.

Table 10. Mass spectral data for $[(EtO)_2PS_2]_2$ at 200°C.

m/z	433	372	370	338	325	310	306	293	281	268	262	261	253	246
%	1	5	25	1	1	1	2	1	1	1	1	1	1	1
m/z	230	221	218	214	198	193	189	188	187	186	185	184	170	169
%	2	1	2	1	2	2	1	3	5	30	30	1	1	1
m/z	161	160	159	158	157	156	155	154	153	144	143	142	141	140
%	1	1	2	4	11	1	2	3	42	2	1	16	4	2
m/z	137	132	131	130	129	128	127	126	125	124	122	121	115	114
%	1	1	4	5	27	1	3	3	79	1	3	56	1	8
m/z	113	112	111	110	109	108	107	99	98	97	96	95	94	93
%	7	6	1	1	14	2	1	5	1	100	3	4	2	30
m/z	92	91	81	80	79	77	76	75	69	66	65	64	63	62
%	4	1	7	6	2	4	2	1	1	2	45	15	8	9
m/z	61	60	59	58	57	55	49	48	47	46	45	44	43	42
%	6	4	3	1	1	1	1	2	16	2	11	2	5	1
m/z	41	36	35	34	33	32	31	30	29	28	27	26	25	19
%	1	1	2	3	1	2	1	1	55	16	28	8	1	1

The disulfide shows an abudant peak for the parent ion (m/z 370). The characteristic ions of the fragmentation of phosphorodithioates are strong ions. The fragmentation patterns are shown in scheme 17.



Sheme 17

Table 11. Mass spectral data for $(EtO)_2P(S)SCH_2CO_2H$ at 200°C.

m/z	245	238	222	214	202	198	194	192	186	177	174	170	160	154
%	4	6	6	5	7	22	7	5	14	5	5	22	8	10
m/z	153	143	142	138	137	128	127	126	125	122	121	115	114	113
%	8	10	26	8	7	5	6	12	14	6	32	20	28	5
m/z	111	110	109	99	98	97	96	94	93	91	90	83	81	75
%	10	5	17	9	6	44	9	8	33	6	8	5	26	12
m/z	66	65	64	63	62	60	59	58	57	56	54			
%	10	33	26	5	100	32	11	15	15	8	5			

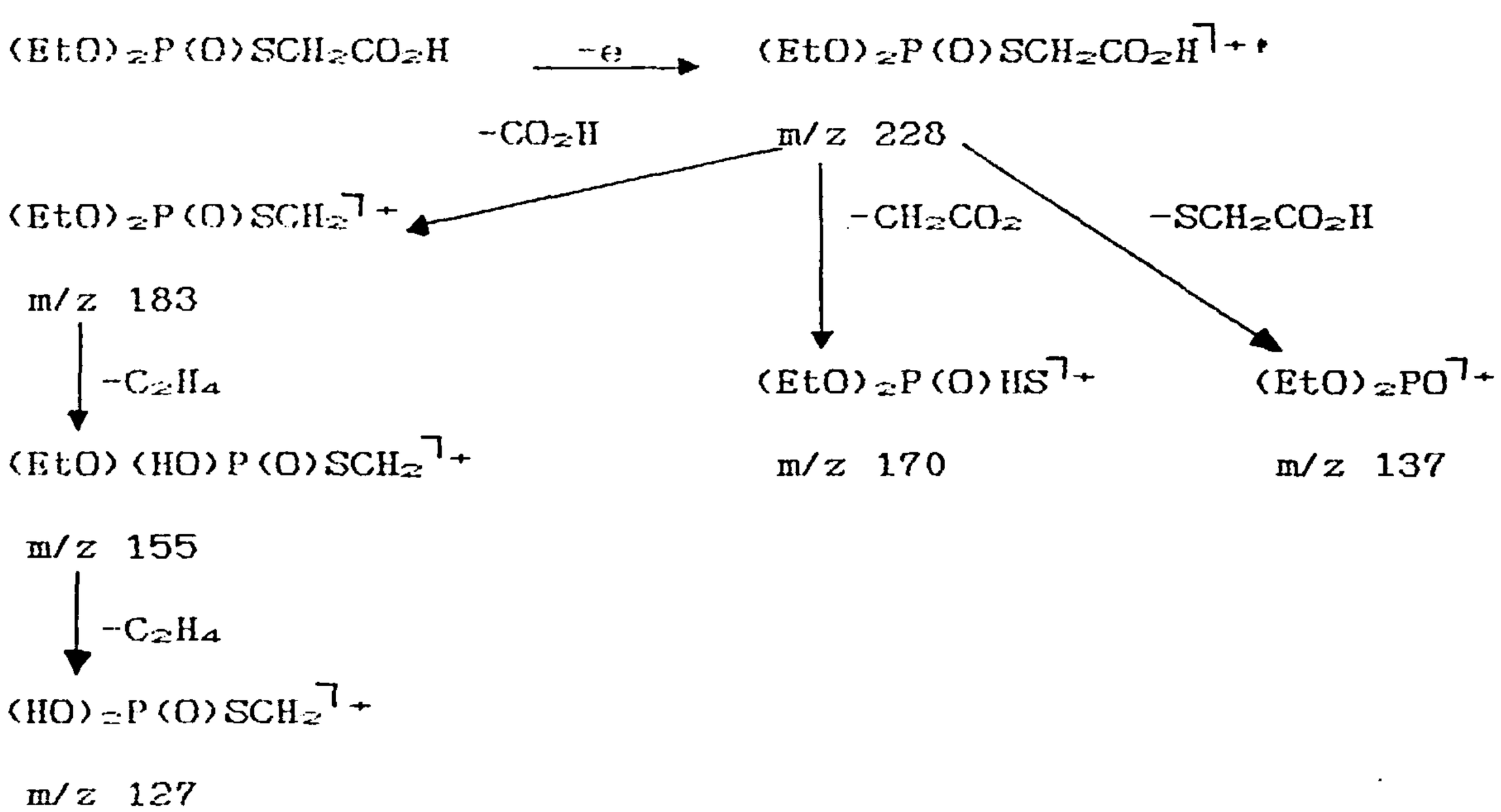
The parent ion at m/z 244 is not observed, and the possibility of thermal decomposition should be considered. This view is supported by the complexity of the spectrum. Certain peaks characteristic of dithiophosphates are however seen, including those as m/z 153, 121, 97, 93, and 65, corresponding to $(EtO)_2PS^+$, $(EtO)_2P^+$, $(OH)_2PS^+$, $(EtO)(OH)P^+$, and $(OH)_2P^+$ respectively.

The ion corresponding to initial cleavage of the thiolo S-C bond, i.e $(EtO)_2PS_2^+$, was not however observed.

Table 12. Mass spectral data for $(EtO)_2P(O)SCH_2CO_2H$ at 200°C.

m/z	228	183	170	155	143	142	137	127	126	120	115	114	111	109
%	1	7	23	15	6	5	4	12	7	28	16	22	7	22
m/z	99	97	93	92	91	83	82	81	77	75	74	65	60	
%	10	16	17	33	6	5	6	23	6	15	41	19	9	

The parent ion m/z 228 is a very weak peak that initially may fragment into three diverse ions (Scheme 1). The M^+ ion by loss of CO_2H gives the m/z 183 ion which loses ethylene in two subsequent steps to give the m/z 155 and m/z 27 ions. The molecular ion may also give by cleavage of the

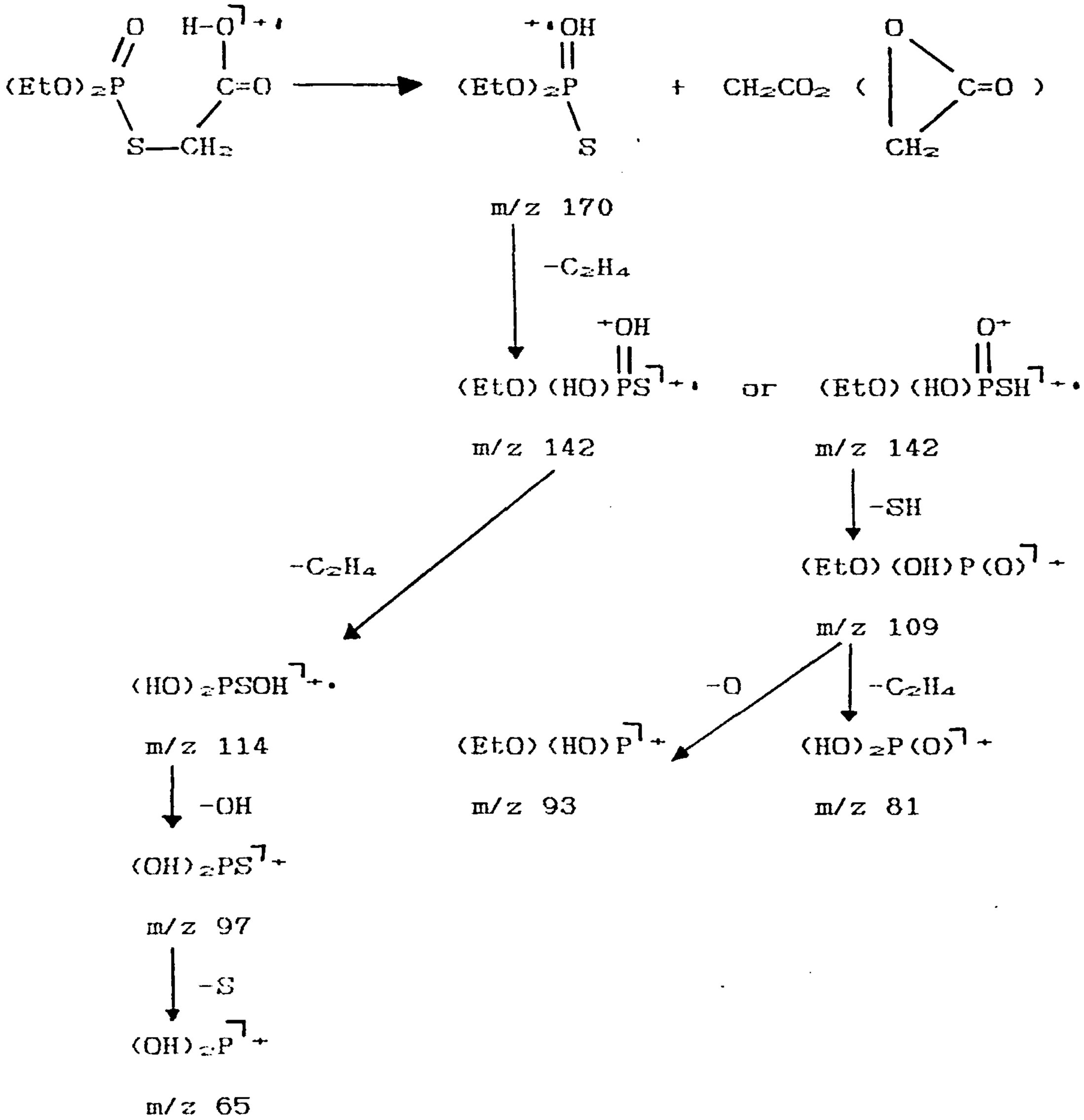


Scheme 18

P-S bond the intense m/z 137 ion while the cleavage of the S-C bond with direct hydrogen transfer to the oxygen or sulphur atoms forms the relatively intense m/z 170 ion.

The formation of m/z 170 ion and a possible cyclization for the CH_2CO_2 fragment are shown in Scheme 19.

The m/z 170 ion by subsequent loss of ethylene forms



Scheme 19

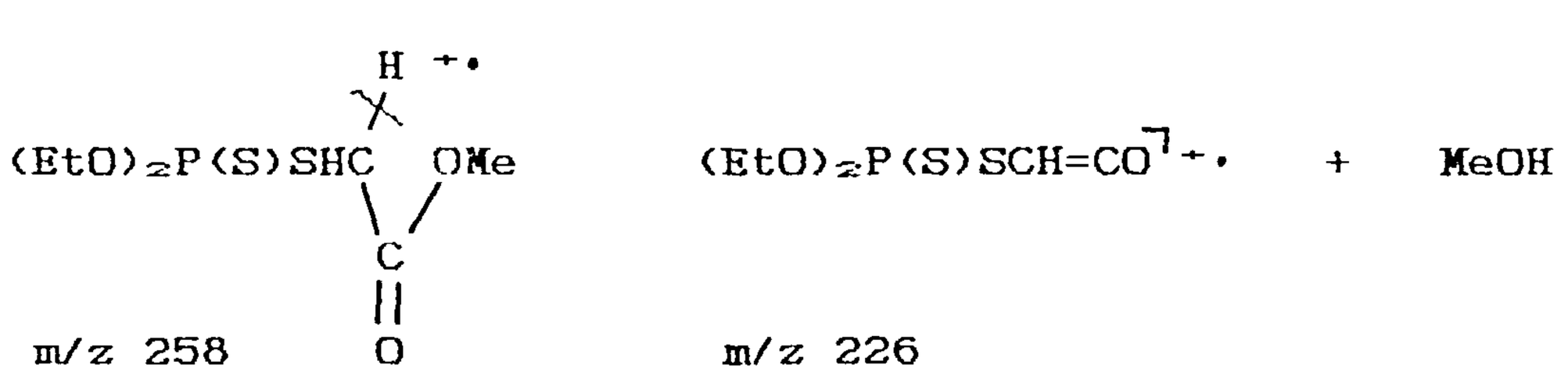
the m/z 142 and m/z 114 ions. The latter by cleavage of the P-OH bond gives the characteristic m/z 97 ion, which may lose the S to give another characteristic m/z 65 ion.

Another alternative mode of fragmentation for the m/z 142 ion explains the m/z 109 ion by loss of SH and the m/z 81 ion by loss of ethylene from the former ion. The m/z 109 ion may also lose O to give the relatively intense m/z 93 peak.

Table 13. Mass spectral data for $(EtO)_2P(S)SCH_2CO_2Me$ at room temperature.

