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THE DECOMPOSITION OF SOME ORGANOPHOSPHORUS INSECTICIDES AND RELATED COMPOUNDS IN PLANTS

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CONTENTS

To discover the reactions undergone in plants by organophosphorus compounds of the type used as insecticides the non-ionizable metabolites of several compounds were studied.

Octamethylpyrophosphoramide (schradan) is degraded in four species of plants. Low yields of heptamethylpyrophosphoramide and a powerful anticholinesterase are obtained besides ionizable compounds. Oxidation of schradan with hydrogen peroxide and with oxygenated liver slices gives the same products in similar proportions. Thus plants probably degrade schradan by oxidation, producing mainly compounds unstable in water, which are hydrolyzed to substituted phosphoric acids. The powerful anticholinesterase is believed to be hydroxymethyl-heptamethylpyrophosphoramide from its chloroform/water partition coefficient and its yield in plants. It may, however, be octamethylpyrophosphoramidic oxide.

Turnip plants also degrade the dimethylamide, monomethylamide, *n*-butylamide, *iso*propylamide and ethyl ester of tetramethylphosphorodiamidic acid. As these compounds contain no acidic group which can be removed by hydrolysis after oxidation, high yields of compounds extractable from water by chloroform are obtained. None of the parent compounds are hydrolyzed, nor are butyl or *iso*propyl groups removed in one step. Probably the dimethylamide is demethylated to the monomethylamide in a way similar to schradan. The physical properties of the degradation products of the other compounds indicate that only the dimethylamido groups are attacked, the other groups being inert.

OO-diethyl *O*-ethylthioethyl phosphorothionate is converted in plants to at least three compounds extractable by chloroform. Their structures are unknown.

OO-diethyl *S*-ethylthioethyl phosphorothiolate is oxidized rapidly in plants to *OO*-diethyl *S*-ethylsulphinylethyl phosphorothiolate and another compound of unknown structure, both of which are more stable in plants than the parent compound. The same products are formed by the action of hydrogen peroxide on the parent compound.

Thus a number of organophosphorus compounds are degraded by oxidation in plants. There is no evidence that their hydrolysis is catalyzed.

Introduction

In recent years numerous organophosphorus compounds have been applied to crops as insecticides. Some of these compounds are absorbed by plants, and it was interesting to find

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that, although they are very different from any which are known to occur naturally in plants, they are broken down by the plant system, even though they are stable to many chemical reagents. It is necessary to know the nature of the metabolites formed in plants in order to be able to assess toxic residues, and it was this that initially led us to investigate the degradation of the parent compounds. The results, however, appeared to be of more general chemical interest. It is solely with the chemical aspects of the degradation that this paper is concerned. Some related non-insecticidal compounds of more simple structure were also studied, because more insight can then be gained into the general mechanisms involved. All the phosphorus compounds used were labelled with the radioactive isotope of phosphorus, ^{32}P, to aid separation and identification of the metabolites.

When this work was started it already seemed very likely that the primary action of plants on schradan (octamethylpyrophosphoramide, $(Me_2N)_4P_2O_3$), the most fully investigated of these compounds, was oxidative, although nearly all the metabolites possessed the properties of hydrolysis products (Bennett & Thomas 1951; Heath, Lane & Llewellyn 1952*a*,*b*). Thus Hall, Stohlmann & Schechter (1951) found that a chloroform extract of treated plants contained an irreversible inhibitor of cholinesterase,* analogous to that produced by the oxidation of schradan by liver slices *in vitro* and *in vivo* (Dubois, Doull & Coon 1950; Gardiner & Kilby 1950, 1952; Casida, Allen & Stahmann 1953; Casida & Stahmann 1953). Schradan and its hydrolysis products exhibit negligible anticholinesterase activity. On this evidence alone the oxidation to an inhibitor could be a side reaction and catalyzed hydrolysis be the most important mode of degradation. Evidence against this was found by Heath *et al.* (1952*b*), who discovered that two plant species degrade schradan and hexamethylphosphoramide at the same rate, although the latter is more stable to chemical hydrolysis at all pH's (Heath & Casapieri 1951). The major plant-produced product from hexamethylphosphoramide is not ionizable and therefore not produced by hydrolysis. Hartley & Heath (1951) concluded that it was an oxidation product formed by attack on the N—C—H system. If schradan was also attacked there, the similarity of the environments of this structural unit in schradan and hexamethylphosphoramide would provide a ready explanation of the similarity in their rates of degradation. The inhibitor reported by Hall *et al.* (1951) was thus supposed to be a hydrolytically unstable oxidation product of schradan. Hexamethylphosphoramide contains no acid anhydride system, so that an analogous metabolite would not be hydrolyzed. It, or a derivative of it, would thus appear as a non-ionizable compound.

