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THE SYNTHESIS AND FUNGICIDAL ACTIVITY OF SOME NOVEL PHOSPHORAMIDATES

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy of The Council for National Academic Awards

by

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School of Chemistry

May 1984

ACKNOWLEDGEMENTS

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DECLARATION

While registered as a candidate for the degree of Ph.D for which this submission is made, I have not been registered as a candidate for any other award.

In partial fulfilment of the requirements for the degree, postgraduate lecture courses in mass spectrometry and in nuclear magnetic resonance spectroscopy have been followed at The Polytechnic of North London.

P.J. Eccles

CONTENTS

ABSTRACT

The synthesis and fungicidal activity of some novel phosphoramidates

P.J. ECCLES

The synthesis of novel fungicidal phosphoramidates was the main objective of the present work. Various fungitoxic substituents were included in their structure together with a trichloromethyl group which has been shown to enhance fungicidal activity in other compounds.

Fourteen compounds containing the 1,1,1-trichloro-2-(diethoxyphosphinylamino)ethyl group were synthesised from the condensation product of chloral and diethyl phosphoramidate. These included one derivative which also contained the 1,1,1-trichloro-2-(bisdimethylaminophosphinylamino)ethyl group. Other fungitoxic substituents introduced into the molecular structure of the compounds include imidazole, 1,2,4-triazole, morpholine, piperazine, dimethyldithiocarbamate, diethyldithiocarbamate, xanthate and 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylate.

Preliminary investigations were carried out into the synthesis of compounds containing the 1,1,1-trichloro-2-(bisdimethylaminophosphinylamino)ethyl group via the condensation of chloral with N,N,N',N'-tetramethylphosphoric triamide but these proved to be unfruitful due to side reactions.

Similar side reactions occurred, but to a lesser extent, when chloral was condensed with O-ethyl N,N-dimethylphosphorodiamidate, and one derivative was prepared containing the 1,1,1-trichloro-2-(ethoxydimethylaminophosphinylamino)ethyl group.

The fifteen potential fungicides were characterised by elemental analysis, nuclear magnetic resonance spectrometry, mass spectrometry, and infra-red spectroscopy.

Their fungicidal activity was assessed *in vitro* against spores of *Fusarium culmorum*, *Fusarium oxysporum*, *Helminthosporium sativum*, *Helminthosporium avenae*, *Septoria nodorum*, *Ophiobolus graminis*, *Piricularia oryzae* and *Rhizoctania solani* and *in vivo* against *Dreschlera teres* and *Septoria nodorum*. These pathogens were chosen because they affect economically important crops, mainly wheat and barley or, in the case of *P. oryzae*, rice. All the compounds showed some fungicidal activity, the best results being shown by O,O-diethyl-N-2,2,2-trichloro-1-(dimethylthiocarbamoylthio)ethyl phosphoramidate against *Fusarium culmorum*.

CHAPTER 1, INTRODUCTION

1. INTRODUCTION

The aim of the work in this thesis is to develop new types of organophosphorus compound for possible use as fungicides in crop protection. A brief survey of some aspects of the development of agricultural fungicides in general and also of organophosphorus pesticides is therefore given first.

1.1 Early Fungicides

Although fungi were not clearly established as causative agents of plant diseases until a study was conducted by Prevost in 1807,[1] their effects have been noticed since time immemorial. Even the Old Testament contains many references to what must have been blights, blasts, rusts, smuts and mildews.[2]

Throughout the ages many substances have been used for the treatment of diseased plants. The first fungicide discovered was probably elemental sulphur, which was very widely used in many preparations, so much so that the period up until 1882 has become known as the 'sulphur era'.[2] Other compounds used successfully as fungicides during this period included ferrous sulphate, mercuric chloride, copper acetate and arsenic compounds.[3]

1.2 Copper-based Fungicides

A major breakthrough in the area of fungicides occurred in 1882, and was triggered by the observations of Alexis Millardet. To discourage petty thieving, the vines along the roadside in the Gironde had for a long time been daubed with a noxious looking mixture of lime and copper sulphate. In 1882 many vineyards in France were affected by downy mildew, which spread with alarming rapidity and against which sulphur preparations were ineffective. Millardet noticed that the vines which had been treated with the lime/copper sulphate mixture remained green and healthy whilst the rest of the crop was affected by downy mildew. Millardet began to experiment with mixtures of lime and copper sulphate. Three years later a satisfactory mixture was announced.[4] This mixture became known as Bouille Bordelaise or Bordeaux Mixture, and found very wide application as a fungicide. Many rival preparations soon appeared on the market, most of them using other alkaline substances in place of lime, such as ammonia (Eau Celeste) or sodium carbonate (Burgundy Mixture).[5] Due to the importance of the discovery by Millardet the period from 1882 to 1934 has become known as the 'copper era' of fungicides.

1.3 Organic Fungicides

Many other substances were introduced as fungicides during the 'copper era' including formaldehyde,[6] lime sulphur,[7] and organomercury compounds.[8] Organomercurials such as phenylmercury acetate are of great

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1.3 Organic Fungicides

Many other substances were introduced as fungicides during the 'copper era' including formaldehyde,[6] lime sulphur,[7] and organomercury compounds.[8] Organomercurials such as phenylmercury acetate are of great

significance since they were the first to find application as seed disinfectants, though mercuric chloride was known as a fungicidal wood preservative as early as 1705.[9] Treatment with organomercurials became standard practice for most kinds of seeds. However, organomercurials are undesirable as fungicides because of their high toxicity to man and wildlife and their cumulative effect. Because of their danger to the environment replacements are required and research is in progress in industrial and academic institutions into organic fungicides that could be used as seed dressings and that would undergo degradation to innocuous compounds.

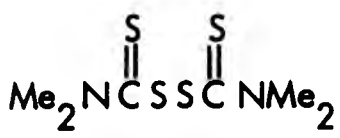
The third phase, which can be termed the 'Organic era' of fungicides began in 1934 when Tisdale and Williams were granted a patent for the use of derivatives of dithiocarbamic acid as seed disinfectants.[10] Their discovery was not followed up until seven years later, however, the delay having been due mainly to the economic depression in America.[2]

Following the discovery of Tisdale and Williams many organic fungicides were released on to the market. Many were highly specific, a characteristic that set them apart from previous fungicides. Only a few of the more notable compounds will be described here as examples.

1.3.1 Dithiocarbamates

The first successful dithiocarbamate derivative to be used as a seed dressing was tetramethylthiuram disulphide, thiram (1). Thiram was

first used as a seed dressing to combat seedling blight in flax. Other early dithiocarbamate fungicides included the iron[11,12] and zinc[13,14] salts of dimethyldithiocarbamic acid, ferbam (2) and ziram (3) respectively, useful for foliage portection of fruit and vegetables.



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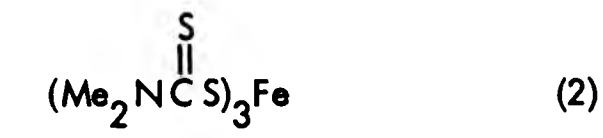

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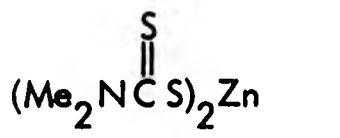

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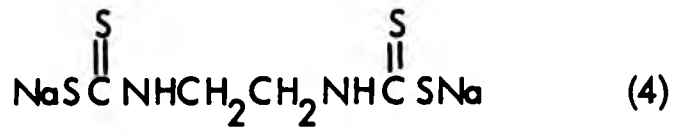
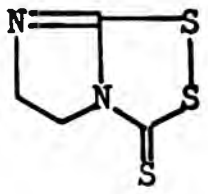


(4)

In 1940 a new type of dithiocarbamate derivative, the ethylenebisdithiocarbamate was discovered. The first example of this type of compound to be used as a fungicide was the disodium salt, nabam (4). Although nabam proved to be somewhat phytotoxic in the field it found wide application as a soil sterilant. It was later shown that the fungitoxic effect was due not to the compound itself but to its decomposition products, mainly sulphur and 5,6-dihydroimidazo [2,1-c]-1,2,4-dithiazole-3-thione (5) which was subsequently patented as the fungicide etem.



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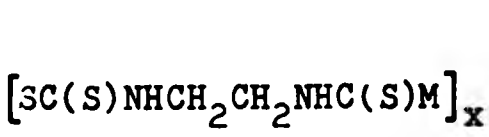
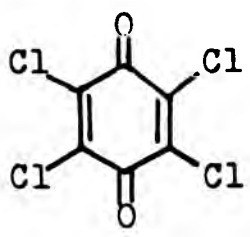


(6)

Other ethylenebisdithiocarbamates used as fungicides include the zinc and manganese salts, zineb (6, M=Zn) and Maneb (6, M=Mn). These are both similar to nabam (4) but are far less phytotoxic. They find wide application for the foliage diseases of many plants and are particularly effective against the blights of potato and tomato.

1.3.2 Quinone derivatives

The first organic fungicide to be produced on a tonnage scale, and which came on to the market just before the first dithiocarbamate was tetrachloro-1,4-benzoquinone, chloranil (7). The compound has a long history as a rubber accelerator but its fungicidal action was discovered in 1940,[15] and it then found application as a treatment for pea seeds,[16] lima bean seeds,[15] and many others.[17,18,19]



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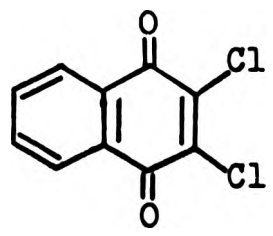
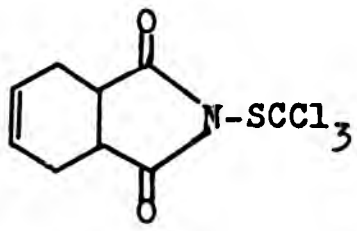


(8)

In 1946 the related 2,3-dichloro-1,4-naphthoquinone, dichlone (8) was introduced and was found to be up to eight times as effective as chloranil.

1.3.3 Chloroalkylthio compounds

3a,4,7,7a-Tetrahydro-N-(trichloromethanesulphenyl)phthalimide (9) was introduced as a fungicide in 1949 and was later given the name captan. The related compound 3a,4,7,7a-tetrahydro-N-(1,1,2,2-tetrachloroethanesulphenyl)phthalimide, captafol (10) was introduced later in 1961. The group of compounds containing the chloroalkylthio chain and represented by captan (9) and captafol (10) is considered to be one of the most important groups of fungicides discovered since the advent of the dithiocarbamates.[2] They are persistent in the field and are virtually non-phytotoxic. They also have a broad spectrum of activity and uses range from the prevention of fruit and foliage diseases to timber preservation.



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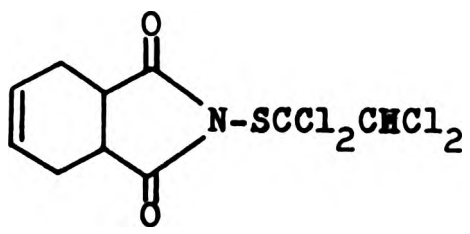

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1.3.4 Guanidines

Dodecylguanidine, dodine (11), previously described as a bactericide, was introduced as a fungicide in 1956,[20] and is usually marketed as the acetate for the control of apple scab and other orchard diseases.

$C_{12}H_{25}NHC(:NH)NH_2$ (11) $[H_2NC(:NH)NH(CH_2)_8]_2NH$ (12)

In 1968 guazatine (12) a compound related to dodine was introduced. Guazatine, 9-aza-1,17-diguanidinoheptadecane, is the most effective of the range of guanidines derived from di-, tri-, and poly-amines.[21] It is marketed as the triacetate and is especially useful as a seed dressing for use in the place of organomercury fungicides. Some of these guanidines also appear to have potential as wood preservatives, and for combatting bacteria and fungi which cause destruction and decomposition of paper, leather and textiles.[22]

1.3.5 Systemic fungicides

The fungicidal compounds described so far have protectant or eradicant properties or both. The use of a protectant fungicide is intended to prevent or protect against infection by a fungal organism. Protectant fungicides which may be applied to seeds, soil or the plant surface cannot penetrate into the plant tissue in effective amounts. They act outside the plant tissue prior to infection of the host cells. Eradicant fungicides are typically fungitoxic compounds which when applied to the site of infection are capable of limited penetration into the plant tissue leading to the elimination of an established infection.

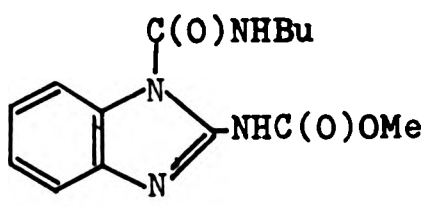
A systemic fungicide is a compound which is absorbed into the plant and is capable of being freely translocated within its vascular system

offering protection to parts of the plant that are distant from the locus of application. Although applications of systemic fungicides are often said to 'protect' new growth from infection, their action is usually therapeutic in that it occurs after penetration of the host even if no visible symptoms of the disease arise.[23] Frequently the fungitoxic action is due to metabolites of the fungicide rather than the parent compound.

The first organic compound to be introduced as a systemic fungicide was "Wepsyn" now known as triamiphos (35) and this will be dealt with in more detail in the section on organophosphorus fungicides.

1.3.5.1 Benzimidazoles and related compounds

Of particular importance as systemic fungicides are derivatives of benzimidazole carbamates and their precursors. Methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate, benomyl (13), was introduced in 1967 and is effective against a wide range of pathogens.[24] Thiophanate



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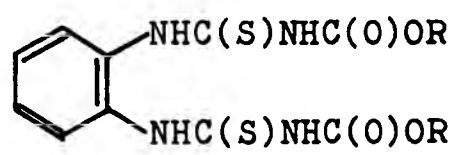
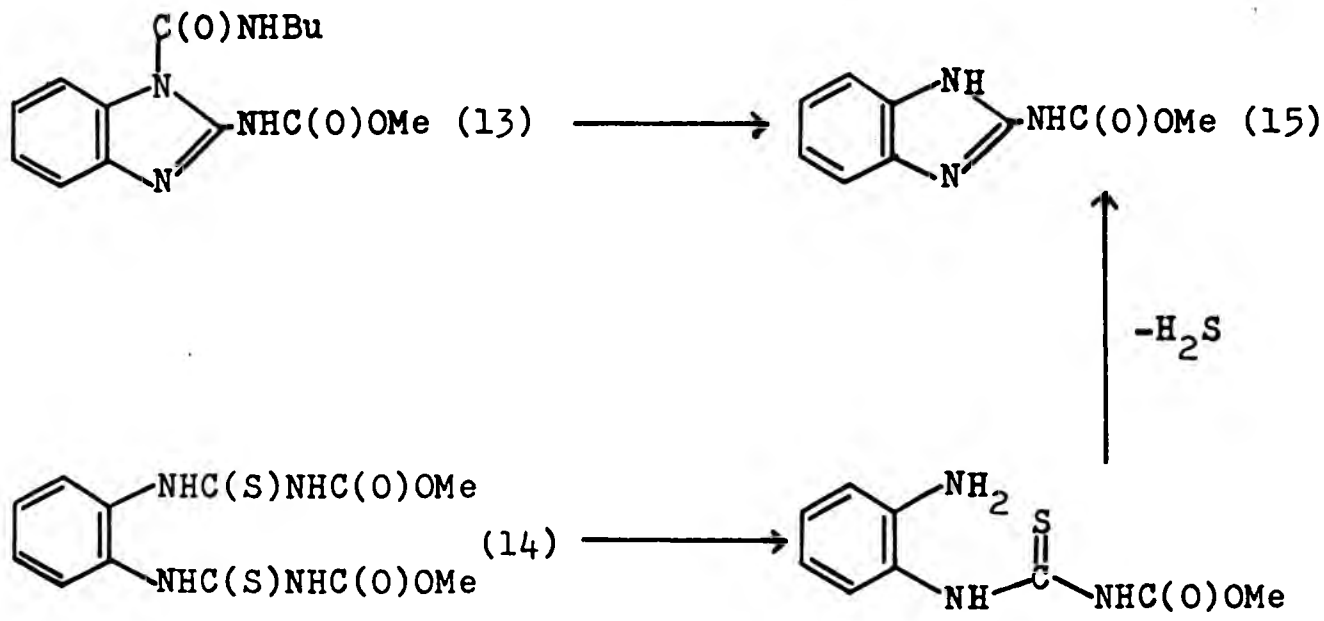


(14)

(14, R=Et) and thiophanate methyl (14, R=Me) introduced in 1970-71 also have good systemic properties.[25]

It was shown later that benomyl is converted in water to methyl benzimidazol-2-ylcarbamate, carbendazim (15),[26] that thiophanate methyl cyclises to carbendazim[27] as in reaction scheme I and that the antifungal spectra of these three compounds are almost identical.[28,29,30] It has been established therefore that benomyl and thiophanate methyl act as 'pro-fungicides' of carbendazim, a compound which has been introduced as a fungicide in its own right.[31]

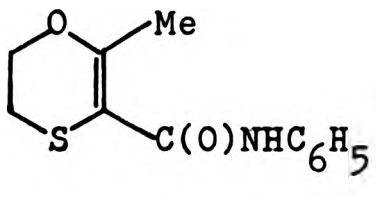
Reaction Scheme I



1.3.5.2 Oxathiins

Certain substituted carboxyanilides also show good systemic fungicidal properties, e.g. 2,3-dihydro-6-methyl-5-phenylcarbamoyl-1,4-oxathiin, carboxin (16), and the corresponding 4,4-dioxide, oxycarboxin were described[32] and introduced in 1966 and are used for the control of

cereal diseases such as rusts, smuts and bunts.



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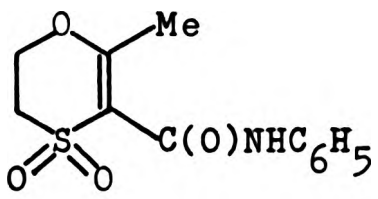

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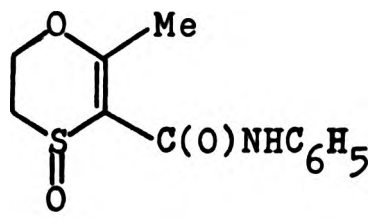
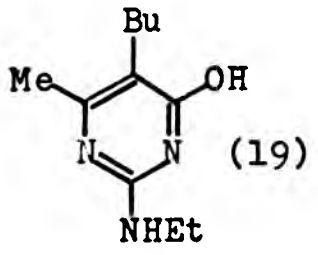


(18)

Certain anomalies which appear in the fungicidal spectrum of carboxin may be due to its oxidation in vivo to the less active sulphoxide (18) but there is no evidence for further oxidation in vivo to the active sulphone, oxycarboxin.[33]

1.3.5.3 Other miscellaneous compounds

Good systemic activity against powdery mildews is exhibited by the substituted pyrimidines 5-butyl-2-ethylamino-6-methylpyrimidin-4-ol, ethirimol (19),[34] and 5-butyl-2-(dimethylamino)-6-methylpyrimidin-4-ol, dimethirimol (20),[35] introduced in 1968. Certain derivatives of 2,2,2-trichloroethylformamide are also effective, viz. piperazine-1,4-diyl-bis 1-(2,2,2-trichloroethyl)formamide, triforine (21), N-2,2,2-trichloro-1-(3,4-dichloroanilino)ethyl formamide, chloraniformethan (22), and N-(1-formamido-2,2,2-trichloroethyl)morpholine (23).



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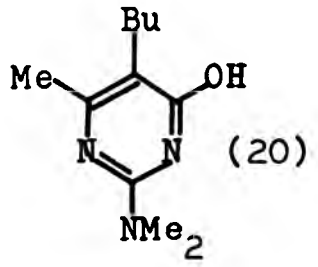

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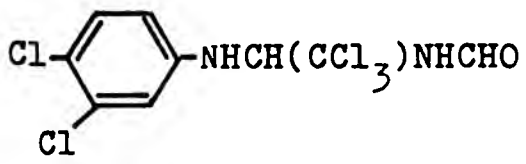
(21)



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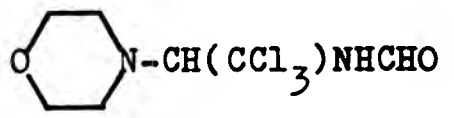
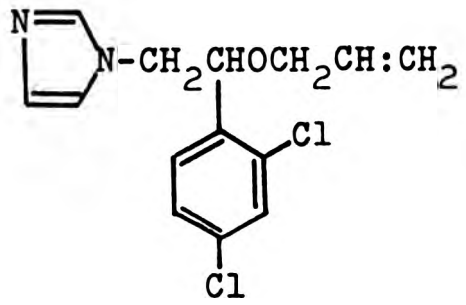


(23)

Many other compounds containing the 2,2,2-trichloroethylformamido group have also been prepared and shown to possess fungicidal activity.[36]

Several imidazole derivatives have been shown to possess systemic fungicidal activity, a particularly noteworthy example being 1-(β allyloxy-2,4-dichlorophenylethyl)imidazole, imazalil (24),[37] which is active against a wide range of fungal pathogens and is recommended for use as a seed disinfectant against Helminthosporium, Fusarium and Septoria diseases of cereals.



(24)

1.4 Organophosphorus Pesticides

Organophosphorus compounds have found important applications in agriculture as insecticides and acaricides, and more recently as fungicides and herbicides. Some of the more important aspects are outlined below.

1.4.1 Organophosphorus insecticides and acaricides

1.4.1.1 Early examples

During the Second World War several biologically active organophosphorus compounds were synthesised as potential nerve gases. The systematic development of these compounds as insecticides and acaricides arose from this research.[38] Much of the early work was carried out by Gerhard Schrader, who discovered in 1938 that tetraethyl pyrophosphate, TEPP (25) possessed aphicidal properties.[39] In 1941 Schrader prepared a

$(EtO)_2P(O)OP(O)(OEt)_2$ (25)

$(Me_2N)_2P(O)OP(O)(NMe_2)_2$ (26)

related compound, octamethyl pyrophosphoramide (26), which later became known as schradan. This was the first organophosphorus compound to be recognised as a systemic insecticide but was later largely replaced by the demeton series of compounds such as demeton-S (27) which was introduced in the early 1950's. Development in this field was quite rapid and by

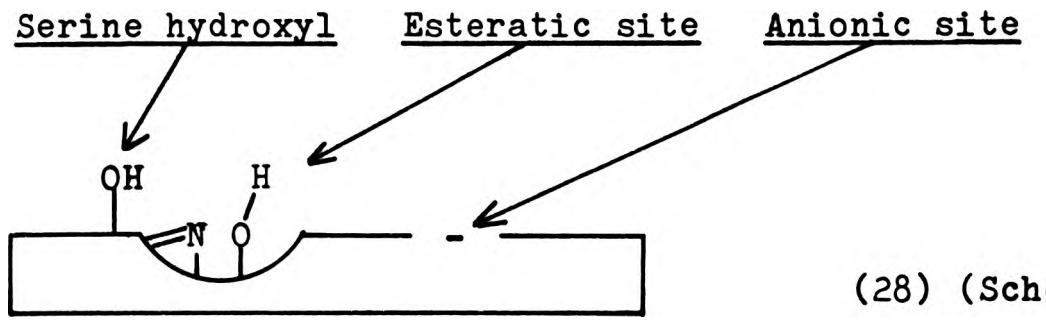
$(EtO)_2P(O)SCH_2CH_2SEt$ (27)

1960 there were some forty or so organophosphorus compounds available as insecticides and acaricides.

1.4.1.2 Anticholinesterase activity

The early organophosphorus insecticides had a very high mammalian toxicity. The insecticidal activity and the mammalian toxicity of organophosphorus compounds are generally considered as being due to their ability to phosphorylate the enzyme acetylcholinesterase (28) which is an enzyme that effects the hydrolysis of acetylcholine (29) to choline (30) and acetic acid (Reaction scheme II). Acetylcholine is released at neuromuscular junctions causing stimulation of the nerve and is then removed by the acetylcholinesterase. The acetylated enzyme is readily hydrolysed back to the free enzyme and acetic acid as in reaction scheme II. Many organophosphorus compounds can react with the enzyme to give a phosphorylated form (32) and thus inhibit the addition of acetylcholine to the enzyme and its hydrolysis, (e.g. demeton-S, reaction scheme III).

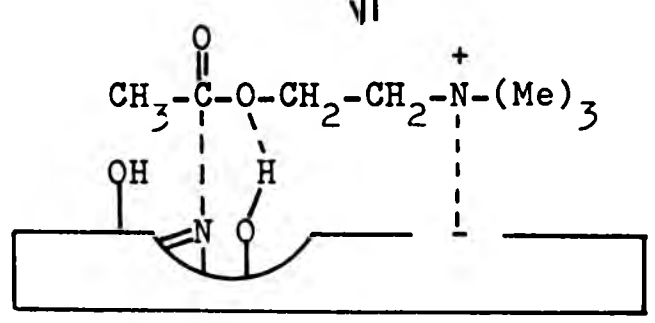
Reaction scheme II

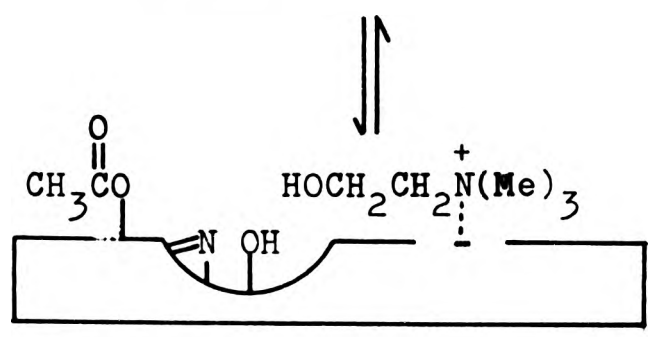


(28) (Schematic)

+

$CH_3C(O)O-CH_2-CH_2-\overset{+}{N}(Me)_3$ (29)



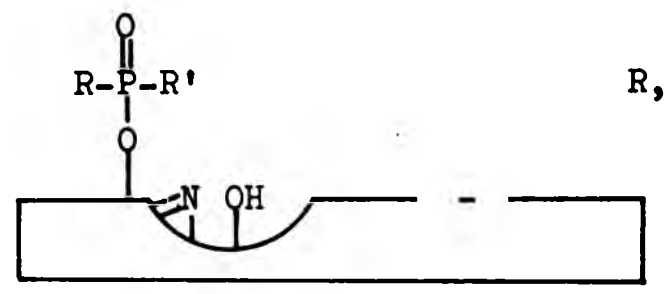


(30)

(31)

H_2O

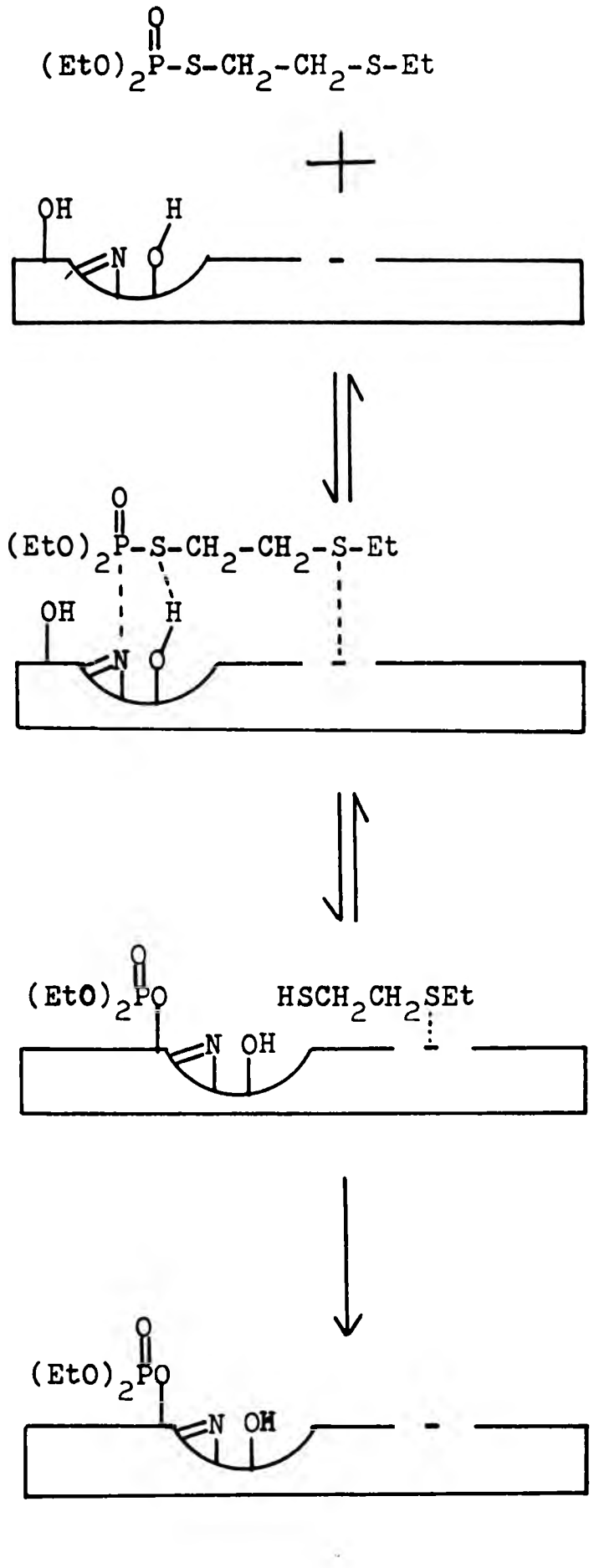
CH_3COOH + (28) + (30)



R,R'= alkyl, alkoxy, alkylamino, etc.

(32)

Reaction Scheme III



+ $EtSCH_2CH_2SH$

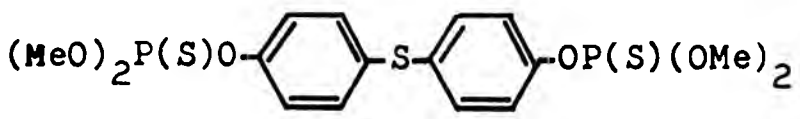
The consequent accumulation of acetylcholine in mammals, due to organophosphorus poisoning, results in paralysis and eventual death.

1.4.1.3 Further developments

The acute oral LD_{50} for rats, which is the dose required to kill 50% of the animals when the substance is administered orally, for schradan is 9.1 mg Kg^{-1}, for TEPP 1.12 mg Kg^{-1} and for demeton-S 1.5 mg Kg^{-1}. The high mammalian toxicity of these compounds led to the search for less toxic organophosphorus derivatives and in 1950 malathion (33) was introduced.

$(MeO)_2P(S)SCH(CH_2COOEt)COOEt$ (33)

This is a good general insecticide with low mammalian toxicity, the LD_{50} for rats being 2800 mg Kg^{-1}. Other organophosphorus insecticides and acaricides with low mammalian toxicity have since been developed, e.g. temephos (34), which is a broad spectrum insecticide introduced in 1965 and which has an acute oval LD_{50} for male rats of 8600 mg Kg^{-1}



(34)

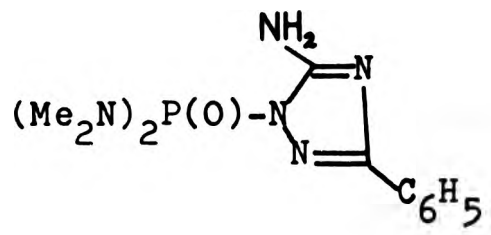
The low mammalian toxicity of some of the more recently developed organophosphorus insecticides opens up new areas of use which could not have been contemplated with the older, more toxic compounds. Organophosphorus compounds can now be used in sheep dips, and against dog and cat fleas. In fact temephos (34) is recommended for the control of human body lice.[40]

1.4.2 Organophosphorus fungicides

The development of organophosphorus compounds as fungicides has progressed at a slower rate than their development as insecticides and by 1979 there were only six such compounds listed in the Pesticide Manual (6th edition).[37]

At present there is no general model for the mode of action of organophosphorus fungicides although progress is being made towards an understanding in the case of Kitazin and its analogues (see below). As noted earlier the insecticidal activity of organophosphorus compounds is related to their anticholinesterase activity, but this cannot be the case for fungicides since fungi possess no nervous system. It has been proposed however that certain organophosphorus fungicides interfere with the synthesis of chitin in the fungal cell wall.[41] Much biochemical research is in progress on the mode of action of fungicides[42] and this should facilitate the future design of fungicidal compounds.

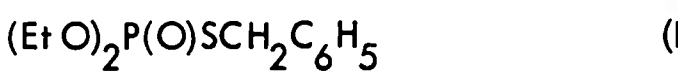
The first organophosphorus fungicide to appear on the market was triamiphos (35), first described as a fungicide in 1960,[43] and found



(35)

to show limited systemic activity. This compound contains the bis-(dimethylamino)phosphoryl group which may be significant in imparting or enhancing fungitoxicity. Unfortunately it is highly toxic to mammals, the acute oral LD_{50} for rats being 20 mg Kg^{-1}.

In 1965 a phosphorothiolate ester (36) was introduced as a fungicide in Japan under the trade name 'Kitazin'. This showed good



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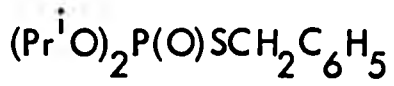
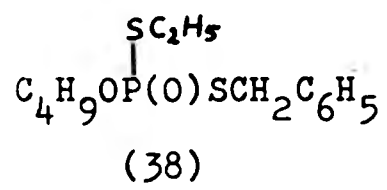


(37)

systemic activity against Piricularia oryzae, the causative agent of rice blast, but was replaced in 1968 by the di-isopropyl homologue, 'Kitazin P' (37). The fungicidal activity of diisopropyl benzyl phosphorothiolate is about the same as that of the diethyl ester but the compound is more stable and less toxic to mammals, the acute oral LD_{50} for rats for Kitazin and Kitazin P being 320 mg Kg^{-1} and 640 mg Kg^{-1} respectively. Increase in

the size of the alkyl groups to butyl reduced the mammalian toxicity still further.[44] Several S-benzyl derivatives related to Kitazin such as 'Conen' (38), 'Cerezin' (39), 'Inezin' (40) and 'Inezin T' (41) have been shown to possess similar fungicidal activity.



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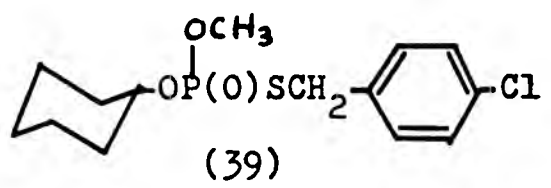

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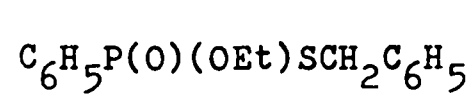

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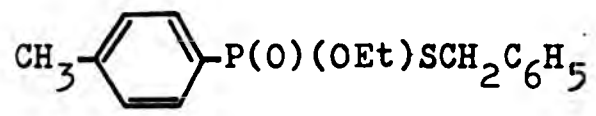

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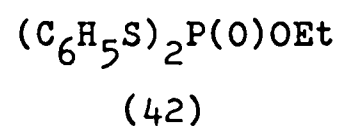


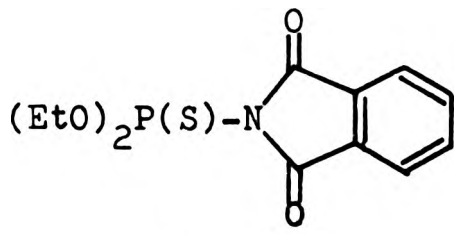
(42)

Edifenphos (42), introduced in 1968 for the control of rice blast is also effective against sheath blight and ear blight of rice, and has an acute oral LD_{50} for rats of about 350 mg Kg^{-1}.

Mode of action studies have shown that Kitazin and similar compounds inhibit the formation of cell wall chitin.[41] This effect appears to result in the cases of Kitazin P and edifenphos from specific inhibition of the conversion of phosphatidylethanolamine to phosphatidylcholine,[45,46] an

important membrane constituent, and to a consequent modification of cell wall permeability.

In 1966 ditalimfos (43) was introduced as a protectant fungicide. The compound gives good control of apple scab and powdery mildew and has



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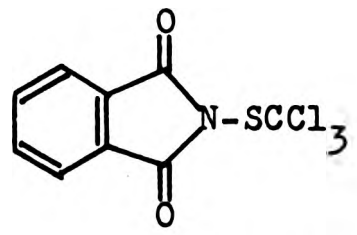
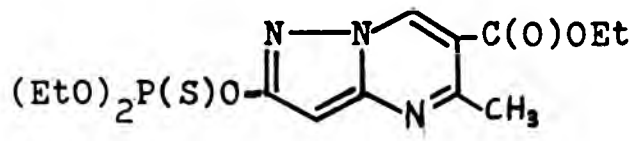


(44)

a very low mammalian toxicity, the acute oral LD_{50} for rats being about 5,000 mg Kg^{-1}. Other phthalimide derivatives such as folpet (44) which is the aromatic analogue of captan (9), also show good fungicidal activity, and it has been postulated that the activity of ditalimfos is due more to the phthalimide part of the molecule than to the phosphorus group.[47]

A further example of a commercially available organophosphorus fungicide is pyrazophos (45),[48] which was introduced in 1971 and gives good control of powdery mildews. Its acute oral LD_{50} for rats is about 500 mg Kg^{-1}.



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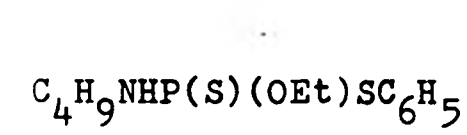

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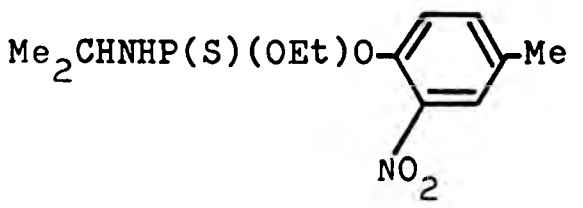

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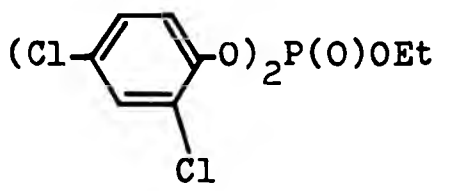
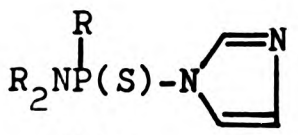


(48)

Other organophosphorus compounds which have been considered as fungicides include 'Phosbutyl' (46), 'Amiprophos' (47) and 'Phosdiphen' (48).

Not many organophosphorus fungicides have reached the marketing stage as yet. This is, however, no reflection on the considerable amount of research that has been carried out in this area of chemistry, since even as long ago as 1972 there were some 450 publications on this subject as enumerated by Grapov and Melnikov.[49] Compounds showing promising fungicidal activity include derivatives of phosphorous and phosphinous acids, phosphines, phosphonium salts, phosphate esters and a considerable number of phosphoramidates. Several phosphoramidates have progressed beyond the experimental stage, e.g. triamiphos (35), ditalimfos (43), 'Phosbutyl' (46) and 'Amiprophos' (47) which have already been mentioned. Many other phosphoramidates (49-56) have been reported as showing high fungicidal activity[49,50,51,52] and it is with novel compounds of this class that the

investigations reported in this thesis are concerned.