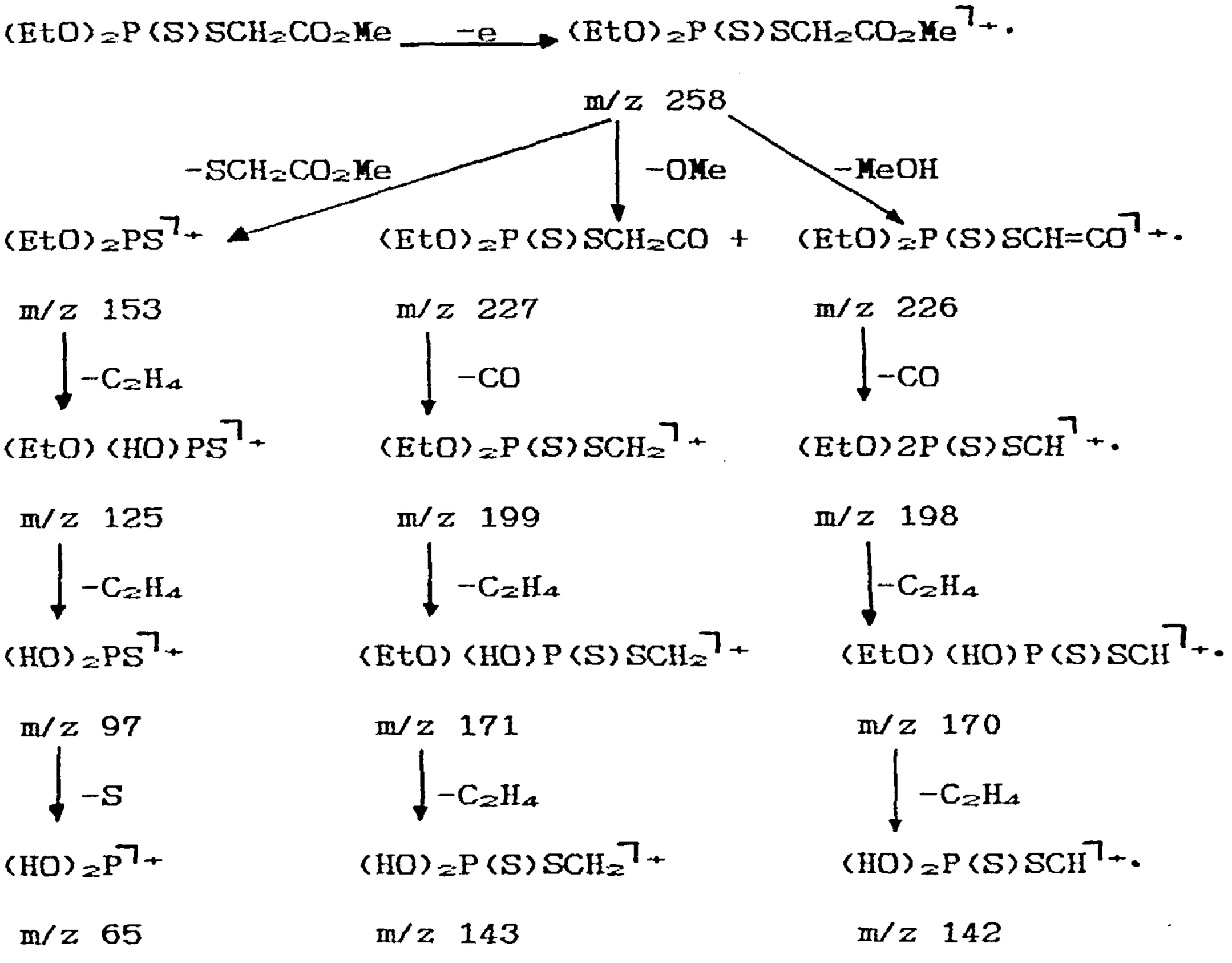
m/z	258	227	226	214	199	198	186	185	181	171	170	169	168	157
%	74	12	35	8	6	18	17	11	36	6	9	12	13	10
m/z	155	154	153	143	142	141	139	129	127	125	121	113	112	111
%	3	6	40	7	5	9	6	25	3	60	53	7	5	16
m/z	109	107	106	97	95	93	81	80	79	74	65	63	59	58
%	7	26	28	100	8	36	5	6	14	25	47	6	3	12

The ions at m/z 227 and 226 have also been reported in the e.i. mass spectrum of mecarbam, the latter being formed by a hydrogen rearrangement. A similar process can be considered to occur in this case.



Scheme 20

The methyl ester, $(EtO)_2P(S)SCH_2CO_2Me$, is considerably more stable than the corresponding acid and gives a strong parent ion at m/z 258 with a relative intensity of 74%. Fragmentation then appears to occur principally by P-S cleavage and the subsequent loss of ethylene and sulphur. The fission of the C-OMe bond with or without hydrogen transfer give less strong fragment ions (Sheme 21).



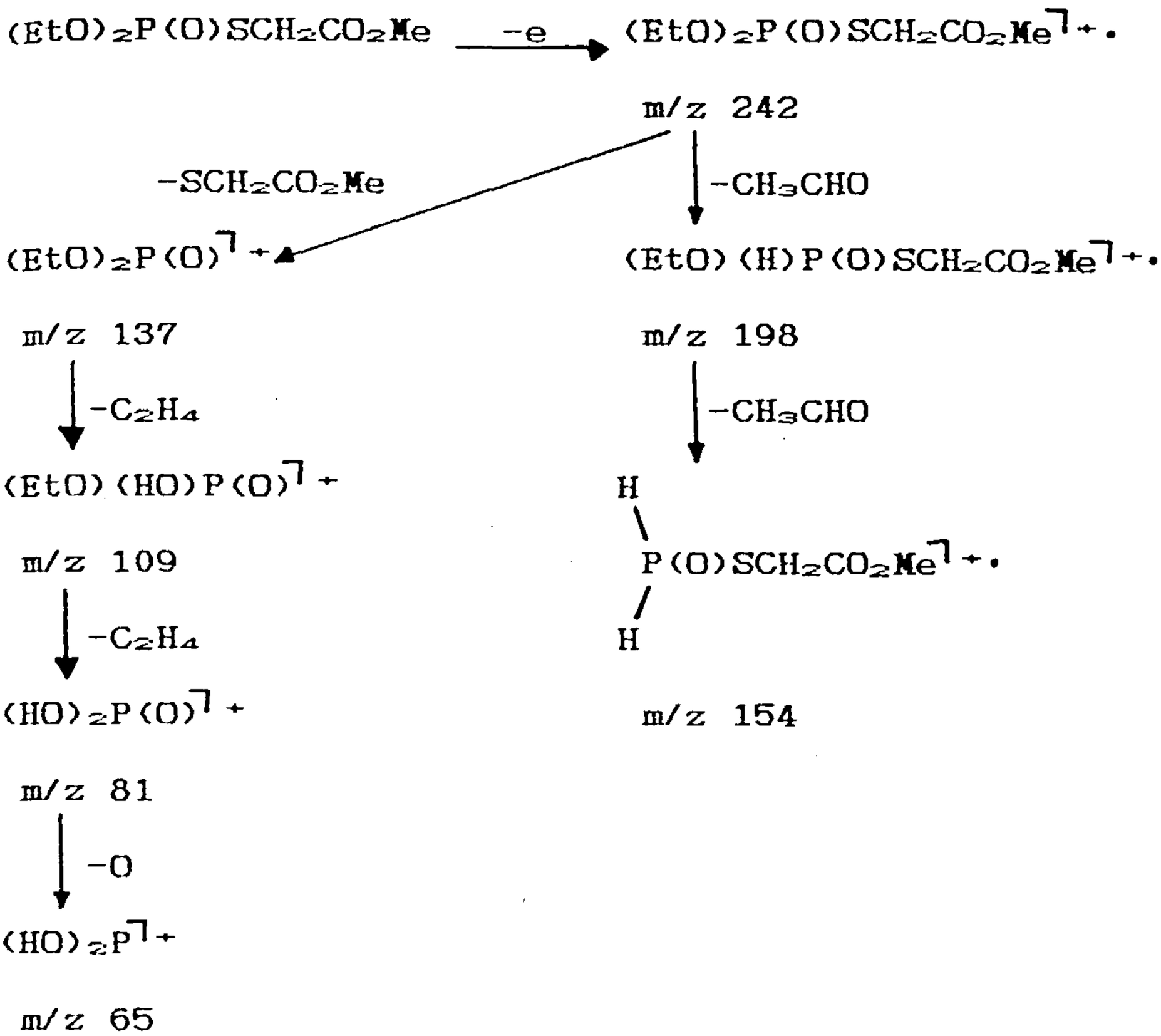
Scheme 21

Table 14. Mass spectral data for $(EtO)_2P(O)SCH_2CO_2Me$ at 240°C.

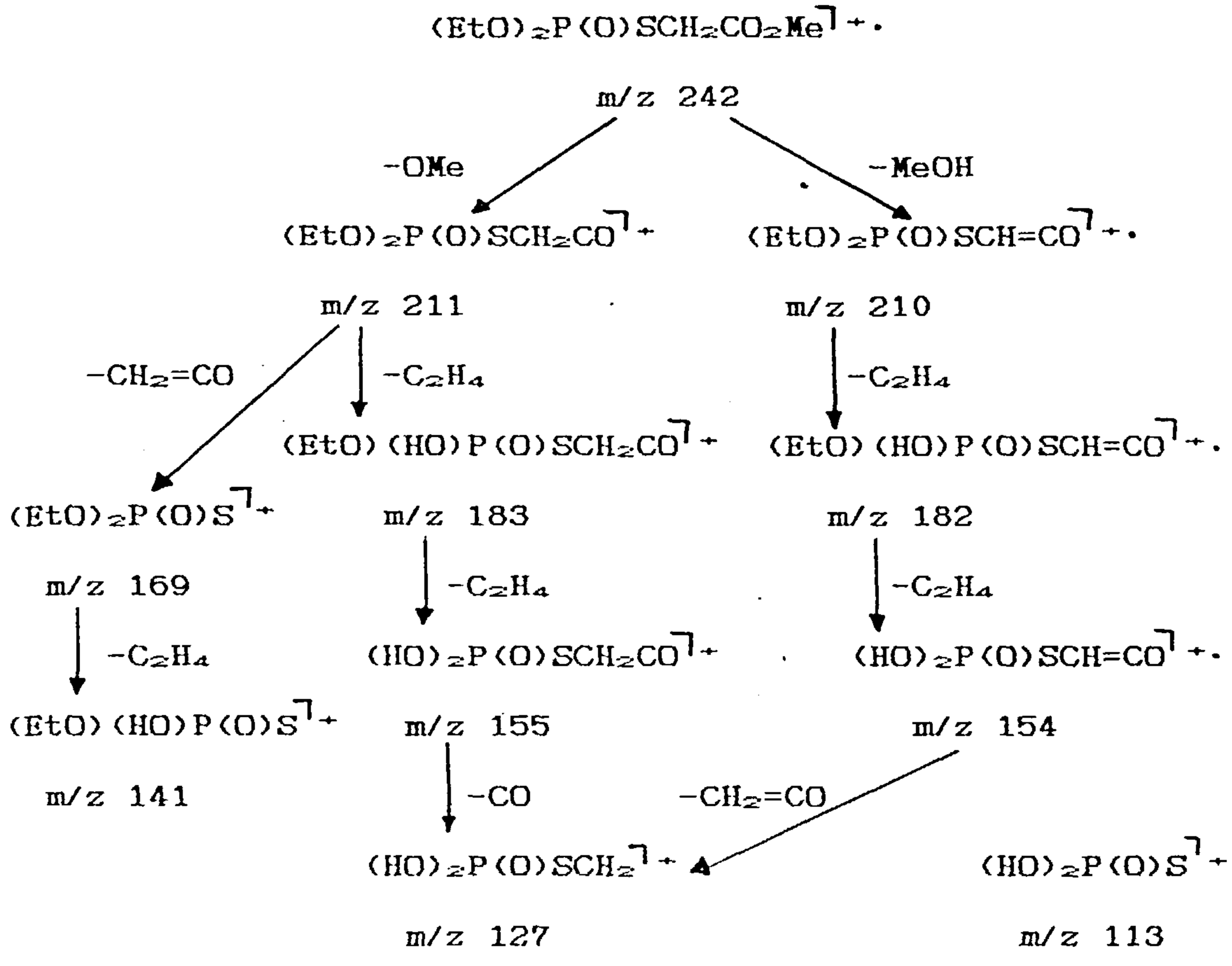
m/z	242	214	211	210	198	197	184	183	182	171	170	169	155	154
%	18	8	19	55	6	7	5	36	74	4	14	51	32	63
m/z	141	139	138	137	127	126	125	123	113	111	110	109	106	99
%	27	11	9	16	26	7	11	6	20	24	7	100	37	7
m/z	97	96	95	93	91	88	84	83	82	81	79	77	75	74
%	17	11	17	21	30	25	12	7	12	81	7	9	14	38
m/z	65	61	59											
%	32	10	22											

The cleavage of the P-S bond and subsequent loss of ethylene and O in the case of the phosphorothioate, $(EtO)_2P(O)SCH_2CO_2Me$, give very intense peaks including the base peak, m/z 109. Direct hydrogen rearrangement to the P atom may give the strong ion at m/z 154 (Scheme 22).

In Scheme 22 the possible loss of OMe or MeOH followed by ethylene may give the important m/z 183 and 182 ions respectively. The strong peak at m/z 182 indicates that the hydrogen transfer is an important process of the fragmentation. The loss of keten from the ion at m/z 211 by cleavage of the S-C bond may give rise to the intense peak at m/z 169.


(EtO)2P(O)SCH2CO2Me
-e
(EtO)2P(O)SCH2CO2Me⁻⁺·
m/z 242
-SCH2CO2Me
-CH3CHO
(EtO)2P(O)⁻⁺
(EtO)(H)P(O)SCH2CO2Me⁻⁺·
m/z 137
m/z 198
-C2H4
-CH3CHO
(EtO)(HO)P(O)⁻⁺
H
P(O)SCH2CO2Me⁻⁺·
H
m/z 109
m/z 154
-C2H4
(HO)2P(O)⁻⁺
m/z 81
-O
(HO)2P⁻⁺
m/z 65

Scheme 22



Scheme 23

Table 15a. Mass spectral data for $(EtO)_2P(S)SCH_2OH$ at room temperature.

m/z	188	187	186	158	154	153	144	142	141	140	131	130	129	125
%	14	12	100	14	5	31	6	69	16	19	11	26	19	51
m/z	121	114	113	112	109	99	97	96	95	93	81	80	79	65
%	8	35	32	13	69	10	100	6	19	11	37	14	7	46
m/z	64	63	61	44	42	39								
%	6	17	8	44	20	20								

Table 15b. Mass spectral data for $(EtO)_2P(S)SCH_2OH$ at 200°C.