The final products appear to be substituted phosphoric acids, and a study of these would throw little light on how the degradation is initiated. We have therefore largely ignored them and concentrated on the non-ionizable metabolites such as Hall's inhibitor, although it was plain from the work referred to above that those from schradan are present in minute quantities and might prove very unstable. Chloroform is, however, an exceptionally good solvent for non-ionizable organophosphorus compounds, so that, using it as an extractant, we have been able to obtain concentrates of various metabolites and to prove or infer their constitution.

The degradation of schradan is the subject of the first section of the paper, starting with chemical oxidation. A comparison of the products of these reactions with those from

* Such irreversible inhibitors are referred to as 'inhibitors' throughout.

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several plant species and from oxygenated liver slices confirms that the primary attack in plants is oxidative. The major non-ionic metabolite, heptamethylpyrophosphoramide, has not been reported previously. Physico-chemical considerations lead to a different view of the structure of Hall's anticholinesterase from that put forward by Casida, Allen & Stahmann (1954). The difference is fully discussed.

The following two sections are devoted to the metabolism in plants of some amides and the ethyl ester of tetramethylphosphorodiamidic acid ($(Me_2N)_2PO.OH$); and of *OO*-diethyl *O*(ethylthioethyl) phosphorothiolate ($(EtO)_2PO.SC_2H_4.SC_2H_5$) and its thionate isomer, $(EtO)_2PS.OC_2H_4SC_2H_5$. It is shown that these also are oxidized in plants.

Preparations of compounds are given in the appendix.

Methods

Methods of extraction and concentration

It was expected that some metabolites would be unstable in water. Methods were therefore designed to obtain extracts quickly without using high temperatures.

Extraction of products from chemical oxidation

The reaction mixtures were diluted with water and extracted five times with an equal volume each time of chloroform. The chloroform solution was concentrated either by evaporation *in vacuo*, or, for volumes exceeding 1 l., by flash evaporation *in vacuo*. The flash evaporator was an all-glass modification of the descending film evaporator widely used in chemical industry.

Extraction of products from treated plants

Plant samples varied in size from a few grams to over 100 kg. All but one very large sample were macerated with water and each macerate filtered. It was shown that the filtrate contained about 98 % of the total radioactive phosphorus in the plants, in agreement with the previous findings of Bennett & Thomas (1951) and Heath *et al.* (1952*a*, *b*) The filtrate was then extracted with an equal volume of chloroform, usually only once, the layers centrifuged and the chloroform layer concentrated as before.

For most purposes it was necessary to free the chloroform concentrates from chlorophyll and other natural products. Each concentrate was evaporated down *in vacuo* to a small volume, water added and the evaporation continued until the remainder of the chloroform was removed. The aqueous residue was then filtered and the filtrate extracted five times with chloroform, and this solution was reconcentrated. The residue was dissolved in a little chloroform and the re-evaporation in the presence of the water and the rest of the process repeated. Five extractions of the residue recovered more than 80 % of the radioactivity from it. In a few early experiments the chlorophyll was extracted once only and recoveries as low as 20 % were obtained. A 120 kg sample of turnip leaves was extracted directly with chloroform as follows: 1 kg portions were boiled with 2 l. of chloroform each for 1 h. The chloroform was filtered off and the residue washed with 0·5 l of chloroform. The bulked chloroform solutions were flash evaporated to a small volume. The concentrate was freed from natural products by evaporating off the chloroform in the

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presence of water and filtering, as above, dissolving the natural products in benzene and extracting the benzene solution twice with water. All radioactivity was now in the aqueous solutions and these were bulked and extracted five times with chloroform. The chloroform solutions were then concentrated.

Extraction of products from reaction mixtures after incubating with liver slices

The product was centrifuged, the supernatant layer extracted five times with chloroform and the chloroform solution concentrated as before.