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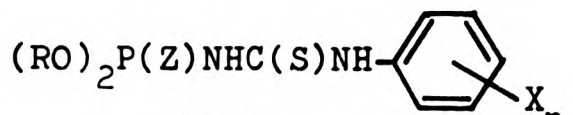

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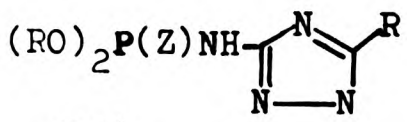

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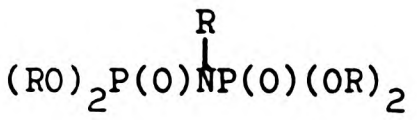

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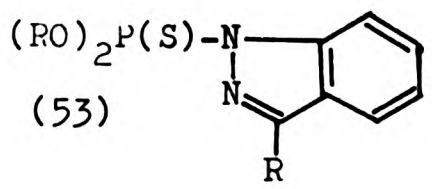

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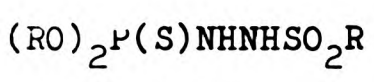

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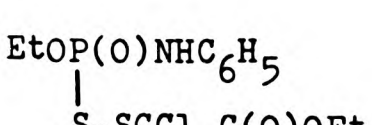

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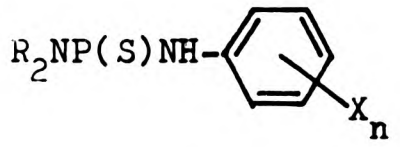


(56)

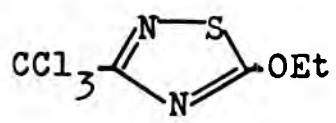
Z = O or S , R = alkyl , X = halogen

CHAPTER 2, DISCUSSION

2. DISCUSSION

2.1 Objectives

The synthesis and evaluation of the fungicidal activity of novel compounds containing the phosphoramidate group, in combination with other fungitoxic groups, has been a principal aim of the present work. The incorporation of a trichloromethyl group into such compounds was another objective since this group is present in a number of successful fungicides such as triforine (21) and trimorfamid (23), which were mentioned earlier, and also in others such as 5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, 'Terrazole' (57) which is a soil fungicide[53] and 2-chloro-6-methoxy-4-trichloromethylpyridine (58).[54]



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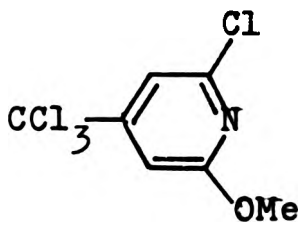
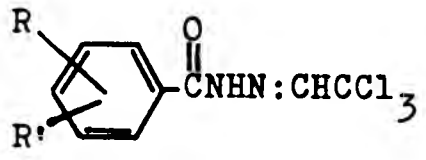
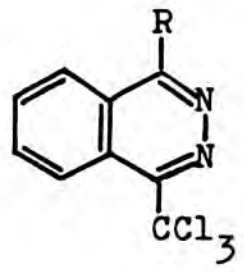


(58)

A number of fungicidal compounds of the general formulae (59)[55] and (60)[56] have also been described in the patent literature.



(59) R, R' = H, halogen, Me, MeO, OH, NO_2



(59) R = halogen or alkoxy

$Me_2NC(S)SCH(R)NHC(O)R'$ (61) R = H or CCl_3

R' = CH_2Cl, C_6H_5,

$CH_2C(S)NMe_2$

$CH_2NHC(S)SCH(R)NHC(O)R"_2$ (62) R = H or CCl_3

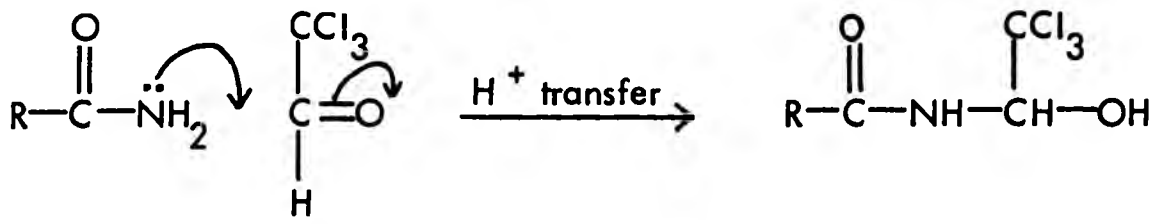
R" = CH_2F or CH_2Cl

In a series of compounds (61 and 62) synthesised by Pianka et al.[57] the compounds containing a trichloromethyl group showed consistently higher fungicidal activity than the corresponding compounds without this group. In many cases the activity was greater by a factor of 10 and in some cases it was greater by a factor of 100. The trichloromethyl group may enhance fungicidal activity, by increasing lipid solubility and penetration through the cuticle of fungal spores.[57]

2.2 Chemical Outlines

Carboxylic acid amides and carbamates are known to react with chloral to give N-(1-hydroxy-2,2,2-trichloroethyl) derivatives (63). The hydroxyl group in these compounds is easily replaced by a chlorine

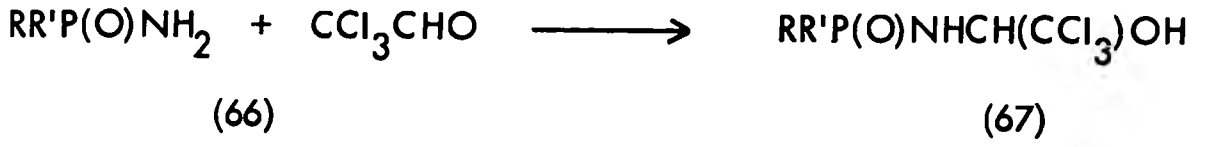
Reaction scheme IV



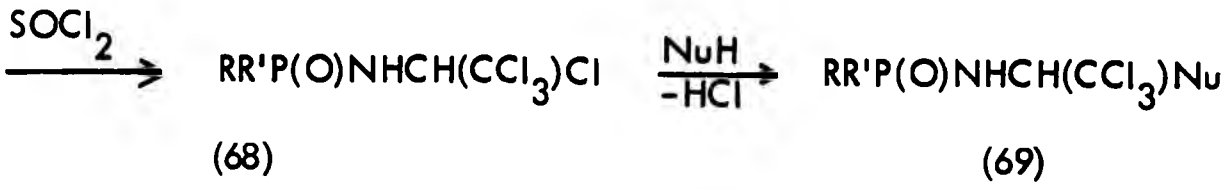
atom to give N-(1,2,2,2-tetrachloroethyl) derivatives (64) and the chlorine in the 1- position is readily displaced by nucleophiles to give derivatives (65), as in reaction scheme IV. Many compounds of this type have been prepared previously and some of these show definite biological activity as insecticides[58] and fungicides.[57] It was therefore considered likely that the organophosphorus analogues would have interesting biological activity.

By analogy with the carboxamides it should be possible to prepare such compounds by a sequence involving the reaction of a phosphoramidate or phosphoramide (66) with chloral, chlorination of the resulting N-(1-hydroxy-2,2,2-trichloroethyl) derivative (67) and reaction of the N-(1,2,2,2-tetrachloroethyl) derivative (68) as in reaction scheme V with various nucleophiles (NuH), preferably those which would introduce a fungitoxophore into the product (69).

Reaction scheme V



(66) (67)



(68) (69)

R,R' = ethoxy or dimethylamino

2.3 Preparations of Diethoxyphosphinyl Derivatives

The condensation of phosphoramides with chloral was first reported by Maeder[59] who tentatively assigned the structure 70 to the products obtained from the di(2-chloroethyl), diethyl and di(p-chlorophenyl) esters and claimed a wide range of applications as pesticides, flame-retardants, textile assistants, lubricant additives, plasticisers and chemical intermediates. A range of adducts (70, R=Me, Et, Pr, Pr^i, Bu) was also reported by Alimov

(70)

R=$ClCH_2CH_2$, C_2H_5 or p-ClC_6H_4O

who stated that the dimethyl and diethyl derivatives were active as insecticides against flies.[60]

In the present work diethyl phosphoramidate was prepared by the reaction of anhydrous ammonia with diethyl phosphite and carbon tetrachloride according to the method of Atherton et al.[61] who proposed two possible routes for the reaction [Scheme VI, (a) and (b)]. It is now

Reaction scheme VI

a) $(EtO)_2P(O)H + CCl_4 + NH_3 \longrightarrow [(EtO)_2P(O)CCl_3] + NH_4Cl$

$[(EtO)_2P(O)CCl_3] + NH_3 \longrightarrow (EtO)_2P(O)NH_2 + CHCl_3$

b) $(EtO)_2P(O)H + CCl_4 \xrightarrow{NH_3} (EtO)_2P(O)Cl + CHCl_3$

$(EtO)_2P(O)Cl + 2NH_3 \longrightarrow (EtO)_2P(O)NH_2 + NH_4Cl$

generally accepted that the reaction proceeds by route (b) since the reaction of the phosphite in bromotrichloromethane proceeds more readily than with

$^{\delta-}CCl_3 - {}^{\delta+}Cl$ (71)

$^{\delta+}CCl_3 - {}^{\delta-}Cl$ (72)

carbon tetrachloride indicating that the carbon tetrachloride acts as a

positive halogen source (71) rather than generating a halide anion (72).[62]

Condensation of diethyl phosphoramidate with anhydrous chloral proceeded readily, as reported,[59,60] to give N-(1-hydroxy-2,2,2-trichloroethyl)phosphoramidate which was readily converted to N-(1,2,2,2-tetrachloroethyl)phosphoramidate (73) on heating with thionyl chloride under reflux (scheme VII).

Reaction scheme VII

$$(EtO)_2P(O)NH_2 + CCl_3CHO \longrightarrow (EtO)_2P(O)NHCH(CCl_3)OH$$

(70)

$$\xrightarrow{SOCl_2} (EtO)_2P(O)NHCH(CCl_3)Cl$$

(73)

The chloride (73) was expected to react with nucleophiles as shown in reaction scheme VIII. Thus with water one would expect the hydroxy compound (70) to be formed. To investigate the ease with which this conversion would occur the reaction was performed in an nmr tube by

Reaction scheme VIII

$$(EtO)_2P(O)NHCH(CCl_3)\text{–}Cl \longrightarrow (EtO)_2P(O)NHCH(CCl_3)Nu$$

(Nu–H)

shaking a deuterochloroform solution of the chloride (73) with water and monitoring the disappearance of the C<u>H</u>CCl$_3$ signal which appears as a doublet of doublets at δ = 5.95 ppm (J_{PNCH} = 9.5 Hz, J_{HNCH} = 12.7 Hz). The results were rather unexpected. Instead of the hydroxy compound (70) being formed directly diethyl <u>N</u>-(2,2,2-trichloroethylidene)phosphoramidate (74) was first obtained as shown by the characteristic methine doublet at δ = 8.45 ppm (J_{PNCH} = 27.1 Hz). This intermediate was then slowly converted to the hydroxy compound (70) as shown by the appearance of the C<u>H</u>CCl$_3$ signal at δ = 5.2 ppm (dd, J_{PNCH} = 8.0 Hz, J_{NHCH} = 11.3 Hz). ^{1}H nmr spectra for the three authentic compounds are shown in figs. 1, 2 and 3. The water had obviously dehydrochlorinated the chloride (73) and then added across the double bond thus formed as shown in reaction scheme IX. The ease with which a relatively weak base such as water can bring about this elimination has not been reported before and

<u>Reaction scheme IX</u>

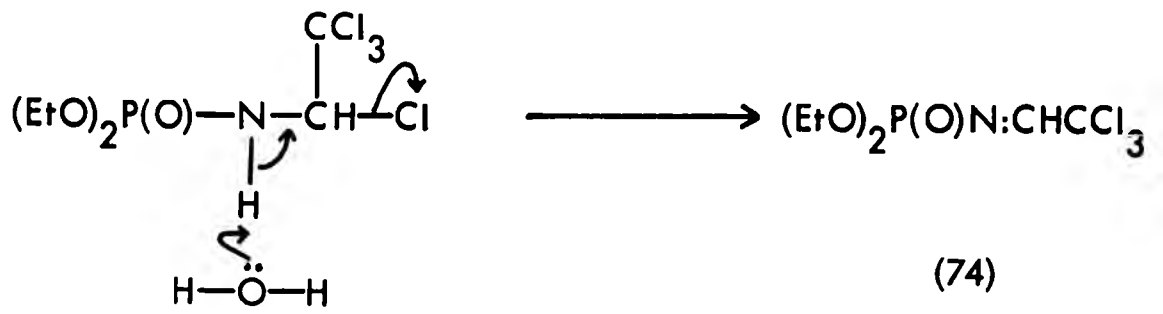


Fig. 1. Nmr spectrum of $(EtO)_2P(O)NHCH(CCl_3)Cl$ (73)

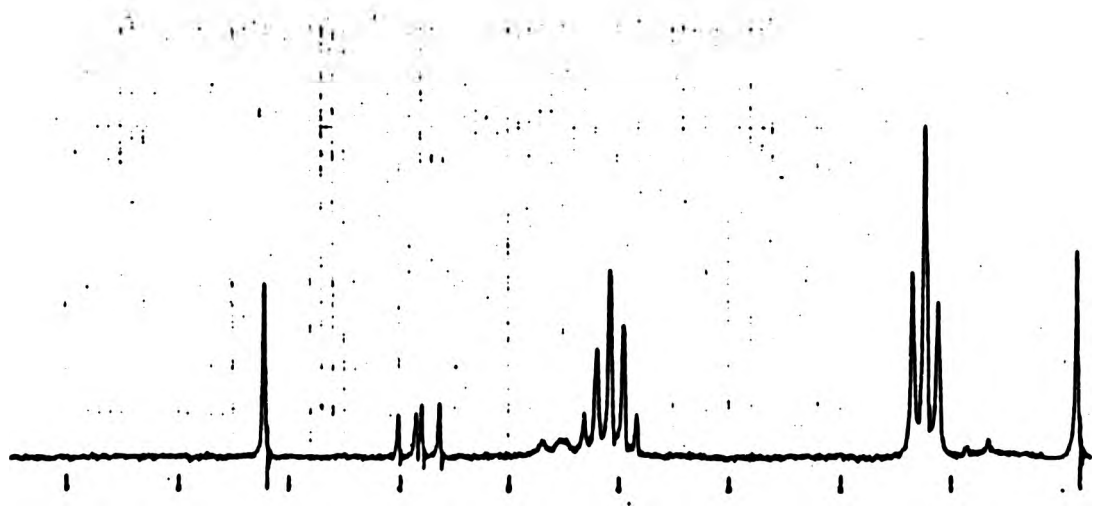


Fig. 2. Nmr spectrum of $(EtO)_2P(O)N{:}CHCCl_3$ (74)

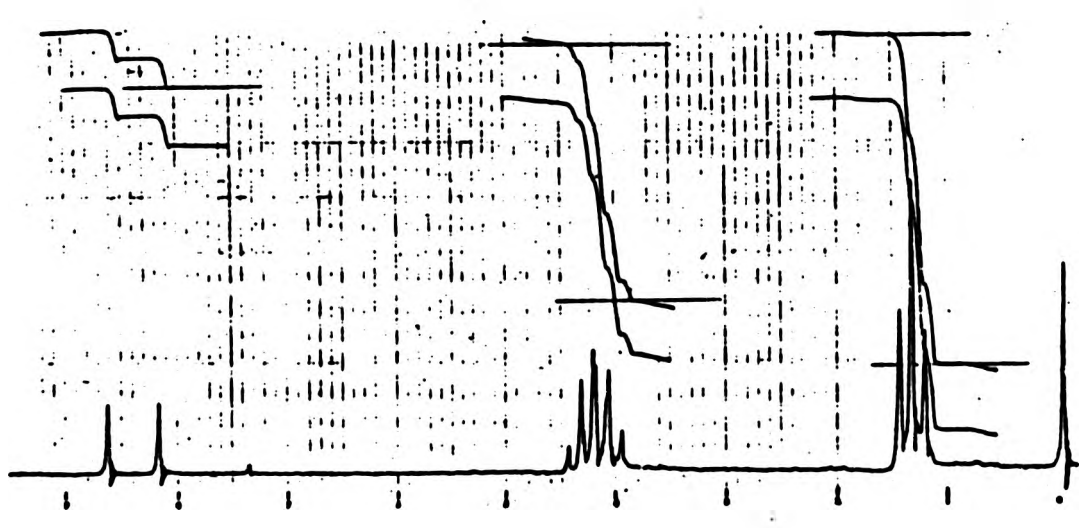
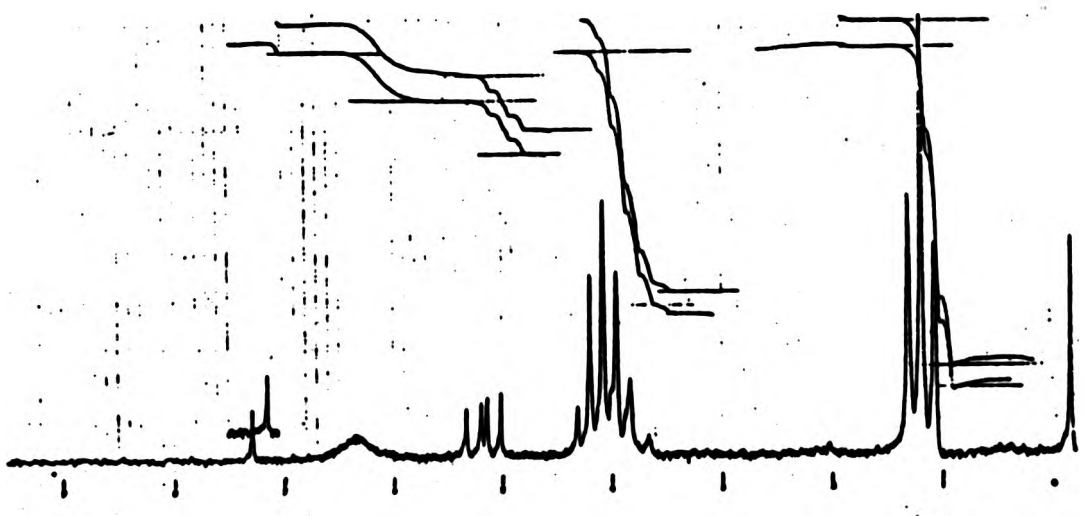


Fig. 3. Nmr spectrum of $(EtO)_2P(O)NHCH(CCl_3)OH$ (70)



can probably be attributed to the inductive effect of the trichloromethyl group. No reports can be found on the dehydrochlorination of similar compounds in which the trichloromethyl group is absent even by stronger bases, whereas a similar reaction of the chloride (73) with tertiary bases has been reported by Drach et al. in 1968.[63] These authors isolated the unsaturated compound (74), characterised it, and found that it undergoes addition reactions with many compounds containing an active hydrogen such water, hydrogen chloride, alcohols, phenols and thiols.

Similar methods have also been reported for the prepration of carboxy-analogues using the elimination-addition reaction shown in Scheme X.[64]

Reaction scheme X

$$CH_3C(O)NHCH(CCl_3)Cl \xrightarrow{Et_3N} CH_3C(O)N{:}CHCCl_3$$

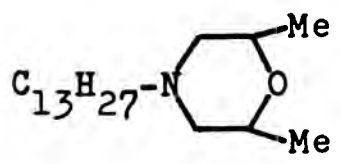
$$\xrightarrow{NuH} CH_3C(O)NHCH(CCl_3)Nu$$

This type of elimination-addition sequence proved very convenient in the present work for the prepration of the diethyl phosphoramidate derivatives since the unsaturated compound (74) could be prepared in solution and subsequent reaction with nucleophiles could be carried out without isolation of this intermediate.

2.3.1 Derivatives of heterocycles and thiols

One molecular equivalent of triethylamine was thus added to a solution of the chloride (73) in benzene. The reaction mixture was agitated gently to mix the reagents and was left to stand for about ten minutes. The precipitated triethylammonium chloride was then filtered off and one molecular equivalent of a substance containing an active hydrogen atom was added, either as a solution in benzene or neat. After leaving the reagents to react for about ten minutes the products were isolated by removal of the solvent and were recrystallised from appropriate solvents. The new compounds prepared in this manner are shown in Table 1.

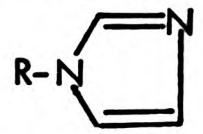
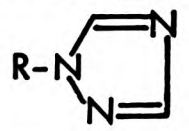
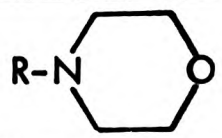
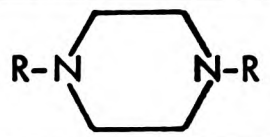
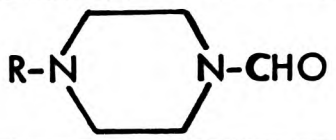
Most of the substituents were chosen because they show activity in other fungicidal compounds, and some are indeed substituents in proprietary fungicides. Thus the morpholino group for example appears in the fungicide tridemorph (75) in which the morpholine is substituted with tridecyl and



(75)

methyl groups in the 4-, 2- and 6- positions respectively. This is an eradicant fungicide with systemic action. The piperazine derivative, triforine (21), the imidazole derivative, imazalil (24), and the triazole

Table 1 $R = (EtO)_2P(O)NHCH(CCl_3)-$

Compound		Derived from	M.P. (°C)	Yield (%)
	(76)	Imidazole	118 - 120	93
	(77)	1,2,4-Triazole	123 - 126	48
	(78)	Morpholine	127 - 129	78
	(79)	Piperazine	162 - 165 (dec.)	78
	(80)	1-Formylpiperazine	102 - 107	60
$R\text{-}S\text{-}CH_2\text{-}CH_2\text{-}OR$	(81)	2-Mercaptoethanol	178 - 180	57
$R\text{-}S\text{-}CH_2\text{-}CH_2OH$	(82)	2-Mercaptoethanol	91 - 100	81

derivative, triamiphos (35), were mentioned in chapter 1.

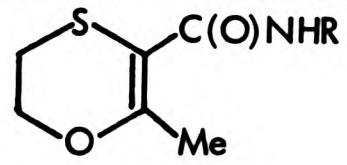
2.3.2 Derivatives of amides

The reactivity of the trichloroethylidene derivative (74) was found to be such that not only amines, but also amides add across the nitrogen-carbon double bond, though at a slower rate. This reaction has been reported previously in the case of acetamide and diethyl phosphoramidate[65] but has not been exploited further.

The compounds prepared in the present study are listed in Table 2 and were obtained using conditions similar to those described above, allowing a longer reaction time or heating under reflux. By this method it was possible to obtain a phosphorus containing analogue (86) of carboxin, and also a derivative (87) in which the bisdimethylaminophosphinyl group is attached to the trichloroethyl moiety. This type of structure was unobtainable *via* the interaction of chloral with *N,N,N',N'*-tetramethylphosphoric triamide, $(Me_2N)_2P(O)NH_2$ (see later).

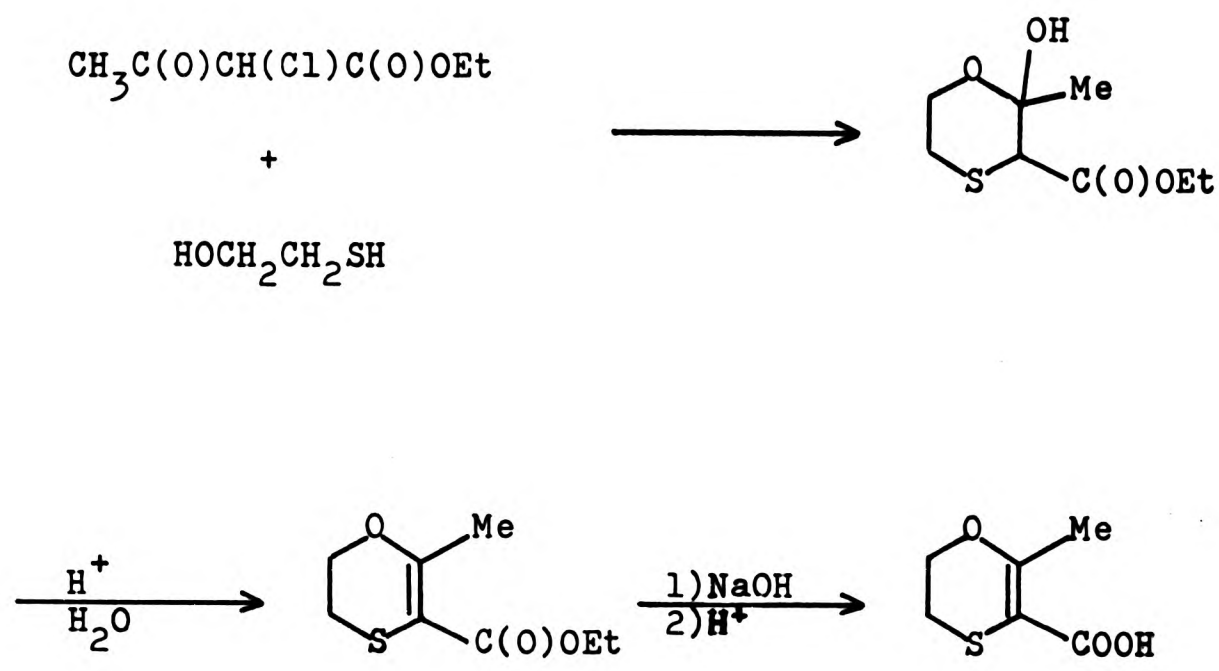
For use in the synthesis of compound (86), 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide (88) was first prepared by the condensation of 2-mercaptoethanol with α-chloroethylacetoacetate, dehydration by acid catalysis under Dean-Stark conditions, and conversion of the resultant ester (83) to the amide *via* the acid and acid chloride[66]

Table 2 $R = (EtO)_2P(O)NHCH(CCl_3)-$

Compound		Derived from	M.pt. (°C)	Yield (%)	Reaction Conditions
$CH_3C(O)NHR$	(84)	Acetamide		66	2 weeks @ room temp.
$(EtO)_2P(O)NHR$	(85)	Diethyl phosphoramidate	186 - 189	12	48 h. @ room temp.
			198 - 199	36	6h. under reflux in benzene
	(86)	5,6-Dihydro-2-methyl-1,4-oxathiin-3-carboxamide	156 - 159	76	3h. under reflux in benzene
$(Me_2N)_2P(O)NHR$	(87)	N,N,N',N'-tetramethyl-phosphoric triamide	219 - 221	83	1h. under reflux in benzene

as shown in Reaction scheme XI.

Reaction scheme XI



(88)

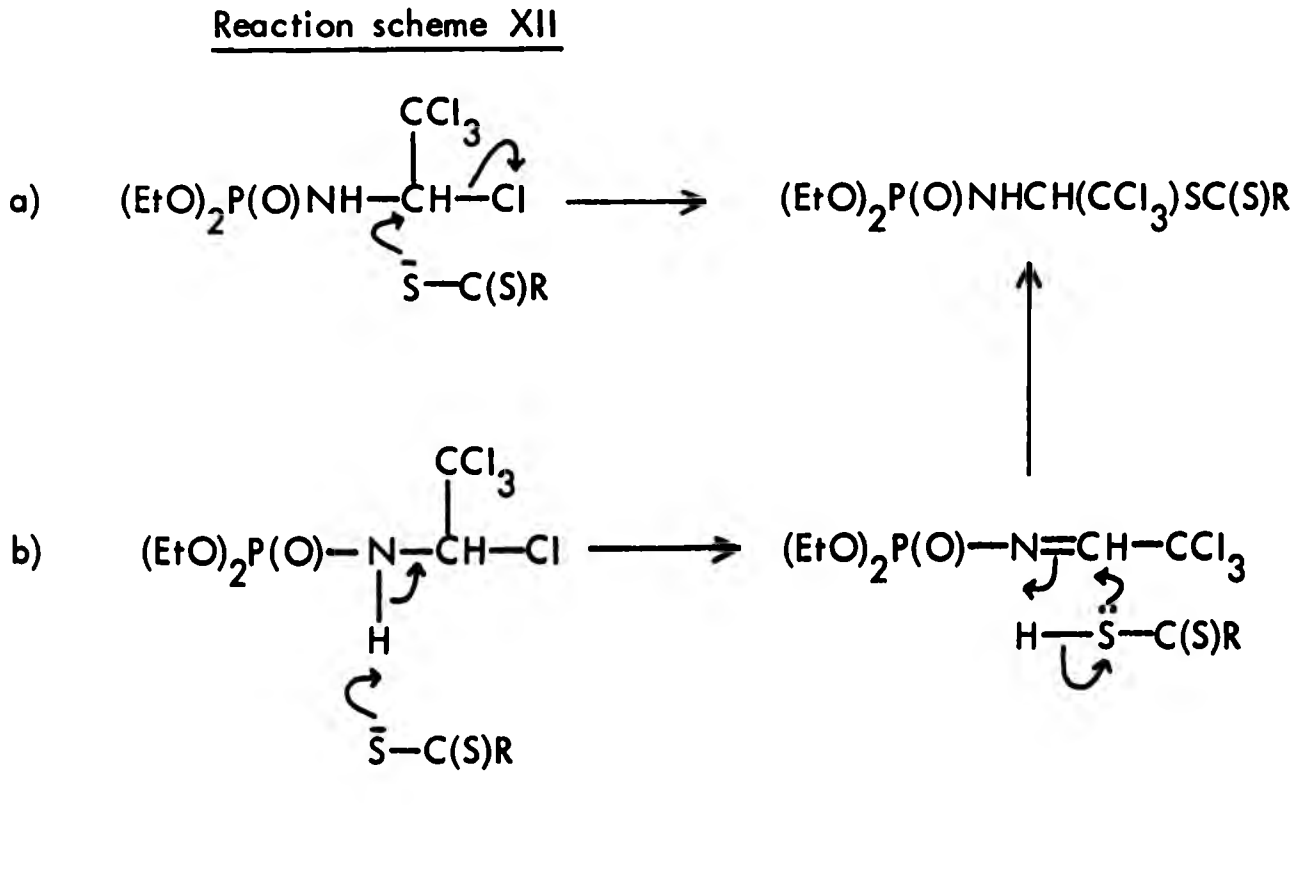
2.3.3 Derivatives of dithiocarbamates and xanthates

Since certain dithiocarbamates and xanthates possess good fungicidal properties it was of interest to synthesise compounds containing these groups. This would be difficult to achieve by the above method because of the instability of the dithiocarbamic and xanthic acids that

would add across the azomethine group of compound (74). It was however found that the desired products could be prepared by reaction of the chloro-compound (73) with the sodium or potassium salts of the appropriate acid.

Two mechanisms appear to be possible for this reaction [Reaction scheme XII (a) and (b)]. The reaction could proceed either according to scheme (a) involving the direct nucleophilic displacement of chloride by the dithiocarbamate or xanthate anion or according to scheme (b) by an elimination-addition sequence similar to that for the reaction

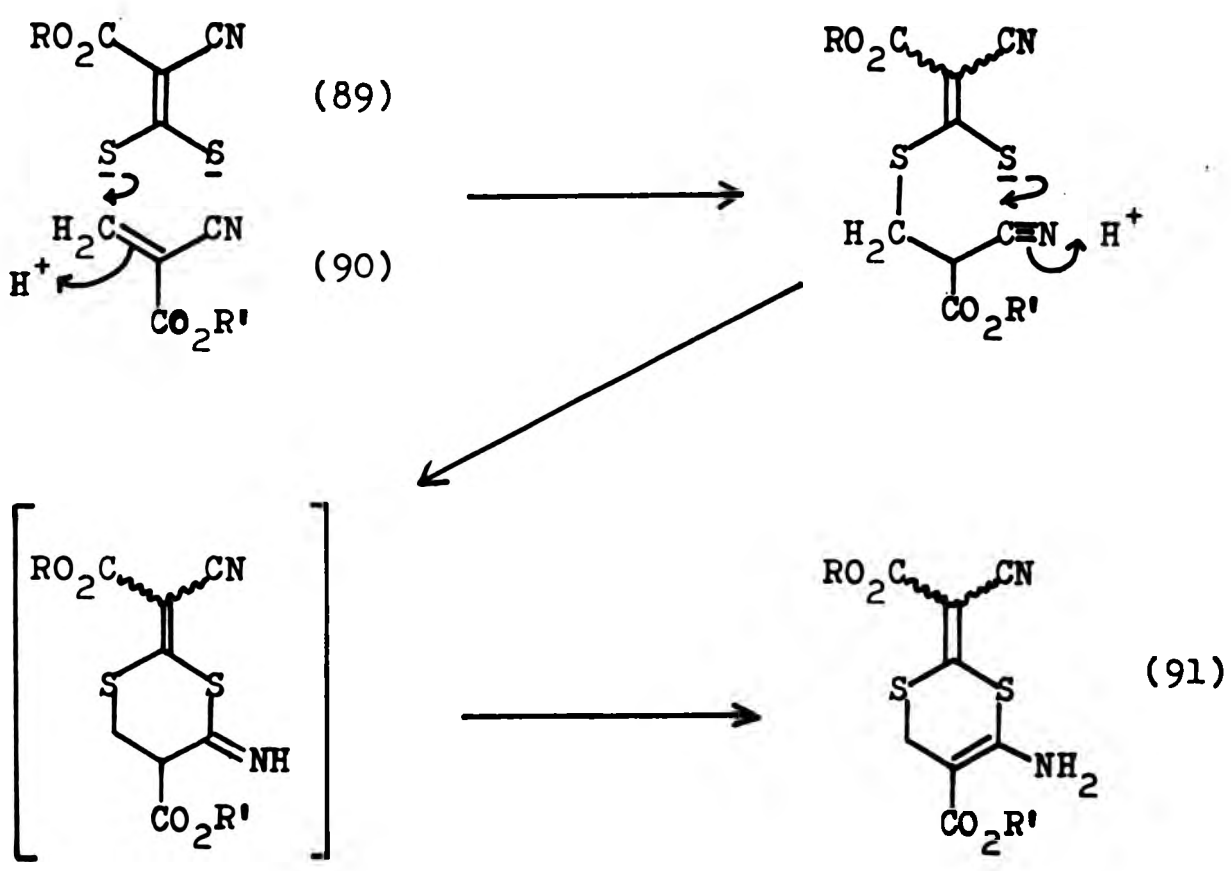
Reaction scheme XII



with water (reaction scheme IX). This second mechanism would involve the addition of the free acids to the trichloroethylidene phosphoramidate (74). Although these acids are unstable they could be involved as transient

intermediates. Scheme (b) is reminiscent of the mechanism involved in the reaction between the unstable dithiolic acid (89) and the cyanoacrylate (90) to give the amino dithiin carboxylate (91)[67] as in reaction scheme XIII. The dithiolic acid (89) is related to dithiocarbamic and xanthic acids as it is derived from carbon disulphide and a cyanoacetate just as dithiocarbamic

Reaction scheme XIII



and xanthic acids are derived from carbon disulphide and an amine or alcohol respectively.

The compounds prepared by this method are listed in Table 3.

Table 3 $R = (EtO)_2P(O)NHCH(CCl_3)-$

Compound		Derived from	M.p. (°C)	Yield (%)
$Me_2NC(S)SR$	(92)	Sodium dimethyldithiocarbamate	103 - 105	80
$Et_2NC(S)SR$	(93)	Sodium diethyldithiocarbamate	99 - 102	85
$EtOC(S)SR$	(94)	Potassium ethyl xanthate	91 - 93	73

The dithiocarbamate derivatives appear to be sensitive to light. On storage in stoppered glass containers the surface exposed to light darkened after a few weeks and became very dark after a few months whereas the bulk of the product not exposed to light remained white.

2.4 Preparation of Bis(dimethylamino)phosphinyl and Ethoxydimethylaminophosphinyl Derivatives

Derivatives containing one or two dimethylamino groups on phosphorus (95 and 96) were also sought in order to compare their

$Me_2N(EtO)P(O)NHCH(CCl_3)R$ (95)

$(Me_2N)_2P(O)NHCH(CCl_3)R$ (96)

fungicidal activity with that of the diethoxy analogues (69; R=R'=EtO).

The bis(dimethylamino)phosphinyl group is a feature of a number of active fungicides, including triamiphos (35) and Drabek et al.[68] found that in a series of fifty dinitroaryl phosphates those containing at least one dimethylamino group showed the highest fungicidal activity.

Initial investigations were carried out using N,N,N',N'-tetramethylphosphoric triamide (97) which was prepared by the reaction of dimethylamine with phosphoryl chloride followed by treatment of the resultant tetramethylphosphorodiamidic chloride with anhydrous ammonia as in reaction

scheme XIV. The compound has been reported by a few workers[69,70] but

Reaction scheme XIV

$$4Me_2NH + POCl_3 \longrightarrow (Me_2N)_2P(O)Cl + 2Me_2NH_2Cl$$

$$(Me_2N)_2P(O)Cl + 2NH_3 \longrightarrow (Me_2N)_2P(O)NH_2 + NH_4Cl \quad (97)$$

is otherwise not well known. It was hoped that it would be possible to condense the amide with chloral as in reaction scheme XV, but the reaction did not proceed as anticipated. The products of various experiments

Reaction scheme XV

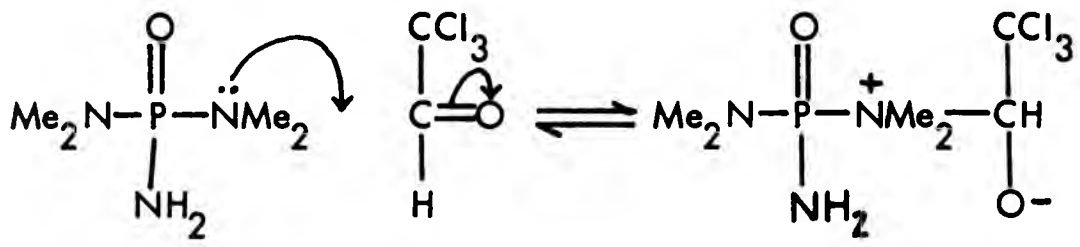
$$(Me_2N)_2P(O)NH_2 + CCl_3CHO \longrightarrow (Me_2N)_2P(O)NHCH(CCl_3)OH \quad (98)$$

carried out under different conditions were intractable oils. Their nmr spectra showed them to be mixtures of many products which may have contained a small amount (ca. 20%) of the desired product, calculated on the basis of a weak doublet of doublets centred at δ = 5.2 ppm. This signal occurs in the spectrum of the diethoxy analogue and is characteristic of the $C\underline{H}CCl_3$ proton. In addition, the Me_2N signal (originally a simple doublet centred at δ = 2.59 ppm) became complex.

All attempts at separation of the desired product by distillation, extraction or crystallisation failed.

The formation of a multi-component mixture could possibly be explained by an initial attack on chloral, not only by the amino nitrogen as in reaction scheme XV, but also by the more basic dimethylamino nitrogen as in reaction scheme XVI. Evidence for this type of side

Reaction scheme XVI

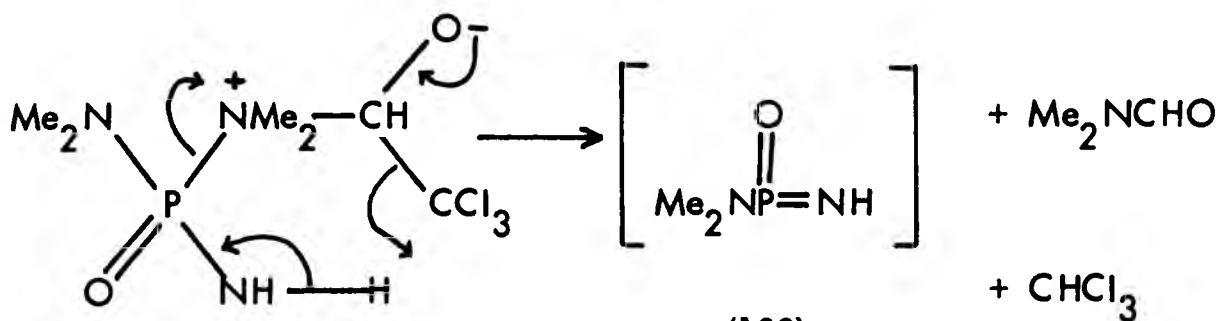


(99)

reaction is discussed later in the chapter and appears to lead to P-N fission (cf. reaction scheme XXII).