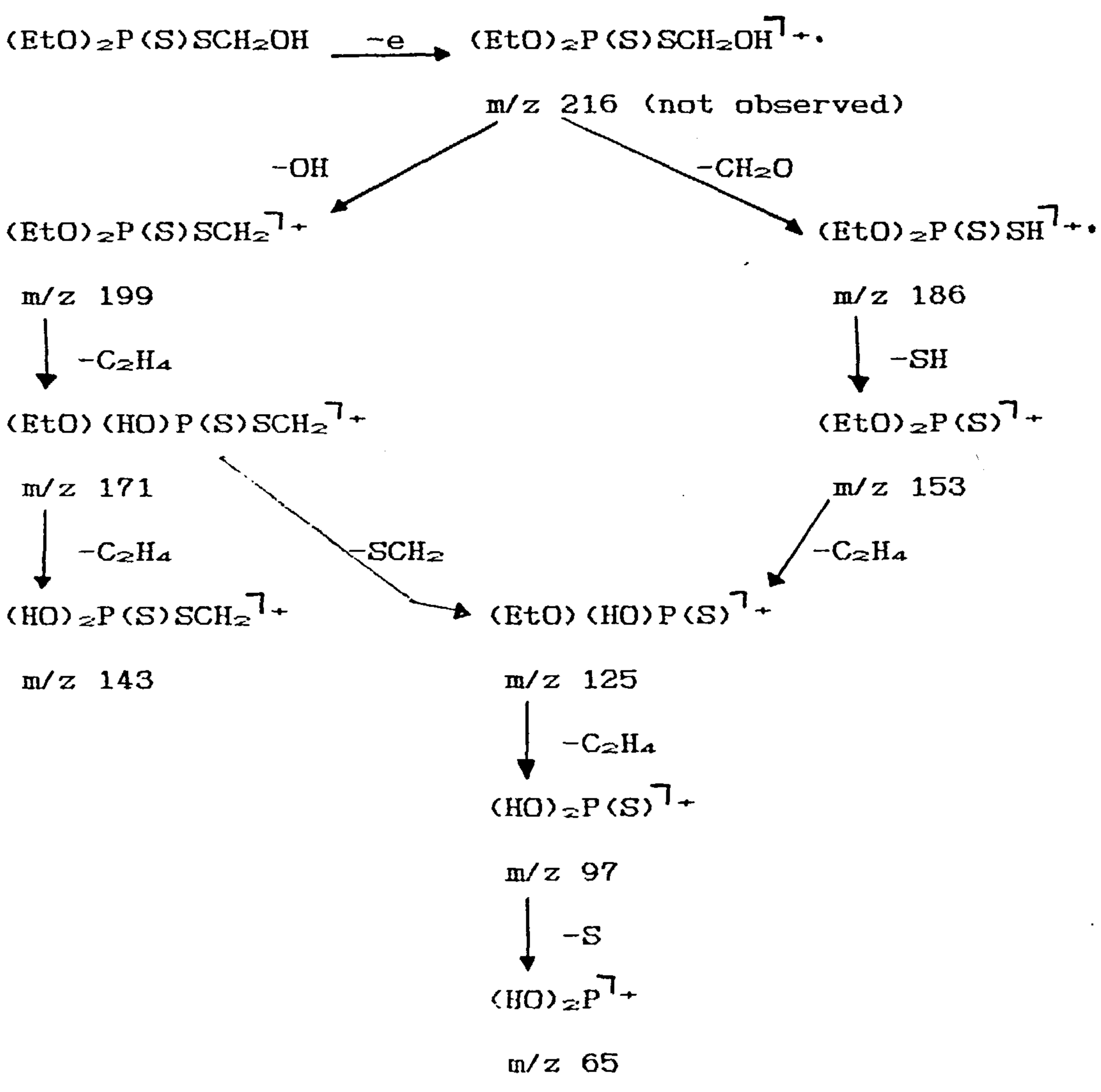
m/z	232	228	214	201	199	189	188	187	186	159	158	157	153	143
%	6	49	7	8	8	6	20	96	100	15	9	9	18	9
m/z	142	141	140	139	131	130	129	125	121	115	114	113	112	109
%	53	100	100	21	10	11	13	40	8	7	34	28	12	34
m/z	99	98	97	95	93	86	85	83	81	80	73	69	67	65
%	21	6	100	8	9	9	29	6	14	8	12	15	22	26
m/z	63	62	61	59	54	52	44	42	41	39	37	34	27	
%	7	6	8	9	7	14	24	36	8	100	35	25	7	

The observation of different ion intensities at room temperature and at 200°C in the mass spectrum of $(EtO)_2P(S)SCH_2OH$ indicates that thermal decomposition may occur in the inlet system. The strong peak at m/z 186 could be accounted for in this way. Decomposition

$$(EtO)_2P(S)SCH_2OH \longrightarrow (EtO)_2P(S)SH + CH_2O$$

to yield formaldehyde is the reverse of the reaction used to prepare the hydroxymethyl compound. The possibility of fragmentation of the molecular ion in this way should also be considered although the molecular ion itself was not detectable. Fragmentation of the molecular ion by loss of the hydroxyl radical is however necessary to account for the strong peak at 199. This then undergoes further fragmentation by the stepwise loss of ethylene, thio-formaldehyde, and sulphur (Scheme 24).

The strong peak at m/z 186 (originating either after thermal decomposition or by fragmentation of the molecular ion as discussed above) gives rise to the peak at m/z 153 and further fragments as discussed earlier for DETA. A number of relatively intense ions at m/z 187, 141, and 113 have not been assigned but might originate from products of thermal decomposition.

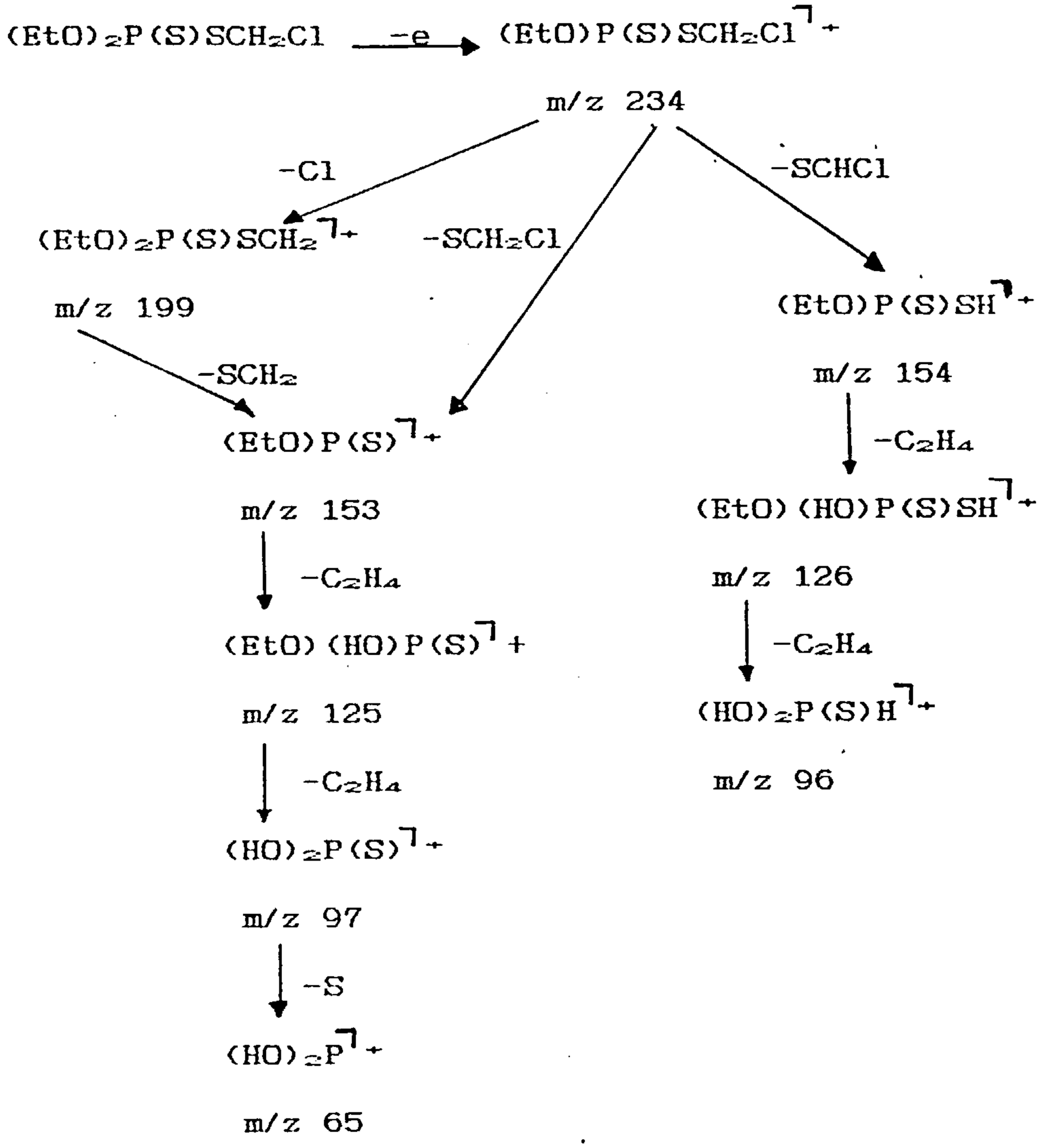


Scheme 24

Table 16. Mass spectral data for $(EtO)_2P(S)SCH_2Cl$ (chlormephos) at room temperature.

m/z	236	234	199	188	161	154	153	144	141	129	126	125	121	115
%	20	47	2	5	7	54	15	6	5	10	8	26	93	7
m/z	113	109	98	97	93	81	80	65	63					
%	8	11	5	100	29	9	6	27	5					

The parent ion for chlormephos appears at m/z 234 and m/z 236 for the ^{35}Cl and ^{37}Cl isotopes respectively and is a fairly abundant peak. The loss of Cl gives an insignificant peak at m/z 199(2%) which by cleavage of the P-S bond may give the m/z 153 ion although this could also be formed directly from the parent ion. Stepwise loss of ethylene then gives the base peak at m/z 97. The peak at m/z 154 could be explained by initial P-S cleavage with hydrogen transfer (Scheme 25).



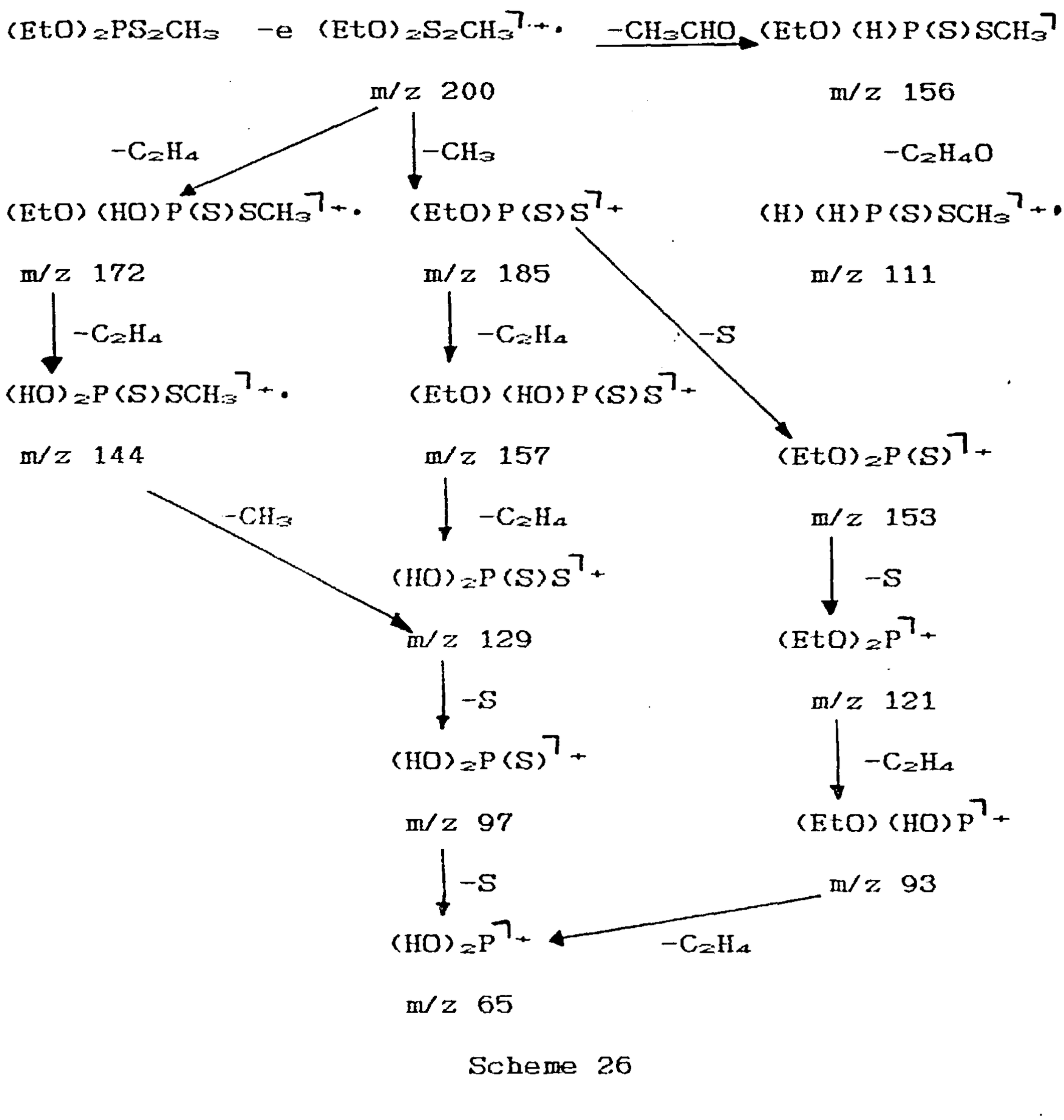
Scheme 25

Table 17. Mass spectral data for $(EtO)_2PS_2CH_3$ at 200°C.

m/z	202	201	200	172	156	155	153	144	139	129	128	127	125	123
%	10	7	100	20	31	13	10	17	12	7	15	24	22	51
m/z	121	111	109	97	93	91	81	80	79	65	62	56		
%	21	20	6	64	19	14	8	9	14	28	7	5		

The molecular ion of $(EtO)_2PS_2CH_3$ corresponds to the base peak which by initial loss of C_2H_4, or CH_3, or CH_3CHO

gives several possible routes of fragmentation (Scheme 26). The ions in such routes are very common in the process of fragmentation of phosphorodithioates.



Scheme 26

CONCLUSION ABOUT THE MASS SPECTRA PRESENTED

For the electron impact mass spectra the ions of highest relative intensity were those resulting from the cleavage of the bond between phosphorus and the thiolo-sulphur atom, $(RO)_2O(X)^+$, followed by dealkylation to give $(HO)_2P(X)^+$, or loss of sulphur to give $(RO)_2P^+$, as would be expected by previously reported fragmentation patterns for phosphorodithioates and phosphorothioates [10,16,64].

The FAB spectra also give similar fragment ions as the most intense peaks although some compounds show $[M+H]^+$ and $[M+H-H_2O]^+$ as the base peaks or very strong peaks. For instance the $[M+H]^+$ is the base peak for $(EtO)_2P(S)SCH_2CO_2H$ and $[M+H-H_2O]^+$ is the base peak for $(EtO)_2P(S)SCH_2OH$. The FAB mass spectrum for mecarbam gave the base peak by cleavage of the bond CO-NMe, with formation of the ion $(EtO)_2P(S)CH_2CO^+$, m/z 227.

The ammonium salt of diethyl monothiophosphoric acid and the potassium salt of diethyl dithiophosphoric acid, which had been prepared for the previously mentioned synthetic work, were also examined and showed some novel features. For the ammonium compound, significant peaks were observed corresponding to $[M+H]^+$, which in this case results from protonation of the anion, i.e. $[(EtO)_2P(O)SH + NH_4^+]$, the protonated acid $[(EtO)_2P(OH)SH]^+$ (the base peak), and

others resulting from stepwise loss of ethylene, water, sulphur and ammonia. $(EtO)_2P(O)SNH_4$ also gave the peak [NH_4^+ + glycerol] of very high relative intensity (62.8%).