Partition-chromatographic techniques

Columns were prepared from 20 g of kieselguhr and 10 ml. of water packed under the eluent phase (70/30 v/v trichloroethylene/chloroform) by the method of Martin (1948) into tubes 40 cm long and 12 mm bore. Samples were prepared for chromatography by evaporating chloroform concentrates *in vacuo* to a small volume, adding about 20 ml. of trichloroethylene and re-evaporating the solution to 1 to 2 ml. This was introduced on to the column. Eluent fractions were 'counted' in a liquid-counting Geiger–Mueller tube, and the position of the bands corresponding to the various products determined.

Several concentrates contained quantities too large to be separated on such columns. These were separated on a partition chromatography cascade separator, consisting of thirty-six cells in cascade each containing about 18 ml. of static phase (chloroform) and 5 ml. of moving phase (water). The contents of each cell were stirred magnetically. The separations obtained agree with those calculated from the equations of Johnson (1950) for such machines.

Exhaustive partitioning

In some instances no suitable chromatographic technique was developed and analyses of plant extracts were carried out by the allied technique of exhaustive extraction (Heath *et al.* 1952*b*; Gardner & Heath 1953). Each analysis consisted of partitioning a filtered aqueous plant extract with successive equal volumes of an immiscible solvent. The partition coefficient of the radioactive phosphorus was determined after each extraction. The first solvent extract was also extracted successively with water in the same way. Sometimes a series of extractions with one solvent was followed by a series with another. Twelve to twenty partition coefficients were determined for each analysis. The concentrations and partition coefficients of the compounds in the extract were determined by mathematical trial and error.

The sensitivity of the method depends on the ratios of the partition coefficients (k) of the solutes and how many solutes there are. From a series of trial calculations it appears that:

(*a*) Every solute of k equal to 10 or more in favour of water has to be recorded as non-extractable, as its k cannot be found accurately enough for it to be estimated as an extractable. The non-extractable compounds as a group can, however, be determined more accurately than any extractable.

(*b*) If the k's of any two solutes are unknown, and differ by a factor of less than 2, the analysis will record only one solute, with k between the k's of the two solutes and in a concentration close to the sum of the concentrations of the two.

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(*c*) If there are three extractable solutes, and k for the solute partitioning most heavily in favour of the water-immiscible solvent is known, then only if the ratio between each pair of k's is at least 3 and the middle k is near 1 can each solute be determined to 5 % of the total extractables.

(*d*) In practice the ratio of the k's proved to be about 10, and the k of the parent compound, which in each case partitioned most heavily in favour of the water-immiscible solvent, was known approximately. Errors in the results therefore arose either from faulty manipulation during the many transfers in each analysis, or, at the beginning of the experiment, from using unsatisfactory extracting solvents. Results have only been quoted where the errors are not more than 5 %. Most of the errors are in fact less than 3 % of the total extractables.

Determination of anticholinesterase activity

The potentiometric method of Michel (1949) was used throughout, using freeze-dried human plasma as a source of cholinesterase. The anticholinesterase activity of a compound is given as an $IN/50$, which is defined here as the molar concentration inhibiting 50 % of the esterase after incubation with it for 1 h at 25° C.

Samples in non-aqueous solvents were evaporated down *in vacuo* in the presence of water to obtain solutions for the determination of activity. The concentration of inhibitor in an eluent fraction is expressed as the number of ml. of an $IN/50$ solution per ml. of eluent.

Determination of hydrolysis rates

Hydrolysis rates were determined by the method used for radioactive schradan by Heath & Casapieri (1951).

Schradan

Chemical oxidation with hydrogen peroxide

1 to 2 g samples of schradan were oxidized with 2 to 4 ml. of 100 vol. hydrogen peroxide at 100° C for 3 to 6 h and a chloroform extract of the products was analyzed in the cascade separator. The chromatogram (figure 1) showed three bands, the first of ionic

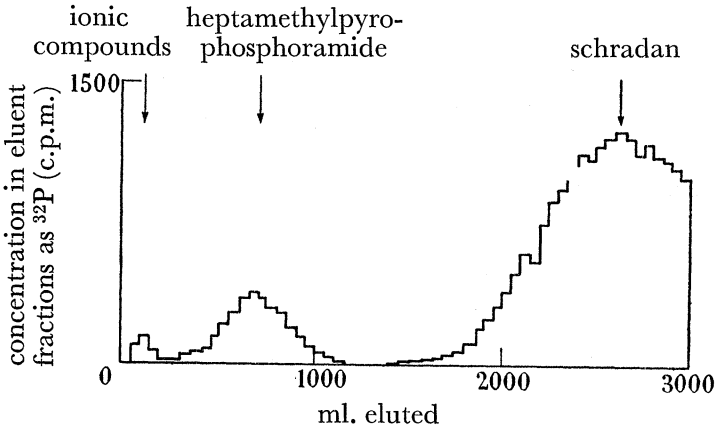

Figure 1. Chromatogram from cascade separator of the chloroform extract of schradan oxidized with hydrogen peroxide. Static phase, chloroform; eluent phase, water.