More recent studies[71] have shown that dimethylformamide and chloroform are produced, along with other more complex products, but the overall reaction sequences are not yet clear. The absence of interaction between hexamethylphosphoramide and chloral would lead one to assume that a proton on the amino nitrogen must be involved, e.g. as shown in reaction scheme XVII. Although monomeric species such as 100 are not known, it is a possible precursor of the polymeric by-products which this reaction appears to produce. Similar three-coordinate phosphorus (V)

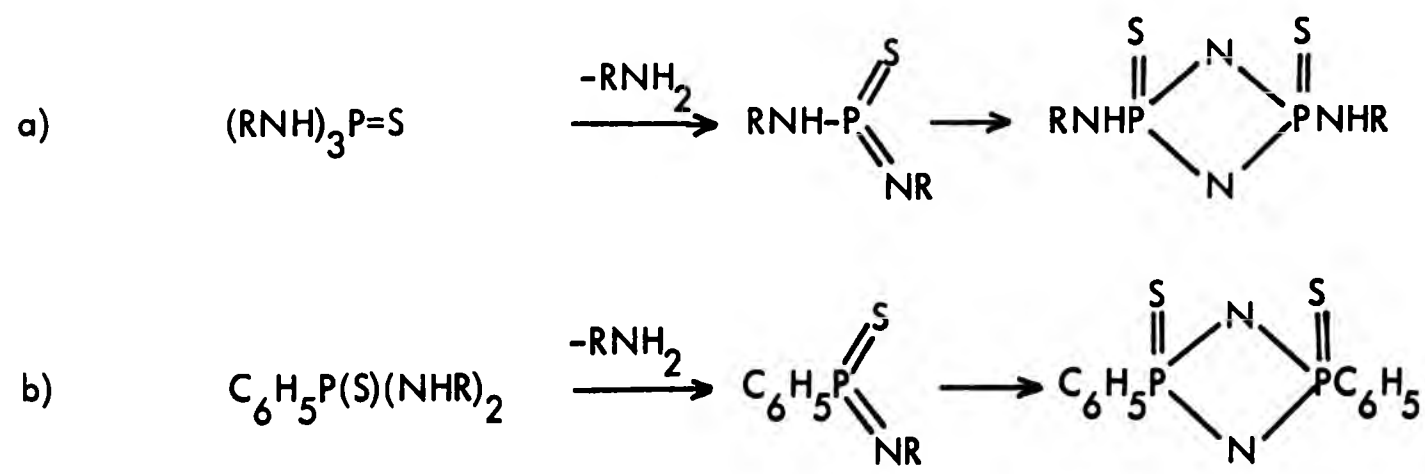
Reaction scheme XVII



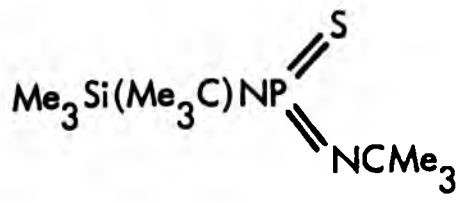
(100)

species have been postulated as intermediates in the formation of cyclodiphosphazanes by the thermolysis of trialkylphosphorothioic triamides[72] and phenyl phosphorothioic diamides[73] as shown in reaction scheme XVIII a) and b). This type of intermediate is also involved in the reaction of

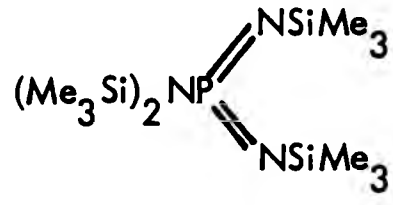
Reaction scheme XVIII



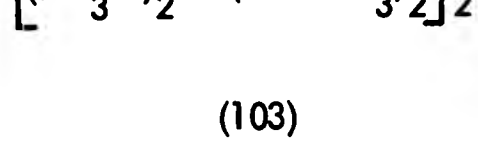
aromatic amines with thiophosphoryl chloride.[74] The intermediates (101),[75] (102)[76] have been isolated and the latter has been shown to dimerise to the cyclodiphosphazane (103).[77]



(101)



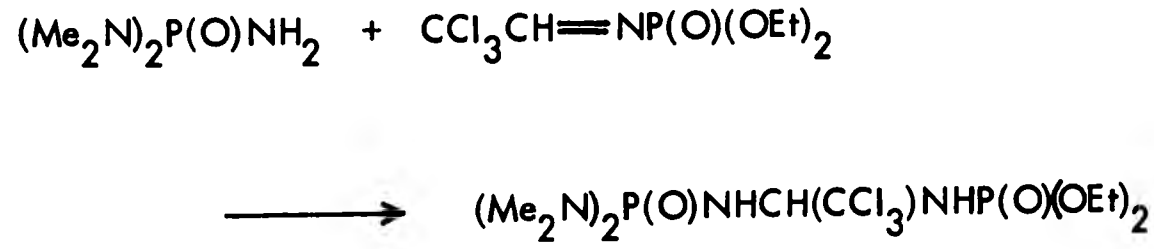
(102)



(103)

One derivative containing the $(Me_2N)_2P(O)CHCCl_3$- group was nevertheless obtained as described in the previous section by the reverse reaction involving the addition of N,N,N',N'-tetramethylphosphoric triamide to diethyl N-(2,2,2-trichloroethylidene)phosphoramidate as in reaction scheme XIX and it is likely that similar additions could be

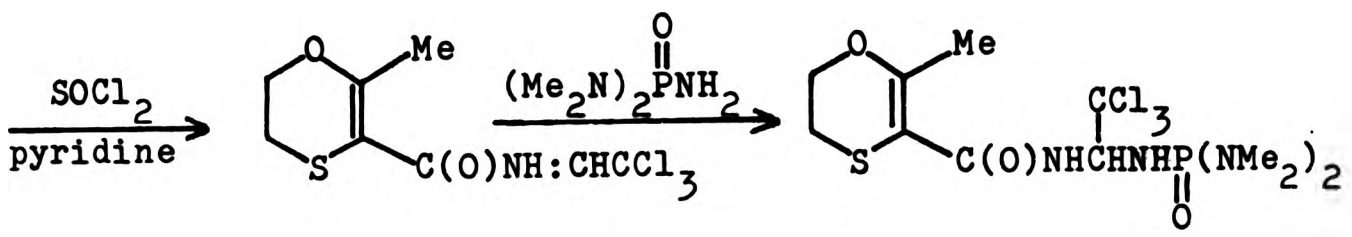
Reaction scheme XIX



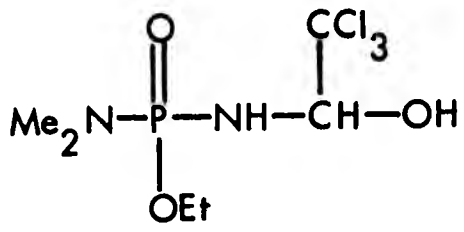
carried out with other trichloromethyl-substituted imines. This could provide a possible approach, for instance, for the preparation of an oxathiin derivative (analogue of carboxin) which would involve condensation of chloral with the amide (88), followed by chlorination, dehydrochlorination,

and addition of tetramethylphosphoric triamide as in reaction scheme XX.

Reaction scheme XX



Some success was achieved in obtaining an example of a compound having a dimethylamino and an ethoxy group on phosphorus via the intermediate O-ethyl-N,N-dimethyl-N'-(1-hydroxy-2,2,2-trichloroethyl) phosphorodiamidate (104).



(104)

O-Ethyl-N,N-dimethylphosphorodiamidate (106) was prepared according to reaction scheme XXI. Phosphoryl chloride was condensed

with ethanol to give ethyl phosphorodichloridate (105) and this was then treated successively with dimethylamine and anhydrous ammonia. The

Reaction scheme XXI

$$POCl_3 + EtOH \longrightarrow EtOP(O)Cl_2 + HCl$$

(105)

$$EtOP(O)Cl_2 + Me_2NH \xrightarrow{Et_3N} Me_2N(EtO)P(O)Cl$$

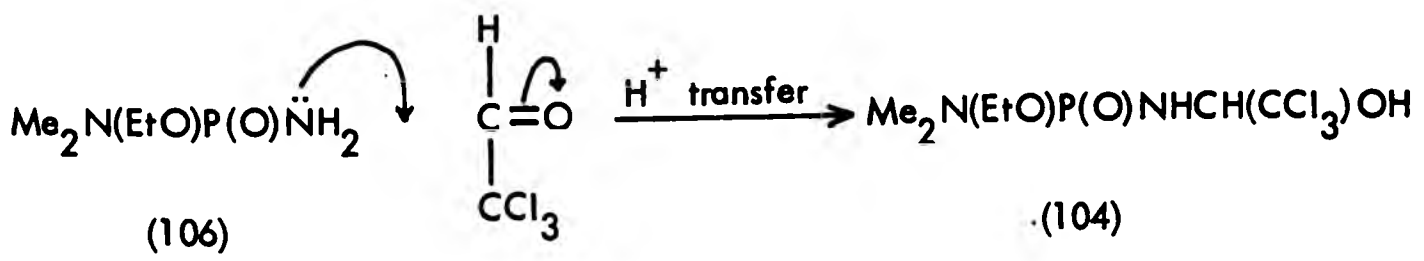
$$Me_2N(EtO)P(O)Cl + 2NH_3 \longrightarrow Me_2N(EtO)P(O)NH_2 + NH_4Cl$$

(106)

amide (106) which has not been reported previously was obtained as a low-melting, deliquescent white solid and was characterised by ir, nmr and mass spectroscopy.

It was found that the reaction of this diamidate with chloral was best carried out at 0-5°C in benzene by slow addition of anhydrous chloral (Reaction scheme XXII).

Reaction scheme XXII



(106) (104)

Nmr spectroscopy showed a multiplet in the region δ = 4.9-5.3 ppm probably corresponding to C<u>H</u>CCl_3 which suggested that the desired product (104) was present (<u>ca</u>. 50%) but by-products were indicated by a large singlet in the dimethylamino region of the spectrum (δ = 2.68 ppm) and also what appeared to be the signal of another ethyl group superimposed on that of the desired compound. On shaking the mixture in carbon tetrachloride with deuterium oxide the peaks due to the impurities were substantially reduced. Since it seemed unlikely that all these protons had exchanged with deuterium it was concluded that the D_2O must have extracted the impurities from the solution.

Bearing this in mind the total product from the reaction was dissolved in carbon tetrachloride and the solution was washed thoroughly with water. This procedure left the desired product in the carbon tetrachloride in a substantially pure state ($>$ 90%). (Fig 4).

The nmr spectrum of the aqueous extract in D_2O (Fig 5) was very similar to that of compound (104) except that the dimethylamino protons appeared as a singlet. The spectrum showed a triplet at δ = 2.2 (3H), a singlet at δ = 2.7 (6H), a quintet at δ = 3.8 (2H) and a small multiplet at δ = 5 (1H). From these data it appears that the extracted material contains an ethoxyphosphoryl group and a dimethylamino group, but the latter is no longer attached to phosphorus. It may be reasonably assumed that nucleophilic attack by the lone pair of electrons of the

Fig. 4. Nmr spectrum of $EtO(Me_2N)P(O)NHCH(CCl_3)OH$ (104)

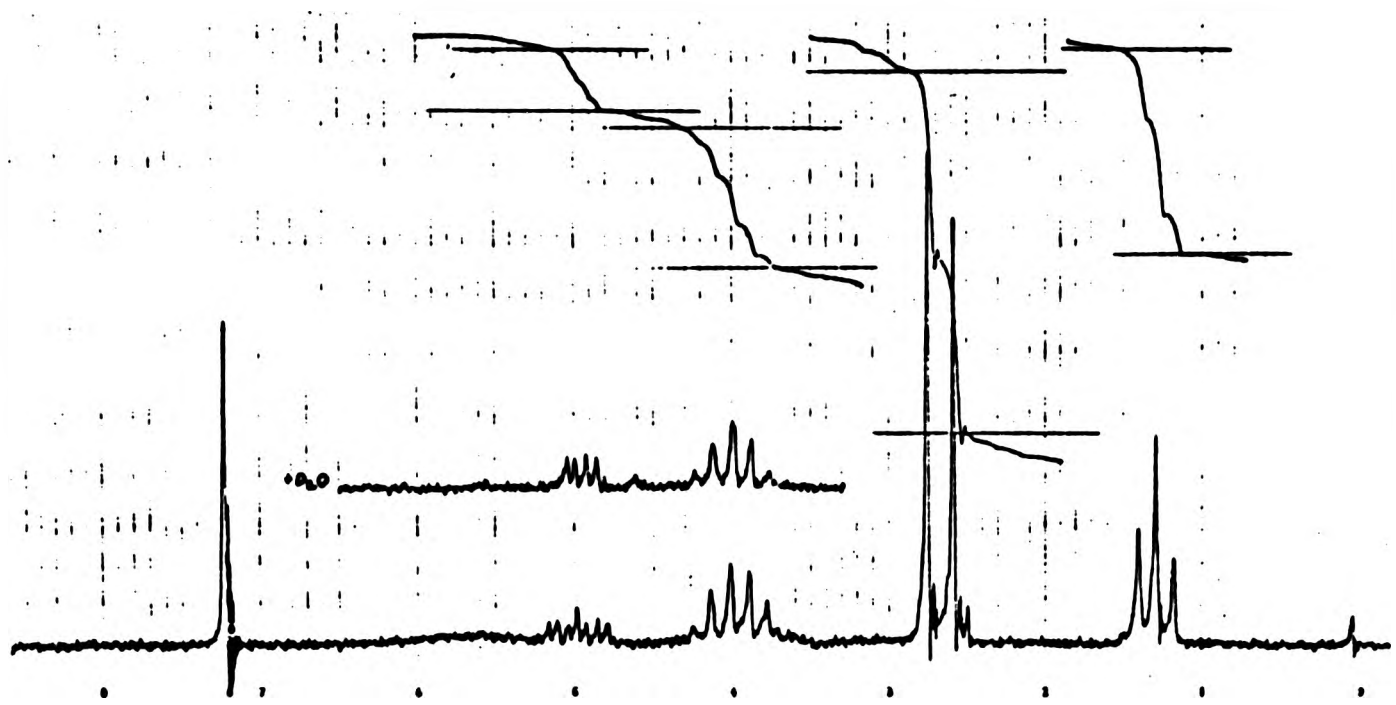
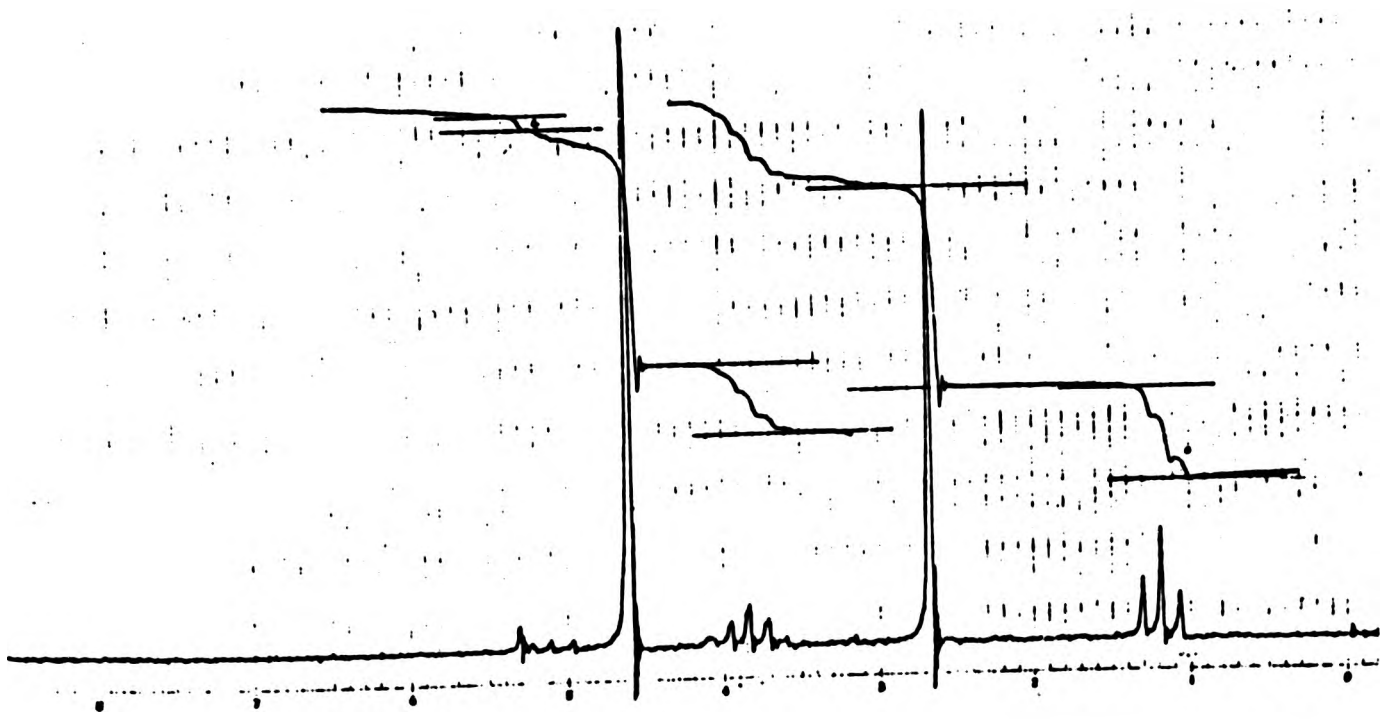
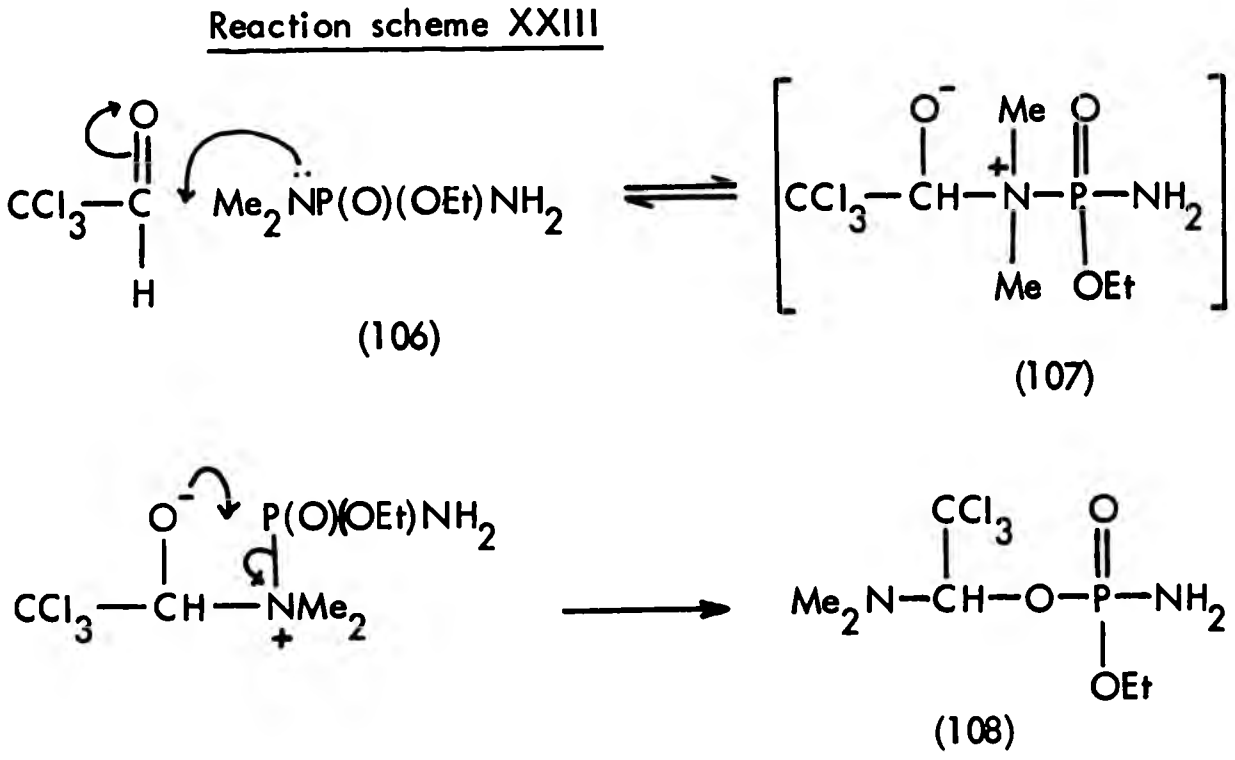


Fig. 5. Nmr spectrum of reaction by-product from the condensation of diethyl phosphoramide with chloral.



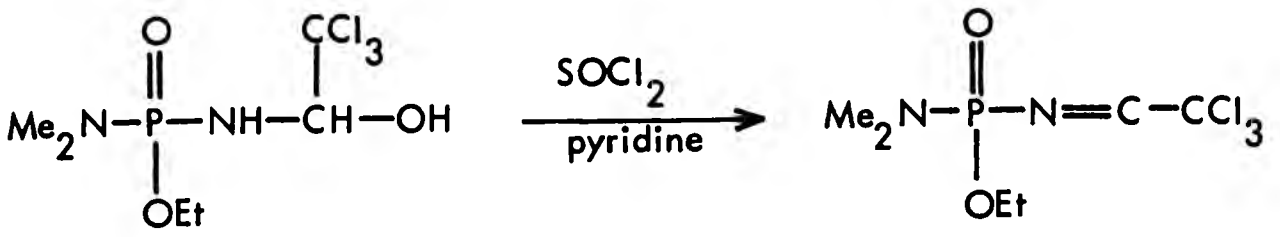
dimethylamino group at the carbonyl group of chloral has occurred and that this is followed by phosphorus-nitrogen fission. The results are consistent with a product such as that shown (108, reaction scheme XXIII) but it was not possible to confirm this speculative structure.

Reaction scheme XXIII



Chlorination of the O-ethyl-N,N-dimethyl-N'-(1-hydroxy-2,2,2-trichloroethyl)phosphoramidate (104) in the normal way using thionyl chloride was unsuccessful. However, the reaction with thionyl chloride in the presence of a large excess of pyridine gave ethyl N,N-dimethyl-N'-(2,2,2-trichloroethylidine)phosphoramidate (109) directly as in reaction scheme XXIV. Condensation with one molecular equivalent of imidazole then gave O-ethyl-N,N-dimethyl-N'- 1-(imidazol-1-yl)-2,2,2-trichloroethyl)-phosphorodiamidate (110) which was characterised by microanalysis, nmr, ir and mass spectrometry.

Reaction scheme XXIV



(104) (109)

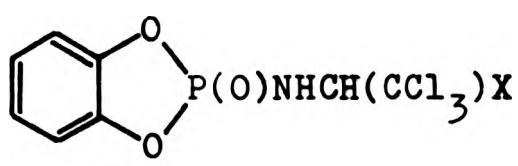
(110)

This type of compound is interesting in that its main skeleton contains two optical centres, the methine carbon and the phosphorus atom. Because of this the $C\underline{H}CCl_3$ proton appears in the nmr spectrum as an octet instead of a quartet since its chemical shift is slightly different in the two diastereoisomeric forms. Similarly the protons of the dimethylamino group appear as a quartet.

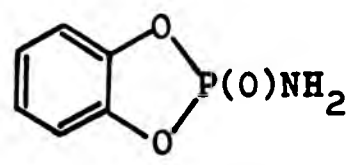
2.5 Attempted Preparations of o-Phenylene Phosphoramidate Derivatives

In order to extend the range of compound types derivatives such as (111) were sought. It was hoped that it would be possible to synthesise the amide (112), which has not been reported previously, then

condense this with chloral to give the hydroxy derivative (111, X=OH) and other derivatives by similar routes to those employed for the diethoxy-phosphoramidate analogues.



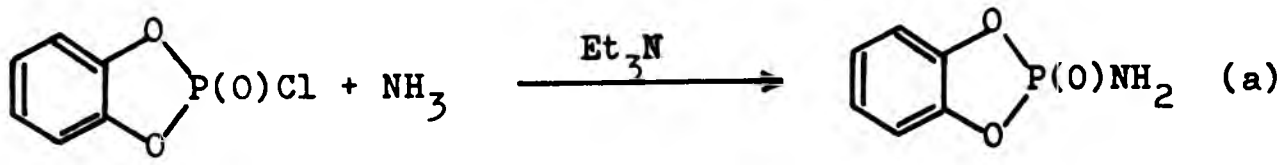
(111)

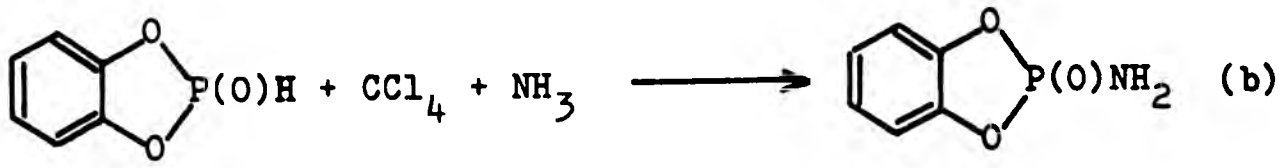


(112)

Two possible routes were investigated for the preparation of the amide (112) as shown in reaction scheme XXV a) and b).

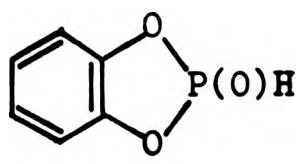
Reaction scheme XXV



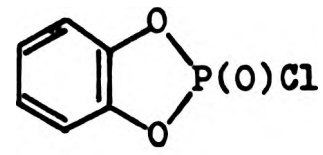


Route a) gave a mixed product which elemental analysis showed may have contained ca. 60% of the desired product together with ammonium chloride but attempts at separation were unsuccessful.

Route b) requires the phosphite (113) as a reagent which is also unknown and attempts were made to synthesise this from the chloride (114)

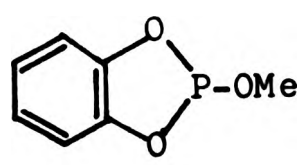


(113)

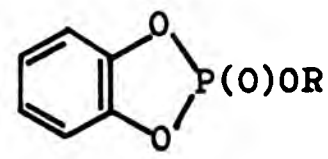


(114)

and water. However this reaction did not proceed as anticipated but P-O cleavage by the water readily gave catechol in high yield. Similar P-O cleavage by water has been reported previously for the phosphorochloridite (114)[78] and also for compounds of the type (116).[79] Further attempts were made to synthesise the phosphite (113) by the action of anhydrous hydrogen



(115)



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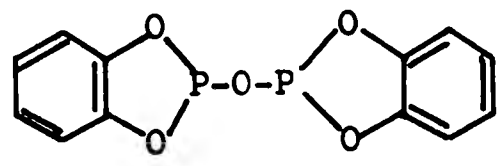


(117)

chloride on the methyl ester (115). The nmr spectrum of the product did show a characteristic phosphite signal at δ = 9.35 ppm (J_{PH}=906 Hz), and integration indicated a purity of ca. 35% but attempts at high vacuum distillation led to decomposition. Difficulty in the isolation of pure o-phenylene phosphite has been reported previously[78] when attempts to prepare it led to the pyrophosphite (117).

CHAPTER 3, SPECTROSCOPIC STUDIES

For reference structures of all novel compounds are shown in Appendix I

3 SPECTROSCOPIC STUDIES

3.1 Infrared Spectroscopy

Infrared spectra were obtained mainly for 'fingerprint' identification and the spectra of the novel compounds are reproduced in the thesis for future reference.

A limited amount of structural information can be obtained from the spectra. The compounds containing the group $(EtO)_2P(O)NHCH(CCl_3)$- all show characteristic bands at 3130-3200 cm^{-1} due to P(O)N-H stretching, 1230-1290 cm^{-1} due to P=O stretching and 1030-1050 cm^{-1} due to P(O)-OEt stretching. Certain of the individual compounds show other characteristic bands. The N-formylpiperazine (80), the acetamide (84) and the oxathiin (86) derivatives show strong carbonyl absorptions at 1677, 1675 and 1658 cm^{-1} respectively. The acetamide derivative (84) also shows a C(O)N-H band at 3325 cm^{-1}, the oxathiin derivative shows two such bands at 3277 and 3397 cm^{-1} and the thioethanol derivative (82) shows an O-H band at 3310 cm^{-1}. The main characteristic bands for each compound are shown in Table IV.

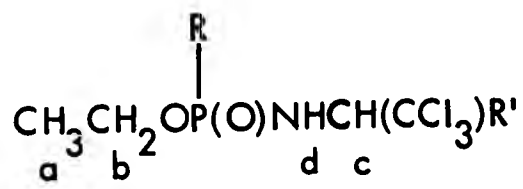
Table IV

Characteristic infrared absorptions (cm^{-1})

Derivative		P=O	P-OEt	PN-H	C=O	C(O)N-H	O-H
Imidazole	(76)	1260	1047	3140			
Triazole	(77)	1249	1026	3130			
Morpholine	(78)	1247	1047	3200			
Piperazine	(79)	1250	1039	3180			
Formylpiperazine	(80)	1240	1054	3150	1677		
Thioethanol	(81)	1233	1050	3160			
Thioethanol	(82)	1228	1050	3125			3310
Acetamide	(84)	1295	1045	3200	1675	3325	
Phosphoramidate	(85)	1247	1045	3170			
Oxathiin	(86)	1250	1046	3180	1658	3397 3277	
$Me_2NC(S)S$-	(92)	1237	1027	3130			
$Et_2NC(S)S$-	(93)	1240	1030	3140			
Xanthate	(94)	1250	1050	3140			
$Me_2N(EtO)P(O)$	(110)	1242	1048	3100			
$(Me_2N)_2P(O)$	(87)	1250	1045	3175			

3.2 ^{1}H Nmr Spectra

Nmr spectroscopy was found to be very useful in the present study both for structural confirmation and for monitoring of reactions. All the compounds described in this section have the basic structure (118), thus the



(118)

R = CH_3CH_2O, $(CH_3)_2N$ R', see text

nmr spectra of the compounds share many common features though some spectra depart from them (see pp. 139-145 for nmr data).

3.2.1 Nmr spectra of the diethoxyphosphinyl derivatives

3.2.1.1 Basic spectrum

The basic spectrum of the diethoxy compounds is in most cases similar to, and well represented by that of the chloride (73, cf. Appendix I). Protons of the methyl group (a) of the compounds appear in the high field region (δ 1.2-1.4 ppm) of the spectrum as a triplet, being coupled to the two protons (b). Protons of the methylene group (b) resonate at a lower field (δ 3.9-4.3 ppm) and usually appear as a 'quintet' though this consists of two superimposed quartets since protons (b) are coupled both to protons (a)

and to phosphorus, but the two quartets were not separated at the resolution of the instrument used to obtain the spectra (ca. $1:10^8$). Proton (c) resonates over a wide field range (4.4-6.9 ppm) depending on the nature of R' and generally appears as a doublet of doublets being coupled to proton (d) and to phosphorus. Proton (d) usually resonates at ca. 4 ppm but in certain cases it can appear at up to 6 ppm. Its position in the spectrum is not always constant and seems to be affected by concentration, which in turn affects intermolecular hydrogen bonding and hence the chemical shift. In most cases one cannot 'see' this proton in the spectrum because it is swamped by the CH_3CH_2O resonances but its presence is confirmed by the decrease in integration on shaking with D_2O. In certain cases the resonances of protons (c) and (d) appear close together in the spectrum under which circumstances a typical ABX pattern is obtained since each is coupled to the other and to phosphorus.

The nmr spectrum for the phosphoramidate part of the molecules is as described above for the morpholine (78), piperazine (79), formylpiperazine (80), thioethanol (81), phosphoramide (85) and xanthate (94) derivatives. For the remaining compounds further complication of the spectra arises.

3.2.1.2 Complication of the basic spectrum

Further complication of the basic spectrum can be seen for the remaining compounds. This could be due to either or both of two factors.

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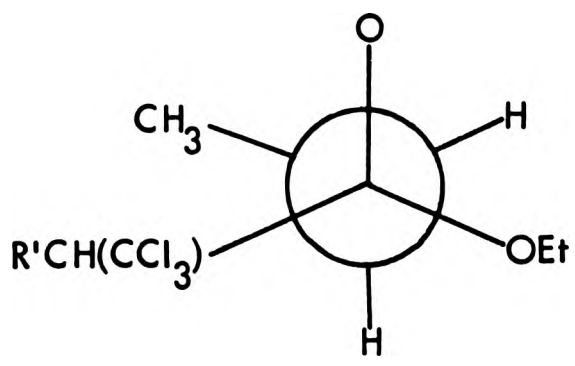
3.2.1.2 Complication of the basic spectrum

Further complication of the basic spectrum can be seen for the remaining compounds. This could be due to either or both of two factors.

a) Non equivalence of the methylene protons. This arises because the two protons are in different environments (see fig. 6) which can lead to coupling between them and should give rise to an AB pattern

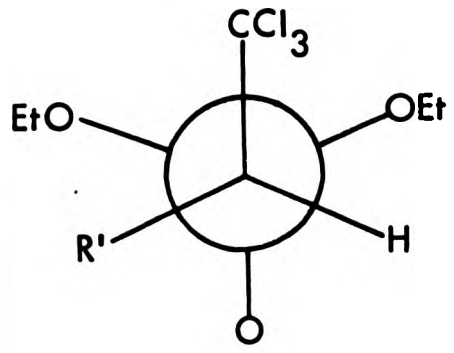
Fig. 6, looking along C-O-P bond of (118)



amongst the other coupling. This type of non equivalence has been reported for the methylene protons of alkyl chlorosulphites.[80]

b) Non equivalence of ethyl groups due to optical isomerism. This arises because in all the compounds the C-CCl_3 carbon is an asymmetric centre which means that the two ethyl groups attached to phosphorus are in different environments (see fig. 7) thus both the methylene and the methyl protons may

Fig. 7, looking along P-N-C bond of (118)



have a different chemical shift in each group, depending on the nature of R', which would lead to a doubling of the ethyl signals of the basic spectrum.

Splitting of the methyl signals of the ethyl groups must arise due to factor (b) but in the case of the methylene signals it is difficult to say which mechanism is in operation since both would lead to a similar pattern. The splitting of the methylene 'quintets' and methyl triplets are shown in table V.

Table V, splitting (Hz) of ethyl signals

R'	deriv. No.	CH_3CH_2	CH_3CH_2
Imidazole	(76)	4.7	1
Triazole	(77)	0	4.7(a)
SCH_2CH_2OH	(82)	1	1.5
Acetamide	(84)	1	2.5
$SC(S)NMe_2$	(92)	4.7	4.7
$SC(S)NEt_2$	(93)	0	1.5
subst. oxathiin	(86)	1	3.3

(a) further fine splitting can be seen giving a very complex pattern.

3.2.2 Bis(dimethylamino)phosphinyl and ethoxydimethylaminophosphinyl derivatives

The bis phosphoramidate 118, R=EtO, R'=$(Me_2N)_2P(O)NH$ shows

only the basic spectrum of the other diethoxy analogues together with a doublet due to the resonance of the dimethylamino groups which are coupled to phosphorus. There is very fine splitting (<1 Hz) of the $C\underline{H}_3CH_2O$ signal.

The two compounds containing both ethoxy and dimethylamino groups or phosphorus (118, $R'Me_2N$, R'=OH or imidazole) are interesting in that they contain two asymmetric centres, the $\underline{C}$-CCl_3 carbon and the phosphorus atom and one would predict that the existence of different diastereoisomers should complicate the nmr spectra of the two compounds but this turns out to be the case for only one of them. For the hydroxide (118, $R=Me_2N$, R'=OH) the ethyl signal appears in the spectrum as a triplet and a 'quintet' and the dimethylamino signal as a doublet. For the imidazole derivative the methyl signal of the ethyl group appears as a sextet (1:1:2:2:1:1), the methylene signal as a complex multiplet (basically a quintet showing further splitting) and the dimethylamino signal as a quartet (1:1:1:1). The fine splitting of the resonances is shown in table VI.

Table VI Fine splitting in spectra of (118) (Hz)

R	R'	$C\underline{H}_3CH_2$	$CH_3C\underline{H}_2$	$(C\underline{H}_3)_2N$
EtO-	$(Me_2N)_2P(O)NH$-	1	0	0
Me_2N-	-OH	0	0	0
Me_2N-	imidazole	3.3	2.3	5.2

3.3 Mass Spectrometry

Mass spectra were obtained for the new compounds reported, firstly for confirmation of molecular structure and also to provide a 'fingerprint' identification for future use.

Mass spectral data, which are shown in the tables on pages 146-161, were obtained at 70 e.v. with an inlet temperature of 200°C. Molecular ions were observed for only five of the compounds viz. the morpholino- (78), 4-formylpiperazinyl- (80), acetamido- (84), 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamido- (86), and bisdimethylphosphinylamino- (87) derivatives and in each case were very weak (0.05-1.5% relative abundance). Repeated scans at 150°C for the imidazolyl- (76) and the dithiocarbamate- (92 and 93) compounds gave similar results to those at 200°C thus to some extent excluding thermal decomposition in the inlet system. For the compounds which gave no molecular ion the mass spectral fragmentation patterns provided evidence of specific groupings within the molecules as discussed below.

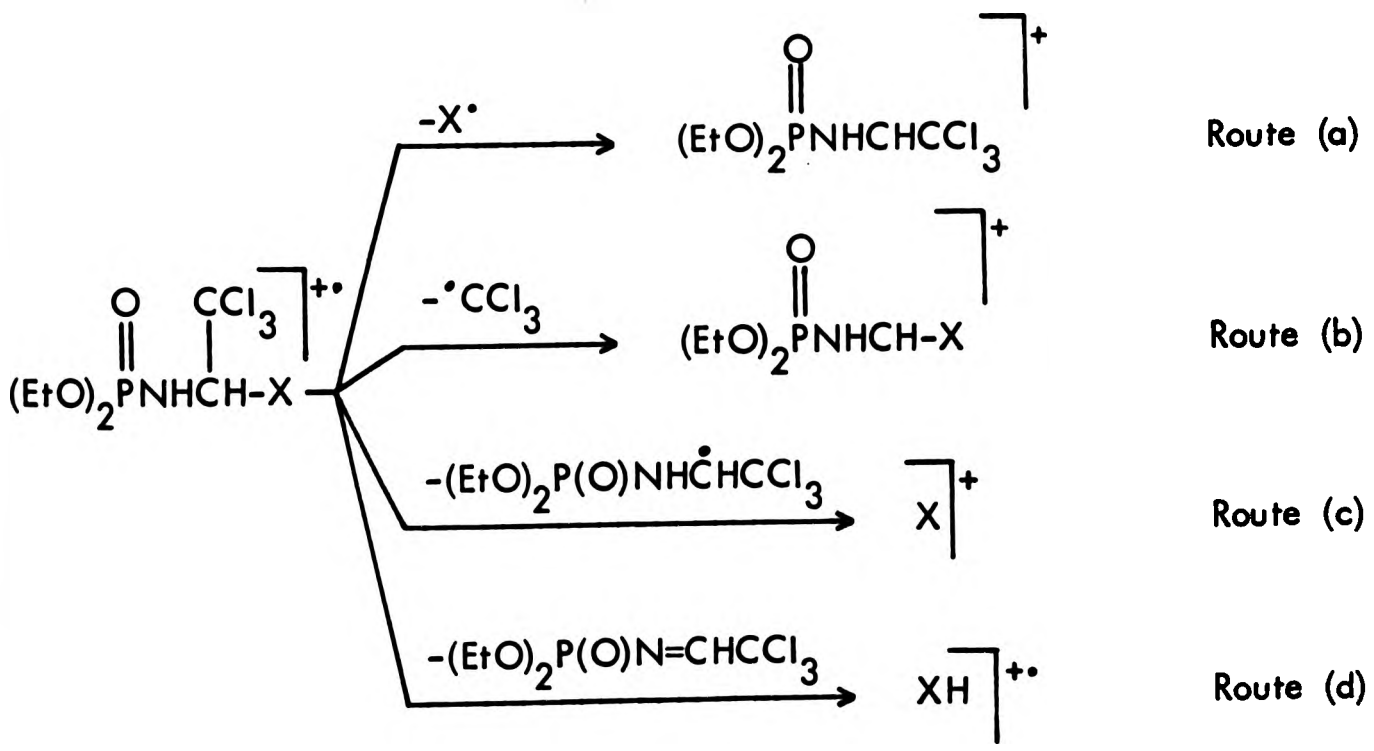
Many of the ions arising from these compounds contain one or more chlorine atoms. These ions appear in the mass spectra as multiplets since naturally occurring chlorine contains two isotopes: ^{35}Cl and ^{37}Cl with an abundance ratio of approximately 3:1 respectively. Thus fragments containing one chlorine atom appear in the mass spectrum as a doublet with an abundance ratio of 3:1. Those containing two chlorine atoms appear as a triplet with abundance ratio 9:6:1 and those containing three chlorine atoms appear as a

quartet with abundance ratio 27:27:9:1. Each peak in these multiplets is separated from the next by two mass units. When quoting m/z values for these ions the number quoted is that for the fragment containing solely ^{35}Cl atoms.