The potassium salt showed different features and did not give a peak due to the protonated form of the corresponding acid. In this case, two sulphur atoms are present and there is no phosphoryl oxygen which can easily undergo protonation. The base peak was seen to correspond to the aggregate [$(EtO)_2PS_2^-$ $2K^+$], with a much weaker peak resulting from the loss of two ethylene molecules, i.e. [$(OH)_2PS_2^-$ $2K^+$]. A peak assigned to [$(OH)_2P(S)SH$ + K^+] was also noted, together with others that were unidentified.

FAB has clear advantages in the examination of ionic and thermally labile compounds but is less advantageous for compounds that are more easily vapourised, such as mecarbam, for which electron impact spectroscopy is satisfactory.

INVESTIGATION INTO THE POSSIBLE USE OF GC-MS (GAS CHROMATOGRAPHY-MASS SPECTROMETRY) FOR THE IDENTIFICATION OF METABOLITES OF MECARBAM AND OF CHLORMEPHOS

With the aim of investigating the possibilities of identifying the metabolites of mecarbam and chlormephos in the study of the degradation of these organophosphorus pesticides in liver and plants, a known sample of probable metabolites (or their methylated derivatives) was analysed by gc-ms using the service provided by PCMU (Physicochemical Measurements Unit, Harwell). The sample contained:

$(EtO)_2PS_2H$, $(EtO)_2PS_2CH_3$, $(EtO)_2PS_2CH_2CO_2Me$, $(EtO)_2P(O)SCH_2CO_2Me$, and the oxygen analogue of mecarbam, $(EtO)_2P(O)SCH_2CON(Me)CO_2Et$.

Analysis was carried out on a capillary column containig silicone oil, with an initial temperature of 55°C, final temperature of 250°C, and ramp rate of 5.0°/minute. The following results were obtained.

Table 1-A

Peak/Compound	Retention time(min:sec)	SCAN number	Ion Current intensity(%)
$(EtO)_2PS_2H$	14:58	855	45.0
$(EtO)_2PS_2CH_3$	18:20	1048	100.0
$(EtO)_2POSCH_2CO_2Me$	25:08	1436	6.0
$(EtO)_2PS_2CH_2CO_2Me$	26:55	1538	30.0
$(EtO)_2PS_2CH_2CH_3$	19:58	1141	13.0

The compound $(EtO)_2PS_2CH_2CH_3$ was probably formed by thermal decompostion of the methoxycarbonyl derivative, $(EtO)_2PS_2CH_2CO_2Me$

$$(EtO)_2PS_2CH_2CO_2Me \xrightarrow{-CO_2} (EtO)_2PS_2CH_2CH_3$$

The principal routes of fragmentation for these compounds in electron impact mass spectrometry (except for the S-ethyl compound), have already been studied in chapter II. The mass spectra obtained by the direct insertion method are compared in the following table to these obtained by GC-MS to clarify the identification of the compounds.

Table 2-A

Compounds	Relative Intensity by GC-MS (%)	Relative Intensity by direct insertion (%)
$(EtO)_2PS_2H$	47(100), 65(30), 97(65), 109(10), 125(19), 153(7), 186(15)($M^{+\cdot}$)	65(49), 97(100), 109(13), 125(50) 153(21), 186(16)
$(EtO)_2PS_2CH_3$	47(100), 65(35), 79(13), 97(28), 123(15), 139(<5), 144(<5), 156(<5), 172(<5), 200(8)($M^{+\cdot}$)	65(28), 79(14), 97(64), 123(51), 139(12), 144(17), 156(31), 172(20), 200(100)
$(EtO)_2PS_2CH_2CO_2Me$	47(100), 59(10), 65(30), 74(15), 81(20), 93(10), 97(35), 109(20), 121(<5), 127(20), 155(25), 169(<5), 198(trace), 258(trace)($M^{+\cdot}$)	59(3), 65(47), 74(25), 81(5), 93(36), 97(100), 109(7), 121(53), 127(3), 155(3), 169(12), 198(18), 258(74)
$(EtO)_2P(O)SCH_2CO_2Me$	45(100), 65(15), 74(18), 81(18), 91(25), 109(17), 127(<10), 141(<5), 154(17), 169(<10), 182(10), 197(trace), 210(<5), 242(trace)($M^{+\cdot}$)	65(32), 74(38), 81(81), 91(30), 109(100), 127(26), 141(27), 154(63), 169(51), 182(74), 197(7), 210(55) 242(18).
$(EtO)_2PS_2CH_2CH_3$	47(100), 59(28), 65(68), 97(75), 109(12), 125(22), 169(trace), 186(25), 214(10)($M^{+\cdot}$).	not determined

In all cases the molecular ion was detectable and the same principal fragments were observed by the gc-ms technique as for the same compounds by direct insertation. The relative intensities of the ions however differed.

On the basis of the present information, the best interpretation is as follows:-

The compound $(EtO)_2P(S)SCH_2CH_3$ (not discussed above) appears to fragment mainly by fission of the P-S bond (Scheme 1). In addition the peak at m/z 186 indicates initial loss of ethylene, but whether this originates from the ethoxy or ethhylthio group is not certain (Scheme 2). Cleavage of the P-O bond appears to be less important as the peak at m/z 169 is very weak, although this ion could be an intermediate en route to the formation of the moderately intense ion at m/z 109, tentatively assigned the structure $EtOPSH^+$ (Scheme 2),

$(EtO)_2P(S)SEt]^{+\bullet}$ $\xrightarrow{-SEt}$ $(EtO)_2PS]^+$ $\xrightarrow{-C_2H_4}$

m/z 214 m/z 153

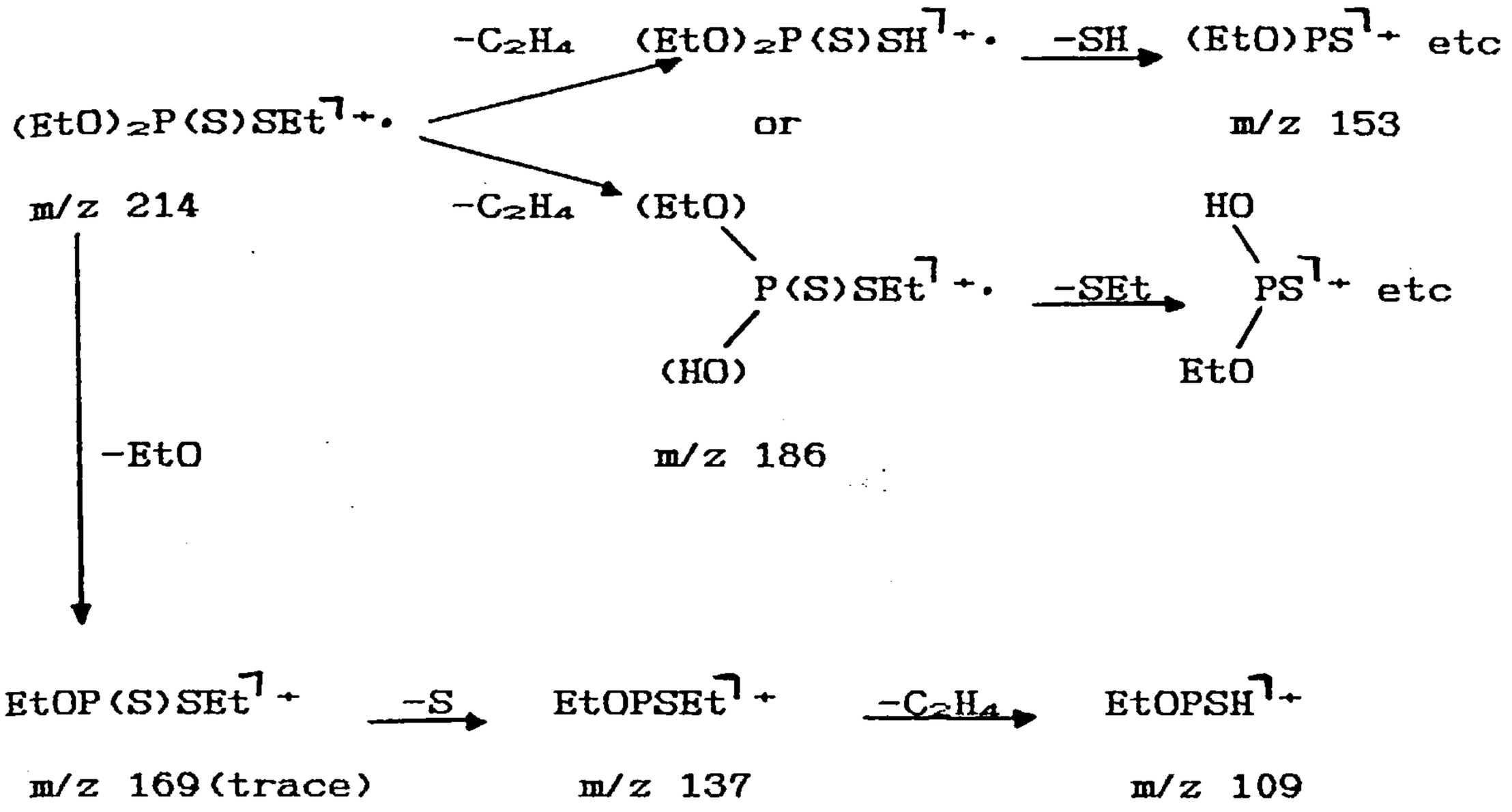
$(EtO)(HO)PS]^+$ $\xrightarrow{-C_2H_4}$ $(HO)_2PS]^+$ $\xrightarrow{-S}$

m/z 125 m/z 97

$(HO)_2P]^+$ $\xrightarrow{-H_2O}$ $PO]^+$

m/z 65 m/z 47

Scheme 1



Scheme 2

It was not possible to identify the oxygen-analogue of mecarbam in the above mixture, but individual samples of mecarbam and its oxygen-analogue were analysed by gc-mass spectrometry on the silicone oil capillary column in order to obtain information about their peaks and fragmentations. By using an initial temperature of 55°C with temperature programming at 20.0°C/min up to 200°C, the following results were obtained.

Table 1-B

Peak/Compound	Retention time(min:sec)	SCAN number	Ion Current intensities(%)
(EtO)2P(S)SCH2CONMeCO2Et	15:15	871	<10.0
(EtO)2P(O)SCH2CONMeCO2Et	13:24	766	<1.5

The main peaks in the mass spectra of mecarbam and its oxygen-analogue by GC-MS are compared in Table 2-B with those previously reported for the direct insertation method.[19]

Table 2-B

Relative intensity by GC-m/s (%)	Relative intensity by direct insertation (%)
$(EtO)_2P(S)SCH_2CONMeCO_2Et$	
42 (100), 46(78), 58(75),	42(27), 46(17), 58(46),
64 (68), 74(18), 86(30),	- , 74(11), 86(39),
97 (88), 104(15), 116(28),	97(95), 104(19), 116(36),
125(45), 131(80), 143(17),	125(51), 131(100), 143(9),
153(26), 159(48), 160(40),	153(31), 159(49), 160(50),
171(10), 185 (4), 198(8),	171(10), 185(5), 198(11),
226(3), 252 (1), 283(1),	226(14), - -
296(3), 329 (8)($M^{+\cdot}$)	296(14), 329(35).
$(EtO)_2P(O)SCH_2CONMeCO_2Et$	
42 (100), 43(20), 44(35),	42(16), 43(7), 44(5),
59 (58), 65(20), 76(<10)	59(2), 65(10), 76(6),
81 (40), 86(35), 91(15),	81(6), 86(39), 91(5).
109(30), 131(90), 137(5),	109(24), 131(100), 137(8)
154(30), 159(35), 177(1),	154(25), 159(78), 177(2)
182(18), 196(1), 210(15)	182(17), 196(2). 210(22)
268(1), 313(1)($M^{+\cdot}$).	268(4), 313(10).

This technique gives many fragment peaks in common for the compounds under study, but the relative intensity varies as can be observed in Table 2-B. The GC-MS method is however clearly siutable for the identification of these compounds in mixtures, on the basis of the peaks that are observed.

GAS-CHROMATOGRAPHIC ANALYSIS AND HYDROLYSIS STUDIES

Separation and identification of some metabolites or their methyl derivatives by gas-chromatography have been investigated under the conditions that follow.

A Varian model 3700 gas-chromatograph fitted with a thermionic detector (phosphorus mode) and a system of direct capillary injection was used. The column used was a capillary wall-coated open tubular (WCOT) column, containing SE 30 on vitreous silica, 12 m in length and 0.2 mm in diameter. In the detector, the air flow-rate was 175 cm^3/min, the make-up gas N_2 flow-rate was 35 cm^3/min and the hydrogen flow-rate was 4 cm^3/min; and in the column, the carrier gas was nitrogen with a flow-rate of 5.0 cm^3/min.

The analysis was carried out with a temperature program starting at 55°C for 5 min and heating at 5°C/min to 147°C. The detector and injector temperature were 220°C and 90°C respectively.

Compounds identified and separated using the conditions above are shown in Table 1 and a typical chromatogram is shown in Figure 1.