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products derived from very unstable chloroform extractable products, the second a non-ionizable derivative and the third schradan. The second band was rechromatographed on a column, using 30 % chloroform/70 % trichloroethylene v/v as eluent, and was shown to consist of heptamethylpyrophosphoramide by comparing some of its physico-chemical properties with those of the known compound (see Appendix for preparation). The comparison is made in table 1.

TABLE 1. A COMPARISON OF THE PHYSICO-CHEMICAL PROPERTIES OF HEPTAMETHYLPYROPHOSPHORAMIDE WITH COMPOUNDS PRODUCED BY THE OXIDATION OF SCHRADAN WITH HYDROGEN PEROXIDE AND BY THE ACTION OF PLANTS ON SCHRADAN

property	heptamethylpyrophosphoramide	ex H_2O_2	ex plants
partition coefficient at 18° C: chloroform/water	$1{\cdot}43 \pm 0{\cdot}02$	$1{\cdot}45 \pm 0{\cdot}02$	$1{\cdot}43 \pm 0{\cdot}03$
partition coefficient at 18° C: water/amyl alcohol	$1{\cdot}95 \pm 0{\cdot}04$	$1{\cdot}91 \pm 0{\cdot}04$	$1{\cdot}88 \pm 0{\cdot}06$
hydrolysis rate: k (min/(OH^-) in N-NaOH at 25·1° C	$3{\cdot}09 \pm 0{\cdot}02 \times 10^{-2}$	$3{\cdot}11 \pm 0{\cdot}02 \times 10^{-2}$	$3{\cdot}05^* \times 10^{-2}$

* Only four points were found on the graph of $\log_{10}(\text{concn}_{t=0}/\text{concn}_t)$ against time, so no standard error can be given.

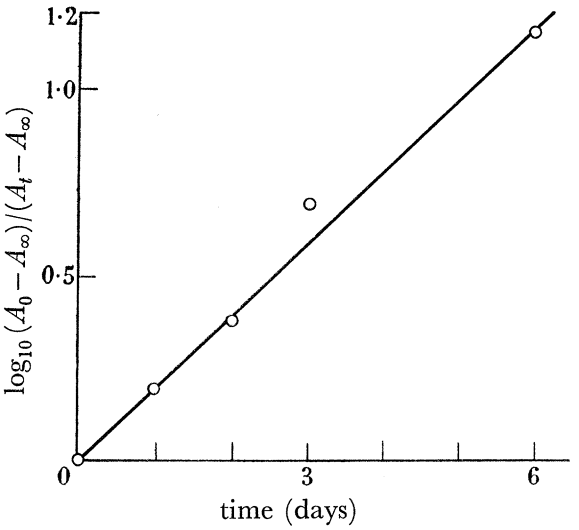

FIGURE 2. The hydrolysis at 25° C of the powerful inhibitor produced by the action of hydrogen peroxide on schradan.

The heptamethylpyrophosphoramide band contained traces of a powerful inhibitor, which decomposed in water in a few days and which could not be separated from heptamethylpyrophosphoramide by partition chromatography. Its hydrolysis rate was, however, determined as follows.

A little highly radioactive schradan (1 mc/g) was oxidized as before, extracted with chloroform and the chloroform replaced by water. This solution was stored for several days at 25° C and its anticholinesterase activity determined from time to time until it reached a steady value. $\text{Log}_{10}\ (A_0 - A_\infty)/(A_t - A_\infty)$ was plotted against t, where A_0, A_t and A_∞ are the total activities at times 0, t and infinity respectively. Figure 2 shows that the graph is a straight line within experimental error, showing that there is only one transient inhibitor and that this decomposes according to first-order kinetics with a

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half-life of 37 h. By chromatographic analysis of samples taken at 0 and 11 days it was shown that only negligible quantities of heptamethylpyrophosphoramide had decomposed during the run.