3.3.1 General fragmentation patterns

All but one of the compounds contain the group $(EtO)_2P(O)NHCH(CCl_3)$- (119) and these share many common fragments in their mass spectra. The fragmentation patterns are frequently complex but four principal cleavages of the molecular ion can be recognised (see Fragmentation scheme 1).

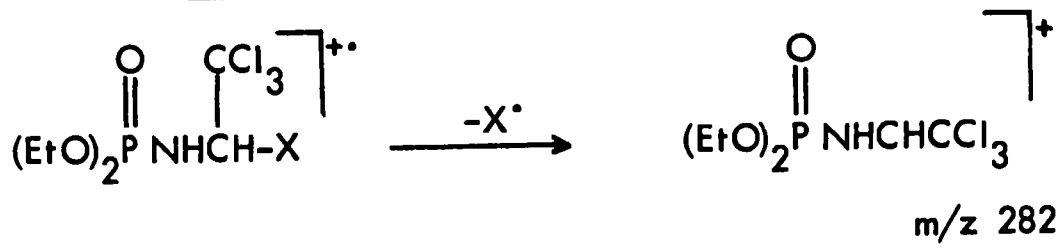
Fragmentation scheme 1

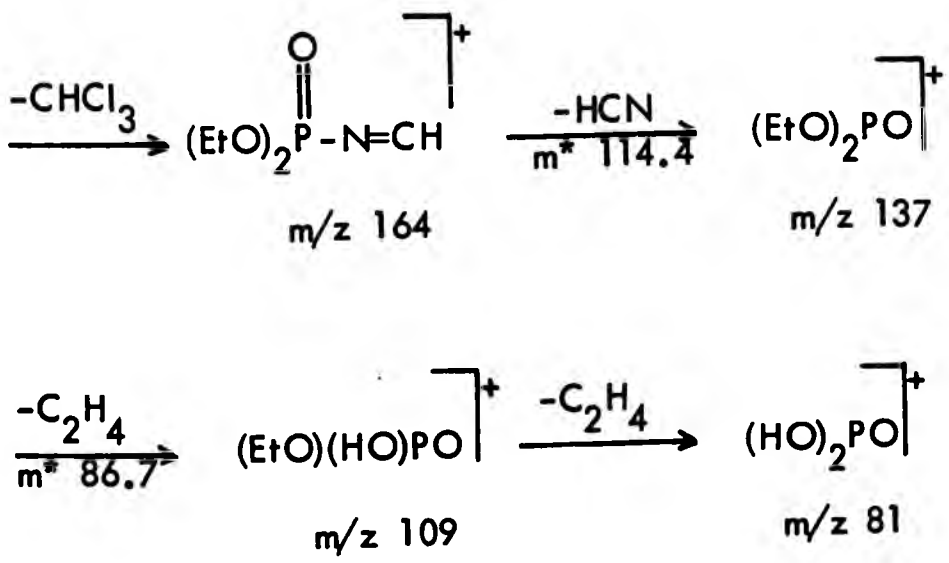


3.3.1.1 Fragmentation by route (a)

Notable ions are found with m/z values 282, 164, 109 and 81 due to this fragmentation pathway in which the substituent group X (imidazolyl, etc.) is initially lost as a radical. The ion with m/z 282 is usually of low abundance but is present in the spectra of all the compounds except that in which X = diethoxyphosphinylamino (85); it is noticeable also that this ion, corresponding to group (119) has the highest m/z value in many of the spectra. The ions at m/z 164, 137, 109 and 81 which occur in all spectra arise from this fragment by successive loss of chloroform, HCN, and two molecules of ethylene (see fragmentation scheme 2). In ten of the fifteen compounds studied it is one of the latter ions which gives rise to the base peak in the mass spectrum. In most of the mass spectra the transitions 164 to 137 and

Fragmentation scheme 2





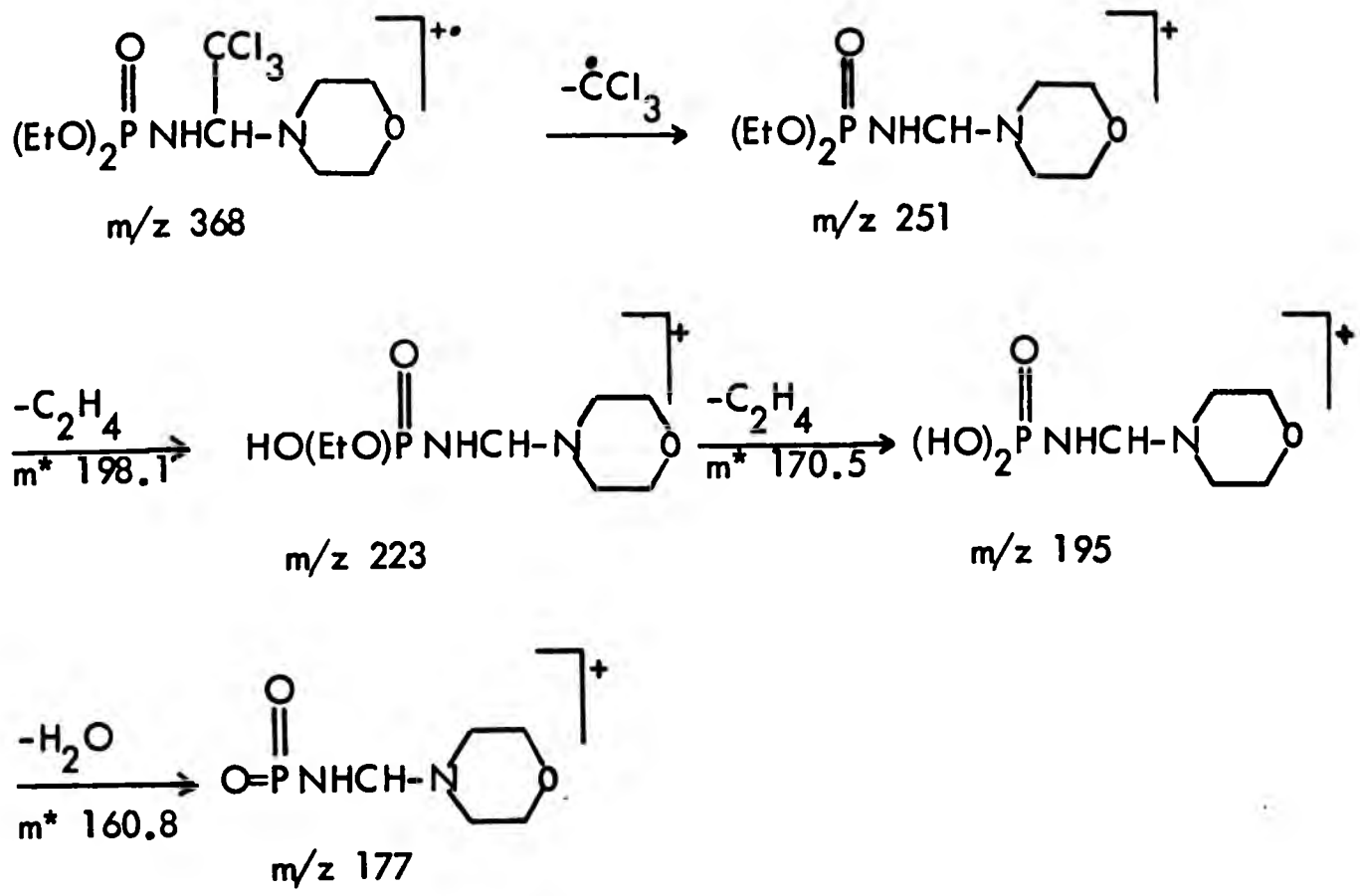
137 to 109 are supported by metastable ions at 114.4 and 86.7 respectively.

Most compounds containing the group $(EtO)_2P(O)$- (120) give rise to ions at m/z 137 (low intensity), 109 (high intensity) and 81 (variable intensity).[81] It is interesting to note that the ion at m/z 137 for the present series of compounds is usually of high intensity, being the base peak in three instances. This probably implies that the phosphorus-nitrogen bond in this series of compounds is relatively easily cleaved which is in contrast to most other compounds containing a similar bond.[81,82,83] The ease of P-N bond cleavage in this case is probably due to the stability of HCN which is lost from the parent ion during its cleavage (see fragmentation scheme 2).

3.3.1.2 Fragementation by route (b)

In the case of the morpholino-compound (78) the base peak (m/z 251) can be attributed to the ion arising from the loss of the trichloromethyl group, and this process is followed by the stepwise loss of two molecules of ethylene and one of water (see fragmentation scheme 3).

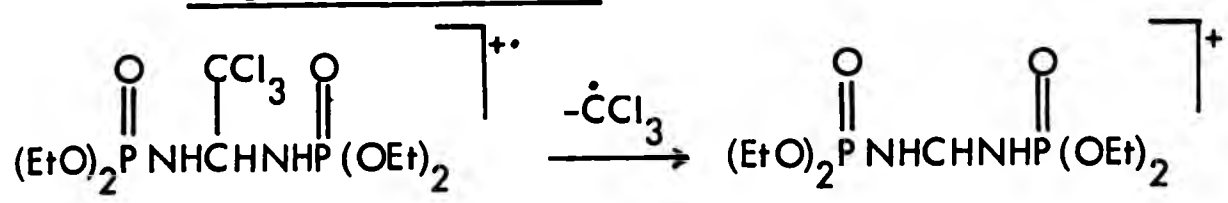
Fragmentation scheme 3

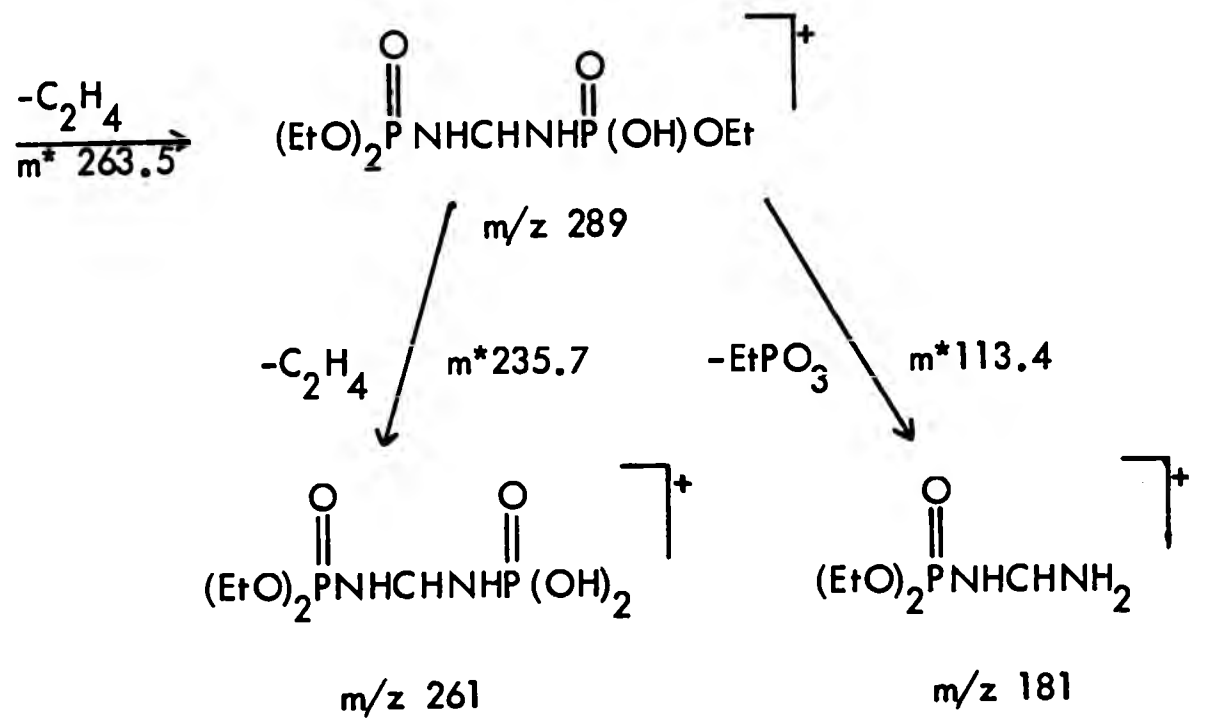


An intense ion (82%) corresponding to $\left[M-CCl_3\right]^+$ is also observed in the spectrum of bis(diethoxyphosphinylamino)ethane (85). This ion goes on to lose two molecules of ethylene and there is evidence that rearrangement may also occur after the loss of one molecule of ethylene with the elimination of an ethyl metaphosphate moiety (see fragementation scheme 4).

The acetamido derivative (84) gives rise to abundant fragments at m/z 223, 181, 153 and 125 which arise from the molecular ion by the successive loss of $\cdot CCl_3$, ketene, and two molecules of ethylene (see

Fragmentation scheme 4

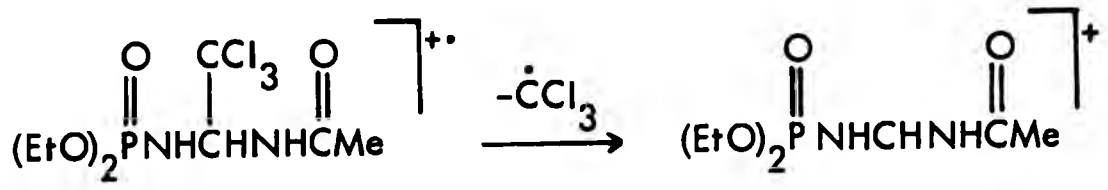




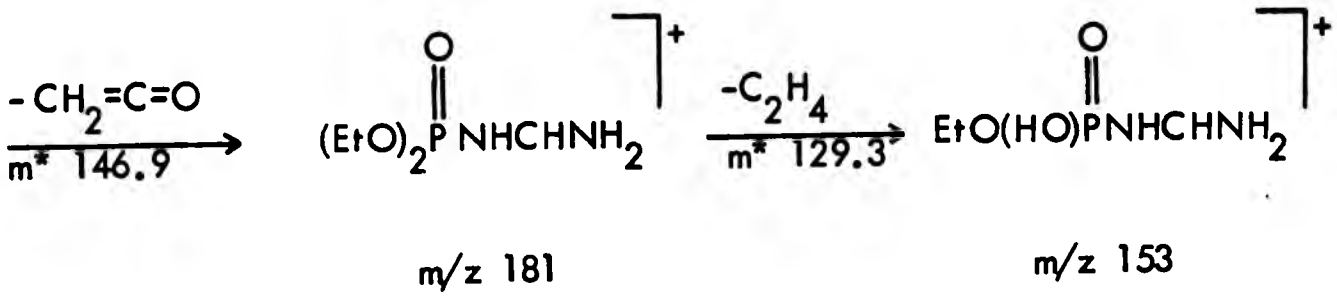
fragmentation scheme 5). The loss of ketene is characteristic of N-acetylated compounds.[84] An intense ion at m/z 43, which is in fact the base peak for this compound (84), is another characteristic of acetyl compounds in general.[84]

Initial loss of the trichloromethyl radical also occurs from the molecular ion of the triazolyl derivative (77), the 4-formylpiperazinyl derivative (80), the bis(dimethylamino)phosphinyl derivative (87) and the oxathiin (86). In the case of the triazolyl compound (77) the $\left[M-CCl_3\right]^+$ ion, which appears at m/z 233, probably goes on to eliminate 1,2,4-triazole since no other ions containing the triazole residue can be seen in the mass spectrum apart from

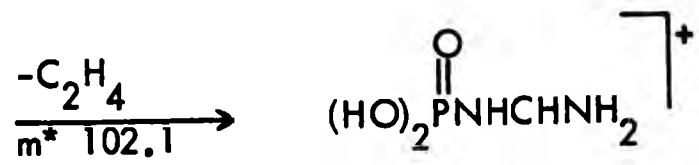
Fragmentation scheme 5



m/z 340 m/z 223



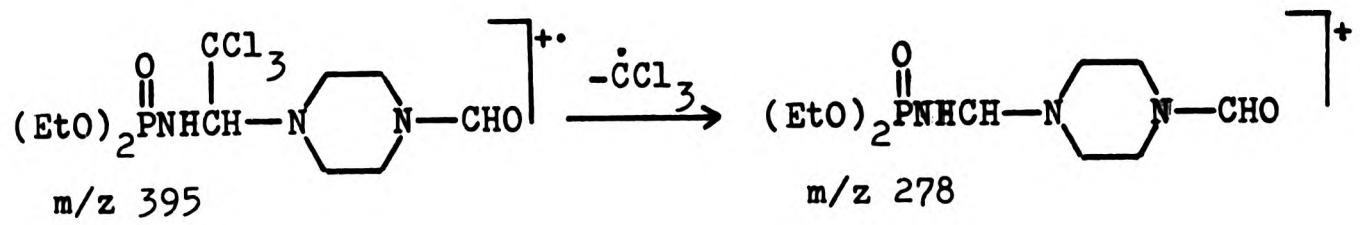
m/z 181 m/z 153

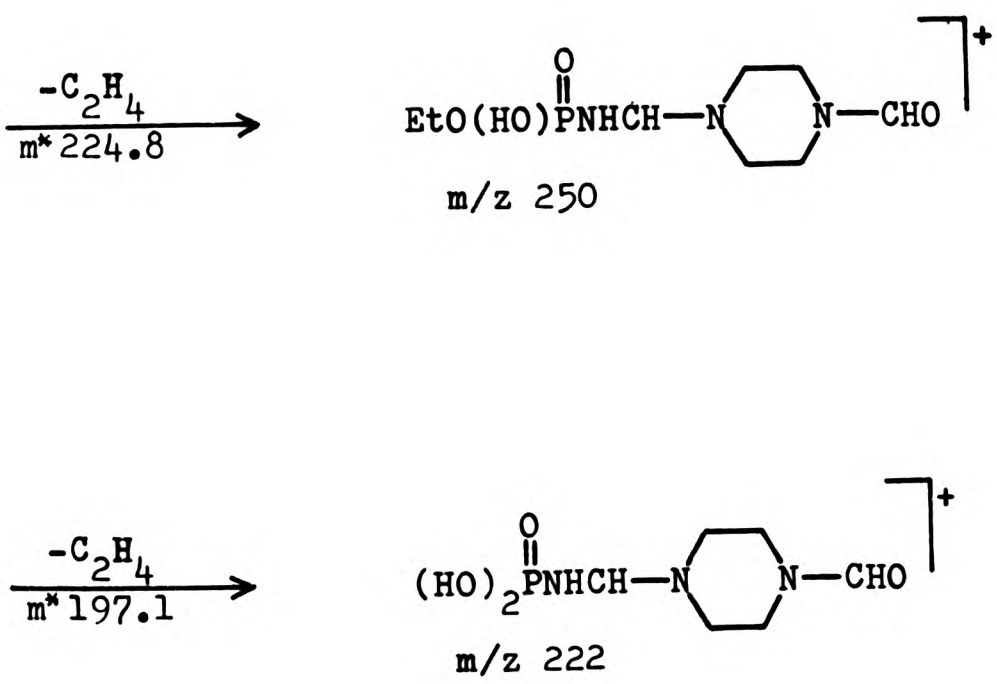


m/z 125

that of the free triazole itself at m/z 69. The loss of $\cdot CCl_3$ from the 4-formylpiperazinyl compound (80) is followed by the successive loss of two molecules of ethylene to give ions with m/z 278, 250 and 222 (see fragmentation scheme 6) whilst the $\left[M-CCl_3\right]^+$ ion from the oxathiin (86)

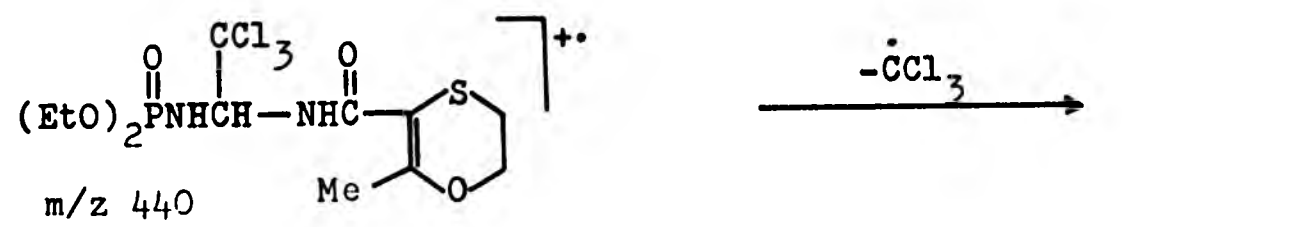
Fragmentation scheme 6

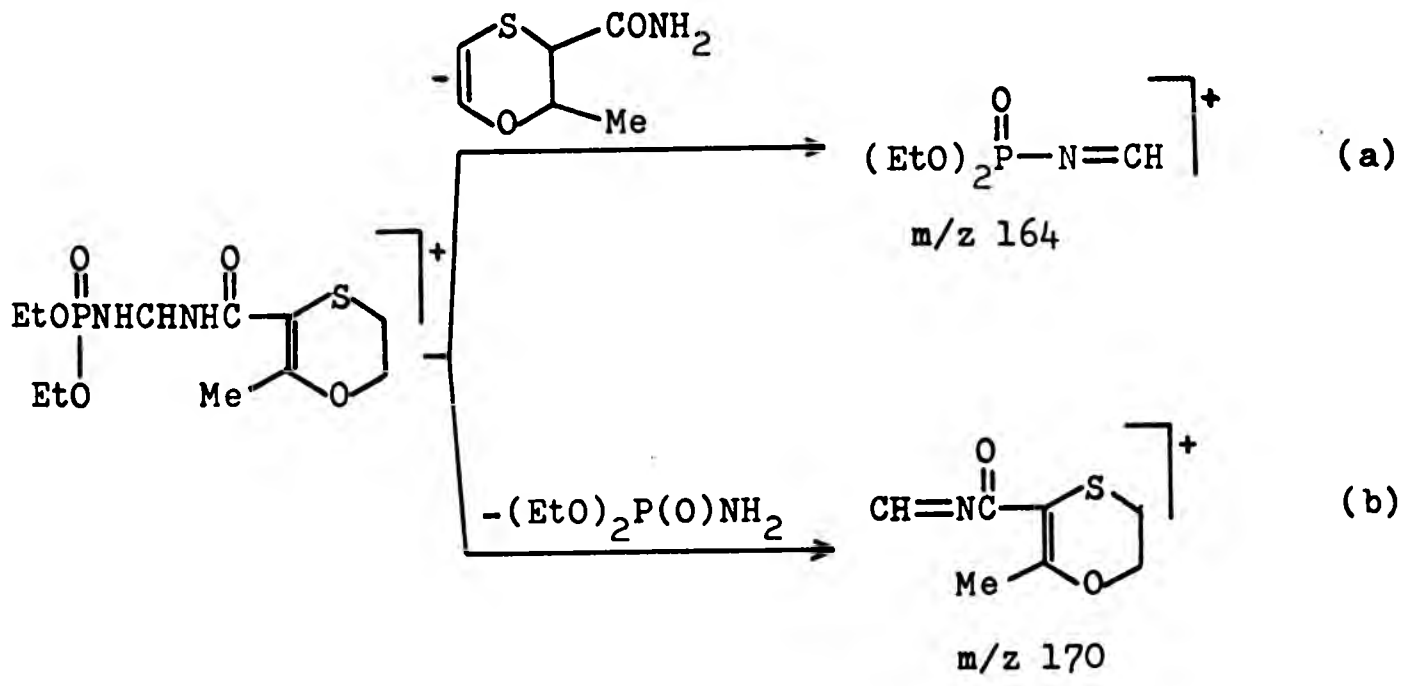




appears to undergo loss of either oxathiincarboxamide, or diethyl phosphoramidate as neutral species (see fragmentation scheme 7).

Fragmentation scheme 7



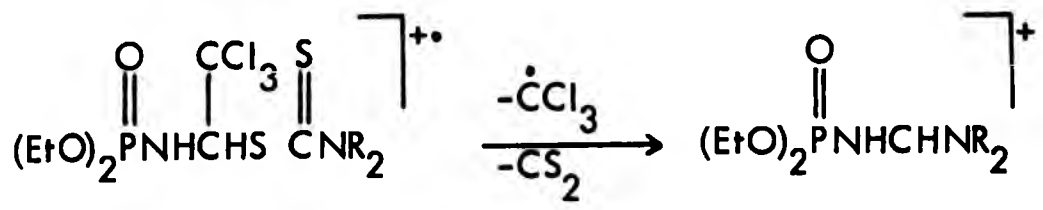


(a) Continues as in scheme (2)

(b) Continues as in scheme (14)

Weak but noticeable ions in the mass spectra of dithiocarbamates (92 and 93) can possibly be explained on the basis of loss of trichloromethyl and of carbon disulphide (see fragmentation scheme 8) although the order in which the processes occur is not certain.

Fragmentation scheme 8



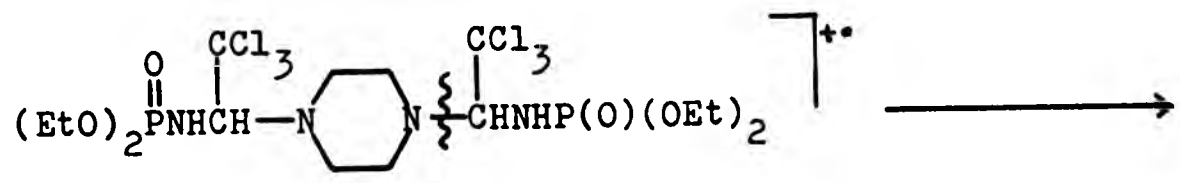
m/z 209 (R=Me)

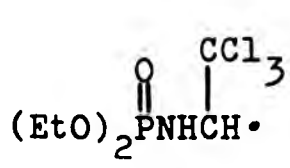
m/z 237 (R=Et)

3.3.1.3 Fragmentation by route (c)

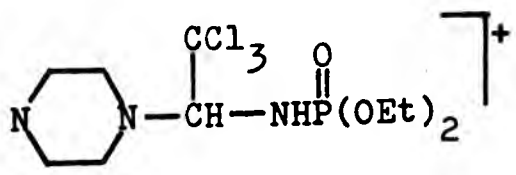
Cleavage of the $(EtO)_2P(O)NHCHCCl_3$ group as a neutral radical is observed in the 1,4-disubstituted piperazine (79) and in the O,S-disubstituted 2-hydroxyethanethiol (81), as shown in fragmentation schemes 9 and 10 respectively.

Fragmentation scheme 9



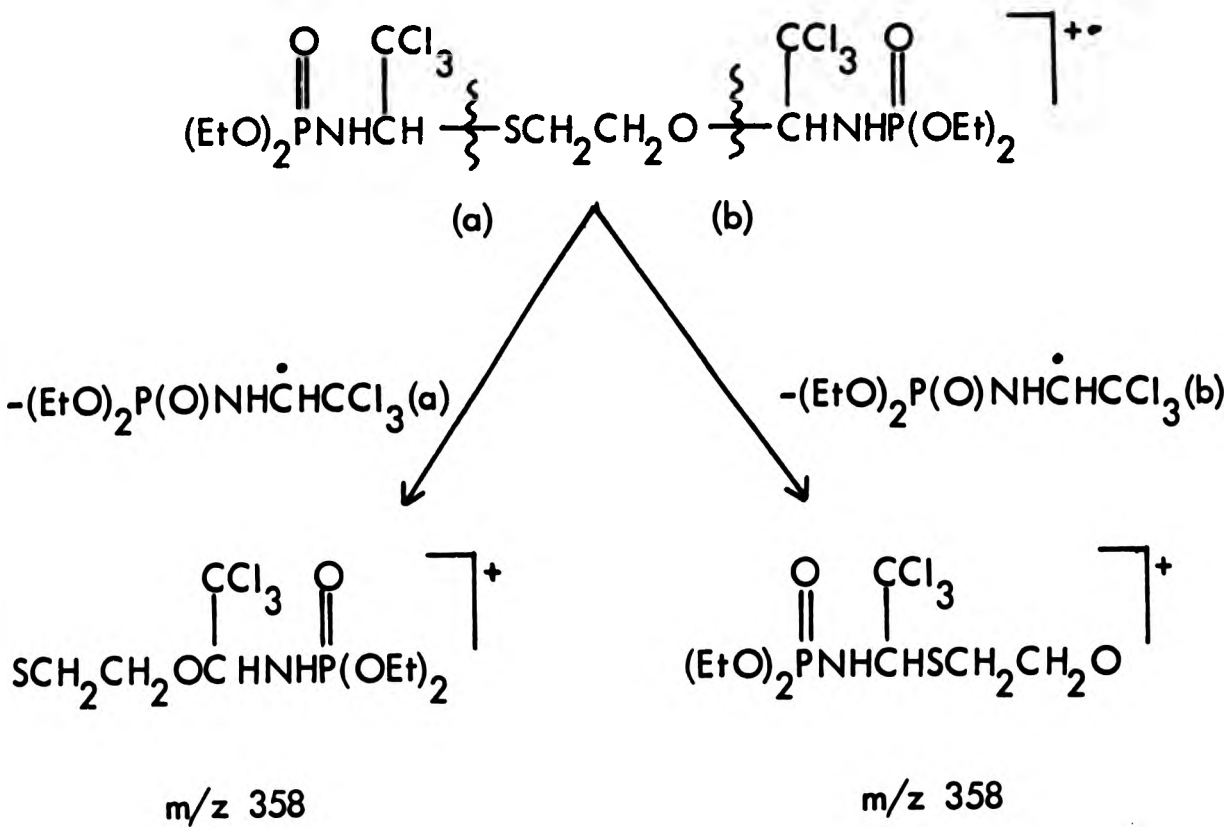


+

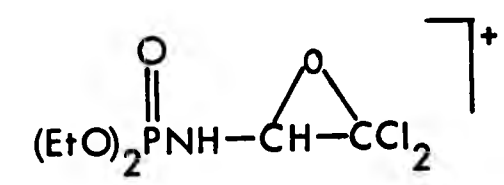


m/z 366

Fragmentation scheme 10



In the case of the 2-hydroxyethanethiol derivative (81), cleavage could occur at either the C-S or the C-O bond, giving in each case an ion of m/z 358. Cleavage of the C-S bond can be assumed to have occurred in the formation of a multiplet at 263, 265 and 267 with an abundance ratio suggesting that these correspond to one type of fragment containing two chlorine atoms. The structure (121) can possibly be assigned to this ion although the exact mode of its formation is not clear.

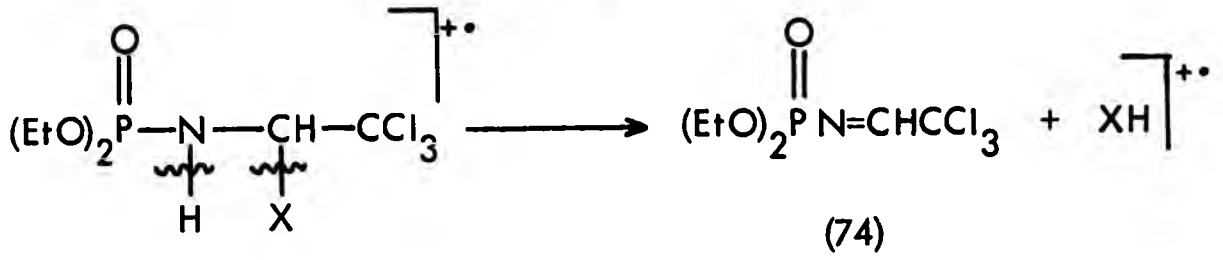


(121)

3.3.1.4 Fragmentation by route (d)

The formation of an ion $\overline{XH}^{+\bullet}$ was observed in the mass spectra of the imidazolyl (76), triazolyl (77), dithiocarbamate (92, 93) and xanthate (94) derivatives, and can be rationalised by assuming the loss of the trichloromethylimine (74) as a neutral molecule (see fragmentation scheme 11).

Fragmentation scheme 11



(74)

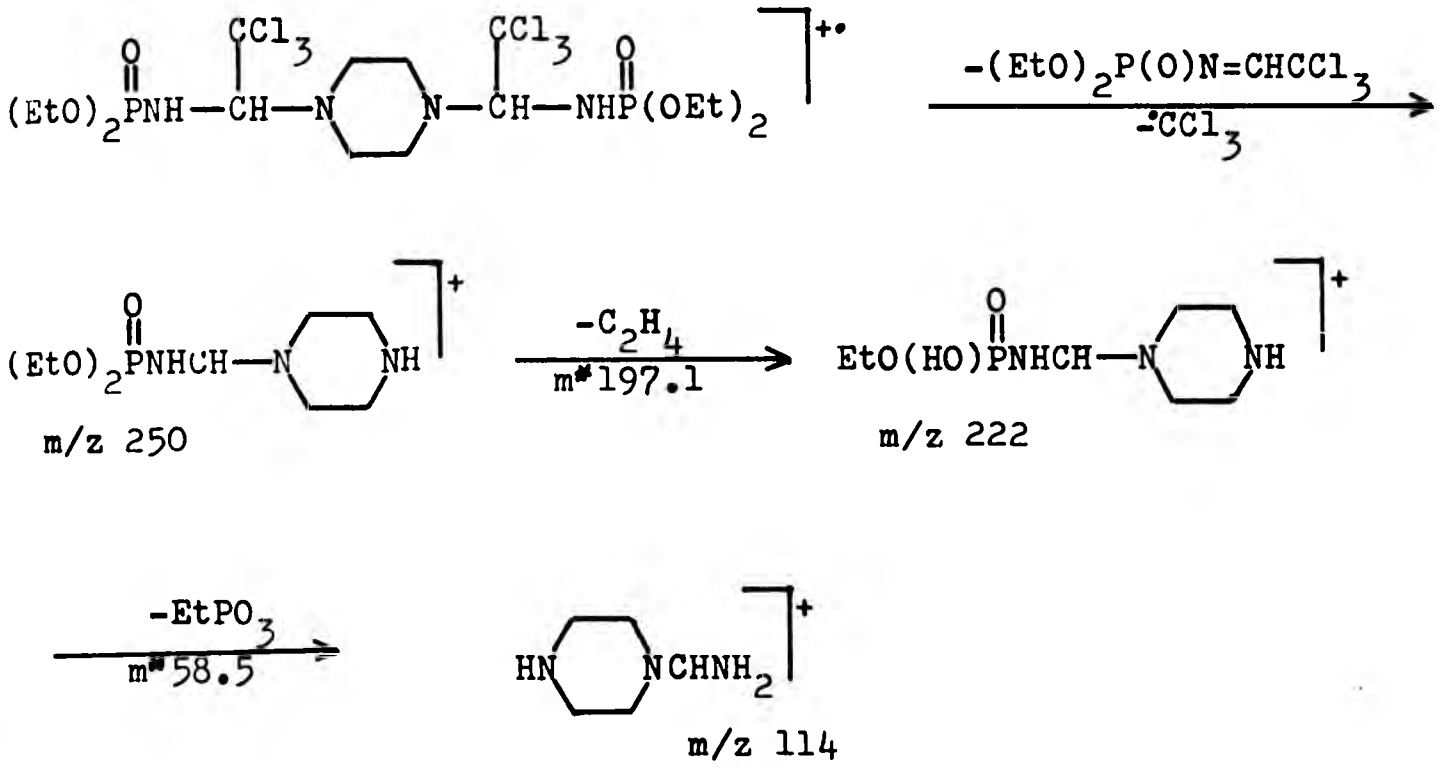
XH = imidazole (m/z 68), triazole (m/z 69),

Me_2NCS_2H (m/z 121), Et_2NCS_2H (m/z 149)

or $EtOCS_2H$ (m/z 122)

A similar rearrangement with hydrogen transfer presumably occurs in the case of the symmetrically 1,4-disubstituted piperazine derivative (79) and will account for the series of ions shown (see fragmentation scheme 12), after the loss also of $\bullet CCl_3\bullet$.

Fragmentation scheme 12



3.3.1.5 Other significant fragmentations

In addition to those noted above, the dithiocarbamate compounds (92, 93) gave characteristic ions corresponding to the structures: R_2NCS^+ (R=Me, m/z 88; R=Et, m/z 116), $RNHCS^+$ (R=Et, m/e 88), and CS_2^+ (m/z 76). The ion at m/z 88 is a characteristic fragment of several dimethyldithiocarbamates such as ferbam, ziram and thiram.[85]

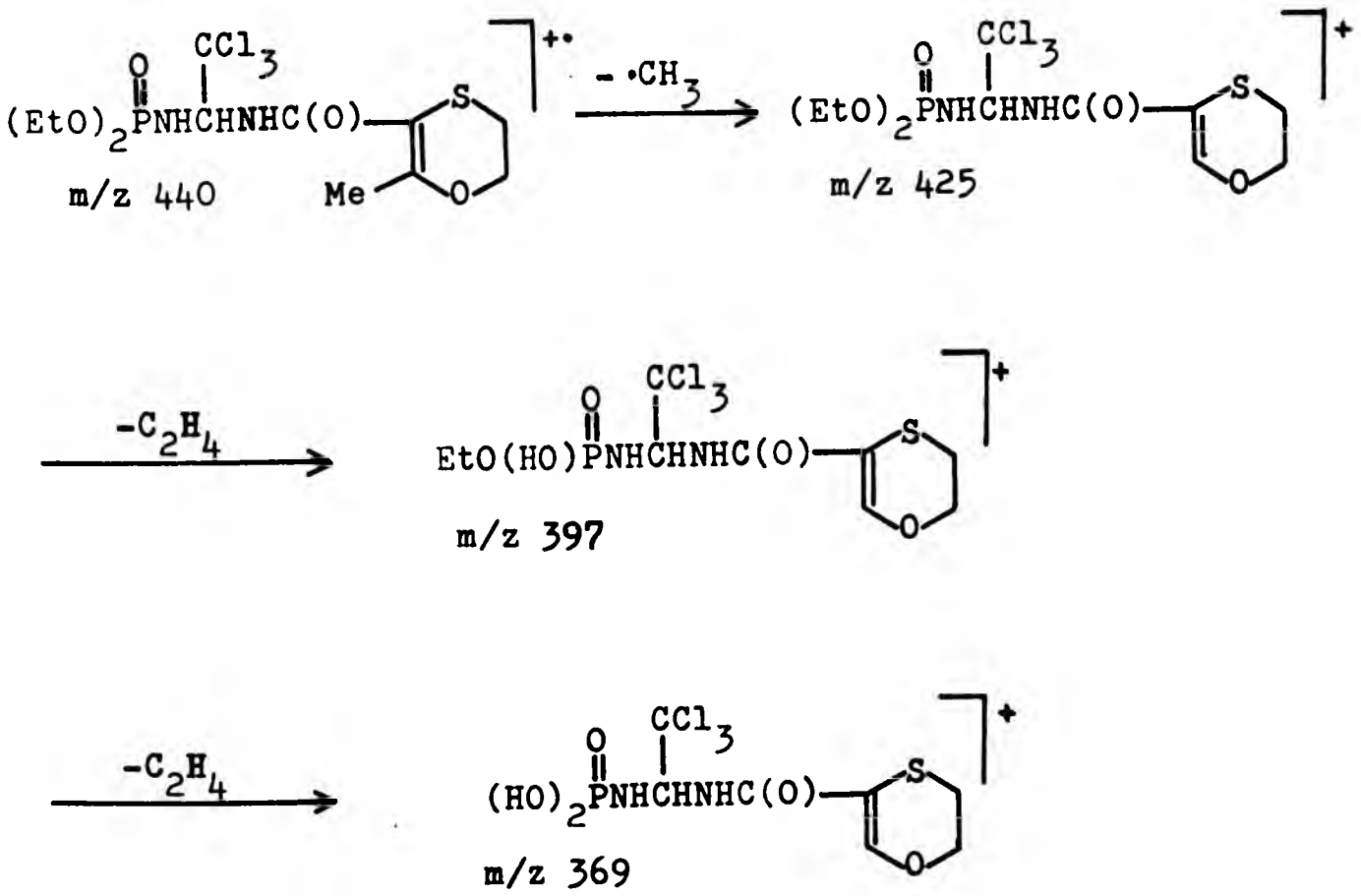
From the xanthate derivative (94), peaks assigned to CS_2^+ (m/z 76) and COS^+ (m/z 60) were obtained.