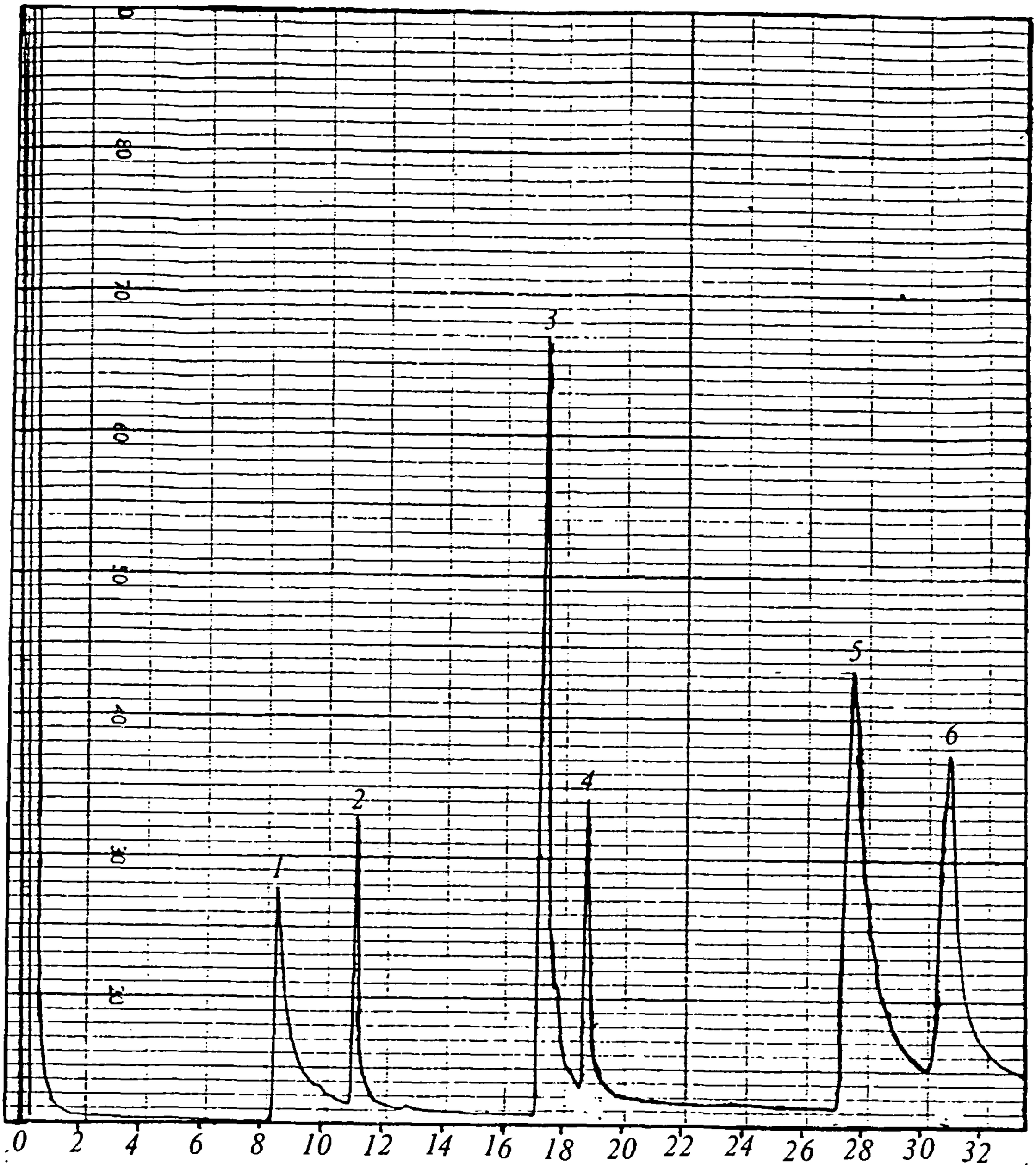


Figure 1
Separation of metabolites and methyl derivatives by gas chromatography - see also Table 1.

Table 1

COMPOUND	Retention time (min)
1. $(EtO)_2PS_2H$	8.6
2. $(EtO)_2PS_2CH_3$	11.3
3. $(EtO)_2P(O)SCH_2CO_2Me$	17.9
4. $(EtO)_2PS_2CH_2CO_2Me$	19.0
5. $(EtO)_2P(O)SCH_2CONMeCO_2Et$	27.7
6. $(EtO)_2PS_2CH_2CO_2NMeCO_2Et$	31.0

On the non-polar stationary phase the compounds are seen to elute in order of increasing molecular weight, and presumably of boiling point.

Hydrolysis Studies

The importance of the study of hydrolysis of pesticides is due to the fact that pesticides usually undergo hydrolytic reactions under natural conditions. So a knowlegde of rates and products of hydrolysis of a pesticide provides useful information about its level of toxicity.

The final products of hydrolysis of organo-phosphorus pesticides normally are non-toxic residues, but before detoxification takes place, reactions of oxidation

and isomerization can give very poisonous metabolites. Reactions of oxidation are normally carried out by enzymes present in insects and via soil organic matter catalyzed by clay surfaces, metal oxides, metal ions, etc. Oxidation occurs in plant but at a slower rate.

To improve the method for determining the rates of hydrolysis the investigation of a possible new procedure for determining the concentration of organophosphorus pesticides in solution, without extraction, was carried out at different values of pH and in two solvent systems.

The basis of the method was to use a suitable reference compound as an internal standard against which mecarbam could be measured. This avoids the inaccuracies that would result from either incomplete extraction by a solvent, or variation in the volume injected on each occasion by the microlitre syringe. Benzophenone was found to be a suitable reference compound for this purpose. However, because of the low solubility of mecarbam (and of benzophenone) in water, hydrolysis was carried out in mixtures of ethanol-water or dioxane-water.

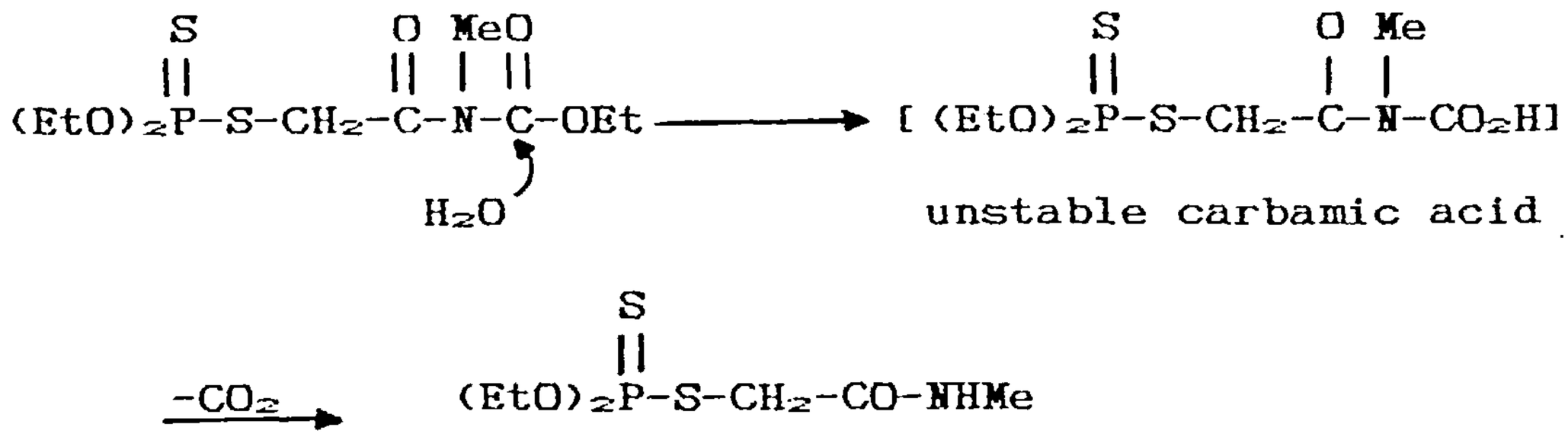
The details of the procedure are given in the experimental section. The results are shown in Table 2.

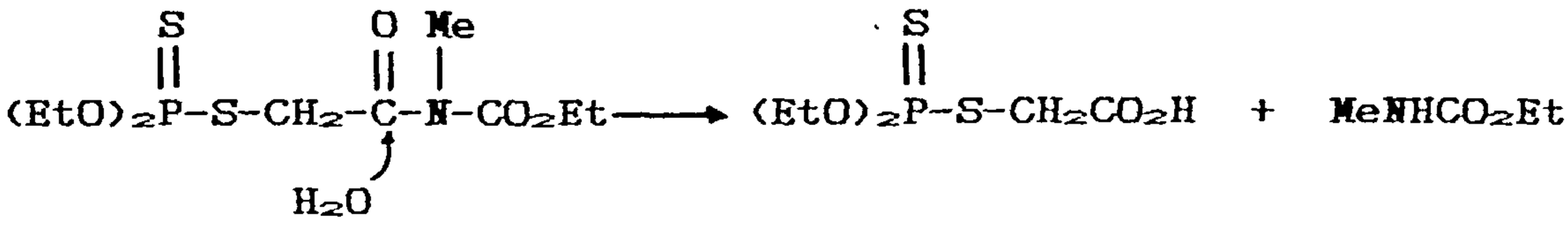
Table 2

Exp.	pH	Solvent	Total time/h	K_1/h^{-1}	t½/h	r
A	7.3	50% EtOH/				
		50% buffer 6.8	5.5	0.2544	2.72	0.9914
B	7.1	50% EtOH/				
		50% buffer 6.6	8.0	0.1834	3.77	0.9840
C	8.4	50% dioxane/				
		50% buffer 7.0	6.0	0.3064	2.26	0.9926

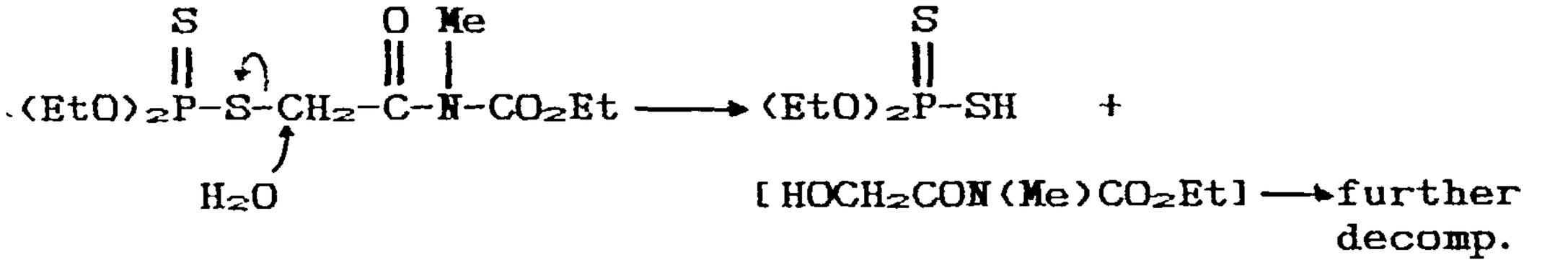
Initial concentration of mecarbam (g/dm^3) = 0.002(exp.A); 0.0086(exp.B); 0.0016(exp.C).

The rate of hydrolysis increases somewhat with increasing pH, as would be expected for nucleophilic attack by water on mecarbam. Only a narrow range of pH values has so far been studied however. Possible modes of reaction with water are as follows.





As hydrolysis proceeded, a number of degradation products were observed by g.l.c., one of which appeared to be diethyl dithiophosphoric acid (DETA), formed by cleavage of the S-CH_2 bond



Under similar conditions chlormehos was found to be more stable and underwent no detectable hydrolysis in 70 hours.

Futher identification of the hydrolysis products was achieved by methylation followed by g.c.-m.s. as described in the following section.

GC-MS ANALYSIS FOR HYDROLYSIS STUDIES

In two experiments, as described in the experimental section, alkaline hydrolysis was used to cause almost total degradation (Sample 1) or only partial degradation (Sample 2) of mecarbam.

Gas chromatography - mass spectrometry analysis was carried out on an OV1 column (25 m x 0.2 mm, film thickness 0.1 μm) with an initial temperature of 40°C for 5 minutes, final temperature 250° for 10 minutes, and a ramp rate of 15°C/minute. The carrier gas was He, flow rate 30 cm/s, head pressure 10 psi. and injector temperature 200°C.

The results for sample 1 are shown in Table 1.

Table 1

Retention Time (min:sec)	Scan number	Ion current intensity (%)
12:57	606	25
16:39	811	100
16:50	848	5
20:15	995	45

The compounds scan number 606, 811, 848, 995 gave the fragment ions in tables 2, 3, 4 and 5 respectively.

Table 2. Mass spectral data for compound scan number 606

m/z	184	156	139	128	111	93	81	65	47
%	18	18	45	35	80	60	95	50	100

Table 3. Mass spectral data for compound scan number 811

m/z	258	226	198	181	168	153	141	125	106	97	74	65
%	45	25	15	28	10	30	8	48	28	100	38	48
m/z	45											
%	35											

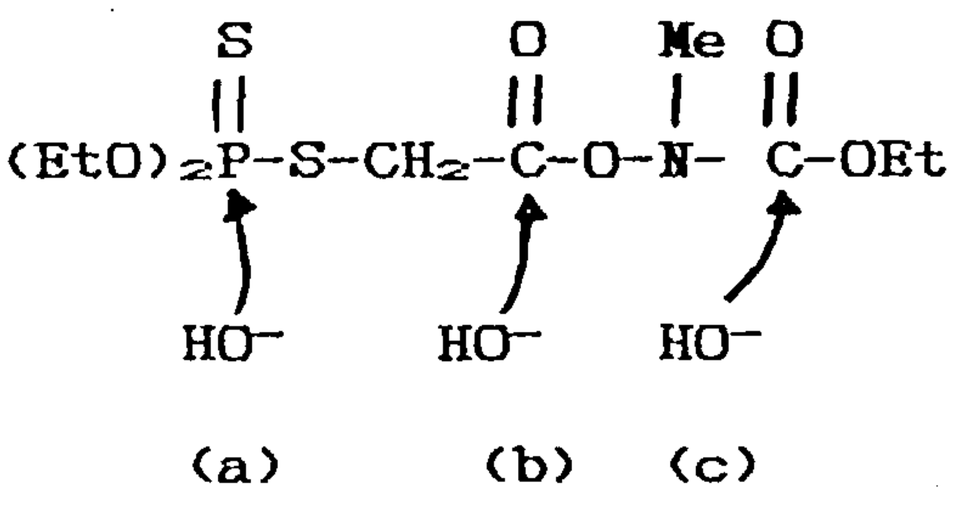
Table 4. Mass spectral data for compound scan number 848

m/z	272	227	195	171	153	121	97	88	74	65	40
%	18	8	15	9	20	55	100	12	12	55	52

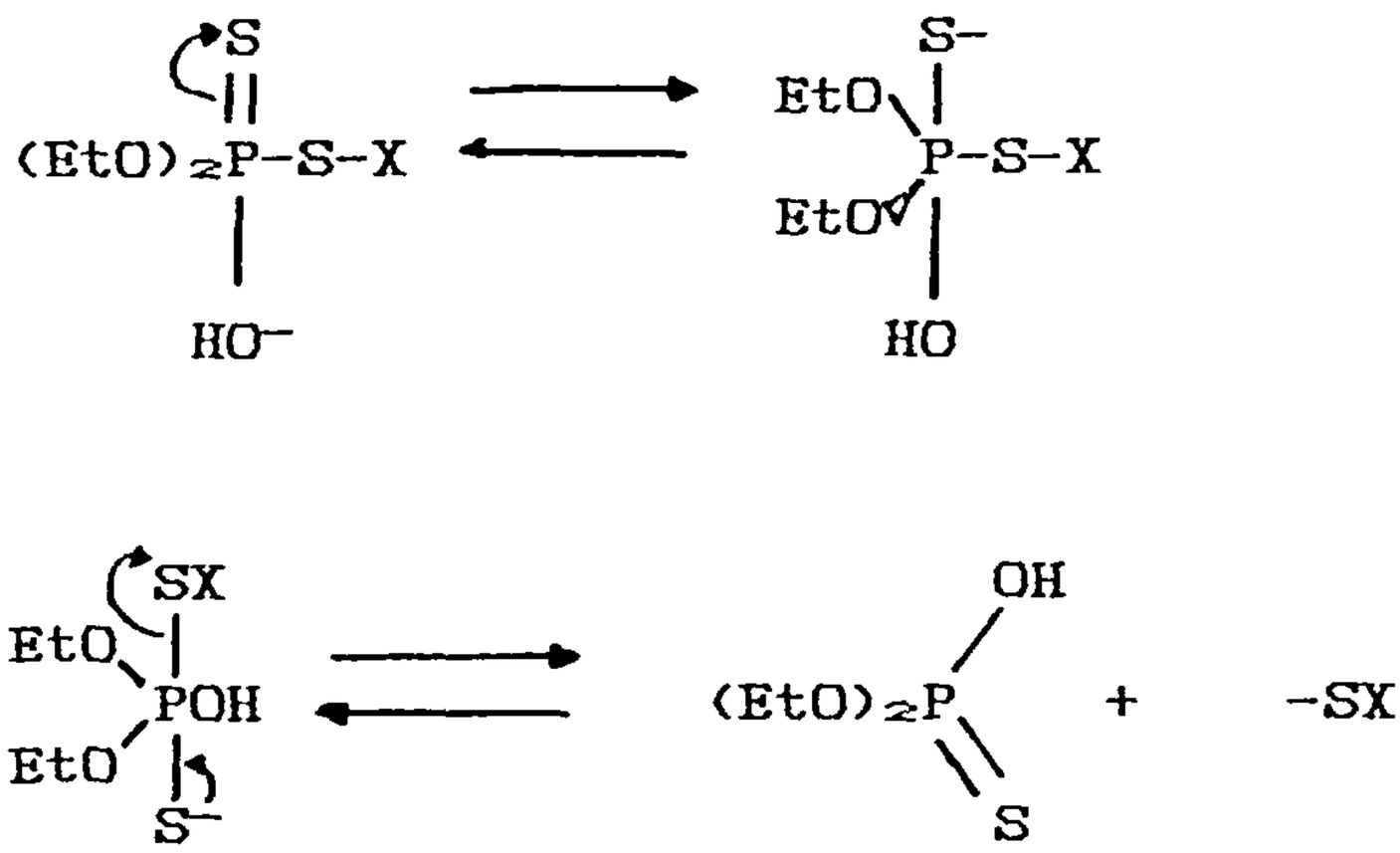
Table 5. Mass spectral data for compound scan number 995

m/z	329	296	226	198	159	144	131	116	97	86	74	59
%	18	10	8	8	40	12	85	30	100	35	28	52
m/z	42											
%	40											