The oxathiin (86) gave a molecular ion at m/e 440 which underwent a number of fragmentations in addition to the loss of X• (fragmentation scheme 2) and of $\cdot CCl_3$ described above, which were useful for the confirmation of its structure:

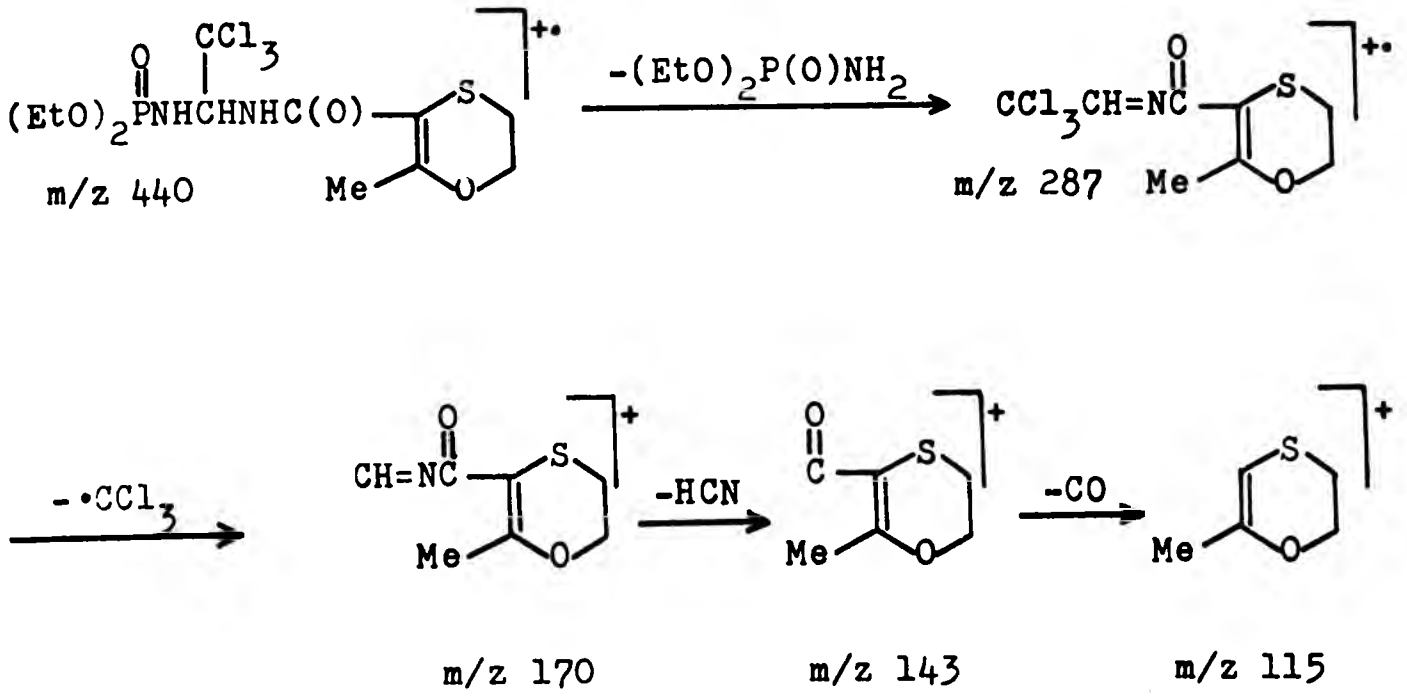
i) Loss of the methyl group from the oxathiin substituent followed by the loss of two molecules of ethylene (see fragmentation scheme 13);

ii) Rearrangement to eliminate diethyl phosphoramidate with the subsequent loss of $\cdot CCl_3$, HCN, and CO (see fragmentation scheme 14).

Fragmentation scheme 13



Fragmentation scheme 14



The peaks which appear at m/z 143 and 115 are also characteristic of the mass spectrum of carboxin (16).[85]

It is difficult to follow these fragmentation pathways any further because of the large number of different ions at lower m/z values and the complete absence of metastable ions to confirm the transitions that actually occur.

The mono-substituted 2-hydroxyethanethiol derivative (82) gave an ion at m/z 341 which can be assumed to arise from the dehydration of the molecular ion, although the latter was not detectable. This dehydration

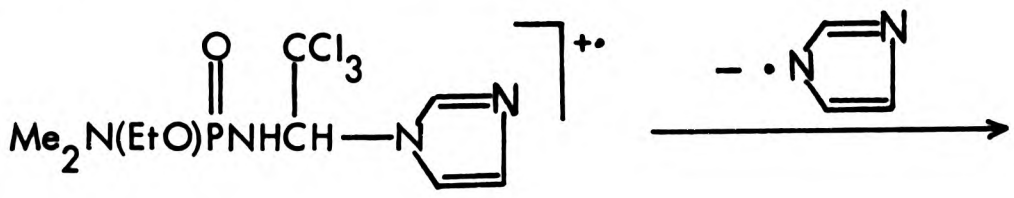
is commonly observed for alcohols and would seem to confirm that the compound contains a free hydroxyl group and therefore that it is the sulphur atom and not oxygen which is bonded to the trichloroethyl group.

3.3.2 Fragmentation of bis(dimethylamino)phosphinyl and ethoxydimethylaminophosphinyl derivatives

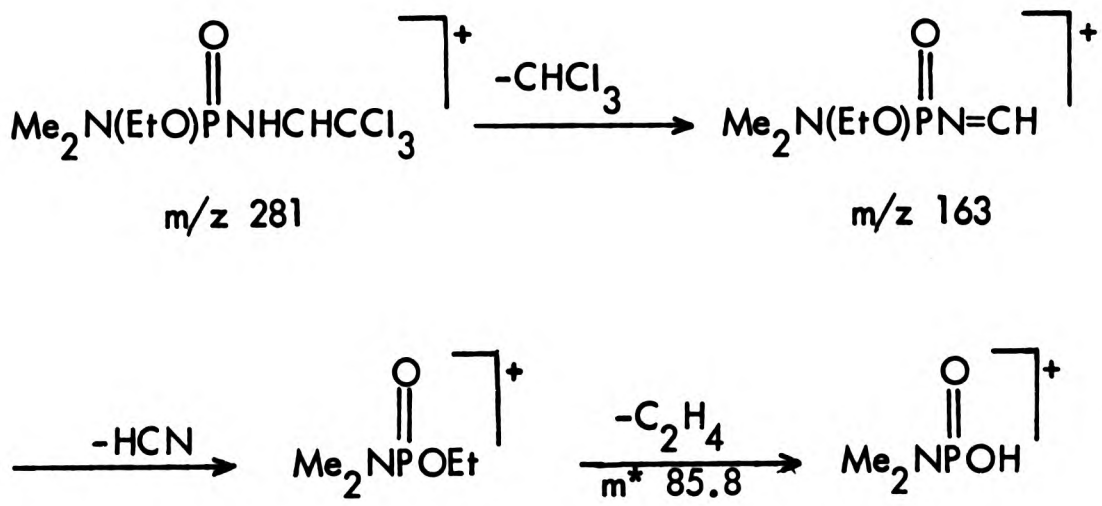
Certain fragmentations of the bisdimethylamino compound (87) are referred to above Fragmentation scheme 1, X = $NHP(O)(NMe)_2$; and also loss of $\cdot CCl_3$ which gives an ion with m/z 315 . In addition, at the high mass end of the spectrum one can see $\left[M-Cl\right]^+$ at m/z 397 and $\left[M-Me_2N\right]^+$ at m/z 388 whilst in the mid-mass range the ions $(Me_2N)_2P(O)N=CH^+$ at m/z 162 and $(Me_2N)_2P=O^+$ at m/z 135 are observed.

The compound containing both dimethylamino and ethoxy groups attached to phosphorus (110) fragments giving rise to ions at m/z 281, 163, 136 and 108 (see fragmentation scheme 15). These correspond to the ions found at m/z 282, 164, 137 and 109 in the diethoxy analogues, however, there is no ion corresponding to that at m/e 81 in the mass spectra of the diethoxy analogues since the fragment at m/z 108 has the structure $\left[Me_2NP(O)OH\right]^+$ and cannot lose another molecule of ethylene. In addition ions at m/z 312 corresponding to the loss of HCl and at m/z 280, corresponding to the loss of imidazole were observed, the latter being more abundant than that at m/z 281.

Fragmentation scheme 15



m/z 348



m/z 281

m/z 163

CHAPTER 4, BIOLOGICAL ACTIVITY

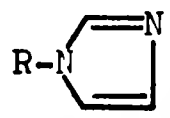
For reference, structures of all novel compounds are shown in Appendix I.

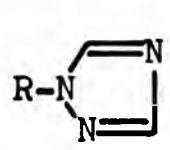
4. BIOLOGICAL ACTIVITY

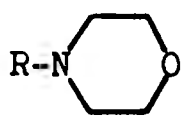
4.1 Subdivision into Compound Types

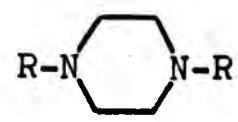
The compounds under investigation can be grouped together into various types as indicated below in which R = $(EtO)_2P(O)NHCH(CCl_3)$- and R' = $(Me_2N)(EtO)P(O)NHCH(CCl_3)$-

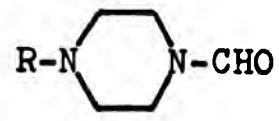
Group I : based on nitrogen heterocycles

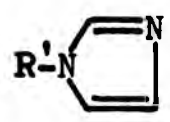
76 

77 

78 

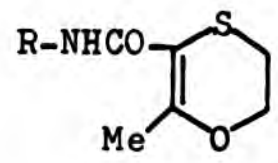
79 

80 

102 

Group II : carboxamide derivatives

84 $R\text{-}NHCOCH_3$

86 

Group III : thio compounds

81 $R\text{-}SCH_2CH_2O\text{-}R$

82 $R\text{-}SCH_2CH_2OH$

92 $R\text{-}SC(S)NMe_2$

93 $R\text{-}SC(S)NEt_2$

94 R-SC(S)OEt

Group IV : bisphosphoramides

85 $R-NHP(O)(OEt)_2$ 87 $R-NHP(O)(NMe_2)_2$

4.2 Fungicidal Activity

The compounds were screened for fungicidal activity as described in the Experimental section. The organisms against which the compounds were screened are shown in table VII. The names of the pathogens have been abbreviated as shown in parentheses for use in the activity tables.

Table VII

Pathogen		Disease
Fusarium culmorum	(F.c.)	Damping off, brown foot rot and ear blight
Fusarium oxysporum	(F.o.)	
Ophiobolus graminis	(O.g.)	Take all
Helminthosporium sativum	(H.s.)	Foot rot of wheat and barley
Septoria nodorum	(S.n.)	Glume blotch of wheat
Helminthosporium avenae	(H.a.)	Oat leaf spot or stripe
Piricularia oryzae	(P.o.)	Rice blast
Rhizoctania solani	(R.s.)	Sharp eyespot
Dreschlera teres	(D.t.)	Barley net blotch

The results of *in vitro* testing carried out at the Polytechnic of North London are shown in table VIII. Germination was assessed by comparing each plate with the controls and standards. The extent of germination was recorded on a five point scale as shown below:-

0 No inhibition of growth

1 Slight but noticeable inhibition of growth

2 Extensive inhibition of growth

3 Very slight growth hardly visible to the naked eye but visible under the microscope (X100)

4 Complete inhibition of growth

\- A dash indicates that no results were obtained for these tests.

Table VIII

Fungicidal activity of phosphoramidates in vitro at 500 ppm

Compound (group)		F.c. (a)	F.c. (b)	F.o. (a)	F.o. (b)	O.g. (a)	O.g. (b)	H.s. (a)	H.s. (b)	S.n. (a)	S.n. (b)	H.a. (a)	H.a. (b)
76	(I)	1	0	0	0	0	0	1	-	0	0	0	0
77	"	2	0	0	0	0	0	1	-	0	0	0	0
78	"	0	0	0	0	0	0	0	-	0	0	0	0
79	"	0	0	0	0	0	0	0	-	0	0	0	0
80	"	1	0	0	0	0	0	1	-	0	0	0	0
102	"	2	0	1	0	0	0	1	-	0	0	0	0
84	(II)	0	0	0	0	0	0	0	-	0	0	0	0
86	"	0	0	0	0	0	0	0	-	0	0	0	0
81	(III)	1	0	0	0	0	0	0	-	0	0	0	0
82	"	2	0	0	0	1	0	1	-	0	0	0	0
92	"	4	4	4	0	4	1	4	-	0	0	4	3
93	"	3	0	3	0	3	1	4	-	2	0	4	3
94	"	2	0	0	0	3	1	4	-	3	0	0	0
85	(IV)	1	0	0	0	0	0	0	-	0	0	0	0
87	"	-	-	0	0	0	0	-	-	0	0	0	0

(a) After incubation for one week

(b) After incubation for one month

Additional screening carried out by KenoGard AB is reported in table IX. For both *in vitro* and *in vivo* tests the results are expressed as a percentage inhibition.

Table XI

Further screening of phosphoramidates

Compound (group)		*In vitro* 300 ppm P.o.	R.s.	*In vivo* 20% D.t.	S.n.
76	(I)	18	24	26	28
77	"	18	0	9	21
78	"	13	33	6	23
79	"	22	0	0	23
80	"	16	0	14	2
102	"	47	4	30	16
84	(II)	27	40	21	0
86	"	21	0	23	8
81	(III)	14	0	-	-
82	"	19	3	15	22
92	"	26	46	27	18
93	"	48	51	33	5
94	"	21	17	22	19
85	(IV)	9	0	7	8

4.2.1 Overall performance of the compounds

From the results of the screening carried out by both P.N.L. and KenoGard certain trends emerge, with all the compounds showing at least some fungicidal activity. All were active against *P. oryzae in vitro* and most were against *D. teres in vivo*. Contrasting results were obtained for *S. nodorum*, with most compounds showing activity *in vivo*, but very little activity *in vitro*.

Group I

This group of compounds showed activity against *F. culmorum*, *H. sativum* and *P. oryzae in vitro* and against *S. nodorum in vivo* but no activity *in vitro*.

Group II

This group showed activity against *P. oryzae in vitro* and *D. teres in vivo* but in general there was little activity against the other organisms, except for the acetamide derivative (84) which showed moderate activity against *R. solani in vitro*.

Group III

This group showed the greatest fungicidal activity, particularly

the dithiocarbamate derivatives (92, 93). In nearly all cases it was one of these two compounds which showed the highest activity against any given organism, except for S. nodorum in both of the independent tests. The xanthate (94) showed the highest activity against S. nodorum in vitro and was also better than the two dithiocarbamates in vivo, though the best control of this pathogen in vivo was shown by the compounds of group I.

Group IV

These compounds showed only slight activity in the tests.

4.3 Phytotoxicity

The results of the phytotoxicity tests are presented in table X. Germination (column a) is expressed as a percentage relative to untreated seeds. Plant development (bonitet, column b) is expressed on a scale of 1-5 and is relative to that of untreated seeds which is taken as 4 on the same scale.

Table X Phytotoxicity

Compound (group)		Winter wheat (a)	(b)	Spring barley (a)	(b)
76	(I)	-20	2	-5	2.5
77	"	-20	3	0	4
78	"	-15	2	-5	3
79	"	0	3.5	-5	3
80	"	-10	3	0	4
102	"	+ 5	3	0	4
84	(II)	- 5	4	-5	4
86	"	- 5	4	0	4
82	(III)	0	4	0	4.5
92	"	- 5	3	-5	3
93	"	- 5	3	0	3.5
94	"	- 5	3	-5	3
85	(IV)	- 5	4	0	4

Group I

Most of these compounds badly affected both germination and early plant development though it is interesting to note that the ethoxy dimethylamino derivative of imidazole (102) gave a 5% improvement in the germination of Winter wheat whereas its diethoxy analogue (76) gave a 20% decrease.

Group II

This group was not as phytotoxic as group I and early plant development was unaffected though germination was on the whole slightly suppressed.

Group III

This group, whilst not as phytotoxic as group I was more so than group II and on the whole badly affected both germination and early plant development.

Group IV

Compound (85) had no effect on early plant development but slightly suppressed the germination of Winter wheat.

4.4 Anticholinesterase Activity

Diethyl N-(1-hydroxy-2,2,2-trichloroethyl)phosphoramidate (70) and its homologues (methyl, propyl etc) have been referred to in the literature as insecticidal.[60] Since many of the insecticidal organophosphates, including some phosphoramidates are cholinesterase inhibitors it was decided to examine the anticholinesterase activity of the present series of compounds. Results are presented in table XI in which: 'Conc' is the concentration of the test

compound in μg/ml; '% I' is the percentage acetylcholinesterase inhibition at that concentration; 'A' is a rough estimate of the anti-cholinesterase activity relative to parathion = 100, based on the formula A = 100 x (% I/Conc.) x (2.4/50).

Table XI Anticholinesterase activity

Compound (group)		Conc.	% I	A
76	(I)	488	9.9	0.10
77	"	492	50.0	0.49
78	"	640	20.8	0.16
79	"	109	7.3	0.32
80	"	131	8.0	0.29
102	"	760	35.8	0.23
84	(II)	56	10.4	0.89
86	"	75	3.2	0.20
81	(III)	128	49.2	1.82
82	"	185	7.7	0.20
92	"	200	53.2	1.28
93	"	103	27.4	1.28
94	"	123	58.2	2.27
85	(IV)	732	16.5	0.11
87	"	152	6.1	0.19
Parathion		2.4	50.0	100

As can be seen from the results the compounds of group III are the most potent inhibitors of acetylcholinesterase (being on average about five times more active than compounds of the other groups) though even these show very low in vitro inhibition compared to parathion. The higher anticholinesterase activity of the compounds of group III may be due to their easier hydrolysability (and therefore their greater potential of phosphorylation of the cholinesterase enzyme) than that characterising other compounds.

CHAPTER 5, EXPERIMENTAL

5. EXPERIMENTAL

5.1 Purification of Solvents and Reagents

Solvents

Hydrocarbon solvents were distilled and stored over sodium wire. Diethyl ether was dried over sodium wire. Carbon tetrachloride was distilled from a little phosphorus pentoxide. Chloroform was heated under reflux with phosphorus pentoxide to remove ethanol added by the suppliers as a preservative; it was then decanted, distilled from fresh phosphorus pentoxide and stored in an amber glass bottle in the dark.

Reagents

Phosphorus chloride, thionyl chloride and chloral were distilled. Triethylamine, pyridine and morpholine were fractionally distilled through a 25 cm column packed with glass helices. Ethyl potassium xanthate was recrystallised from acetone/diethyl ether. Imidazole was recrystallised from chloroform. Sodium diethyldithiocarbamate and sodium dimethyldithiocarbamate were dried at 120°C *in vacuo*. Other reagents were used as supplied.

5.2 Analytical techniques

Chlorine analysis

Chlorine was determined by the Shoniger oxygen flask method which involves burning the sample in an atmosphere of oxygen, absorption into aqueous sodium hydroxide then potentiometric titration with silver nitrate.[86]

Phosphorus analysis

Phosphorus was determined by digestion of the sample in a mixture of concentrated nitric and sulphuric acids then estimation as magnesium ammonium phosphate.[87]

Carbon, hydrogen and nitrogen analysis

Carbon, hydrogen and nitrogen were determined by The Polytechnic of North London microanalytical services, using a Perkin-Elmer 240 instrument.

Spectra

Nuclear magnetic resonance (nmr) spectra were obtained on a Perkin-Elmer R12B nuclear magnetic resonance spectrometer operating at 60 MHz.

Mass spectra (ms) were obtained on an AEI MS9 mass spectrometer at 70 e.V.

Infra-red (ir) spectra were obtained on a Pye-Unicam SP 2,000 infra-red spectrophotometer.

Melting Points

Melting points were determined on a Gallenkamp melting point apparatus in degrees Celsius and are uncorrected.

5.3.1 Preparation of diethyl phosphoramidate

Diethyl phosphite (53.6g, 0.388 mol) was dissolved in carbon tetrachloride (100 cm^3). The reaction mixture was stirred and anhydrous ammonia was passed through. After about 2 min the reaction mixture became cloudy due to the precipitation of ammonium chloride, and after about 10 min the solvent began to boil due to the heat evolved from the exothermic reaction. After about 1h the evolution of heat subsided and the reaction mixture cooled down. Ammonia was passed through for a further 2h and the precipitate of ammonium chloride was then filtered off under reduced pressure, washed with chloroform (3 x 20 cm^3) and dried (19.2g, 92.6%). The filtrate and washings were combined and the solvent was removed in vacuo. The resultant colourless oil (61.4g) was triturated with light petroleum (b.p. 60-80°C) to give the product as a white deliquescent solid. This was filtered off in a closed sintered glass filter, washed with light petroleum (b.p. 60-80°C) (3 x 20 cm^3) and then dried to give diethyl phosphoramidate (55.5g, 94.3%), m.p. 40-42°C, (lit.[61] 50-51°C) (Found: C, 31.1; H, 7.7; N, 9.1; P, 20.2. Calc. for $C_4H_{12}NO_3P$: C, 31.4; H, 7.9; N, 9.1; P, 20.2%).

In a similar experiment diethyl phosphite (53.6g, 0.388 mol) in carbon tetrachloride (200 cm^3) was treated with anhydrous ammonia to give ammonium chloride (18.9g, 91.2%) and the title product as a sticky white solid (55.7g, 93.8%) m.p. 43-46°C. A portion of the product was recrystallised from cyclohexane/carbon tetrachloride to give a purer specimen

of the phosphoramidate, m.p. 45-46°C (Found: C, 31.1; H, 7.7; N, 9.0%).

5.3.2 Preparation of diethyl N-(1-hydroxy-2,2,2-trichloroethyl)-phosphoramidate (73)

(a) In the absence of solvent

Anhydrous chloral (5.21g, 0.0353 mol) was added to diethyl phosphoramidate (5.41g, 0.0353 mol). The mixture was stirred and heated on an oil bath to 90-95°C for 4h to give a yellow oil. The bulk of the oil was dissolved in diethyl ether and a white solid (0.38g) (Found: C, 12.7; H, 6.0; N, 13.0. Calc. for $C_6H_{13}Cl_3NO_4P$: C, 24.0; H, 4.4; N, 4.7%) was filtered off. The ether was removed from the filtrate and attempts were made to crystallise the remaining oil from methanol, and then chloroform, both of which failed. Each successive solvent was removed under reduced pressure. The oil was then heated under reflux for 15 min with light petroleum (b.p. 30-40°C, 20 cm^3) whence it solidified on cooling to a waxy solid. The light petroleum was decanted from the waxy solid which was then heated under reflux with cyclohexane (20 cm^3). On cooling an oil separated out and crystallised on stirring over the weekend to give off-white crystals together with a small amount of oil. The oil and the solvent were removed by suction filtration and the crystals were washed with cyclohexane (20 cm^3) and dried in air to give the phosphoramidate (4.81g, 45.3%) m.p. ca. 75°C (Found: C, 24.0; H, 4.7; N, 4.3. Calc. for $C_6H_{13}Cl_3NO_4P$: C, 24.0; H, 4.4; N, 4.7%). Recrystallisation from light petroleum (b.p. 60-80°C) gave a purer product m.p.

89-91°C (Found: C, 23.7; H, 4.2; N, 4.4. Calc. for $C_6H_{13}Cl_3NO_4P$: C, 24.0; H, 4.4; N, 4.7%), although the melting point was still below that previously reported (lit.[59,60] 96-97°C).

(b) In chloroform

Diethyl phosphoramidate (14.1g, 0.092 mol) was dissolved in chloroform (100 cm^3) and anhydrous chloral (13.6g, 0.092 mol) was added to the solution which was heated under reflux for 6h and then left to stand for 3 days. The slightly cloudy solution was then filtered and the solvent was removed in vacuo to leave a pale yellowish oil which was crystallised from light petroleum (b.p. 30-40°C) to give the desired product (25.5g, 92.1%), m.p. 93-95°C (lit.[59,60] 96-97°C) (Found: C, 23.8; H, 4.5; N, 4.4. Calc. for $C_6H_{13}Cl_3NO_4P$: C, 24.0; H, 4.4; N, 4.7%).

(c) In alcoholic solution

Diethyl phosphoramidate (6.77g, 0.0442 mol) and chloral (6.52g, 0.0442 mol) were dissolved in 74° o.p. I.M.S. (70 cm^3) and heated under reflux for 6h. The only identifiable product (ir and nmr) was trichloroacetaldehyde ethyl hemiacetal (3.2g) m.p. 47-49°C (lit.[88] 56-57°C) (Found: C, 20.3; H, 3.1. Calc. for $C_4H_7Cl_3O_2$: C, 24.8; H, 3.6%), which was collected as a sublimate during rotary evaporation.

5.3.3 Preparation of diethyl N-(1,2,2,2-tetrachloroethyl)-phosphoramidate (74)

Thionyl chloride (8.2g, 0.069 mol) was added to a solution of diethyl N-(2,2,2-trichloro-1-hydroxyethyl)phosphoramidate (14.2g, 0.0473 mol) in chloroform (80 cm^3). The mixture was heated under reflux for 6h and then left to stand for 4 days. The solution was filtered and the solvent was removed in vacuo to leave a yellow oil containing a little solid. This mixture was crystallised from a mixture of chloroform and light petroleum (b.p. 60-80°C). The crystals were filtered off with the exclusion of moisture, washed with light petroleum and dried under high vacuum to give the desired product (7.32g, 52%), m.p. 104-106°C (lit.[63] 95-110°C) (Found: C, 22.9; H, 3.9; N, 4.4. Calc. for $C_6H_{12}NO_3P$: C, 22.6; H, 3.8; N, 4.4%).

5.3.4 Preparation of diethyl N-(1,2,2,2-tetrachloroethyl)-phosphoramidate (74) without isolation of the hydroxy compound (73)

Diethyl phosphoramidate (39.4g, 0.258 mol) was dissolved in absolute chloroform (110 cm^3). Chloral (40.8g, 0.277 mol) was added and the reaction mixture was stirred overnight after which time it had turned brownish. Decolourising charcoal (ca. 2g) was added and the reaction mixture was stirred for a further 24h and then filtered. Nmr spectroscopy showed that the reaction was complete. The system was allowed to stand over the weekend after which time it was heated under reflux with

decolourising charcoal (ca. 2g) and then filtered. Thionyl chloride (57.4g, 0.482 mol) was added when a small amount of very fine white precipitate formed immediately. The reaction mixture was then allowed to stand for 24h, and was filtered with the aid of a little Kieselguhr to remove the haze, and was heated under reflux for 6h to complete the reaction (monitored by nmr). The solvent was removed under reduced pressure and the resultant orange oil was heated under reflux with light petroleum (b.p. 60-80°C, 200 cm^3) to give off white crystals in an orange solution on cooling. The solid was recrystallised from light petroleum/chloroform to give the title compound (25.8g, 31.4%) m.p. 104-107°C (Found: C, 22.9; H, 3.9; Cl, 44.5; N, 4.1; P, 9.9. Calc. for $C_6H_{12}Cl_4NO_3P$: C, 22.6; H, 3.8; Cl, 44.5; N, 3.8; P, 9.7%).

5.3.5 Nmr studies of the reaction between diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate and water

A small amount of diethyl N-(1,2,2,2-tetrachloroethyl)-phosphoramidate (ca. 20 mg) was placed in an nmr tube and dissolved in sufficient deuterochloroform to ensure that the water added later would be outside the effective region of the instrument probe. The ^1H nmr spectrum was run, water (3 drops) was added and the spectrum was recorded periodically over a time interval of 25 min, with periodic shaking. Results are discussed in Ch.2. (See p. 31-34.)

5.3.6 Preparation of diethyl-N-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl] phosphoramidate (76)

Triethylamine (0.631g, 0.00624 mol) was added to a solution of diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.99g, 0.00624 mol) in benzene (10 cm^3). A white precipitate (triethylammonium chloride) formed immediately and the mixture became warm (ca. 35°C). After 10 min the white precipitate was filtered off and washed with benzene (5 cm^3). To the combined filtrate and washings was added imidazole (0.425g, 0.00624 mol) which dissolved immediately. After about 5 min crystals began to form in the benzene solution which was allowed to stand for 1h. The mother liquor was then decanted from the yellowish crystals which were dissolved in hot toluene/chloroform and the solution was decanted from a small amount of brown oil. The colourless solution crystallised overnight after which time the white solid was filtered off and dried to give diethyl N-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl]phosphoramidate (0.544g, 28.9%) m.p. 113.5-116° (Found: C, 30.7; H, 4.5; Cl, 30.6; N, 12.0; P, 8.4. $C_9H_{15}Cl_3N_3O_3P$ requires: C, 30.8; H, 4.3; Cl, 30.3; N, 12.0; P, 8.8%). Further crops of crystals were obtained from the filtrate and from the original benzene mother liquor by treatment with light petroleum: 0.146g (6.7%) m.p. 111-113°C, 0.757g (34.6%) m.p. 108-112°C, 0.198g (9.1%) m.p. 109-112°C. Total yield 1.645g, 75.2%.

In a similar preparation triethylamine (1.53g, 0.015 mol) was added to a solution of diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate

(4.83g, 0.015 mol) in benzene (30 cm^3). A white precipitate formed immediately. The flask was stoppered, shaken gently and then allowed to stand for 10 min after which the solid was filtered off and washed with benzene (10 cm^3 + 5 cm^3). Imidazole (1.02g, 0.015 mol) was added to the combined filtrate and washings and dissolved quickly evolving a little heat. The reaction mixture was left to stand for 10 min and the solvent was then removed to leave a slightly off white solid which was recrystallised from a mixture of chloroform, benzene and light petroleum (b.p. 60-80°C) to give diethyl N-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl]phosphoramidate (4.91g, 93%) m.p. 118-120°C, (Found: C, 30.7; H, 4.3; Cl, 30.3; N, 12.0; P, 8.8. $C_9H_{15}Cl_3N_3O_3P$ requires: C, 30.8; H, 4.3; Cl, 30.3; N, 12.0; P, 8.8%).

5.3.7 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(morpholin-4-yl)ethyl]phosphoramidate (78)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (2.15g, 0.0067 mol) was dissolved in benzene (15 cm^3). Triethylamine (0.94 cm^3), 0.0067 mol) was added. The solution was shaken gently and allowed to stand for 10 min, and the white precipitate of triethylammonium chloride was then filtered off, washed with benzene and dried (0.33g, 92%). The filtrate and washings were combined, and morpholine (0.58 cm^3, 0.0067 mol) was added. The flask was again stoppered, shaken and allowed to stand for 10 min. The solvent was removed in vacuo to give a slightly off white solid which was recrystallised from a mixture of chloroform and light petroleum (b.p. 60-80°C),

yielding diethyl N-[2,2,2-trichloro-1-(morpholin-4-yl)ethyl]phosphoramidate (1.95g, 78%), m.p. 127.5-128.5°C, (Found: C, 32.0; H, 5.4; Cl, 28.9; N, 7.5; P, 8.4. $C_{10}H_{20}Cl_3N_2O_4P$ requires: C, 32.5; H, 5.4; Cl, 28.8; N, 7.6; P, 8.4%).

5.3.8 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(dimethylthiocarbamoylthio)ethyl]phosphoramidate (92)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (4.73g, 0.0148 mol) was dissolved in dry benzene (20 cm^3), and anhydrous sodium dimethyldithiocarbamate (2.12g, 0.0148 mol) was added. The reaction mixture was heated under reflux for 1h and then allowed to stand overnight. The sodium chloride which precipitated was filtered off and the solvent was removed from the filtrate in vacuo to give a pale yellow semi-solid which was crystallised from a mixture of chloroform, benzene and light petroleum (b.p. 30-40°C) to yield diethyl N-[2,2,2-trichloro-1-(dimethylthiocarbamoylthio)ethyl]phosphoramidate as a slightly off white solid (4.79g, 80.4%), m.p. 110-112°C, (Found: C, 26.5; H, 4.5; Cl, 26.4; N, 6.9; P, 7.6. $C_9H_{18}Cl_3N_2O_3PS_2$ requires: C, 26.8; H, 4.5; Cl, 26.3; N, 6.9; P, 7.7%).

5.3.9 Preparation of O,O-diethyl N-]2,2,2-trichloro-1-(diethylthiocarbamoylthio)ethyl]phosphoramidate (93)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.81g,

0.0057 mol) was dissolved in dry benzene (15 cm^3). Anhydrous sodium diethyldithiocarbamate was then added and the reaction mixture was heated under reflux for 0.5h and allowed to stand at room temperature for 4h. The precipitate of sodium chloride was filtered off and the solvent was removed in vacuo to leave a pale orange oil which was crystallised from a mixture of chloroform and light petroleum (b.p. 30-40°C) to give diethyl N-[2,2,2-trichloro-1-(diethylthiocarbamoylthio)ethyl]phosphoramidate (2.08g, 84.7%), m.p. 103-104.5°C, (Found: C, 30.5; H, 5.3; Cl, 24.6; N, 6.4; P, 7.1. $C_{11}H_{23}Cl_3N_2O_3PS_2$ requires: C, 30.5; H, 5.4; Cl, 24.6; N, 6.5; P, 7.2%).

5.3.10 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(ethoxythiocarbonylthio)ethyl]phosphoramidate (94)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (4.25g, 0.013 mol) was dissolved in benzene (20 cm^3). Potassium ethyl xanthate (2.14g, 0.013 mol) was then added and the reaction mixture was stirred overnight. The solid potassium chloride which precipitated was filtered off and the solvent was removed from the filtrate in vacuo to give a very pale yellow oil which was crystallised from a mixture of benzene and light petroleum (b.p. 60-80°C) to give diethyl N-[2,2,2-trichloro-1-(ethoxythiocarbonylthio)ethyl]phosphoramidate as a white solid (3.94g, 73%) m.p. 97.5-99°C, (Found: C, 26.5; H, 4.4; Cl, 26.3; N, 3.3; P, 7.5. $C_9H_{17}Cl_3NO_4PS_2$ requires: C, 26.7; H, 4.2; Cl, 26.3; N, 3.5; P, 7.6%).

5.3.11 Preparation of 1,1,1-trichloro-2,2,-bis(diethoxyphosphinyl-amino)ethane (85)

Diethyl N-(1,2,2,2-tetrachloroethyl) phosphoramidate (1.36g, 0.00426 mol) was dissolved in dry benzene (10 cm^3) and triethylamine (0.595 cm^3, 0.00426 mol) was added. The flask was stoppered and shaken gently and left to stand for 5 min. The white precipitate of triethylammonium chloride was filtered off, washed with benzene and dried (0.591g, 100.8%). The filtrate and washings were combined and diethyl phosphoramidate (0.65g, 0.0043 mol) was added. The reaction mixture was left to stand for 2 days when it solidified. The solid mass was recrystallised from a mixture of chloroform and light petroleum (b.p. 60-80°C) to give 1,1,1-trichloro-2,2-bis(diethoxyphosphinylamino)ethane (0.234g, 12.6%), m.p. 186-189°C (lit.[65] 190-193°C) (Found: C, 27.6; H, 5.4; Cl, 24.5; N, 6.3; P, 14.3. Calc. for $C_{10}H_{23}Cl_3N_2O_6P_2$: C, 27.6; H, 5.3; Cl, 24.4; N, 6.4; P, 14.2%).

5.3.12 Preparation of ∝-chloro ethyl acetoacetate

Sulphuryl chloride (266g, 1.97 mol) was added dropwise over a period of 3h to stirred ethyl acetoacetate (256g, 1.97 mol) cooled in a water bath. The reaction mixture was stirred over the weekend. The gaseous products were removed in vacuo and the residue was fractionated under reduced pressure to give ∝-chloro ethyl acetoacetate (276.1g, 85.2%), b.p. 104-106°C at 28 mm Hg, (lit.[88] 86-89 at 12 mm Hg) (Found: C, 44.3;

H, 5.7. Calc. for $C_6H_9ClO_3$: C, 43.8; H, 5.5%).

5.3.13 Preparation of ethyl 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylate

A solution of 2-mercaptoethanol (16g, 0.2 mol) and ∝-chloro ethyl acetoacetate (33g, 0.2 mol) in benzene (200 cm^3) was stirred vigorously, and a solution of sodium bicarbonate (22g, 0.26 mol) in water (150 cm^3) was added dropwise over a period of 1h. The aqueous layer was saturated with sodium chloride and the benzene layer was then separated. The aqueous layer was extracted with benzene (2 x 30 cm^3). The combined filtrate and extracts were dried over anhydrous sodium sulphate and then filtered. Toluene-4-sulphonic acid (0.2g) was added to the solution and the reaction mixture was heated under reflux in a Dean and Stark apparatus to remove water (2.9 cm^3, 0.16 mol, 80%). The benzene was then removed and the residue distilled in vacuo to give the title compound (16.7g, 44.3%), b.p. 120-122°C at 1.5 mm Hg (lit.[66] 120°C at 2.5 mm Hg) (Found: C, 51.0; H, 6.5; Calc. for $C_8H_{12}O_3S$: C, 51.1; H, 6.5%).

5.3.14 Preparation of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid

A solution of sodium hydroxide (4.2g, 0.105 mol) in water (25 cm^3) was added to a solution of ethyl 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylate (13.7g, 0.0728 mol) in ethanol (13 cm^3). The reaction mixture was heated for 3h under reflux, and it was then cooled and acidified with

dilute hydrochloric acid. The white precipitate was filtered off, washed with water (3 x 25 cm^3) and dried *in vacuo* to give the acid (9.92g, 85.1%) m.p. 179-181°C, (lit.[66] 183°C) (Found: C, 44.9; H, 5.1. Calc. for $C_6H_8O_3S$: C, 45.0; H, 5.0%).

5.3.15 Preparation of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid amide (88)

Thionyl chloride (9.84g, 0.0827 mol) was added dropwise to a stirred solution of 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid (11.6g, 0.0724 mol) in chloroform (35 cm^3). The reaction mixture was heated under reflux for 3h and the solvent and other volatiles were then removed *in vacuo* to give the acid chloride (13.1g, 101%). This was dissolved in benzene (35 cm^3) and anhydrous ammonia gas was passed through the solution for 0.5h. The slightly off-white solid obtained was filtered off and recrystallised from water (350 cm^3) with the aid of a little charcoal, washed with I.M.S. (2 x 15 cm^3) and dried to give the amide (8.45g, 73.7%) m.p. 174.5-175°C (lit.[89] 172-174°C) (Found: C, 45.4; H, 5.6; N, 8.2. Calc. for $C_6H_9NO_2S$: C, 45.3; H, 5.7; N, 8.8%).

5.3.16 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(5,6-dihydro-2-methyl-1,4-oxathiin-3-carbonamido)ethyl]phosphoramidate (86)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.50g, 0.00470 mol) was dissolved in benzene (10 cm^3). Triethylamine (0.476g,

0.00470 mol) was then added. The reaction mixture was shaken gently and then left to stand for 10 min. The precipitate of triethylammonium chloride was filtered off, washed with benzene (2 x 3 cm^3) and dried (0.65g, 100%). The filtrate and washings were combined, 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxylic acid amide (0.71g, 0.00446 mol) was added, and the mixture was heated under reflux for 3h to give a clear, slightly orange solution which deposited a voluminous white precipitate on cooling. The precipitate was filtered off, washed with benzene (5 cm^3) and dried in vacuo to give diethyl N-[2,2,2-trichloro-1-(5,6-dihydro-2-methyl-1,4-oxathiin-3-carbonamido)ethyl] phosphoramidate (1.50g, 76.1%) m.p. 156-159°C, (Found: C, 33.2; H, 4.8; Cl, 23.8; N, 6.1; P, 7.1. $C_{12}H_{20}Cl_3N_2O_5PS$ requires: C, 32.6; H, 4.6; Cl, 24.1; N, 6.3; P, 7.0%).

5.3.17 Preparation of 1-formylpiperazine

Methyl formate (10.3g, 0.172 mol) was poured on to anhydrous piperazine (12.6g, 0.146 mol). The reaction mixture was heated on an oil bath at 60°C for 1h and was then fractionally distilled to give 1-formylpiperazine (5.72g, 34%) b.p. 111-112°C at 1 mm Hg (lit.[90] 115-120°C at 4 mm Hg) (Found: C, 53.1; H, 8.6; N, 24.6. Calc. for $C_5H_{10}NO$: C, 52.6; H, 8.8; N, 24.5%).

5.3.18 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(4-formyl-piperazin-1-yl)ethyl]phosphoramidate (80)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (2.48g, 0.00778 mol) was dissolved in benzene (10 cm^3) and triethylamine (0.786g, 0.00778 mol) was added. The reaction mixture was swirled gently to mix the reagents and left to stand for 15 min. The precipitate of triethylammonium chloride was filtered off and washed with benzene (2 x 2 cm^3). 1-Formyl-piperazine (0.89g, 0.00779 mol) was added to the combined filtrate and washings and the reaction mixture was stirred for 30 min. The reaction mixture was concentrated to half its volume and the solution was left to stand for 2 days. The white solid which precipitated was filtered, washed with benzene (2 x 2 cm^3) and dried in vacuo to give diethyl N-[2,2,2-trichloro-1-4-formylpiperazin-1-yl)ethyl]phosphoramidate (1.84g, 59·7%) m.p. 102-107°C, (Found: C, 33.7; H, 5.4; Cl, 26.9; N, 10.5; P, 7.6. $C_{11}H_{21}Cl_3N_3O_4P$ requires: C, 33.3; H, 5.3; Cl, 26.8; N, 10.6; P, 7.8%).

5.3.19 Preparation of 1,4-bis[2,2,2-trichloro-1-(diethyloxyphosphinyl-amino)ethyl]piperazine (79)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.50g, 0.00470 mol) was dissolved in benzene (10 cm^3) and triethylamine (0.476g, 0.00470 mol) was added. The reaction mixture was swirled gently to mix the reagents and then left to stand for 15 min. The white precipitate of triethylammonium chloride was filtered off, washed with benzene (2 x 3 cm^3)

and dried (0.65g, 100.4%). The filtrate and washings were combined and anhydrous piperazine (0.203g, 0.00236 mol) was added as large flakes which dissolved immediately. The reaction mixture was left to stand overnight and the white crystals which formed were filtered off, washed with benzene (2 x 3 cm^3), and dried *in vacuo* to give 1,4-bis[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethyl]piperazine (1.15g, 75.1%) m.p. 162-165°C (decomp.) (Found: C, 29.2; H, 4.9; N, 8.5. $C_{10}H_{32}Cl_6N_4O_6P_2$ requires C, 29.5; H, 4.9; N, 8.6%).

5.3.20 Preparation of O,O-diethyl N-(2,2,2-trichloro-1-acetamidoethyl)-phosphoramidate (84)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.37g, 0.00430 mol) was dissolved in benzene (10 cm^3), triethylamine (0.435g, 0.00430 mol) was added, and the reaction mixture was swirled gently to mix the reagents and left to stand for 10 min. The precipitate of triethylammonium chloride was filtered off and washed with benzene (2 x 3 cm^3). Acetamide (0.254g, 0.0043 mol) was added to the combined filtrate and washings and the reaction mixture was left to stand. Periodic checks by nmr showed that the reaction was complete after standing for two weeks at room temperature. After this time the product was filtered off, washed with benzene (2 x 3 cm^3) and dried *in vacuo* to give the title compound (0.97g, 66%) m.p. 182-185°C (lit.[65] 184-185°C) (Found: C, 28.4; H, 5.0; Cl, 31.3; N, 8.2; P, 9.1. Calc. for $C_8H_{10}Cl_3N_2O_4P$: C, 28.1; H, 4.7; Cl, 31.1; N, 8.2; P, 9.1%).

5.3.21 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(1,2,4-triazol-1-yl)ethyl]phosphoramidate (77)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (3.94g, 0.0124 mol) was dissolved in benzene (20 cm^3) triethylamine (1.25g, 0.0123 mol) was added, and the reaction mixture was swirled gently to mix the reagents and left to stand for 15 min. The triethylammonium chloride was filtered off and washed with benzene (2 x 5 cm^3). 1,2,4-Triazole (0.85g, 0.0123 mol) was added to the combined filtrate and washings and the reaction mixture was stirred for 30 min after which the solvent was removed in vacuo to leave a pale yellow oil which was crystallised from a mixture of chloroform and light petroleum (b.p. 60-80°C) to give diethyl N-[2,2,2-trichloro-1-(1,2,4-triazol-1-yl)ethyl]phosphoramidate (20.7g, 48%), m.p. 123-126°C, (Found: C, 27.5; H, 4.3; Cl, 30.5; N, 15.5; P, 9.0. $C_8H_{14}Cl_3N_4O_3P$ requires: C, 27.3; H, 4.0; Cl, 30.0; N, 15.9; P, 8.8%).

5.3.22 Preparation of 1-[2,2,2-trichloro-1-(diethyoxyphosphinylamino)-ethoxy]-2-[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethylthio]-ethane (81)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (1.44g, 0.00451 mol) was dissolved in benzene (10 cm^3) and triethylamine (0.453g, 0.00448 mol) was added. The reaction mixture was swirled gently to mix the reagents, and left to stand for 10 min. The triethylammonium chloride

was filtered off and washed with benzene (2 x 3 cm^3) and 2-mercaptoethanol (0.154 cm^3, 0.00225 mol) was added to the combined filtrate and washings. The reaction mixture was stirred for 15 min, the solution was concentrated to half its volume and light petroleum (b.p. 30-40°C, 10 cm^3) was added dropwise. The white precipitate which formed was filtered off, washed with light petroleum (b.p. 30-40°C), dried and recrystallised from water to give 1-[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethoxy]-2-[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethylthio]ethane. (0.82g, 57%) m.p. 178.5-180°C, (Found: C, 25.6; H, 4.7; Cl, 33.5; N, 3.6; P, 9.7. $C_{14}H_{28}Cl_6N_2O_7P_2S$ requires: C, 26.1; H, 4.4; Cl, 33.1; N, 4.4; P, 9.6%).

5.3.23 Preparation of O,O-diethyl N-[2,2,2-trichloro-1-(2-hydroxyethylthio)ethyl]phosphoramidate (82)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (4.22g, 0.0132 mol) was dissolved in benzene (20 cm^3), triethylamine (1.85 cm^3, 0.0132 mol) was added with swirling and the mixture was left to stand for 10 min after which the triethylammonium chloride was filtered off and washed with benzene (2 x 3 cm^3). 2-Mercaptoethanol (1.04g, 0.0133 mol) was then added to the combined filtrate and washings and the mixture was stirred for 10 min. The reaction mixture was then concentrated to half its volume and the white solid which formed was filtered off, washed with benzene (2 x 3 cm^3) and dried to give diethyl N-[2,2,2-trichloro-1-(2-hydroxyethylthio)ethyl]-phosphoramidate (3.76g, 81%) m.p. 91.5-100.5°C, (Found: C, 25.5; H, 4.9; Cl, 30.9; N, 4.9; P, 8.8. $C_7H_{17}Cl_3NO_4PS$ requires:

C, 24.1; H, 4.9; Cl, 30.5; N, 4.0; P, 8.9%).

5.3.24 Preparation of ethyl phosphorodichloridate

Ethanol (92.1g, 2 mol) was added dropwise to stirred, cooled phosphoryl chloride (306.7g, 2 mol) over a period of 1h. The reaction mixture was left to stand overnight and it was then fractionated under reduced pressure to give ethyl phosphorodichloridate (173.7g, 53.5%) b.p. 62-63°C at 19 mm Hg (lit.[91] 63°C at 19 mm Hg).

5.3.25 Preparation of O-ethyl-N,N-dimethyl phosphorodiamidate

Ethyl phosphorodichloridate (54.3g, 0.333 mol) and triethylamine (50.6g, 0.50 mol) were dissolved in diethyl ether (150 cm^3). The solution was stirred and cooled in an ice/salt bath, and dimethylamine (15.0g, 0.333 mol) was added dropwise over a period of 0.5h. The solution was stirred for a further 0.5h and then triethylammonium chloride was removed by filtration and washed with diethyl ether (2 x 50 cm^3). The filtrate and washings were combined and the solvent and excess triethylamine were removed under reduced pressure. The residue was fractionated through a 15 cm column packed with glass helices to give O-ethyl-N,N-dimethyl phosphoramidochloridate (25.7g, 45%) b.p. 96-98°C at 14 mm Hg (lit.[92] 99°C at 18 mm Hg). This was dissolved in benzene (75 cm^3) and anhydrous ammonia was passed through the solution for 3h. The white precipitate of ammonium chloride was then filtered off and washed and dried (7.53g, 96%). The filtrate

and washings were combined and the solvent was removed under reduced pressure to give O-ethyl-N, N dimethyl phosphorodiamidate (22.0g, 43.4%) m.p. 30°C (Found: C, 29.1; H, 7.7; N, 17.1. $C_4H_{13}N_2O_2P$ requires: C, 31.6; H, 8.6; N, 18.4%).

5.3.26 Preparation of O-ethyl-N, N dimethyl-N'-(2,2,2-trichloro-1-hydroxyethyl)phosphorodiamidate (104)

O-Ethyl-N, N-dimethylphosphorodiamidate (12.3g, 0.0818 mol) was dissolved in benzene (45 cm^3). The solution was stirred and cooled in ice and a solution of anhydrous chloral (11.9g, 0.810 mol) in benzene (15 cm^3) was added dropwise over a period of 0.5h. The mixture was allowed to warm up to room temperature whilst stirring for a further 2h, the slightly cloudy solution was filtered then the solvent was removed under reduced pressure to leave a colourless, slightly cloudy oil (22.2g, 91.4% calculated as the desired product) which was dissolved in carbon tetrachloride (75 cm^3) and extracted with water (3 x 5 cm^3). The organic layer was separated, dried over anhydrous sodium sulphate and filtered and the solvents were removed under reduced pressure to give a colourless oil, estimated by nmr to contain ca. 94% O-ethyl N, N-dimethyl-N'-(2,2,2-trichloro-1-hydroxyethyl)phosphorodiamidate (11.6g, 47.8%) (Found: C, 26.5; H, 5.9; N, 8.0. $C_6H_{14}Cl_3N_2O_3P$ requires: C, 24.1; H, 4.7; N, 9.4%).

5.3.27 Preparation of O-ethyl N,N-dimethyl-N'-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl]phosphorodiamidate (110)

O-Ethyl N,N-dimethyl-N'-(2,2,2-trichloro-1-hydroxyethyl)phosphorodiamidate (11.6g, 0.0387 mol) was dissolved in a mixture of benzene (50 cm^3) and pyridine (14.7g, 0.186 mol). The solution was stirred and cooled in an ice-bath whilst a solution of thionyl chloride (4.62g, 0.0389 mol) in benzene (10 cm^3) was added dropwise over a period of 0.5h to give a black oily precipitate in a yellow solution. The solution was decanted and the solvent was removed under reduced pressure to give a brown oil which was extracted with carbon tetrachloride to give crude O-ethyl N,N-dimethyl-N'-(2,2,2-trichloroethylidene)phosphorodiamidate as a brown oil (4.94g, 45%). After removal of the solvent the phosphorodiamidate was dissolved in absolute chloroform (5 cm^3), and a solution of imidazole (2.0g, 0.0293 mol) in absolute chloroform (10 cm^3) was added dropwise over 5 min. A little decolourising charcoal was added and the solution was heated under reflux for 0.5h, cooled, filtered, and the solvent was removed under reduced pressure. The residue was re-dissolved in chloroform (15 cm^3), washed with water (4 x 3 cm^3), and the chloroform was removed under reduced pressure. The residue was dissolved in a mixture of carbon tetrachloride (15 cm^3) and chloroform (1.2 cm^3), heated under reflux for 0.5h with a little decolourising charcoal, filtered hot, and allowed to cool. Crystallisation began after 3h and was allowed to continue overnight to give a pale yellow solid which was washed with carbon tetrachloride (3 cm^3) and dried to give O-ethyl-N,N-dimethyl-N'-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl]phosphorodiamidate (1.97g,

14.6%) m.p. 118-120°C (Found: C, 30.9; H, 4.7; N, 16.0. $C_9H_{16}Cl_3N_4O_2P$ requires: C, 30.9; H, 4.6; N, 16.0%).

5.3.28 Preparation of tetramethylphosphorodiamidic chloride

Dimethylamine (191g, 4.24 mol) was added dropwise over 90 min to phosphoryl chloride (163g, 1.06 mol) in dry light petroleum (b.p. 30-40°C) (350 cm^3) stirred and cooled in ice/water. The reaction mixture was allowed to warm up to room temperature and was stirred for 1h. The white precipitate was filtered off, washed with light petroleum (b.p. 30-40°C) (3 x 50 cm^3) and dried to give dimethylammonium chloride (178g, 103%). The solvent was removed from the combined filtrate and washings and the residue was distilled through a 15 cm column packed with Fenske glass helices to give the desired product (151.9g, 84%) b.p. 112-116°C at 12 mm Hg (lit.[93] 110°C at 10 mm Hg). (Found: Cl, 20.9; P, 18.1. Calc. for $C_4H_{12}ClN_2OP$: Cl, 20.8; P, 18.2%).

5.3.29 Preparation of N,N,N',N'-tetramethylphosphoric triamide

(a) In chloroform

Tetramethylphosphorodiamidic chloride (5.61g, 0.0329 mol) was dissolved in absolute chloroform (75 cm^3). The solution was stirred and anhydrous ammonia was passed through for 2h. Some heat was given out initially so the temperature was maintained at about 20°C by the use of a

water bath. The reaction mixture was allowed to stand over the weekend after which time the white solid was filtered off, washed and dried to give ammonium chloride (1.35g, 75.8%). The solvent was removed from the combined filtrate and washings to leave a sticky white solid (5.39g, 108% calculated as the required product) which apparently contained some of the starting material judging from the characteristic odour and the chlorine content. (Found: C, 34.0; H, 8.8; Cl, 7.2; N, 23.0% Calc. for $C_4H_{14}N_3OP$: C, 31.8; H, 9.3; Cl, 0.0; N, 27.8%).

A portion of the product (0.75g) was recrystallised from dry light petroleum (b.p. 100-120°C) to give 0.27g (36% recovery) of the title compound as white crystals, m.p. 100-105°C (lit.[70], 108°C) Found: C, 30.4; H, 9.0; N, 28.2. Calc. for $C_4H_{14}N_3OP$: C, 31.8; H, 9.3; N, 27.8%).

(b) In petroleum ether

Anhydrous ammonia was passed through a solution of tetramethylphosphorodiamidic chloride (17.9g, 0.105 mol) in dry light petroleum (b.p. 60-80°C) (200 cm^3) for 3h, at the end of which period no starting material was detected by glc analysis. The white solid was filtered off and washed with light petroleum (2 x 50 cm^3). The combined filtrate and washings were evaporated in vacuo to leave a colourless oil (0.6g) which was shown by ir to be hexamethylphosphoramide. The white solid was extracted with chloroform (1 x 150 and 2 x 75 cm^3). The insoluble component was dried to give ammonium chloride (5.68g, 101%). The extracts were combined

and the volatiles were removed in vacuo to leave a solid white residue which was recrystallised from light petroleum (b.p. 80-100°C) to give the desired product (10.8g, 68%), m.p. 107-109°C, (lit.[70] 108°C) (Found: C, 31.3; H, 9.0; N, 27.7; P, 20.5. Calc. for $C_4H_{14}N_3OP$: C, 31.8; H, 9.3; N, 27.8; P, 20.5%).

5.3.30 Preparation of 1,1,1-trichloro-2-diethoxyphosphinylamino-2-[bis-(dimethylamino)phosphinylamino]ethane (87)

Diethyl N-(1,2,2,2-tetrachloroethyl)phosphoramidate (7.0g, 0.0219 mol) was dissolved in benzene (30 cm^3), triethylamine (2.22g, 0.0219 mol) was added dropwise with gentle swirling, and the mixture was left to stand for 10 min. The precipitate of triethylammonium chloride was filtered off and washed with benzene (2 x 5 cm^3). N,N,N',N'-tetramethylphosphoric triamide (3.3g, 0.0218 mol) was added to the combined filtrate and washings and the mixture was heated under reflux for 1h and allowed to cool. The white solid which precipitated was filtered off, washed with benzene (2 x 5 cm^3) and dried to give 1,1,1-trichloro-2-diethoxyphosphinylamino-2-[bis(dimethylamino)-phosphinylamino]ethane (7.9g, 83%) m.p. 219-221°C (Found: C, 27.6; H, 5.8; N, 12.9. $C_{10}H_{28}Cl_3N_4O_4P_2$ requires: C, 27.7; H, 5.8; N, 12.9%).

5.3.31 Reaction of N,N,N',N'-tetramethylphosphoric triamide with chloral

(a) N,N,N',N'-tetramethylphosphoric triamide (0.56g, 0.0037 mol)

was heated under reflux in dry light petroleum (b.p. 80–100°C, 20 cm^3) and a solution of anhydrous chloral (0.54g, 0.0037 mol) in dry light petroleum (10 cm^3) was added dropwise over 10 min. The reaction mixture was heated under reflux for a further 0.5h and the mother liquor was then decanted from a small amount of orange oil and placed in the freezer overnight whence a semi-solid crystalline mass formed in the bottom of the flask. The mother liquor was decanted and the solid was dried in vacuo to give a residue (0.72g) (Found: C, 24.5; H, 4.4; N, 10.9. Calc. for $C_6H_{15}Cl_3N_3O_2P$: C, 24.1; H, 5.1; N, 14.1%) which was not the desired product.

(b) Anhydrous chloral (0.58g, 0.0039 mol) in chloroform (2 cm^3) was added dropwise to a solution of the phosphoramidate (0.60g, 0.004 mol) in chloroform (5 cm^3). A little heat was evolved during the addition. The solvent was removed in vacuo to give a colourless oil (1.21g) the nmr spectrum of which showed that it was a mixture containing little, if any, of the desired product. Attempted crystallisation of the oil failed and it decomposed on attempted high vacuum distillation.

(c) Anhydrous chloral (0.50g, 0.0034 mol) was added dropwise to the solid phosphoramide (0.52g, 0.0034 mol) in a small flask which was stoppered and agitated gently to mix the reagents. A little heat was evolved. The reaction mixture was allowed to stand overnight and was then placed under high vacuum to remove any volatiles. A yellowish oil was obtained (0.97g) (Found: C, 18.0; H, 6.2; N, 10.3%). Attempts to crystallised the oil failed and it decomposed on attempted high vacuum distillation.

5.3.32 Reaction of hexamethylphosphoramide and chloral

Hezamethylphosphoramide (3.08g, 0.017 mol) was added dropwise to a solution of chloral (2.51g, 0.017 mol) in chloroform (20 cm^3). The reaction mixture was left to stand for 48h and it was then heated under reflux for 6h and left to stand for a further month. The reaction mixture assumed a slight orange colouration and a haze formed on the inside of the flask. The haze was found to be insoluble in chlorinated hydrocarbons, acetone, ethyl acetate, aliphatic hydrocarbons, alcohols and water, but was soluble in ethers, suggesting that it was a polymer of chloral such as that which is formed on the inside of bottles on storage. Nmr spectra of the reaction mixture were run periodically, but no detectable change occurred.

5.3.33 Nmr studies of the reaction between N,N,N',N'-tetramethyl-phosphoric triamide and chloral

N,N,N',N'-tetramethylphosphoric triamide (2.70g, 0.0178 mol) was dissolved in absolute chloroform (17 cm^3), chloral (2.63g, 0.0178 mol) was added and the nmr spectrum of the mixture was determined periodically after standing at room temperature. Results are presented in table IV.

Table IV

Time	^{1}H nmr signals and assignments		
	δ		
0	2.59 (d, J_{PCH} 10.2)	$(\underline{Me}_2N)_2P(O)NH_2$	(12H)
	8.97 (s)	$CCl_3C\underline{H}O$	(1H)
	δ		
4h	2.59 (d, J_{PCH} 10.2)	$(\underline{Me}_2N)_2P(O)NH_2$	
	8.97 (s)	$CCl_3C\underline{H}O$	(83% original)
	δ		
24h	2.59 (d, J_{PCH} 10.2)	$(\underline{Me}_2N)_2P(O)NH_2$	(58%)
	2.62 (d, J_{PCH} 10.2)	$\underline{Me}_2N$-P	(30%)
	2.66 (d, J_{PCH} 10.2)	$\underline{Me}_2N$-P	(12%)
	8.97 (s)	$CCl_3C\underline{H}O$	(20% original)

Table IV (continued)

Time	^{1}H nmr signals and assignments		
	δ		
4 days	2.47 (s)		
	2.59 (d, J_{PCH} 10.2)	$(\underline{Me}_2N)_2P(O)NH_2$	(37%)
	2.62 (d, J_{PCH} 10.2)	$\underline{Me}_2N$-P	(31%)
	2.62 (s)[a]	$\underline{Me}_2N$-	(14%)
	2.66 (d, J_{PCH} 10.2)	$\underline{Me}_2N$-P	(18%)
	2.81 (s)		
	2.90 (s)		
	3.32 (s)[a]		
	4.57 (m) (v. weak)		
	5.11 (m)[b] (v. weak)	$NHC\underline{H}(CCl_3)OH$[c]	
7 days	Little further change except a slight increase in the relative intensities of signals at 2.62 (s) and 4.57.		

a) Removed by shaking with D_2O

b) Collapses to a doublet on shaking with D_2O

c) $\underline{Me}_2N$ to C$\underline{H}$ proton ratio = 38:1, corresponding to ca. 31% of $(Me_2N)_2P(O)NHCH(CCl_3)OH$

5.3.34 Preparation of o-phenylene phosphorochloridate

Catechol (40.8g, 0.371 mol) was added to phosphoryl chloride (100 cm^3, 167.5g, 1.092 mol) and a slow stream of nitrogen was passed through the system which was heated under reflux for 4.5h. Hydrogen chloride (110%) was collected in a KOH scrubber and the excess of phosphoryl chloride (110g, 0.721 mol) was then distilled off at atmospheric pressure. The residue was then distilled to give the phosphorochloridate (23.6g, 25.7%) b.p. 125-127°C at 15 mm Hg (lit.[94] 122°C at 12 mm Hg), m.p. 58°C (lit.[94] 58-59°C) Found: C, 37.6; H, 2.2. Calc. for $C_6H_4ClO_3P$: C, 37.8; H, 2.1%).

5.3.35 Attempted preparation of o-phenylene phosphoramidate

o-Phenylene phosphorochloridate (10.7g, 0.0561 mol) and triethylamine (5.76g, 0.0569 mol) were dissolved in absolute chloroform (25 cm^3). Anhydrous ammonia was passed through the solution for 30 min after which time a sticky white mass had formed at the bottom of the reaction vessel. The reaction mixture was diluted to ca. 300 cm^3 with absolute chloroform and shaken to dissolve any triethylamine hydrochloride. The remaining solid was extracted with a further portion (100 cm^3) of absolute chloroform, filtered off and dried to give a fine white powder (8.36g) m.p. ca. 215°C. Found: C, 27.1; H, 5.7; Cl, 23.2; N, 16.7; P, 12.4. Calc. for the phosphoramidate (5.43g, 0.0318 mol, 56.7% yield) plus NH_4Cl (2.93g, 0.0548 mol, 97.7% yield): C, 27.4; H, 4.9; Cl, 23.3; N, 14.5; P, 11.8%

5.3.36 Preparation of o-phenylene phosphorochloridite

Catechol (110.1g, 1.00 mol) was dampened with water (4.0g, 0.22 mol) as a catalyst and phosphorus trichloride (206g, 1.50 mol) was poured in slowly with stirring. There was vigorous evolution of HCl but the reaction mixture remained cold. After 1.5h more phosphorus trichloride (83.8g, 0.61 mol) was added and the mixture was heated to 80°C for 1h after which time it was filtered and fractionated to give the phosphorochloridite (161.2g, 92.4%) b.p. 88-89°C at 18 mm Hg (lit.[95] 91°C at 16 mm Hg) (Found: Cl, 20.3; P, 17.6. Calc. for $C_6H_4ClO_2P$: Cl, 20.3; P, 17.7%).

5.3.37 Attempted preparations of o-phenylene hydrogen phosphite

(a) o-Phenylene phosphorochloridite (17.6g, 0.101 mol) was added dropwise with stirring to water (10.0g, 0.56 mol) at 0-1°C. A white precipitate formed during the addition. The reaction mixture was stirred for 15 min and the solid was then filtered off, dried and identified by mixed melting point and ir as catechol (8.18g, 73.4%) m.p. 99-102°C.

(b) o-Phenylene phosphorochloridite (16.7g, 0.0958 mol) was dissolved in diethyl ether (25 cm^3), stirred and cooled in ice. Methanol (3.10g, 0.0968 mol) was added dropwise over 20 min. The reaction mixture was allowed to warm up to room temperature and to stand for 1h, after which it was saturated with hydrogen chloride and allowed to stand overnight when

a white precipitate formed. This was filtered and dried to give a white solid (4.24g, 28.4%), m.p. 101.5-108°C. (Found: C, 56.3; H, 4.2. Calc. for $C_6H_5O_3P$: C, 46.2; H, 3.2%), the nmr spectrum of the solid showed a complex group of aromatic resonances (δ = ca. 7.0) and a phosphite proton (doublet, δ = 9.35 ppm, J_{HP} = 906 Hz). Integration of the spectrum showed that the ratio of the area of the aromatic protons to that of the phosphite proton was 11.4:1 indicating a purity of ca. 35% since it should be 4:1 with pure phosphite. Attempts to purify the product by recrystallisation from diethyl ether or from light petroleum (b.p. 60-80°C) failed and the product decomposed on attempted high vacuum distillation.

(c) o-Phenylene phosphorochloridite (18.6g, 0.106 mol) and pyridine (9.81g, 0.124 mol) were dissolved in diethyl ether (20 cm^3), cooled in ice and stirred. A solution of methanol (3.50g, 0.109 mol) in diethyl ether (20 cm^3) was added dropwise over 30 min. After a further 1½h at room temperature pyridine hydrochloride (nmr) was filtered off and washed with ether (3 x 20 cm^3) and the combined filtrate and washings were distilled to give methyl o-phenylene phosphite (5.16g, 28.5%) b.p. 86-88°C at 26 mm Hg (lit.[96] 76-77°C at 15 mm Hg). The nmr spectrum showed a complex group of aromatic resonances (δ = ca. 7.0 ppm) and a methyl group (δ = 3.28 ppm, J_{HCOP} = 9.7 Hz). Integration showed that the ratio of the area of aromatic protons to that of methyl protons to be 4.15:3 indicating a purity of ca. 96% since it should be 4:3 in the pure compound. The distillate was dissolved in anhydrous diethyl ether, cooled to 0°C, saturated with anhydrous hydrogen chloride and the mixture was stirred for 15 min at 0°C and 1.5h

at room temperature. The solvent was removed under reduced pressure to give an oil (5.05g). The nmr spectrum of the oil showed a complex group of aromatic resonances (δ = ca. 7.0 ppm) a doublet (δ = 3.28 ppm, J = 9.7 Hz) a doublet (δ = 3.75 ppm, J = 12.0 Hz) a doublet (3.85 ppm, J = 12.0 Hz) a doublet (δ = 6.7 ppm, J = 702 Hz) and a doublet (δ = 9.35 ppm, J = 906 Hz). It was estimated from the integrations that the oil contained ca. 20% of the hydrogen phosphite.

5.4 Spectroscopic Identification of the New Compounds

Data are presented in the following section for infra-red spectra, nuclear magnetic resonance spectra and mass spectra.

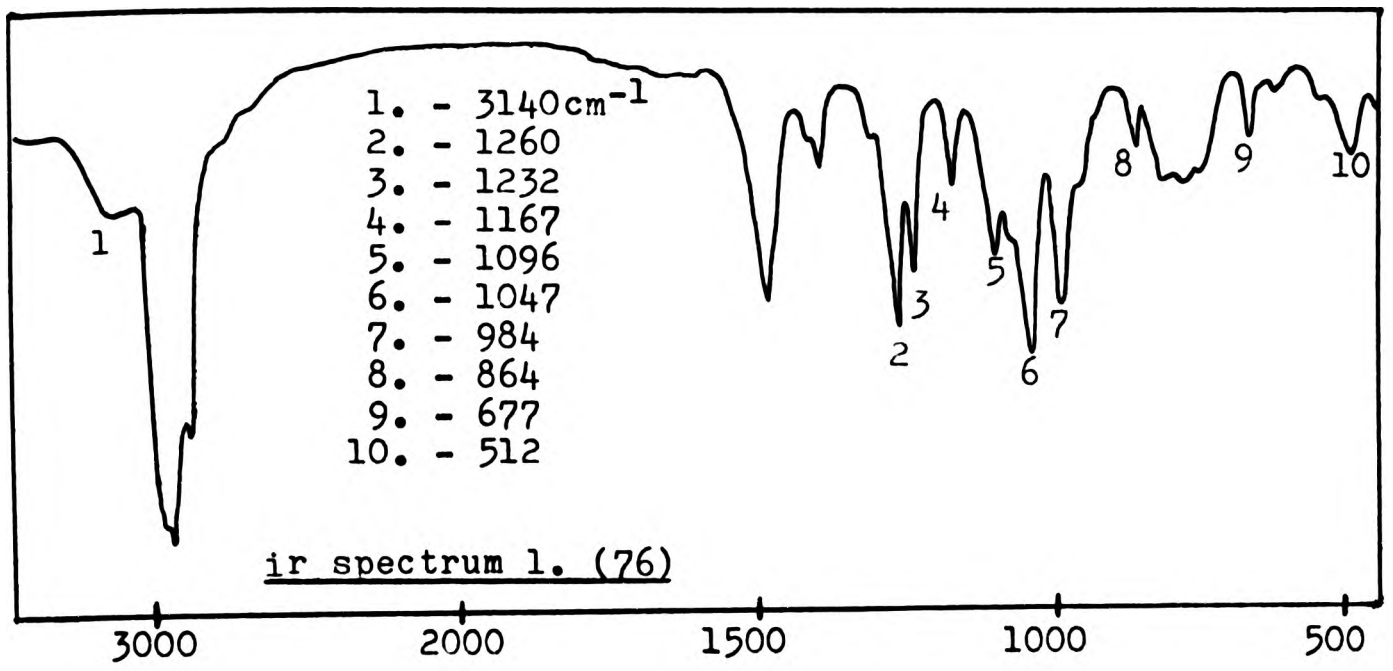
For reference purposes the structures of the novel compounds are shown in appendix 1.

5.4.1 Infra-red spectra

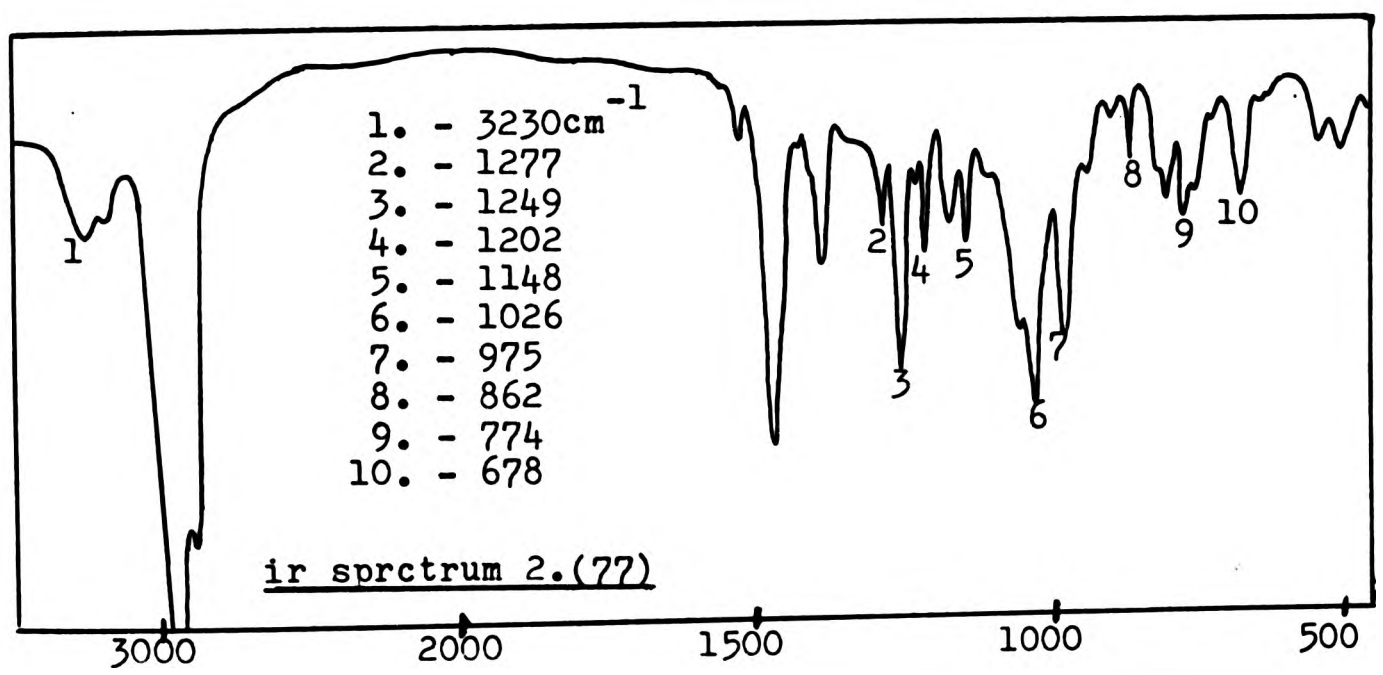
The compounds for which spectra are given are listed below.

Structures are given in Appendix 1.