The compound with retention time 12:57 was identified as $(EtO)_2P(O)SCH_3$ formed after methylation of $(EtO)_2P(O)SH$. This is explained by the fact that one of the three possibilities of hydrolysis of mecarbam is the attack of P by the hydroxide ion, as shown :

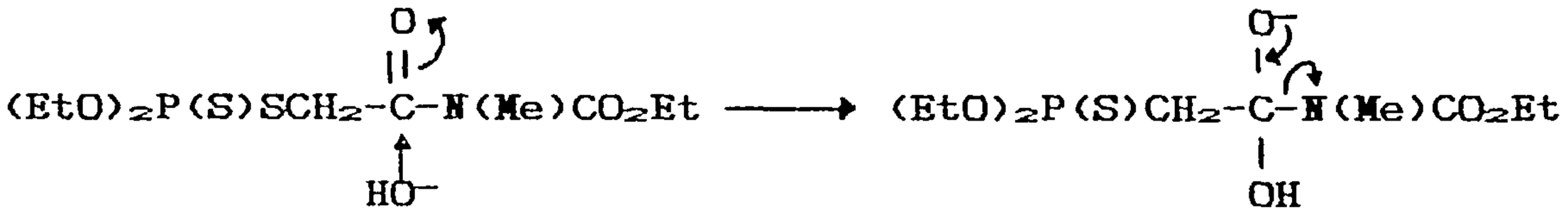


Nucleophilic displacement at phosphorus (a) by a bimolecular $S_N2(P)$ mechanism is well known [68], and proceeds via a five-coordinate intermediate.



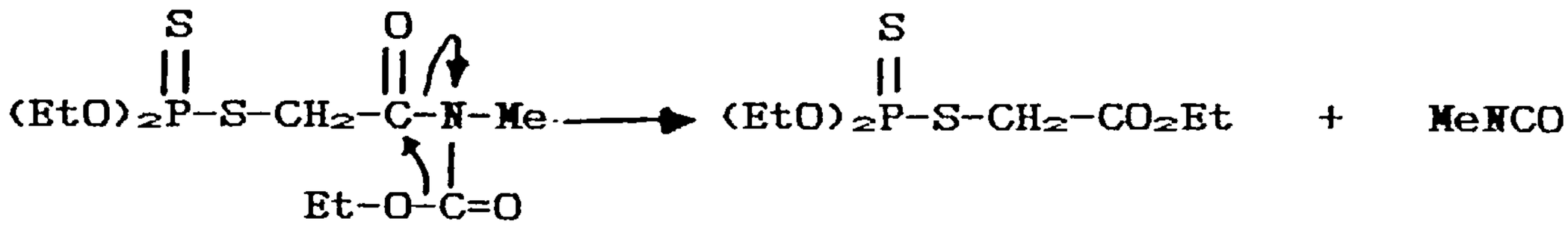
[X = $CH_2CON(Me)CO_2Et$]

The attack at CH_2 by hydroxide, with cleavage of the S-C bond [9] was not confirmed since there is no evidence for the presence of $(EtO)_2P(S)SH$. The second possibility of attack at the carbamoyl carbonyl group (b) is confirmed with the formation of the compound of scan number 811, which is the methylated derivative of $(EtO)_2PS_2CH_2CO_2H$.



There is no evidence for initial hydrolysis of the carbethoxy group which would be expected to yield $(EtO)_2P(S)SCH_2CON(Me)CO_2H$ and hence $(EtO)_2P(S)SCH_2CONHMe$ by loss of CO_2. These products were not detected, although $(EtO)_2P(S)SCH_2CONHMe$ has been observed as a degradation product on the surface of bean leaves or of lucerne [9].

The compound scan number 995 was identified as mecarbam. The product of degradation scan number 848, m/z 272 was present in very low concentration. This is not definitely identified but suggests the formation of the ethyl ester, possibly as follows.



The results for sample 2 are in Table 6.

Table 6

Retention Time (min:sec)	Scan number	Ion current Intensity (%)
3:30	119	14
6:40	272	8
11:30	529	trace
16:39	811	100
17:30	839	5
20:20	1002	100

The compounds scan number 119, 272, 529, 811, 839 and 1002 gave the fragment ions in tables 7, 8, 9, 10, 11 and 12 respectively.

Table 7. Mass spectral data for the compound scan number 119

m/z	100	85	71	57	43
%	18	8	55	56	100

Table 8. Mass spectral data for the compound scan number 272

m/z	103	75	58	40
%	28	45	100	35

Table 9. Mass spectral data for the compound scan number 529

m/z	184	140	129	107	95	79	65	40
%	45	10	30	45	30	65	15	100

Table 10. Mass spectral data for the compound scan number 811

m/z	258	226	198	181	168	153	141	125	106	97	74	65
%	30	18	10	25	10	30	8	50	30	100	40	50
m/z	45											
%	40											

Table 11. Mass spectral data for the compound scan number 839

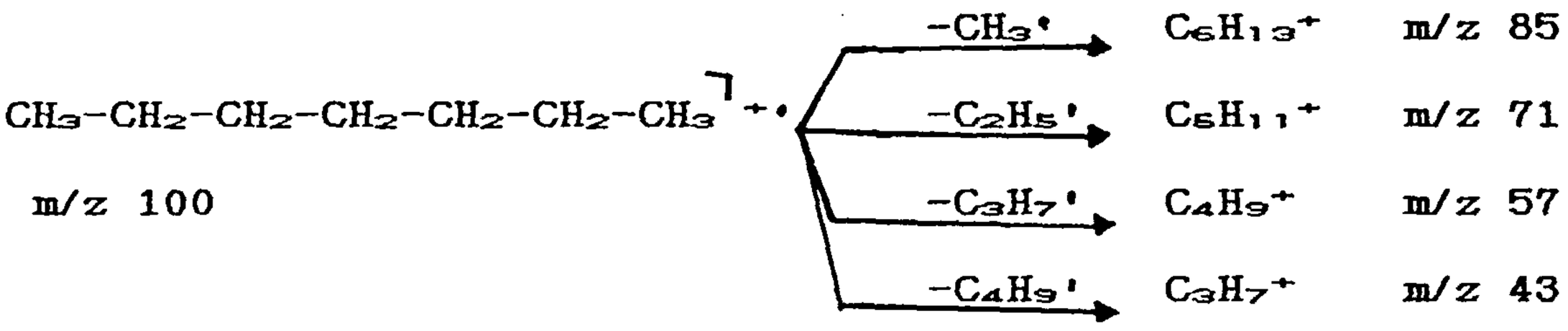
m/z	272	227	195	171	153	121	97	88	74	65	40
%	50	20	25	15	30	60	100	15	10	40	55

Table12. Mass spectral data for the compound scan number 1002

m/z	329	296	226	198	171	159	144	131	116	104	97	86
%	25	18	10	10	10	45	-18	90	40	20	100	40
m/z	76	65	56	42								
%	18	50	18	35								

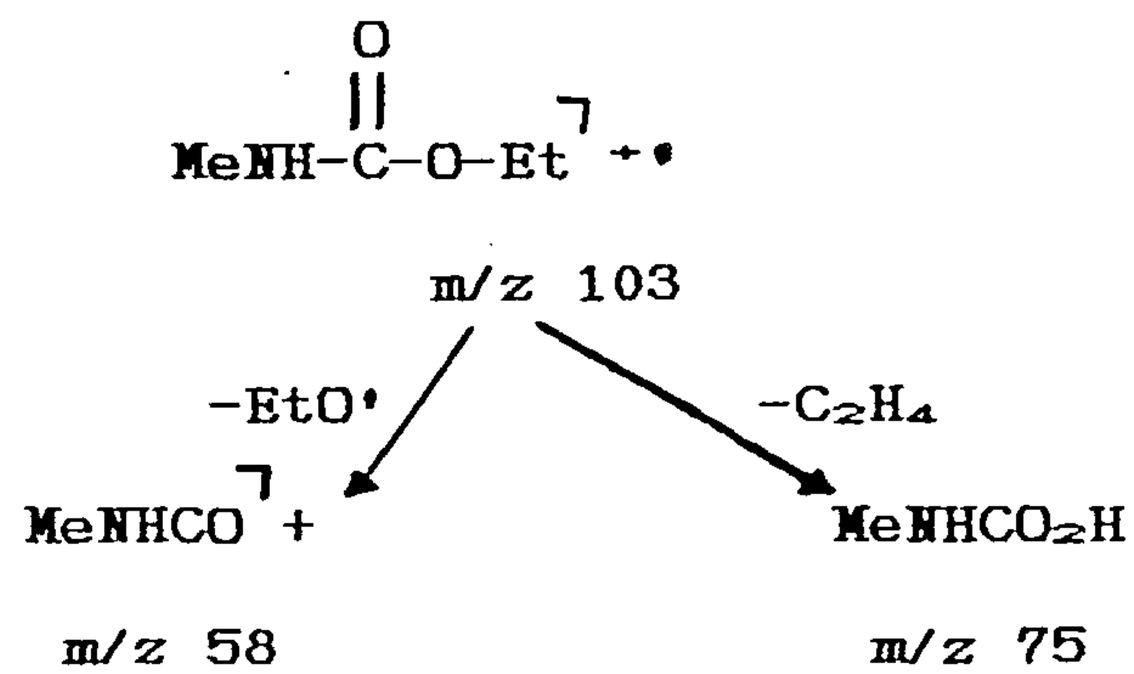
In sample 2, all the four compounds identified in sample 1 ($M^{+\cdot}$ 329, 272, 258 and 184), were present plus two other compounds at scan numbers 119 and 272.

The first of these is seen to be heptane, presumably present in the hexane fraction used in the experiment. Fragmentation of the various C-C bonds in the chain gives the hexyl, pentyl, butyl, and propyl carbonium ions as shown below.



The compound at scan number 272 is ethyl N-methylcarbamate, one of the two products of initial hydrolysis at the carbamoyl carbonyl group. It was not detected in sample 1 as further hydrolysis presumably occurred. Fragmentation occurs by initial loss of ethoxide

radical to give the base peak at m/z 58 or by elimination of ethylene [64].



The alkaline hydrolysis of chlormephos was carried out to reach only a partial level of degradation of this pesticide. The procedure was described in the experimental section.

The g.c.-m.s. analysis was performed with an OV17 capillary column (25 m x 0.32 mm, film thickness 0.52 μm), programmed from 100°C for 1 minute to 300°C for 15 minutes and a ramp rate of 10°C *per* minute, injector temperature 250 °C.

The results are shown in Table 13.

Table 13

Retention Time (min:sec)	Scan number	Ion current intensity %
2:70	91	22
5:40	219	18
7:10	284	100
12:58	598	25
16:37	784	100

The compounds scan number 91, 219, 284, 598, and 784 gave the fragment ions presented in Tables 14, 15, 16, 17, and 18 respectively.

Table 14. Mass spectral data for compound scan number 91

m/z	200	198	172	170	143	121	93	81	65	41
%	5	100	10	15	20	90	80	40	85	18

Table 15. Mass spectral data for compound scan number 219

m/z	214	186	158	121	97	81	65	40
%	20	100	10	70	70	10	65	38

Table 16. Mass spectral data for compound scan number 284

m/z	236	234	189	188	154	121	97	81	65	47
%	18	35	2	2	45	100	95	10	45	38

Table 17. Mass spectral data for compound scan number 598

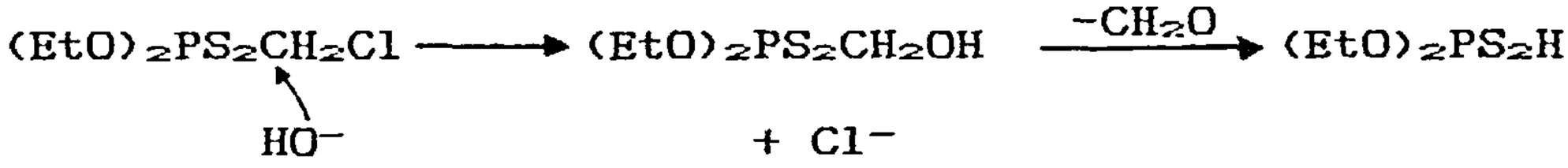
m/z	338	261	207	186	153	121	97	80	65	47
%	25	18	1	10	20	80	100	2	80	10

Table 18. Mass spectral data for compound scan number 784

m/z	384	261	231	199	175	153	125	109	97	79	65	45
%	2	1	80	10	8	70	50	2	100	2	55	22

Unreacted chlormephos gave the scan number 284. The peaks at m/z 234 and 236 showed the isotopic pattern due to ^{35}Cl and ^{37}Cl respectively whilst the peaks at m/z 188, 154, 121, 97, 81 and 65 correspond to the fragmentation pattern of chlormephos.