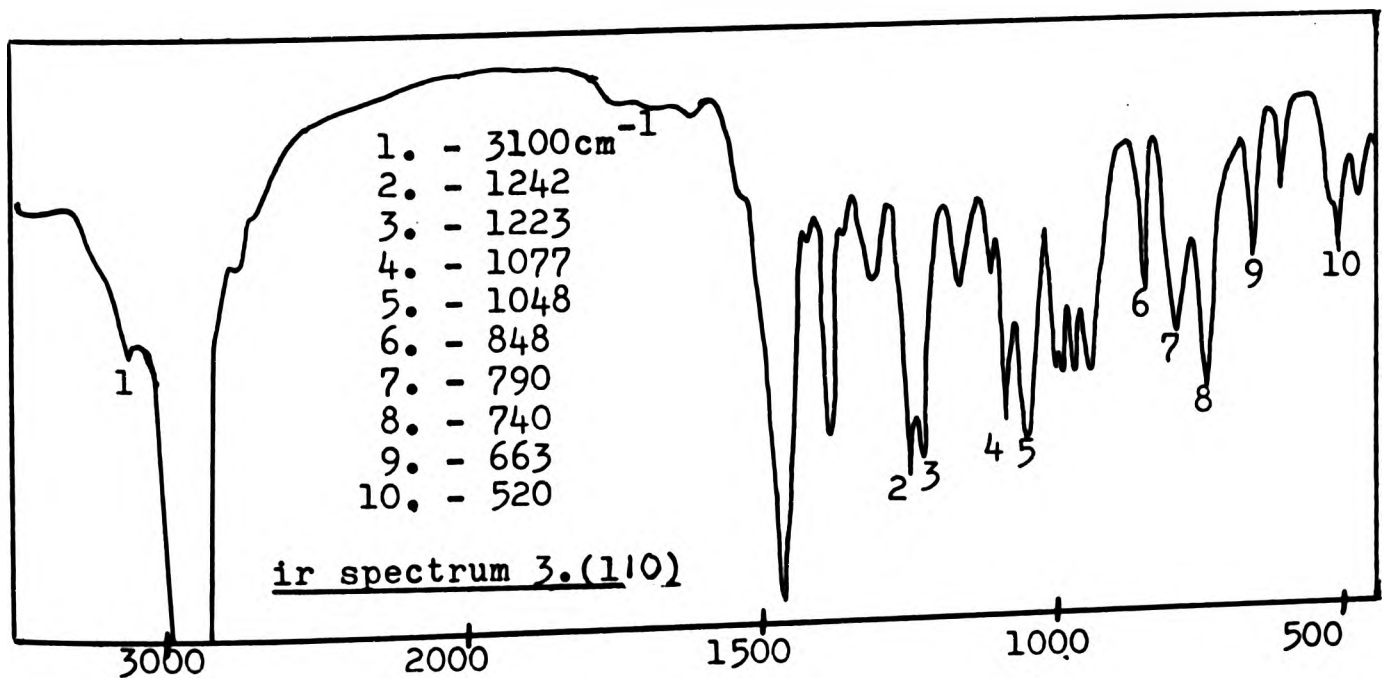
Spectrum	Compound	Derivative	Page
1	76	Imidazole	134
2	77	1,2,4-Triazole	"
3	110	Imidazole	"
4	78	Morpholine	135
5	80	N-formylpiperazine	"
6	79	Piperazine	"
7	92	Dimethyldithiocarbamate	136
8	82	2-Mercaptoethanol	"
9	93	Diethyldithiocarbamate	"
10	86	Substituted oxathiin	137
11	94	Xanthate	"
12	85	Acetamide	"
13	87	Tetramethylphosphoric triamide	138
14	85	Bis(diethyl phosphoramidate)	"
15	81	2-Mercaptoethanol	"



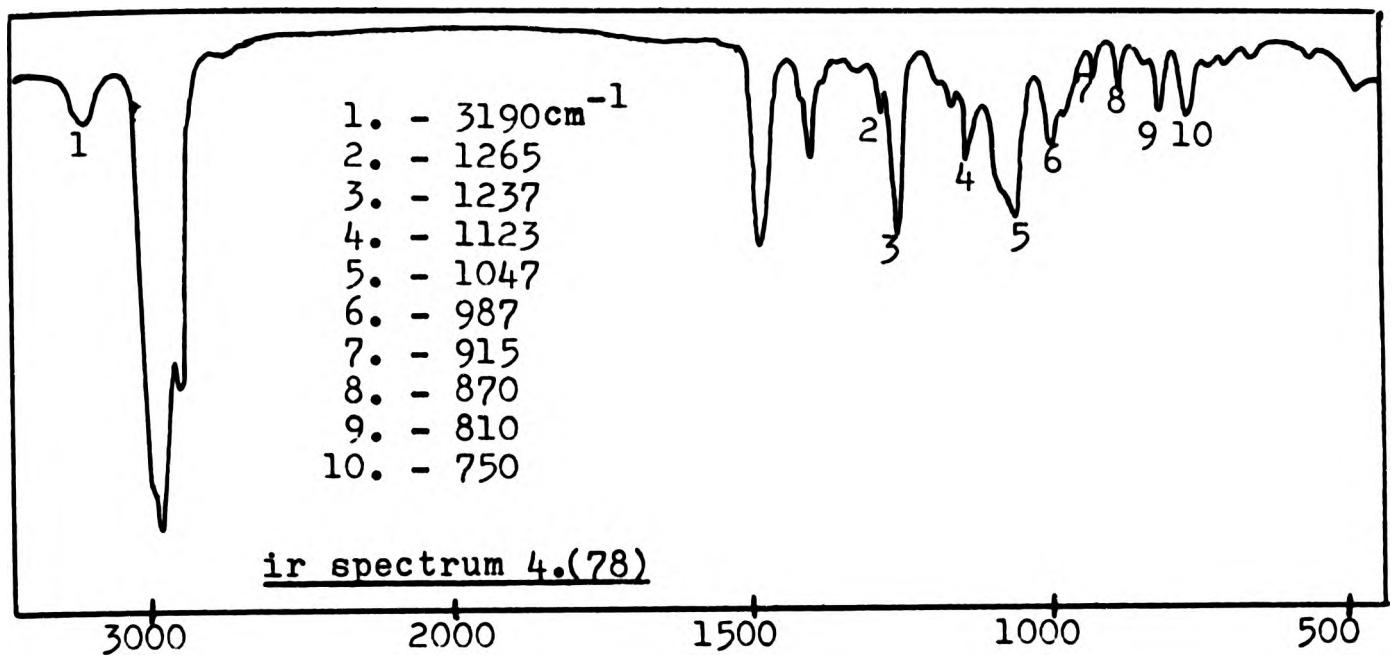
ir spectrum 1. (76)



ir sprctrum 2.(77)



ir spectrum 3.(110)



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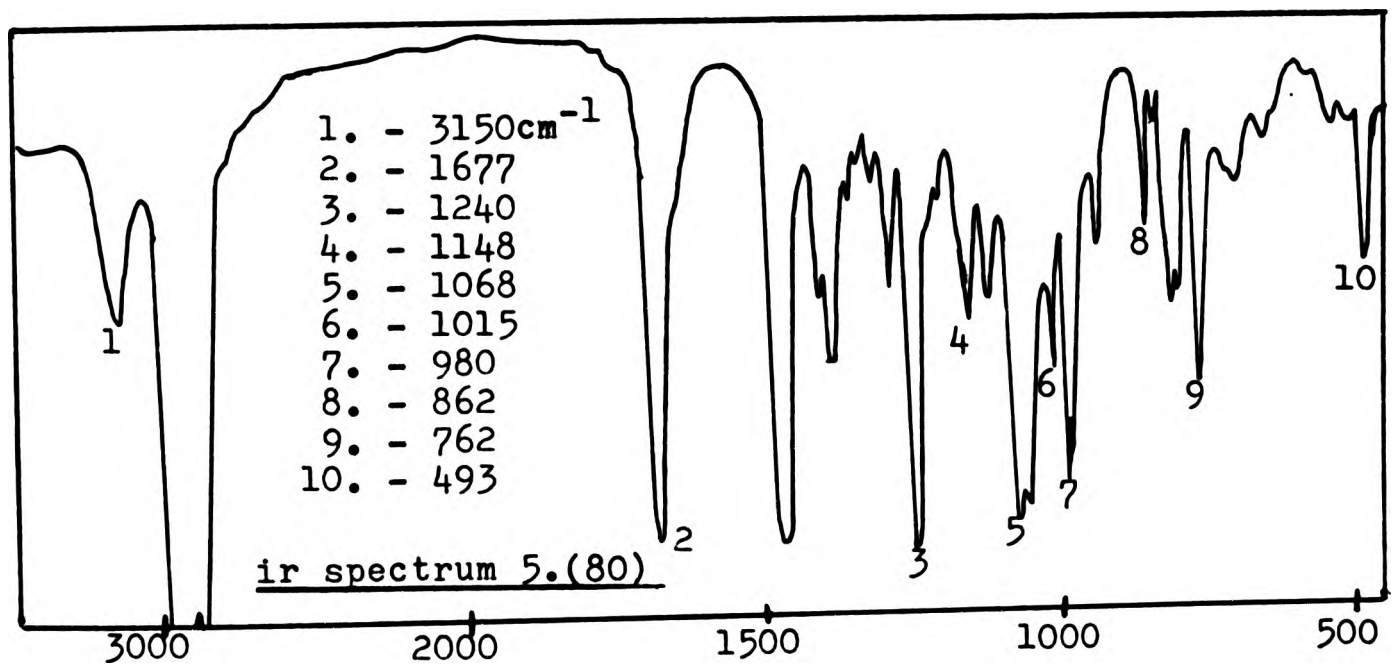
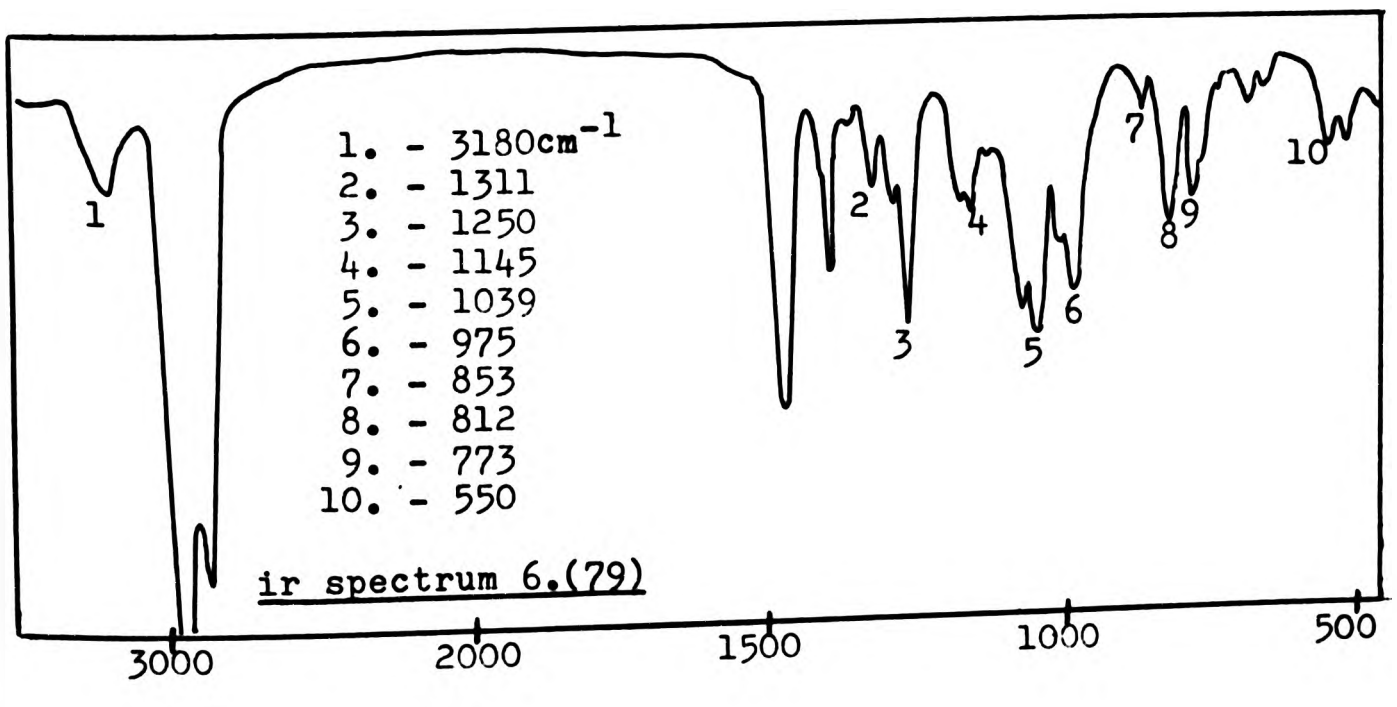
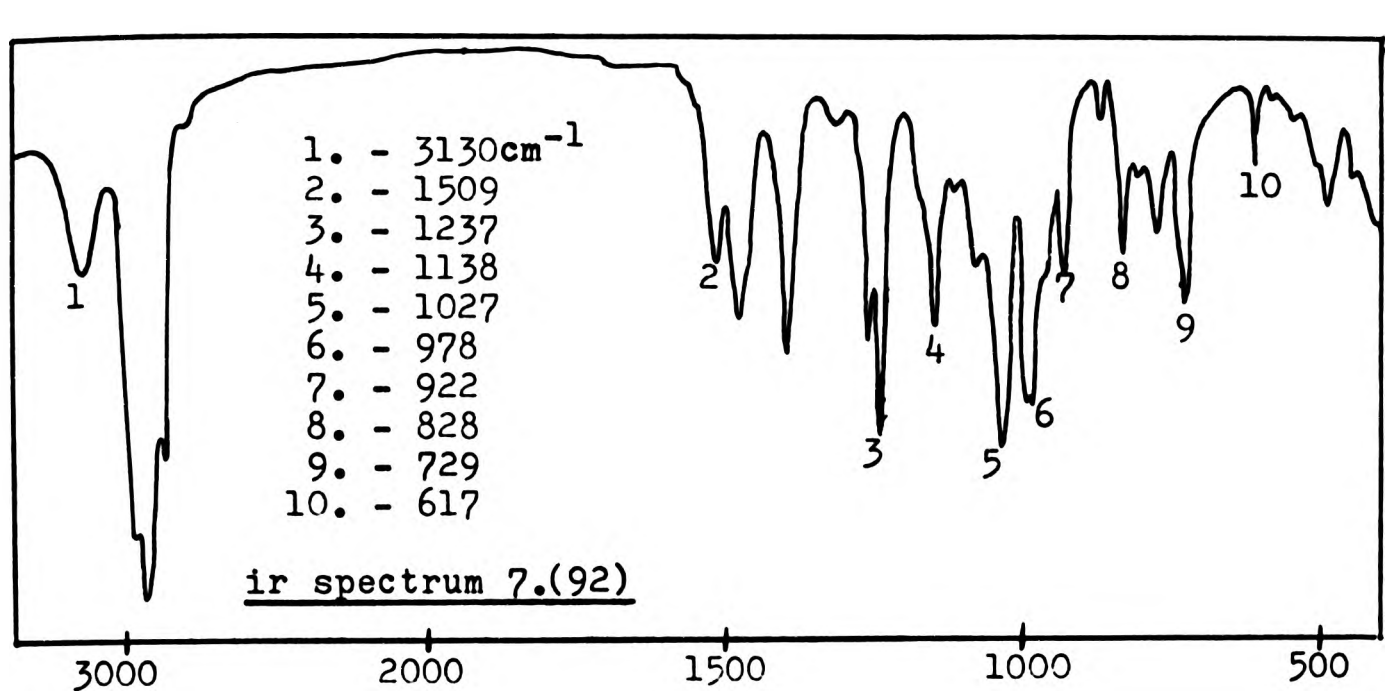


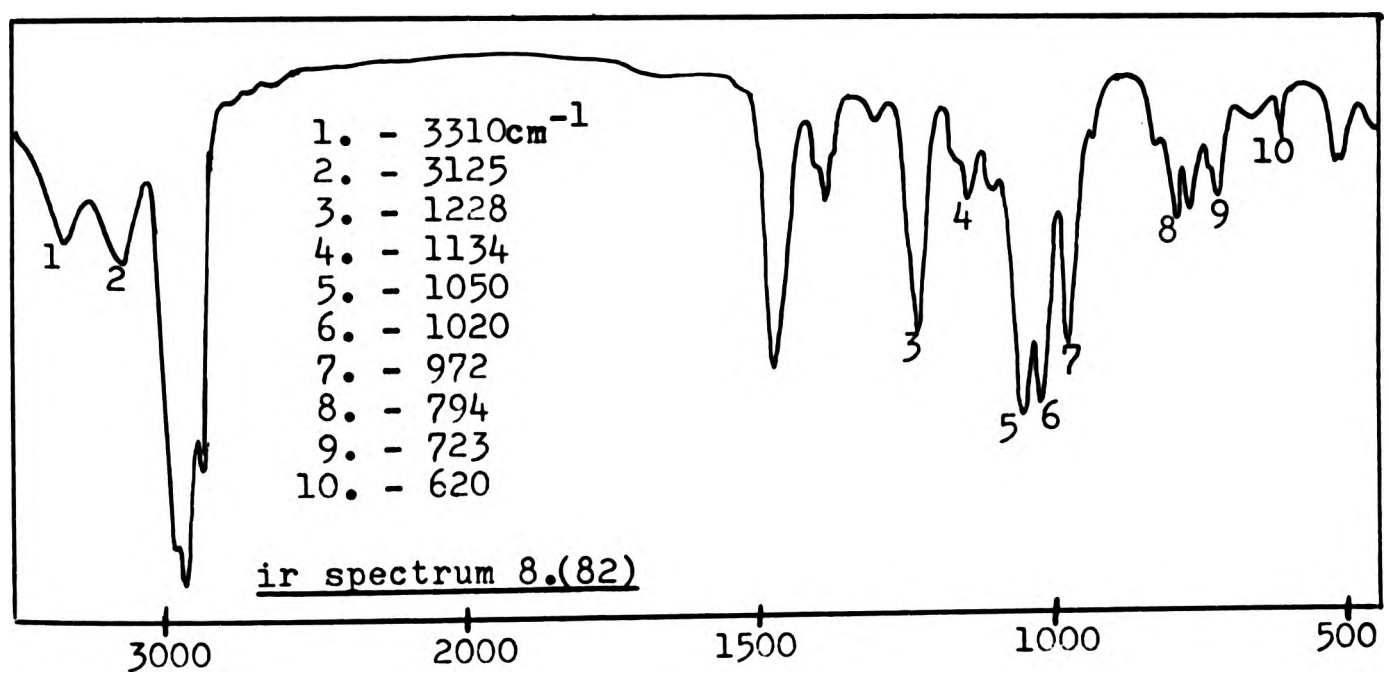
ir spectrum 5.(80)



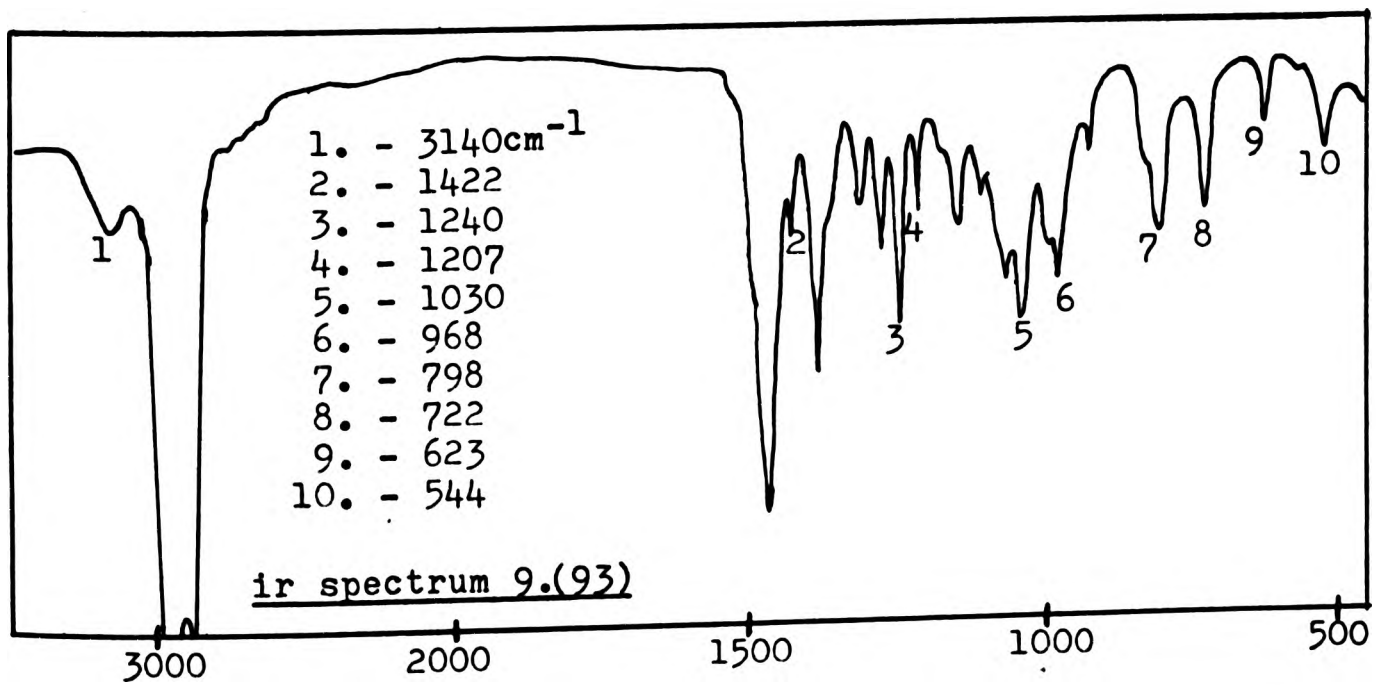
ir spectrum 6.(79)



ir spectrum 7.(92)



ir spectrum 8.(82)



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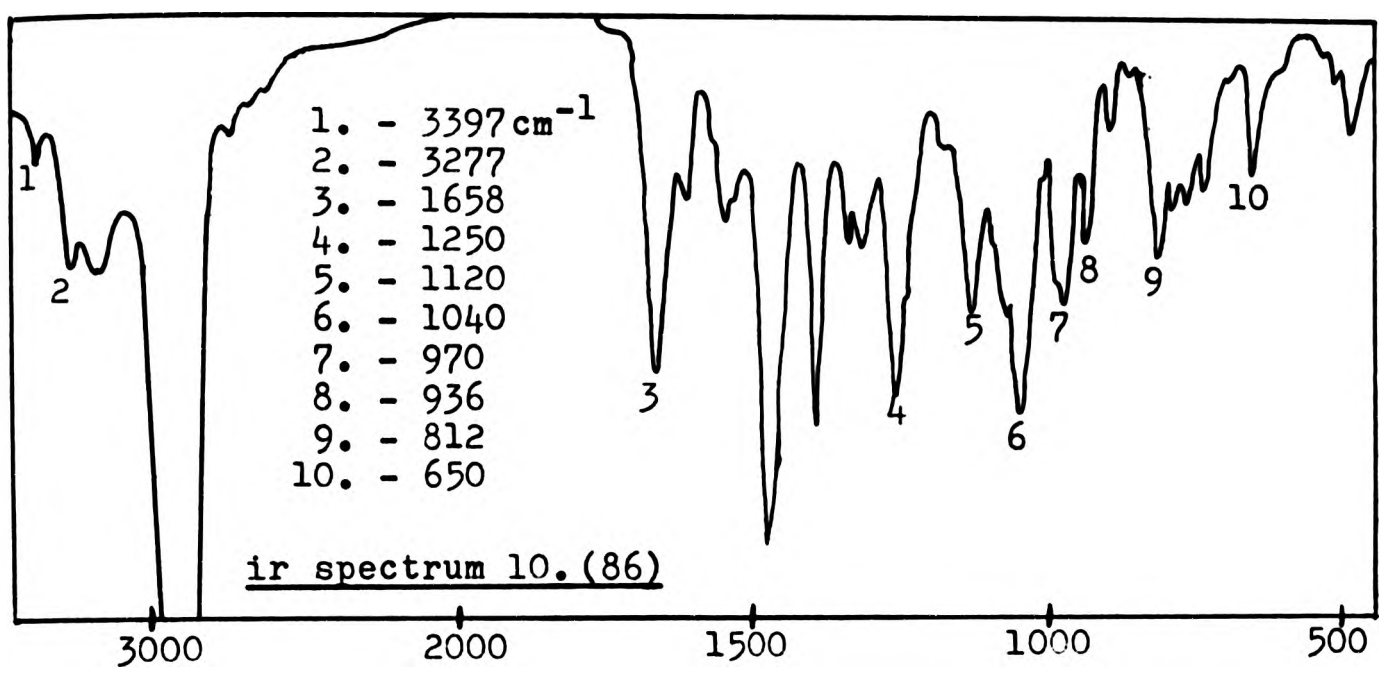

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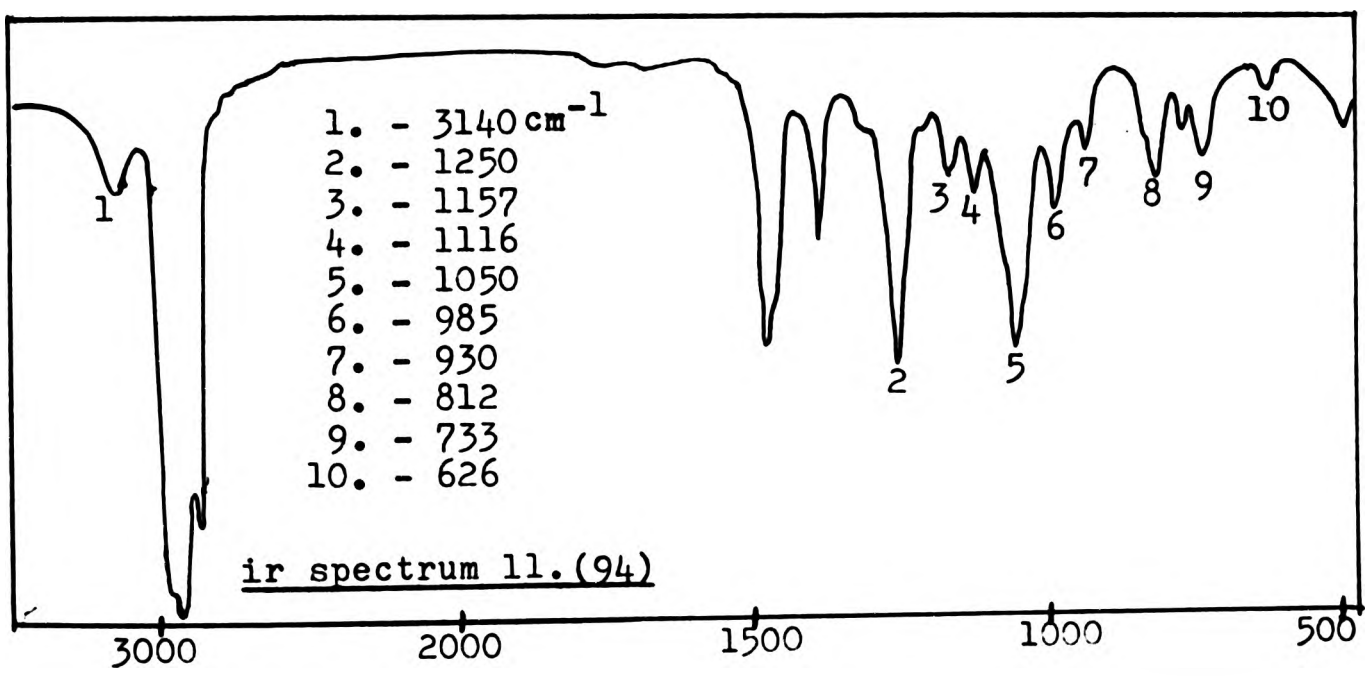

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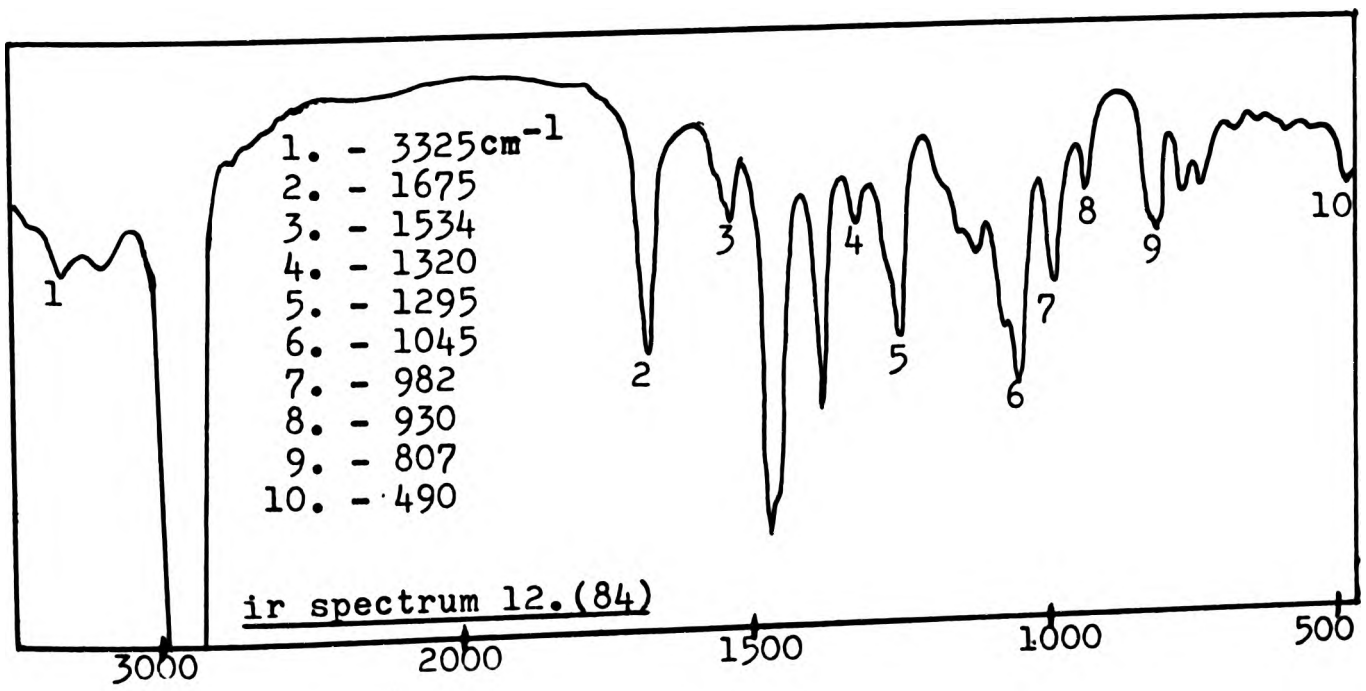
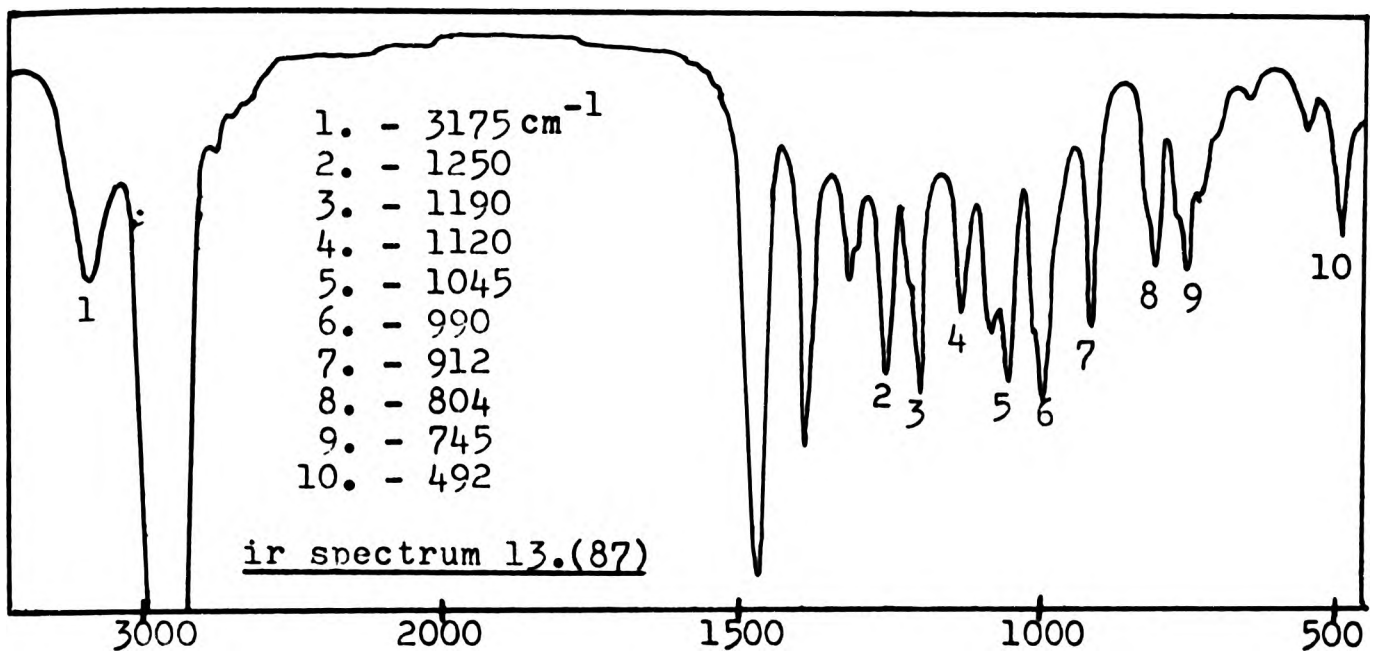
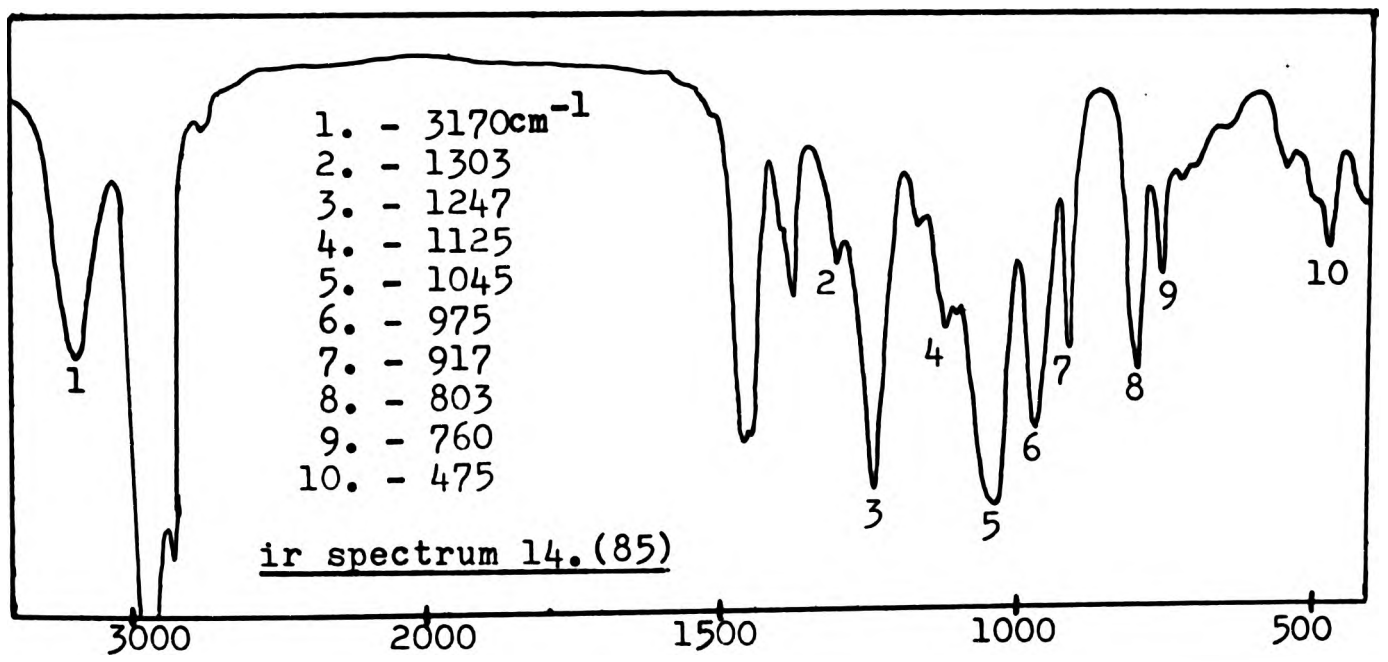


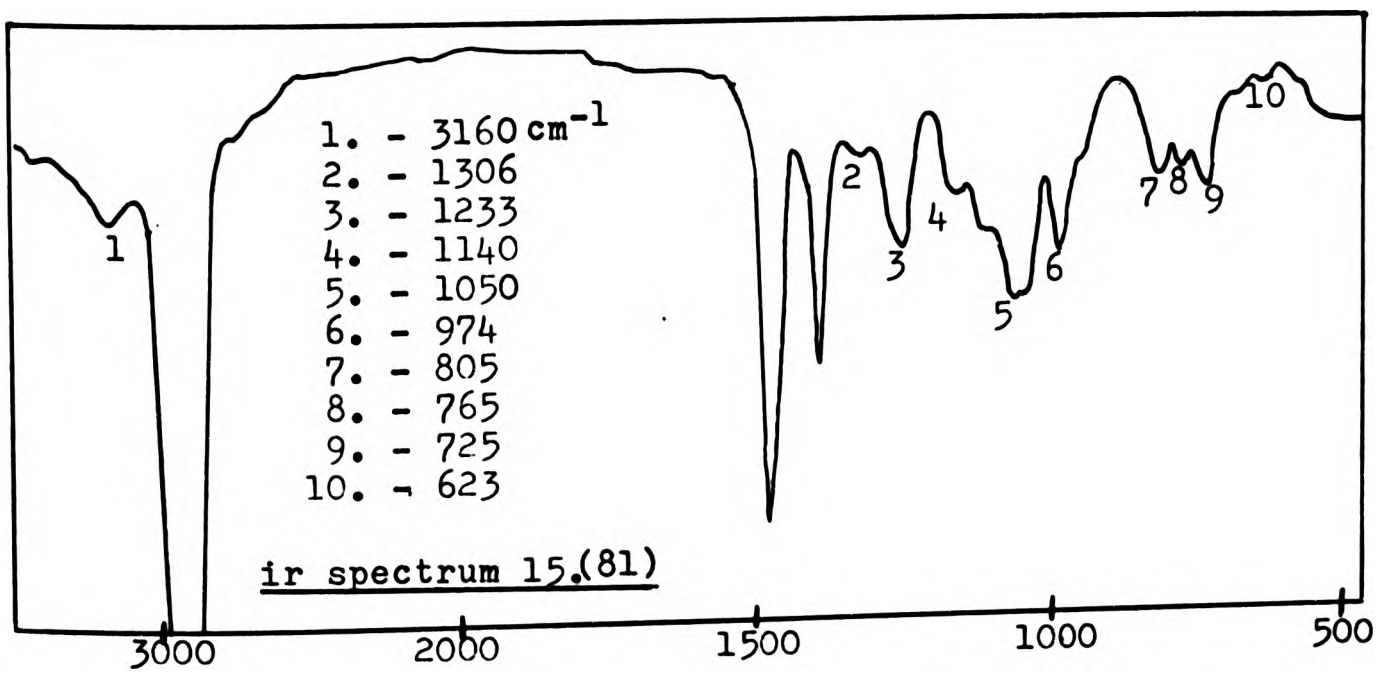
ir spectrum 12.(84)



ir spectrum 13.(87)



ir spectrum 14.(85)



ir spectrum 15.(81)

5.4.2 ^{1}H nmr spectra

Chemical shifts (δ)

These are quoted in ppm relative to tetramethylsilane (TMS=O).

Coupling constants (J)

These are quoted in Hertz for the designated atoms.

Multiplicity

s = singlet

d = doublet

t = triplet

dd = doublet of doublets

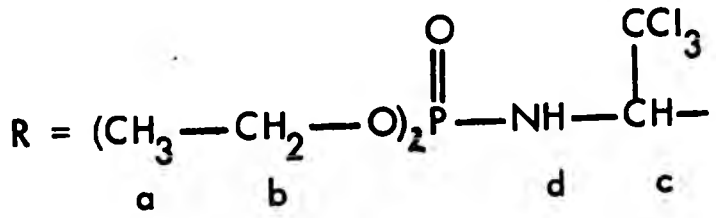
m = indistinct or complex multiplet

b = broad

qa = quartet

Structural diagrams

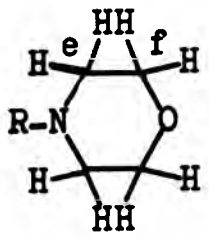
Protons are labelled with lower case letters.