Hydrolysis of chlormephos has previously been shown to give DETA [67] which indicates attack at CH_2Cl as shown.



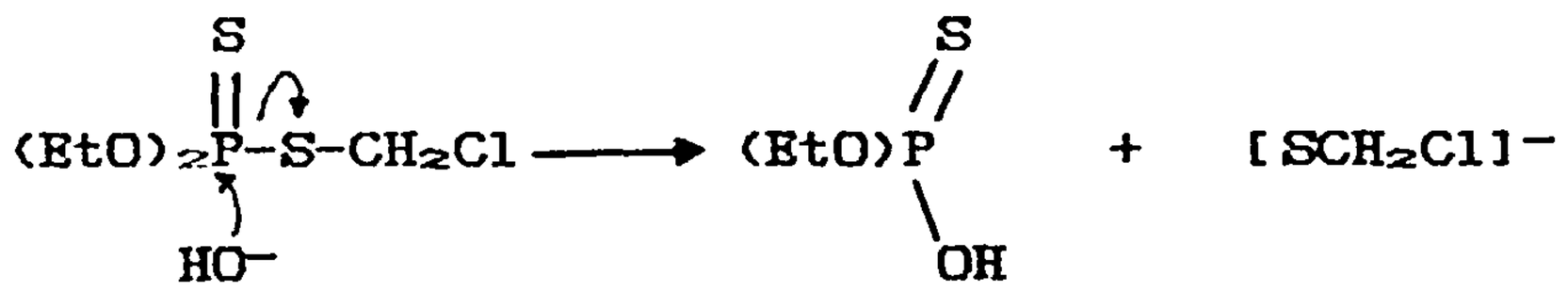
The intermediate hydroxymethyl derivative was not detectable, nor was the methylated form (M.W. 230).

Methylated DETA is suggested by the ion at m/z 200 in scan 91 but it gives only a weak signal. It is however confirmed by m/z 172, corresponding to loss of C_2H_4.

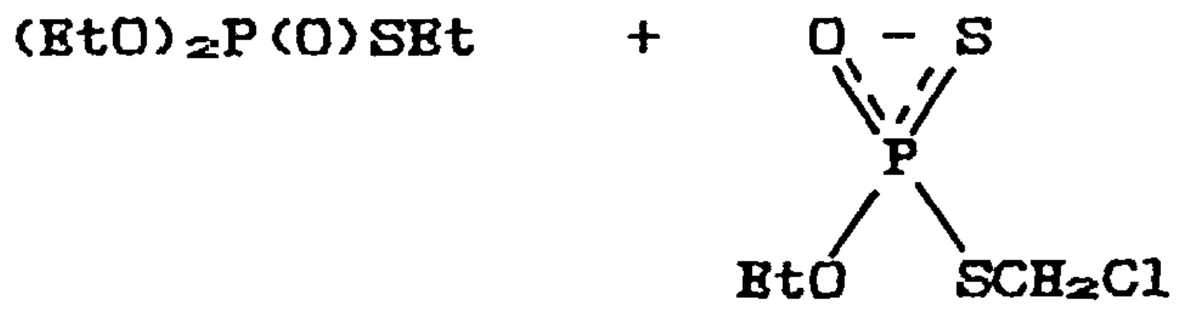
$$(EtO)_2P(S)SCH_3]^{+\cdot} \longrightarrow (EtO)(HO)PS_2CH_3]^{+\cdot} + C_2H_4$$

m/z 200 m/z 172

A very strong peak at m/z 198 also occurs in scan 91 and suggests that two compounds with same retention time are present. The only related structure with molecular weight of 198 is $(EtO)_2P(O)SEt$, which could result from initial hydrolysis by attack at P:-

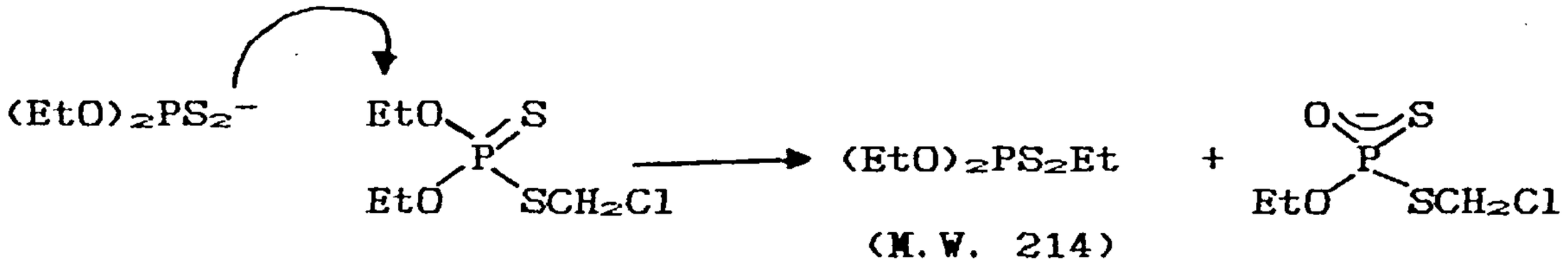


In alkaline solution the anion is formed which could then dealkylate another ester molecule, e.g.



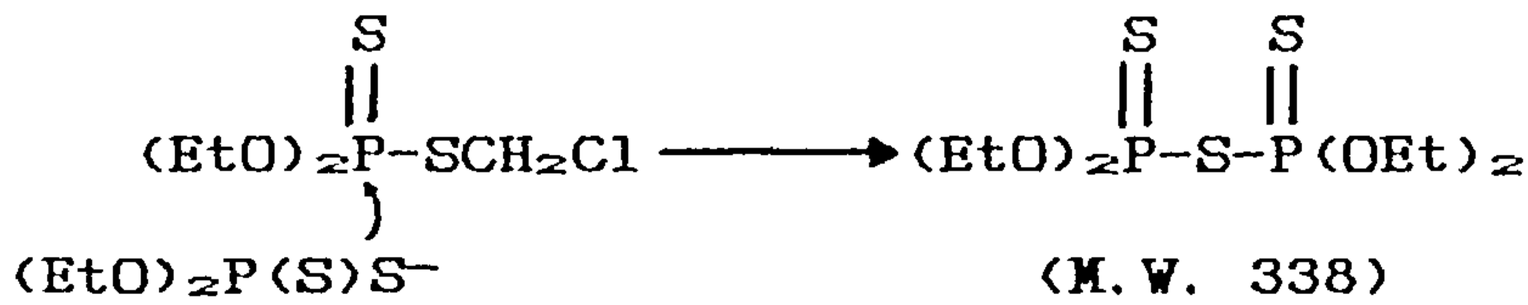
Similarly the formation of $(EtO)_2PS_2^-$ from DETA in the presence of alkali and then attack at ethyl, P, or CH_2Cl in chlormephos, could account for the compounds at scan numbers 219, 598, and 784.

Attack at the ethyl group could give the triethyl ester, scan number 219.

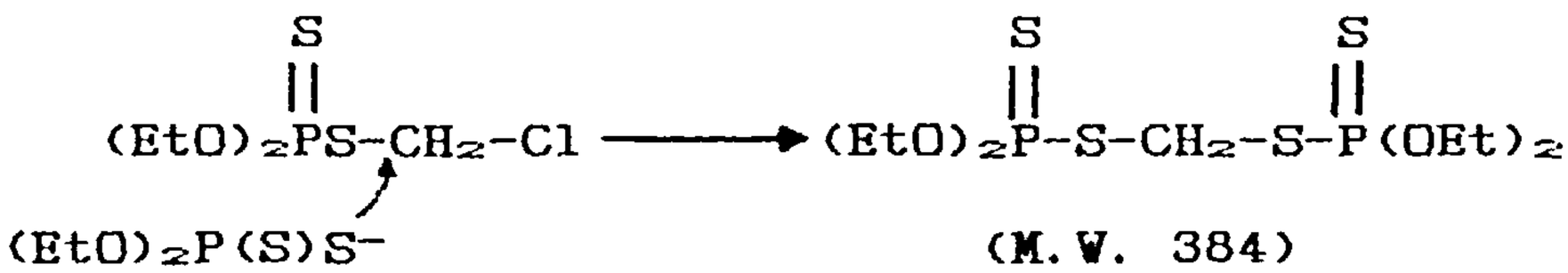


The triethyl ester fragments in mass spectrometry by loss of C_2H_4 to give [DETA] $^{+\cdot}$, m/z 186, and then further as expected.

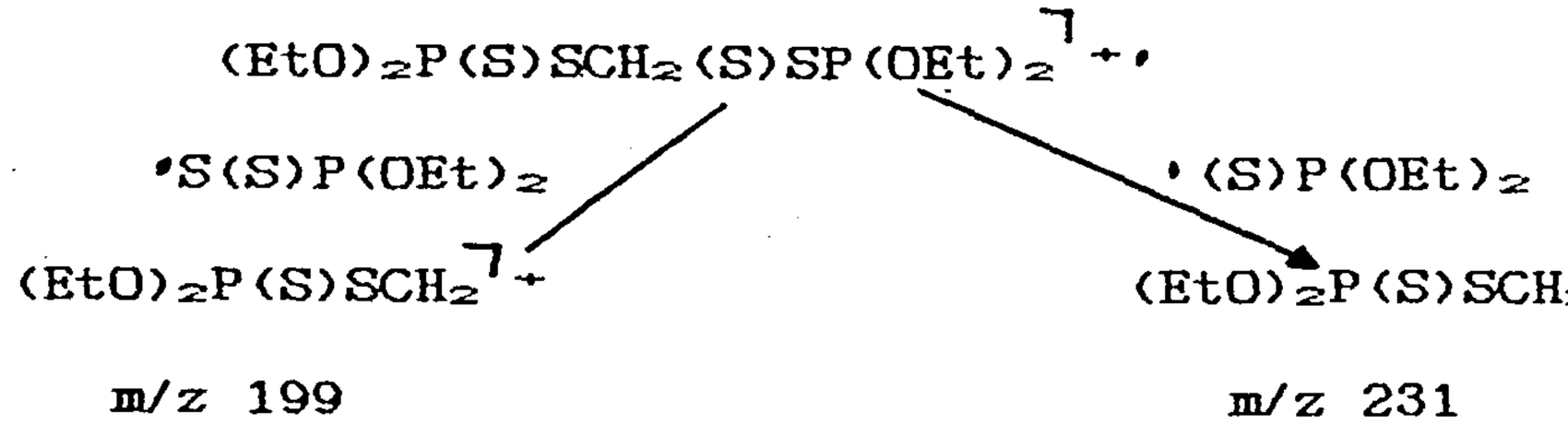
Attack at P would give rise to O,O',O'',O'''-tetraethyl trithiopyrophosphate, scan number 598,



whilst attack at CH_2Cl gives bis(diethoxyphosphinothioylthio)methane, scan number 784.



The parent ion, $(EtO)_2P(S)SCH_2S(S)P(OEt)_2^{+\bullet}$, undergoes fragmentation to give the fragment ions m/z 231 and m/z 199 as shown below.



Also detected are other intense ions at m/z 153, 125 and 97, characteristic of diethyl dithiophosphate derivatives.

DEGRADATION STUDIES OF MECARBAM AND CHLORMEPHOS IN LIVER

The organophosphorus insecticides may reach man's organism through oral, dermal, or respiratory channels and mucous membranes. The velocity of absorption at these points will depend on the physico-chemical properties of the compound, on the environmental conditions, and on the type of formulation.

The absorption by oral channel is of extraordinary importance because it can affect the consumers of fruits, vegetables and grains treated with insecticides of high toxicity. The absorption of insecticides by respiratory channel may occur with persons who work in the formulation industries and persons who work in the application of those insecticides in the form of powder or in domestic use in the form of spray. The absorption by mucous membranes and skin is the principal way of penetration of insecticides for those who apply the product in the form of powder or work in crops.

The majority of the organophosphorus insecticides are transformed in the body into metabolites, in general inactives, and the liver is the principal point of biotransformation, which takes place through the enzymes.

In some case, the metabolites are more toxic than the original products, for instance the oxo form of organophosphorus pesticides.

The biotransformation of insecticides may occur in other tissues of the animal organism. So the thiophosphates may be transformed into the corresponding phosphate triesters in brain, lungs and intestines [69,70,71].

In the biotransformation the liver presents two main functions. The first is transformation of liposoluble compounds into more polar derivatives, making easier their elimination through the kidneys. The formed compounds are more hydrosoluble than the original forms because they have more hydrophilic radicals or because they present themselves in the conjugate form with lipophobic agents. The second function of the liver is a consequence of the first i.e. detoxification. By being converted into a form that can be excreted, the insecticide loses its activity.

Most of the oxidative and reductive enzymes present in the liver that can metabolise drugs, require the reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen for their action.

Another enzyme in the biotransformation of the insecticides is the S-alkyl transferase by the reaction of dealkylation [72,73].

This enzyme is present in the soluble fraction of the homogenate of liver [73]. The reaction occurs mainly with the organophosphorus compounds, and those that show a methyl

group in the molecule are more easily dealkylated than those with an ethyl group.

The microsomal enzymes of the liver not only activate the thiophosphate compounds by transforming the bond P=S into P=O, but also degrade the substances by cleavage of the aryl-phosphate bond [74,75]. For instance, the diethyl ester, parathion is transformed into para-oxon, and then degraded to give diethylphosphoric acid and p-nitrophenol [75].

Degradation Studies on Mecarbam and Chlormephos in Liver

Results obtained for the degradation of mecarbam and of chlormephos with experiment A, as described in the experimental section (see page 55) are shown in Tables 1 and 2 respectively.

Table 1. Concentraton of mecarbam versus time of reaction

Concentration of mecarbam (µg/ml)	Time (minutes)	Peak height (cm)
640.0	0	8
216.0	10	2.7
104.0	20	1.3
16.0	30	0.2

1 µl of solution of mecarbam (680 ppm) corresponds to a peak height of 8.5 cm.

Table 2. Concetration of chlormephos with time of reaction

Concentration of chlormephos (µg/ml)	Time (hour)	Peak height (cm)
13.2	0	9.8
8.5	1	6.3
7.9	2	5.9
8.5	3	6.3
4.5	4	3.3
3.1	5	2.3
2.3	6	1.7

1 µl of solution of chlormephos (8.5 µg/ml) corresponds to a peak height of 6.3 cm

For mecarbam the study was carried out at -10°C because of the high rate of degradation. Under these conditions, the concentration fell from 640 µg/ml to 16.0 µg/ml (97.5% degradation) in 30 minutes. At room temperature the rate was too high for measurement.

Chlormephos was more resistant and the experiment was carried out at 41°C. Under these conditions the concetration fell only from 13.2 µg/ml to 2.3 µg/ml (83.0% degradation) in 6 hours.

Products from similar experiments after short periods of degradation were methylated using diazomethane and examined by g.c.-m.s.

G.C-M.S. analysis of mecarbam in lamb liver homogenate

The parameters used for analysis of experiment A were the same as those used for hydrolysis of chlormephos (see page 150).

Experiment A

It was carried out using 5 g of lamb liver homogenate in 125 ml of water containing 4011.2 ppm of mecarbam. For extraction of the pesticide and its metabolites were used hexane, dichloromethane, anf diethyl ether (see page 55 for details of procedure).

Each of these layers were analysed by gas chromatography (g.c. conditions are described in page 58). The g.c. analysis of the hexane layer showed unreacted mecarbam and other peaks which were further identified by g.c.-m.s. as methyl ester of fatty acids. The dichloromethane and diethyl ester layers did not show the presence of mecarbam or any organophosphorus compound.

The hexane extract layer corresponding to the reaction time of 10 minutes was analysed by g.c.-m.s. and the results in Table 3.

Table 3

Retention Time (min:sec)	Scan number	Ion current intensity %
12:39	582	100
14:23	677	45
14:26	680	100
14:41	693	25
15:41	748	20
16:35	797	25
17:26	844	28
18:16	889	30

The compounds scan number 582, 677, 680, 693, 748, 797, 844, 889 gave the fragment ions present in Tables 4, 5, 6, 7, 8, 9, 10, and 11 respectively.

Table 4. Mass spectral data for compound scan number 582.

m/z	270	227	199	185	171	143	129	97	87	74	55	43
%	1	2	1	1	1	10	2	5	60	100	40	60

Table 5. Mass spectral data for compound scan number 677.

m/z	329	296	263	226	206	160	131	112	97	82	67	55
%	1	1	1	1	1	20	40	20	58	40	50	80
m/z	43											
%	100											

Table 6. Mass spectral data for compound scan number 680.

m/z	265	222	180	166	152	138	113	83	69	55	41
%	10	10	10	5	5	5	20	50	60	100	90

Table 7. Mass spectral data for compound scan number 698.

m/z	298	255	199	185	143	111	87	74	57	43
%	1	5	5	5	10	5	70	100	18	35

Table 8. Mass spectral date for compound scan number 748.

m/z	197	155	126	111	84	71	57	43
%	5	5	5	18	60	90	100	60

Table 9. Mass spectral date for compound scan number 797.

m/z	207	155	133	111	85	71	57	43
%	5	5	5	20	60	80	100	70

Table 10. Mass spectral date for compound scan number 844.

m/z	281	207	191	177	155	126	105	85	71	57	43
%	1	5	5	5	5	10	5	55	80	100	80

Table 11. Mass spectral date for compound scan number 889.

m/z	281	207	191	161	133	119	105	85	71	57	43
%	5	10	5	5	10	10	5	50	80	100	78

The hexane extract of mecarbam in lamb liver showed to contain methyl ester of fatty acids (identified by m.s. library search) and trace of mecarbam were detected with the compound scan number 677 which showed the molecular ion M^+ 329 and characterisctic patterns of the fragmentation of mecarbam. None of the compounds was identified as an organophosphorus compound.

With the aim to reduce extent of degradation and to increase opportunity for detection of its metabolites, a second attempt (experiment B) was made with relatively smaller amount of liver.

The g.c.-m.s. analysis for experiment B was performed with temperature program from 50°C for 2 minutes to 220°C for 10 minutes and a ramp rate of 30°C *per* minute, injector temperature 220°C.

Experiment B

It was carried out using 1.5 g of lamb liver homogenate in 25 ml of water containing 13370.6 ppm. Aliquot of 10 ml was taken after 2 minutes reaction at room temperature. For the extraction of the pesticide and its metabolites was used 10 ml of hexane. The rest of the procedure was carried out as for experiment A.

The g.c. analysis showed unreacted mecarbam and other peaks which were further identified by g.c.-m.s. as methyl esters of fatty acids.

The results of g.c.-m.s. analysis are shown in the Table 12.

Table 12

Retention time (min:sec)	Scan number	Ion current intensity. %
5:06	153-155	25
12:00	537-539	20
14:10	642-644	10
14:20	647-649	12
14:40	664-666	55
16:54	800-802	55
17:20	837-839	15
17:40	854-856	45
18:00	862-864	22
18:34	888-890	25
18:34	899-901	10

The compounds scan numbers 153-154, 537-539, 642-644, 647-649, 664-666, 800-802, 837-839, 854-856, 862-864, 888-890, 899-901 gave the fragment ions presented in Tables 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 23 respectively.

Table 13. Mass spectral data for scan numbers 153-155.

m/z	101	100	85	78	71	70	69	57	56	55	53	51	43
%	2	20	5	5	65	30	3	55	40	15	3	2	100
m/z	42	41	39	32	29	28	27						
%	28	65	22	3	48	21	34						

Table 14. Mass spectral data for scan numbers 537-539.

m/z	242	213	212	211	200	199	185	171	157	144	143
%	4	2	1	5	1	9	2	1	2	1	15
m/z	129	111	101	97	87	83	74	71	70	69	59
%	5	2	5	5	62	8	100	6	2	12	8
m/z	57	56	55	43	41	39	29				
%	12	5	22	28	22	4	13				

Table 15. Mass spectral data for scan numbers 642-644.

m/z	268	237	236	207	195	194	165	152	137	123	110
%	2	7	9	2	2	12	3	12	9	17	20
m/z	97	96	95	91	86	84	83	82	81	79	74
%	40	43	38	6	40	45	48	28	45	18	72
m/z	71	69	68	67	65	55	41	28			
%	25	65	30	55	5	100	87	44			

Table 16. Mass spectral data for scan numbers 647-649.

m/z	268	237	236	207	195	194	179	165	153	152	123
%	2	6	8	1	2	10	1	3	2	10	18
m/z	110	97	87	83	74	69	55	41	29		
%	18	35	40	50	65	77	100	85	33		

Table 17. Mass spectral data for scan numbers 664-666.

m/z	271	270	239	228	213	199	185	171	157	143	129
%	2	8	6	2	2	4	5	5	3	20	8
m/z	115	101	97	87	74	69	55	43	39	29	
%	3	8	10	80	100	18	30	50	5	15	

Table 18. Mass spectral data for scan numbers 800-802.

m/z	329	296	284	252	226	206	198	185	171	159	144
%	20	10	2	3	10	10	10	4	12	51	15
m/z	131	116	97	86	76	71	65	58	42	29	
%	88	36	100	38	15	4	43	35	35	90	

Table 19. Mass spectral data for scan numbers 837-839.

m/z	294	263	220	178	164	150	135	124	109	95	81
%	5	5	2	2	4	7	8	8	24	56	84
m/z	74	67	55	41	29						
%	16	100	77	66	26						

Table 20. Mass spectral data for scan numbers 854-856.

m/z	296	266	235	222	207	194	180	166	137	123	110
%	3	2	2	10	2	2	10	5	10	15	21
m/z	97	83	55	41	29						
%	48	58	100	97	30						

Table 21. Mass spectral data for scan numbers 862-864.

m/z	296	265	264	235	222	193	180	166	151	137	124
%	2	7	10	1	7	2	65	3	5	7	8
m/z	109	83	74	69	55	41	29				
%	40	50	53	81	100	78	25				

Table 22. Mass spectral data for scan numbers 888-890.

m/z	298	267	255	241	227	213	199	185	171	157	144
%	8	4	8	2	1	2	5	2	1	2	2
m/z	143	87	74	69	55	43	29				
%	17	70	100	18	28	40	13				

Table 23. Mass spectral data for scan numbers 899-901.

m/z	295	294	263	220	178	164	150	135	123	109	95
%	2	13	3	3	4	5	8	8	10	24	50
m/z	81	67	55	41	28						
%	70	100	53	51	25						

With the experiment B again many fatty acids (as methyl esters) are present, giving typical fragmentation patterns [76]. For instance:

m/z 298 corresponds to $C_{17}H_{35}CO_2Me$,

m/z 296 " " $C_{17}H_{33}CO_2Me$,

m/z 294 " " $C_{17}H_{29}CO_2Me$,

m/z 268 " " $C_{15}H_{29}CO_2Me$,

m/z 242 " " $C_{13}H_{27}CO_2Me$.

The compound scan numbers 800-802 corresponds to unreacted mecarbam as observed by the presence of the characteristic fragment ions of this pesticide.

With both experiments A and B, no metabolites were deteacted although residual mecarbam was still present. This suggests that the metabolites were themselves degraded as fast as the original pesticides or that they may be combined as conjugates that were not extracted.

G.C.-M.S. analysis of chlormephos in lamb liver homogenate

The parameters of analysis for experiment A and B are described in page 150 and 164 respectively.

Experiment A

This experiment was carried out using 50 g of lamb liver homogenate in 250 ml of water containing 161.3 ppm. Aliquot of 25 ml was taken after diferrent intervals of time. The sample taken after 1 hour was analysed by g.c.-m.s. and the results did not show any compound that could be identified as a product of degradation of chlormephos. Only methyl esters of fatty acids were present.

Experiment B

This experiment was also an attempt to reduce extent of degradation of the pesticide and to increase chance for detection of metabolites.

It was carried out using 1.5 g of lamb liver homogenate in 25 ml of water containing 161.3 ppm. Aliquot of 10 ml was taken after 5 minutes. See page 57 for details

of the procedure. The compounds revealed in this analysis are shown in Table 24.

Table 24

Retention time (min:sec)	Scan number	Ion current intensity %
3:30	162-164	100
6:10	256-258	8
9:20	435-437	12
9:40	461-463	8
9:45	467-463	5
11:20	526-528	7
11:40	537-539	12
13:10	646-648	5
13:15	653-655	5
13:30	661-663	25
17:00	833-835	8
17:20	846-848	20
17:30	856-858	8
17:50	882-884	10

The fragment ions from the compounds present in the liver extracts of chlormephos in hexane are shown in the folowing Tables.

Table 25. Mass spectral data for scan numbers 162-164.

m/z	132	130	103	99	98	83	81	79	77	73	70	68	65
%	1	1	3	4	50	100	2	2	2	10	25	10	2
m/z	59	55	53	51	47	45	41	39	31	29	27		
%	5	65	5	5	2	12	50	28	2	18	17		

Table 26. Mass spectral data for scan numbers 256-258.

m/z	140	130	110	109	97	95	93	80	79	77	65	57	55
%	12	2	100	32	2	30	4	25	35	2	5	3	2
m/z	47	45	43	41	31	28							
%	5	3	5	2	5	13							

Table 27. Mass spectral data for scan nunbers 435-437

m/z	238	237	236	235	234	199	188	161	154	144	125
%	1	1	15	2	37	2	5	10	50	7	25
m/z	121	109	97	93	89	81	77	69	65	57	47
%	95	12	100	35	2	15	2	10	40	12	25
m/z	43	39	29								
%	20	2	38								

Table 28. Mass spectral data for scan numbers 461-463.

m/z	280	214	183	171	161	147	129	119	111	97	87	74
%	1	2	6	8	3	3	7	7	7	18	60	100
m/z	69	55	43	28								
%	25	40	50	25								

Table 29. Mass spectral data for scan numbers 467-469.

m/z	330	213	199	189	173	159	147	133	119	105	91	79
%	1	2	2	2	5	9	10	20	38	42	84	100
m/z	67	55	41	28								
%	73	79	98	68								

Table 30. Mass spectral data for scan numbers 526-528.

m/z	356	355	281	221	208	189	175	166	148	133	119
%	1	4	15	2	6	2	4	10	1	15	30
m/z	105	91	79	67	55	41	28				
%	30	55	90	80	84	100	54				

Table 31. Mass spectral data for scan numbers 537-539.

m/z	242	211	199	185	171	157	145	144	143	129	119
%	3	5	9	3	1	3	2	2	2	5	5
m/z	111	101	97	91	87	83	79	74	69	65	55
%	5	5	10	8	65	12	14	100	20	2	32
m/z	43	39	29								
%	40	8	15								

Table 32. Mass spectral data for scan numbers 646-648.

m/z	386	330	275	237	236	207	194	175	165	152	137
%	1	1	1	1	1	2	7	2	4	8	8
m/z	125	124	111	97	83	81	69	55	41	28	
%	9	8	20	44	52	48	80	100	99	48	

Table 33. Mass spectral data for scan numbers 653-655

m/z	386	275	239	227	213	199	185	159	143	119	108
%	1	1	2	3	2	2	17	3	7	94	9
m/z	97	85	71	57	43	28					
%	29	44	75	94	100	38					

Table 34. Mass spectral data for scan numbers 661-663

m/z	270	239	227	213	199	185	171	157	144	143	129
%	6	5	9	1	3	4	4	2	1	15	8
m/z	111	97	87	74	69	55	43	39	29		
%	4	8	72	100	18	25	42	4	12		

Table 35. Mass spectral data for scan numbers 833-835.

m/z	386	294	279	263	213	191	173	168	163	149	135
%	2	4	2	38	15	1	3	1	5	4	10
m/z	123	109	95	81	67	55	41	28			
%	15	28	67	88	99	96	100	38			

Table 36. Mass spectral data for scan numbers 846-848

m/z	296	280	279	265	235	222	207	193	180	167	149
%	2	1	6	9	2	10	2	2	10	25	77
m/z	137	123	97	83	69	55	41	29			
%	10	15	45	58	74	98	100	30			

Table 37. Mass spectral data for scan numbers 856-858

m/z	386	280	279	264	222	207	193	180	168	167	149
%	1	1	6	6	4	2	2	4	3	30	96
m/z	137	123	109	97	83	69	55	41	28		
%	7	13	20	43	56	78	100	98	37		

Table 38. Mass spectral data for scan numbers 882-884

m/z	299	298	267	255	241	227	213	199	185	171	157
%	1	7	30	76	1	1	2	5	2	1	2
m/z	144	143	129	119	111	97	87	74	69	55	43
%	2	16	7	15	7	16	70	100	25	36	55
m/z	29										
%	14										

The compound scan numbers 435-437 corresponds to unreacted chlormephos, this spectra showed the isotopic ions m/z 236 and 234 and the charactecristic patterns of chlormephos fragmentation.

Again many fatty acids (as methyl esters) are present, showing typical fragmentation patterns [76]. For example:

m/z 386 was identified as $C_{23}H_{51}CO_2Me$,

330 " " " $C_{19}H_{43}CO_2Me$,

298 " " " $C_{17}H_{35}CO_2Me$,

296 " " " $C_{17}H_{33}CO_2Me$,

294 " " " $C_{17}H_{31}CO_2Me$,

270 " " " $C_{15}H_{21}CO_2Me$,

242 " " " $C_{13}H_{27}CO_2Me$.

The scan numbers 435-437 gave a compound with molecular ion, M^+ 140, which appears to be $(MeO)_3P=O$. The fragmentations agree with the literature for the mass spectra of tri-alkyl phosphates [77,78]. The origin of this compound is however not conclusive since it could result from methylation of H_3PO_4, the ultimate degradation product, or may have been derived from the K_2HPO_4 used for breaking down the emulsion.

No metabolite was detectable although residual chlormephos was still present. This indicates that the metabolites themselves were degraded as fast as the original pesticide or that they may be combined as conjugates that were not extracted.

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