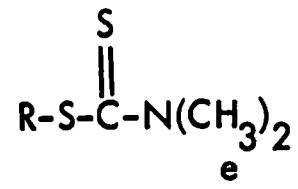


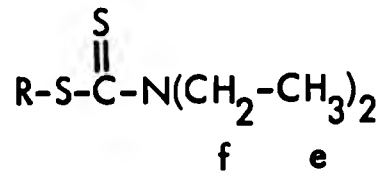
	δ	J
R-OH e	a = 1.35 (t)	H_aCCH_b = 7.2
	b = 4.25 (m)	H_bCOP = 7.2
(70)	c = 5.2 (dd)	H_cCNH_d = 11.3
	d ~ 4	H_cCNP = 8.0
	e = 6.4 (s,b)	

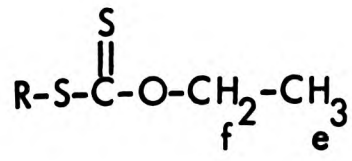
	δ	J
R-Cl	a = 1.35 (t)	H_aCCH_b = 7.2
(73)	b = 4.20 (m)	H_bCOP = 7.2
	c = 5.95 (dd)	H_cCNH_d = 12.7
	d ~ 4.6 (mb)	H_cCNP = 9.5

	δ	J
	a = 1.20 (m)	H_aCCH_b = 6.9
	b = 3.97 (m)	H_bCOP = 6.9
	c = 5.95 (m)	H_cCNP = 10.0
	d ~ 6.4 (m,b)	H_cCNH_d = 11.3
	e = 7.05 (s)	
	f = 7.35 (s)	
(76)	g = 7.92 (s)	

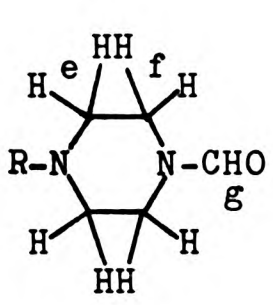
	δ	J	
	a = 1.33 (t)	H_aCCH_b	= 6.9
	b = 4.12 (m)	H_bCOP	= 7.3
	c = 4.45 (dd)	H_cCNH_d	= 11.2
	d ~ 3.7 (m,b)	H_cCNP	= 9.3
	e = 2.89 (m)	H_eCCH_f	= 4.7
(78)	f = 3.69 (t)		

	δ	J	
	a = 1.30 (m)	H_aCCH_b	= 7.1
	b = 4.10 (m)	H_bCOP	= 7.1
	c = 6.68 (dd)	H_cCNH_d	= 12.6
(92)	d ~ 4.5 (m,b)	H_cCNP	= 10.7
	e = 3.46 (s,b)		

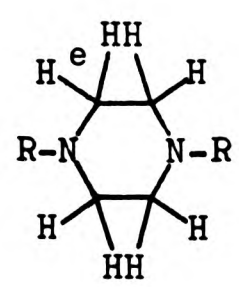
	δ	J	
	a = 1.28 (t)	H_aCCH_b	= 6.7
	b = 4.13 (m)	H_bCOP	= 6.7
	c = 6.87 (dd)	H_cCNH_d	= 12.7
(93)	d ~ 4 (b)	H_cCNP	= 10.5
	e = 1.35 (m)	H_eCCH_f	= 5.1
	f ~ 4 (b)		



	δ	J
	a = 1.38 (m)	H_aCCH_b = 6.8
	b = 4.10 (m)	H_bCOP = 6.9
	c = 6.12 (dd)	H_cCNH_d = 12.5
(94)	d ~ 4.5 (b)	H_cCNP = 10.3
	e = 1.28 (m)	H_eCCH_f = 7.7
	f = 4.68 (qa)	



	δ	J
	a = 1.30 (t)	H_aCH_b = 6.9
	b = 4.09 (m)	H_bCOP = 7.1
	c = 4.52 (dd)	H_cCNH_d = 11.3
	d ~ 3.7	H_cCNP = 10.0
	e = 2.9 (m,b)	
	f = 3.37 (t)	
(80)	g = 8.05 (s)	



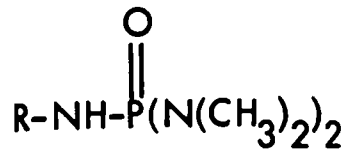
	δ	J
	a = 1.33 (t)	H_aCCH_b = 6.9
	b = 4.12 (m)	H_bCOP = 6.9
	c = 4.48 (m)	H_cCNH_d = 11.2
	d ~ 3.5 (m)	H_cCNP = 9.6
	e = 2.9 (mb)	

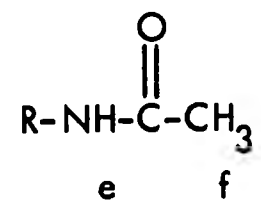
(79)

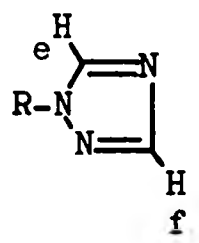
	δ	J
R-S-CH_2-CH_2-O-R e f	a = 1.32 (t)	H_aCCH_b = 6.9
	b = 4.13 (m)	H_bCOP = 6.9
	c = 4.98 (m)	
(81)	d ~ 4	
	e, f ~3.1 (m)	

	δ	J
R-S-CH_2-CH_2-OH e f g	a = 1.32 (m)	H_aCCH_b = 7.0
	b = 4.12 (m)	H_bCOP = 8.3
	c = 5.20 (dd)	H_cCNH_d = 11.2
(82)	d ~ 4	H_cCNP = 9.5
	e = 3.0 (t)	H_eCCH_f = 6.0
	f = 3.86 (m)	
	g ~ 4	

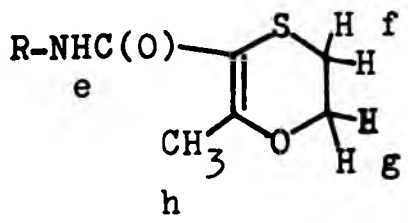
	δ	J
R-NH-P(=O)(OCH_2CH_3)$_2$ d b a	a = 1.33 (t)	H_aCCH_b = 7.1
	b = 4.17 (m)	H_bCOP = 7.1
	c = 5.13 (m)	H_cCNP = 9.5
(85)	d ~ 4	

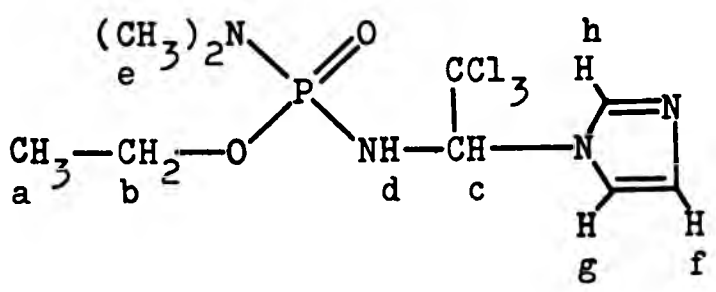
	δ	J
	a = 1.33 (t)	H_aCCH_b = 6.9
	b = 4.11 (m)	H_bCOP = 6.9
	c = 5.18 (m)	H_cCNH_d = 11.3
(87)	d ~ 4	H_cCNP = 8.6
	e ~ 4	H_fCNP = 10.0
	f = 2.65 (m)	

	δ	J
	a = 1.32 (m)	H_aCCH_b = 7.1
	b = 4.13 (m)	H_bCOP = 7.1
	c = 5.80 (m)	H_cCNP = 10.0
(84)	d ~ 4	
	e = 7.1 (m)	
	f = 2.07 (s)	

	δ	J
	a = 1.20 (t)	H_aCCH_b = 6.9
	b = 3.95 (m)	H_bCOP = 6.9
	c = 6.23 (m)	H_cCNH = 11.3
	d ~ 5.7 (mb)	H_cCNP = 10.0
	e = 7.95 (s)	
	f = 8.55 (s)	

(77)

	δ	J
	a = 1.31 (m)	H_aCCH_b = 6.8
	b = 4.12 (m)	H_fCCH_g = 4.7
	c = 5.85 (m)	
	d ~ 4	
	e ~ 7 (mb)	
	f = 2.95 (t)	
	g = 4.35 (t)	
(86)	h = 2.13 (s)	



(110)

	δ	J
	a = 1.20 (m)	H_aCCH_b = 7.3
	b = 3.90 (m)	H_cCNP = 10.0
	c = 5.92 (m)	
	d ~ 6.1 (m)	
	e = 2.55 (m)	
	f = 7.03 (s)	
	g = 7.38 (s)	
	h = 7.96 (s)	

5.4.3 Mass spectra

Electron impact mass spectra are presented in the following tables. Columns headed 'm/z' represent the charge to mass ratio of a particular fragment. Columns headed '%' represent the intensity of a fragment at that m/z value. The largest peak in the spectrum has been assigned an intensity of 100%. Structures are shown in appendix 1.

Mass spectrum of O,O diethyl N-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl] phosphoramidate (76), M. Wt. = 349, Temp. 200°C.

m/z	%	m/z	%	m/z	%
29	45	76	2.7	138	4.2
35	4.1	81	64	144	3.3
36	17	82	5.6	146	4.8
38	5.5	83	8.9	164	24
39	7.3	84	2.5	172	1.8
40	4.7	85	10	190	3.0
43	3.5	91	21	192	3.8
45	8.2	92	4.4	208	1.9
47	11	108	2.6	210	2.5
48	4.4	109	91	212	1.1
49	3.3	110	5.0	218	1.1
63	2.6	111	2.4	220	0.80
65	12	117	3.1	247	0.57
67	2.5	119	2.4	249	0.68
68	39	126	2.3	280	0.17
69	3.0	127	1.1	282	0.39
74	4.1	128	1.7	284	0.22
75	3.0	137	100		

Metastable ions were observed at M* 165.5, 114.4, 86.7, 76.0 and 60.2.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(1,2,4-triazol-1-yl)-ethyl]phosphoramidate (77) M.Wt. = 350, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
35	10	70	56	112	3.8	192	4.2
36	14	74	3.8	113	5.8	193	1.5
37	2.9	75	2.9	117	5.8	208	1.3
38	6.7	76	5.0	119	5.4	210	1.3
39	6.7	80	19	126	11	212	1.3
40	9.6	81	100	127	5.8	218	1.7
41	9.6	82	25	136	2.5	220	1.4
42	38	83	43	137	51	226	1.7
43	16	84	12	138	5.0	228	2.1
44	5.4	85	50	146	2.9	233	2.5
45	13	86	7.1	154	3.8	247	0.83
47	62	86	3.3	155	7.1	249	0.83
48	15	87	4.6	156	6.7	254	1.3
49	13	91	20	164	16	256	1.3
50	4.2	92	5.8	172	1.7	282	4.2
55	2.9	93	2.5	173	1.3	284	4.2
56	3.3	98	24	174	1.7	286	1.3
58	2.9	99	11	175	1.3	304	0.15
63	2.5	108	4.6	182	1.7	306	0.17
64	4.6	109	75	184	2.1	308	0.05
65	15	110	5.0	190	5.0		
69	58	111	5.8	191	2.9		

Metastable ions were observed at M* 114.4, 86.7 and 76.0.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(morpholin-4-yl)ethyl] phosphoramidate (78) M. Wt = 368, Temp. 200°C

m/z	%	m/z	%	m/z	%
31	2.8	98	20	218	6.0
36	24	109	52	223	16
38	8.1	110	4.4	226	3.2
41	5.2	115	6.5	228	3.1
42	15	122	4.8	230	0.97
43	5.2	123	4.8	247	5.2
44	23	124	3.7	249	3.4
45	10	125	3.1	251	100
47	10	126	17	252	10
54	3.6	134	8.1	254	1.0
55	5.9	137	53	256	0.97
56	17	155	3.8	282	6.7
57	27	164	16	284	6.5
65	7.3	177	8.1	286	2.0
69	4.9	179	3.8	297	1.7
70	3.8	180	3.5	299	0.56
80	20	181	5.8	325	7.3
81	40	182	3.2	327	6.9
83	28	183	3.2	329	2.1
85	18	190	4.3	368	0.24
86	27	191	3.4	370	0.24
87	40	192	3.1	372	0.08
88	29	195	27		
91	8.1	216	5.2		

Metastable ions were observed at M* 229, 198, 170.5, 160.8, 114.4, 86.7 and 76.0.

Mass spectrum of 1,4-bis[2,2,2-trichloro-1-(diethoxyphosphinylamino)-ethyl]piperazine (79) M. Wt. = 648, Temp. 200°C

m/z	%	m/z	%	m/z	%
31	5.5	68	7.7	156	6.4
35	5.9	69	23	164	23
36	13	70	6.4	190	3.2
37	2.3	71	9.1	192	1.8
38	2.3	72	43	194	2.3
39	18	73	6.8	212	1.4
40	5.9	76	5.0	222	2.7
41	51	80	5.0	250	14
42	26	81	100	278	3.2
43	41	82	11	282	2.3
44	34	83	16	284	3.6
45	11	84	8.6	286	0.91
47	17	85	25	299	0.32
48	5.9	86	10	301	0.18
53	7.7	87	5.9	325	0.32
54	7.7	91	22	327	0.23
55	30	92	5.9	329	0.05
56	36	93	5.5	342	0.05
57	49	97	14	344	0.05
58	7.3	98	8.2	346	0.02
59	82	109	77	366	0.23
60	10	114	13	368	0.23
65	19	126	5.5	370	0.09
67	10	137	54		

Metastable ions were observed at M* 197.1, 114.4, 86.7, 58.5.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(4-formylpiperazin-1-yl)ethyl]phosphoramidate (80) M.Wt. = 395, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	5.5	80	33	154	2.5	256	0.88
35	6.5	81	41	155	5.5	278	26
36	26	82	8	156	4.5	282	2.0
37	2.0	83	17	164	17	284	1.8
38	7.5	85	16	177	3.0	286	0.55
41	15	87	7.0	179	3.0	324	0.88
42	65	91	24	190	5.1	325	0.71
43	22	92	5.5	192	5.1	326	0.43
44	24	98	23	204	3.0	327	0.68
45	8.5	99	16	206	3.5	337	0.03
49	22	108	8.0	208	4.5	339	0.03
54	10	109	65	210	1.5	341	0.01
55	25	110	5.0	212	2.5	350	0.18
56	100	114	21	218	2.0	352	0.14
57	17	125	9.0	220	1.5	354	0.06
58	10	126	17	222	7.1	361	0.04
65	12	127	7.0	226	2.0	363	0.03
68	5.5	136	4.5	228	1.5	395	0.05
69	20	137	70	247	3.5	397	0.05
70	5.0	138	3.0	250	8.5	399	0.02
72	20	142	19	254	0.90		

Metastable ions were observed at M* 224.8, 197.1, 114.4, 86.7 and 76.0

Mass spectrum of 1-[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethoxy]-2-[2,2,2-trichloro-1-(diethoxyphosphinylamino)ethylthio]ethane (81)
M.Wt. = 640, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	12	81	100	146	3.0	219	1.3
35	11	82	7.8	154	3.9	220	2.2
36	15	83	70	155	8.7	226	5.2
38	3.5	84	5.2	156	4.8	228	4.8
41	7.4	85	31	157	2.2	230	0.87
42	6.1	87	4.8	158	3.0	247	3.0
43	12	89	3.9	164	14	249	1.7
44	4.3	91	31	172	2.2	254	3.9
45	12	92	7.4	173	1.3	256	2.2
47	41	93	3.9	174	1.3	263	0.87
48	7.4	98	2.0	175	0.87	265	0.43
49	8.7	99	5.2	176	0.87	282	11
55	7.4	100	14	182	2.2	284	10
57	4.3	108	5.7	184	2.7	286	2.6
59	6.1	109	89	190	7.0	300	0.25
60	8.3	110	7.0	191	3.0	302	0.30
61	12	117	7.4	192	9.1	323	0.45
63	3.5	119	4.3	193	1.7	324	0.20
65	17	126	4.8	194	1.7	325	0.37
69	6.1	127	8.3	208	1.3	326	0.10
70	5.7	136	3.5	210	3.0	358	0.17
74	5.7	137	78	212	1.7	360	0.15
76	4.8	138	4.8	214	0.87		
80	8.3	144	2.2	218	4.8		

Metastable ions were observed at M* 230.8, 228.8, 114.4, 86.7 and 76.0.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(2 hydroxyethylthio) ethyl]phosphoramidate (82) M.Wt. = 359, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	29	81	9.3	136	3.0	214	1.7
35	7.3	82	11	137	53	218	6.7
36	22	83	21	138	3.7	220	5.0
38	6.3	84	6.3	155	10	226	15
41	7.7	85	13	156	12	228	20
43	13	86	11	158	4.3	230	5.3
44	10	89	7.0	164	12	247	4.3
45	36	91	17	172	1.9	249	2.7
46	10	92	10	173	2.1	254	7.7
47	25	98	5.3	174	2.2	256	11
48	17	99	4.7	182	3.0	258	2.7
59	13	108	7.0	184	2.3	282	43
60	17	109	100	190	28	284	33
61	9.3	110	14	191	6.7	286	15
64	5.6	111	9.3	192	22	341	0.3
65	18	112	10	193	4.7	343	0.3
75	5.0	117	5.0	194	5.0	345	0.1
76	11	126	6.0	208	1.7		
78	8.3	127	3.0	210	3.3		
80	4.3	128	4.0	212	5.0		

Metastable ions were observed at M* 230.8, 228.8, 114.4, 86.7 and 76.0.

Mass spectrum of O,O-diethyl N-(2,2,2-trichloro-1-acetamidoethyl) phosphoramidate (84) M.Wt. = 340, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
29	32	83	4.2	181	79	262	8.9
35	1.3	85	2.9	182	6.3	263	5.5
36	5.8	91	5.0	190	3.9	264	7.4
37	0.79	92	2.9	192	2.4	265	4.2
38	2.1	98	15	204	2.4	268	2.1
41	2.9	107	5.0	206	3.7	269	2.1
42	7.1	108	7.6	207	3.7	270	1.7
43	100	109	16	208	2.4	271	1.0
44	79	110	5.3	209	2.4	282	1.8
45	7.4	111	5.8	218	1.3	284	1.8
47	4.2	117	2.9	220	1.1	286	0.53
60	6.1	119	2.4	223	19	295	1.0
62	2.1	125	32	225	1.8	297	1.8
64	4.2	126	5.3	227	2.9	305	2.2
65	6.6	136	5.0	232	3.4	307	0.81
66	1.6	137	12	234	3.4	340	0.13
76	2.6	138	1.8	236	1.8	342	0.15
78	1.6	153	21	245	1.1	346	0.08
80	13	155	2.6	247	1.1		
81	29	156	2.6	254	2.4		
82	3.9	164	7.6	256	2.4		

Metastable ions were observed at M* 146.9, 129.3, 102.1, 86.7 and 76.0.

Mass spectrum of 1,1,1-trichloro-2,2-bis(diethoxyphosphinylamino)ethane (85)
M.Wt. = 434, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	6.0	63	3.0	98	57	154	30
33	18	64	5.8	99	11	155	7.0
36	7.4	65	16	105	4.0	156	6.7
38	2.5	67	16	107	7.4	160	18
39	10	68	4.6	108	10	164	6.8
40	3.5	69	30	109	56	181	40
41	42	70	5.4	110	4.3	182	4.7
42	9.1	71	14	111	12	187	15
43	58	72	14	113	4.2	188	5.5
44	14	77	9.3	117	5.7	190	4.3
45	30	80	40	119	5.8	199	3.3
46	4.0	81	100	123	10	206	5.3
47	8.8	82	8.4	124	4.5	233	3.0
51	6.3	83	20	125	40	234	3.0
53	3.3	84	4.9	126	33	261	5.7
54	3.9	85	14	127	5.0	262	2.6
55	2.4	86	3.3	136	7.3	277	11
56	16	91	16	137	27	278	5.3
57	44	92	5.4	144	3.2	289	9.2
58	4.5	93	5.7	146	3.6	290	11
59	32	95	11	151	6.9	317	82
60	3.1	96	6.4	152	3.7	318	11
62	3.2	97	19	153	38	319	2.8

Metastable ions were observed at M* 129.3, 113.4, 103.1, 86.7, 76.0, 65.3 and 59.8.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(5,6-dihydro-2-methyl-1,4-oxathiin-3-carbonamido)ethyl]phosphoramidate (86) M.Wt. = 440, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
29	15	72	14	142	38	258	0.29
35	3.2	80	5.3	143	23	275	0.10
36	8.2	81	9.4	144	4.1	282	1.8
37	0.88	83	4.4	154	16	284	1.8
38	2.6	87	22	159	35	286	0.60
39	5.3	88	7.4	160	3.5	287	0.60
41	16	89	68	164	0.35	289	0.88
42	7.6	90	3.2	169	1.2	291	0.29
43	100	91	2.9	170	1.5	323	2.4
44	29	98	6.5	181	1.2	324	0.44
45	20	99	3.5	190	2.4	325	0.29
46	7.4	100	6.0	192	1.8	369	0.24
47	4.4	103	2.9	218	0.20	371	0.12
55	13	108	4.0	220	0.30	397	0.15
56	3.2	109	6.8	226	1.8	399	0.12
58	11	114	8.2	228	2.1	401	0.06
59	23	115	5.2	230	0.60	425	0.03
60	4.4	126	5.3	247	0.17	427	0.03
61	6.5	130	0.57	249	0.07	440	1.6
67	4.7	131	0.30	250	0.11	442	1.5
69	5.9	132	0.14	254	0.88	444	0.44
71	7.6	137	2.9	256	1.5		

No metastable ions were observed.

Mass spectrum of 1,1,1-trichloro-2-diethoxyphosphinylamino-2-bisdimethyl-aminophosphinylaminoethane (87) M. Wt. = 432. Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
35	4.6	90	7.8	187	29	346	14
36	29	91	9.7	199	6.3	347	19
37	1.3	92	24	200	4.6	348	4.9
38	9.7	98	25	214	17	349	6.7
42	27	107	38	215	18	351	23
43	19	108	25	227	11	352	10
44	100	109	28	242	19	353	15
45	61	110	4.4	243	12	354	8.2
46	19	126	19	270	12	360	1.7
47	14	134	8.3	279	2.4	362	1.6
48	6.8	135	98	281	2.4	372	0.29
57	5.8	136	7.3	282	4.4	374	0.48
58	7.7	137	23	284	4.4	376	0.27
59	6.9	150	4.3	295	2.4	388	14
64	6.3	151	9.7	296	2.5	390	12
65	4.9	152	3.9	297	2.6	392	3.9
76	6.7	153	5.0	315	3.4	397	1.8
80	36	154	18	323	7.8	399	1.4
81	25	155	3.4	324	5.8	417	0.19
83	85	164	7.3	325	5.3	419	0.18
85	49	172	3.9	326	3.3	432	1.5
86	12	173	4.8	344	9.8	434	1.5
87	7.3	181	6.8	345	21	436	0.5

Metastable ions were observed at M* 231.4, 216.9, 189.2, 162.6, 114.4 and 86.7.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(dimethylthiocarbamoyl-thio)ethyl]phosphoramidate (92) M. Wt. = 402, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	2.9	77	10	122	3.5	190	6.0
35	1.5	78	4.4	123	4.9	191	3.4
36	10	80	3.2	127	4.0	192	4.0
37	0.51	81	64	136	2.5	193	2.2
38	6.0	82	6.5	137	92	196	1.8
40	2.4	83	9.0	138	8.5	208	4.5
41	4.2	84	4.1	144	2.2	209	6.5
42	22	85	4.8	146	2.4	210	4.6
43	7.0	88	58	148	1.0	212	1.9
44	32	89	3.9	153	3.6	218	3.4
45	13	90	3.4	154	3.6	220	2.3
46	2.8	91	25	155	4.9	222	0.45
47	12	92	5.3	156	5.4	226	0.63
48	2.7	93	4.7	157	1.6	228	0.63
49	1.6	98	2.6	158	3.1	240	0.51
56	6.6	99	4.8	164	52	247	2.7
61	2.1	108	4.9	165	2.0	249	1.8
63	2.3	109	100	172	2.0	251	0.34
64	2.4	110	6.5	174	3.0	282	2.5
65	12	111	2.9	176	1.1	284	2.1
72	6.0	112	3.1	181	1.8	286	0.57
73	13	117	4.7	182	1.6		
74	5.5	119	3.8	183	0.68		
75	3.6	120	2.2	184	2.0		
76	40	121	46	186	0.68		

Metastable ions were observed at M* 114.4 and 86.7.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(diethylthiocarbamoylthio)ethyl]phosphoramidate (93) M.Wt. = 430, Temp. 200°C.

m/z	%	m/z	%	m/z	%	m/z	%
31	2.6	64	2.6	110	5.6	164	33
35	3.5	65	10	111	6.5	165	2.5
36	14	72	8.5	112	3.2	171	1.9
37	1.1	73	8.5	113	4.0	172	2.4
38	5.8	74	2.9	116	16	173	2.2
39	2.0	75	3.2	117	4.5	174	1.6
40	2.1	76	26	119	3.5	182	5.9
41	2.5	77	4.3	126	15	184	4.2
42	4.7	78	2.6	127	7.1	186	1.6
43	3.9	80	20	136	2.3	190	3.0
44	15	81	62	137	100	191	6.7
45	6.4	82	12	138	5.5	192	2.6
46	1.2	83	16	146	2.6	193	4.5
47	8.8	84	8.2	147	2.8	208	2.8
48	3.7	85	9.8	148	1.9	210	2.8
49	2.5	86	3.9	149	21	212	4.4
55	3.4	87	2.3	150	1.9	214	1.7
56	7.3	88	20	151	2.6	237	0.53
57	2.2	91	12	153	3.2	247	5.4
58	29	92	5.6	154	2.5	249	3.5
59	4.5	93	2.8	155	8.8	282	1.0
60	20	98	17	156	11	284	0.71
61	2.5	99	15	157	2.9	286	0.21
62	2.3	108	6.8	158	4.0		
63	2.1	109	86	160	1.1		

Metastable ions were observed at M* 114.4, 86.7, 66.8 and 40.9.

Mass spectrum of O,O-diethyl N-[2,2,2-trichloro-1-(ethoxythiocarbonylthio) ethyl]phosphoramidate (94) M.Wt. = 403, Temp. 200°C

m/z	%	m/z	%	m/z	%	m/z	%
31	24	65	32	112	3.9	191	3.9
35	6.3	74	11	117	10	192	8.0
36	12	75	4.4	119	6.3	208	4.4
38	4.2	76	22	122	36	210	3.0
41	6.3	77	13	126	7.1	212	1.7
42	4.6	78	7.1	128	3.1	218	3.4
43	14	81	64	136	2.2	220	1.9
44	10	82	22	137	100	226	2.5
45	35	83	12	138	6.3	228	3.2
47	46	84	8.0	144	4.4	230	0.89
48	4.0	85	8.5	146	5.4	247	1.9
49	3.6	89	8.9	154	3.4	249	1.0
55	3.8	91	26	155	8.0	254	1.4
56	2.9	92	8.0	156	7.8	256	1.9
57	3.2	93	8.0	158	2.5	258	0.49
60	12	99	2.8	164	36	282	5.0
61	16	108	6.3	172	2.8	284	4.4
62	4.2	109	89	173	2.5	286	1.3
63	6.3	110	8.0	174	3.8	288	0.18
64	8.0	111	3.3	190	12		

Metastable ions were observed at M* 114.4 and 86.7

Mass spectrum of O-ethyl N,N-dimethyl-N'-[2,2,2-trichloro-1-(imidazol-1-yl)ethyl]phosphoramidate (110) M.Wt = 348, Temp. 200°C

m/z	%	m/z	%	m/z	%
35	4.3	91	1.7	211	0.93
36	7.2	92	2.4	213	0.54
37	0.56	106	2.0	235	0.18
38	4.8	107	2.0	237	0.18
39	6.7	108	100	244	0.93
40	13	109	3.0	246	0.93
41	30	126	4.1	280	0.93
42	15	128	1.3	281	0.15
43	19	136	26	282	0.93
44	30	137	24	284	0.33
45	8.5	163	3.7	290	0.03
47	6.7	181	7.4	292	0.02
68	39	182	0.56	312	0.03
83	5.6	183	0.56	314	0.02
85	2.0	207	0.56		
90	1.7	209	0.93		

A metastable ion was observed at M* 85.8.

5.5 Biological Screening Tests

5.5.1 Fungicidal Activity

5.5.1.1 In vitro tests carried out at P.N.L.

Each compound was screened by observing the germination of spores of various fungi (Fusarium culmorum, Fusarium oxysporum, Ophiobolus graminis, Helminthosporium sativum, Septoria nodorum and Helminthosporium avenae) suspended in nutrient agar into which the test compound had been incorporated at a concentration of 0.05% w/v (500 ppm).

Samples were prepared by dissolving the test compounds (0.05g) in absolute ethanol (0.5 cm^3) in a sterile test tube, on heating if required. The solutions were then diluted to a volume of 10 cm^3 with sterile water. If precipitation of the compounds occurred, or they were insoluble in ethanol the samples were used as suspensions.

A homogenous spore suspension of the fungus under examination was prepared by agitating a mature culture of the fungus in nutrient agar with sterile water. A portion of the spore suspension (2 cm^3) was added to the molten nutrient agar (100 cm^3) at 40°C using a sterile pippette. The bottle containing this mixture was stoppered and rotated gently by hand to disperse the spores before the agar set, then portions of the culture (ca. 10 cm^3) were poured into sterile plastic Petri dishes in which had been

placed a sample (1 cm^3) of the solution (or suspension) of the test compounds prepared as described earlier using a sterile pippette. The components were mixed immediately by gentle rotation of the petri dishes in a horizontal plane on a flat surface and were then left undisturbed until the agar had set. Control plates were similarly prepared containing only the nutrient agar spore suspension. As standards a number of plates containing phenyl mercuric acetate at various concentrations (100 ppm, 10 ppm and 1 ppm) were similarly prepared. After the agar had set the cultures were stored in an incubator at 25°C and checked periodically for spore germination. The extent of germination was assessed by comparison of the test plates with the controls and standards. Results are given on page 85.

5.5.1.2 In vitro tests carried out by KenoGard AB

The fungi Pyricularia oryzae (subdivision Deutermycotina) and Thanatephorus cucumeris conidial stage Rhizoctania solani were cultivated on PDA agar oxford CMI 139. The substances were dissolved in the agar (to give 300 ppm) after sterilizing it and shaken thoroughly to distribute the substances evenly before pouring into standard 9 cm petri plates. To each petri plate a 5 mm agar plug containing actively growing mycelia was placed upside down in the centre. The plates were incubated at 28°C. After 7 days for R. solani and 14 days for P. oryzae, the growth diameter was measured and compared to untreated and Panoctine (300 ppm treated plates. The results are given on page 86.

5.5.1.3 In vivo tests carried out by KenoGard AB

Seeds of spring-barley (Tellus 374) infected with Pyrenophora teres (subdivision Ascomycotina) conidial stage Dreschlera teres and winter-wheat (Holme 3055) infected with Leptosphaeria nodorum (subdivision Ascomycotina) conidial stage Septoria nodorum were treated (10 min in a lab seed-treatment machine) with formulations containing 20% (w/v or w/w) of the active ingredient. The dosage rate was 2 ml (2g) per kilo of seeds. A number of seeds (200) of each treatment were placed on a moistened filter paper (distilled water, pH 5.4) and were incubated for 3 days at 10°C then 4 days at 20°C. The coleoptile and roots of the seeds were thereafter examined for disease symptoms and compared with untreated seeds and seeds treated with 20% Panoctine, the commercial products Panoctine 35 (S. nodorum) and Panoctine Plus (D. teres). The results are given on page 86.

5.5.2 Phytotoxicity (tests by KenoGard AB)

Seeds of winter-wheat and spring-barley from the seed treatment test (above) were planted in moist sand and placed in a growing chamber for 4 days at 10°C then 6-7 days at 20°C. Thereafter germination and plant development (bonitet) was observed. In the germination test each of the 200 seeds were examined and those germinating in the normal way were counted. In the bonitet test the early plant development was assessed by visual examination and graded on a scale of 1-5 where 4 represents normal plant development and 5 represents an improvement. Results are given on page 89.

5.5.3 Anticholinesterase activity (Tests by Murphy Chemical Ltd.)

Tests were carried out by a method based on that of Ellman et al.[97] In each case a whole blood sample (10μl) was diluted with buffer solution (6 cm^3, pH3) and a portion of the blood-buffer mixture (3 cm^3) was transferred to a 1 cm cuvette into which acetylcholine iodide solution (2μl) and dithonitrosobenzoate solution (2μl) were added. The test compound (up to ca. 750 g/cm^3 depending on activity) was added and the liberation of thiocholine was followed spectrophotometrically as it reacted with the dithionitrobenzoate ion to give a yellow colouration due to formation of the 5-thio-2-nitrobenzoate anion (λ412 nm). Results are given on page 91.

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APPENDIX 1

Numbering of structures which are frequently referred to in the text.

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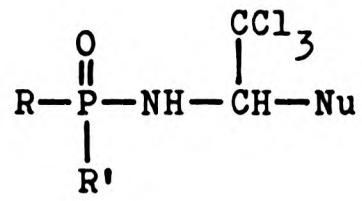

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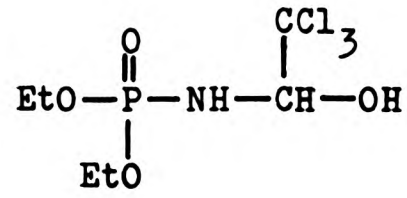

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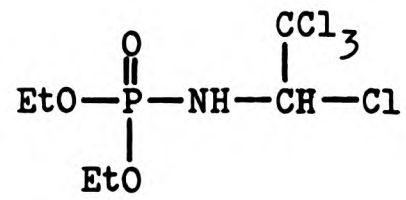

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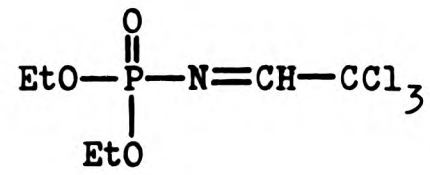

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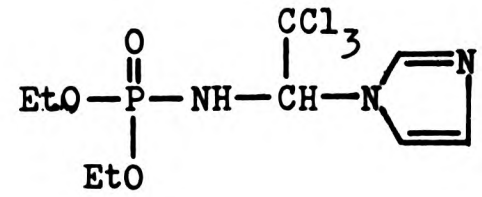

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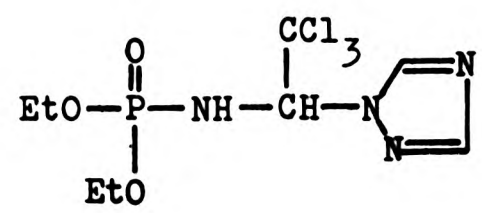

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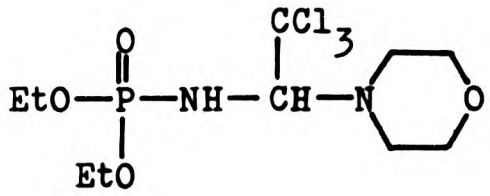

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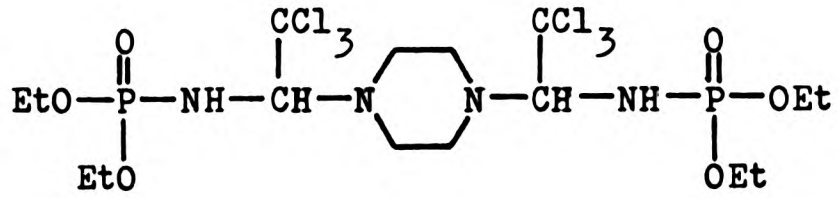

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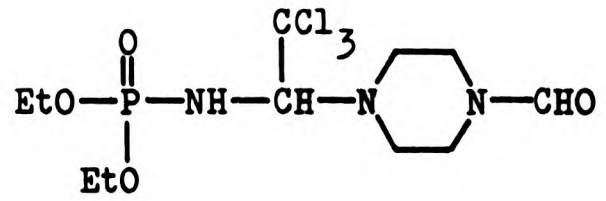

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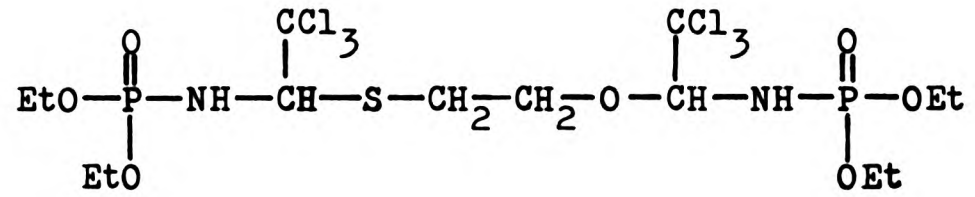

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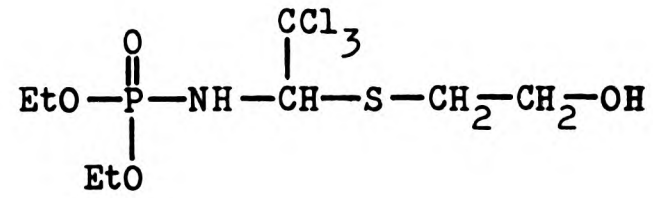

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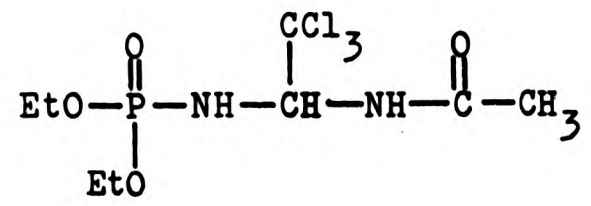

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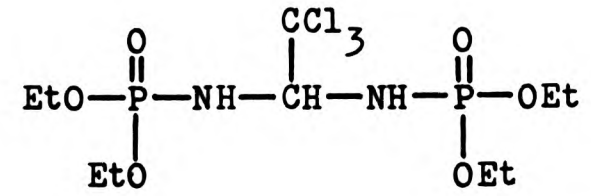

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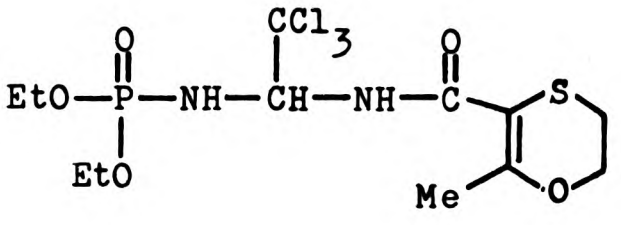

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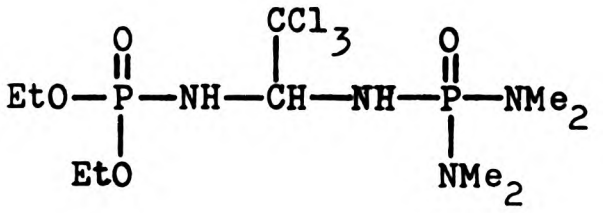

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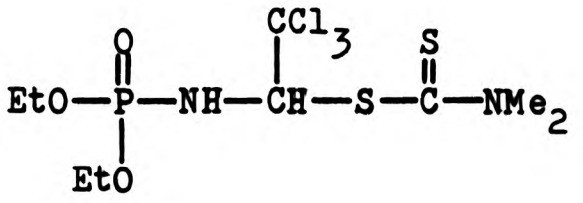

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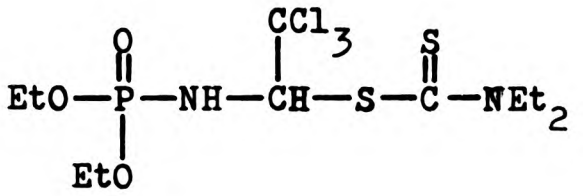

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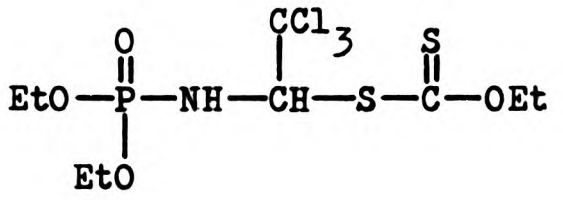

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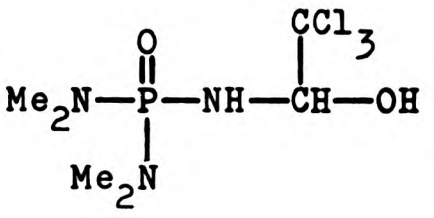

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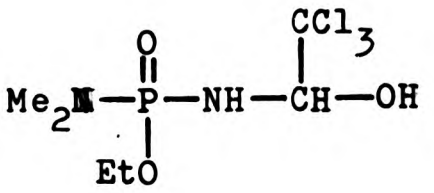

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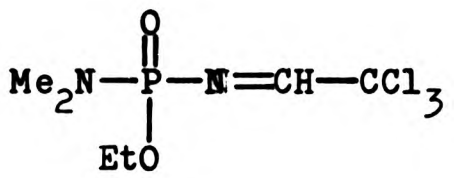

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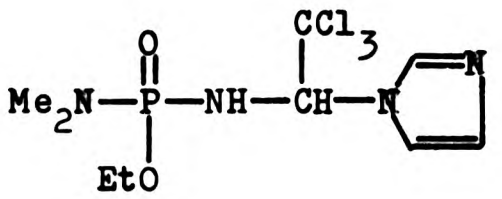

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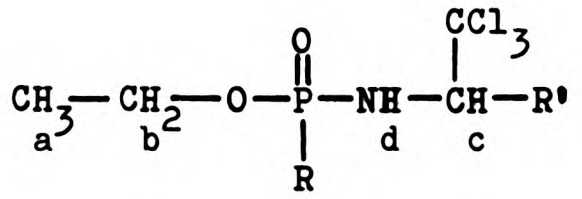

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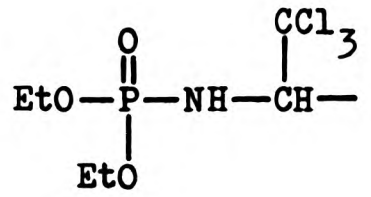

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