The *IN*/50 of heptamethylpyrophosphoramide was calculated from the *IN*/50 of the solution at the end of the run ($1{\cdot}5 \times 10^{-3}$M) and the concentration of heptamethylpyrophosphoramide, allowing for the contribution made by the activity of the schradan (*IN*/50, 7×10^{-2}M). It is $3{\cdot}6 \times 10^{-4}$M.

The chemical oxidation is very inefficient, yields of heptamethylpyrophosphoramide ranging from 3 to 17 %. Most of the products are not extractable from water by chloroform. Thus oxidation with hydrogen peroxide yields heptamethylpyrophosphoramide, a water-unstable inhibitor with a very similar chloroform/water partition coefficient, and ionizable compounds not extractable by chloroform. Very similar compounds are produced by plants and liver slices as shown in the experiments now described.

The metabolites of schradan in plants and oxygenated liver slices: heptamethylpyrophosphoramide from plants

White clover (*Trifolium repens*, S100 strain), seedling and fully grown turnips (var. Early Snowball), Brussels sprouts seedlings and French beans were all sprayed with dilute radioactive schradan. Extracts from every crop gave chromatograms similar to that shown

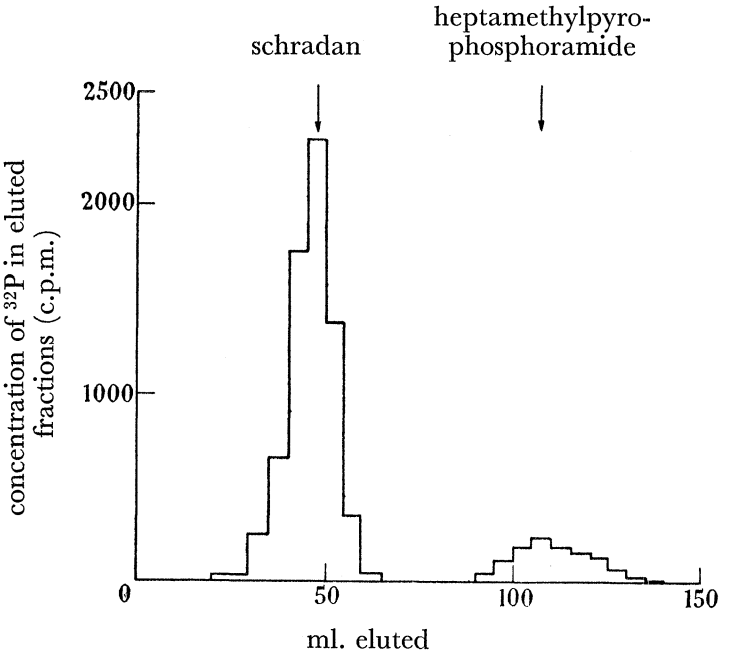

FIGURE 3. Chromatogram of the chloroform extract from a schradan treated plant. Eluent phase, 30 % $CHCl_3$/70 % C_2HCl_3, v/v; static phase, water.

in figure 3. Partition coefficient determinations confirmed that the first ^{32}P band was schradan. The second was shown to consist of heptamethylpyrophosphoramide by extracting 120 kg of turnip foliage, sprayed 25 to 35 days previously, in the way already described. The extract was separated on the cascade separator and the heptamethylpyrophosphoramide band rechromatographed in a column. The 34 mg yield (60 % of

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that in the original 120 kg sample) was used to determine the physical constants given in table 1. The agreement between these and those for a sample of known constitution proves the structure.

Some of the concentrations of heptamethylpyrophosphoramide found are given in table 2. The percentage of total ^{32}P as heptamethylpyrophosphoramide in Brussels sprouts and French beans 3 weeks after treatment was about 5 %. 10 to 15 % were found in the foliage of turnips grown in a cold greenhouse in winter. However, these lost radioactivity by guttating freely, so that the significance of these higher yields is not known. Generally the yields appear to be very close to 5 %. The major part of the products of degradation are not chloroform-extractable, as can be seen from table 2.

TABLE 2. HEPTAMETHYLPYROPHOSPHORAMIDE (A) IN PLANTS TREATED WITH SCHRADAN (B)

crop	date of treatment	days between treatment and sampling	concn in samples in p.p.m. B	concn in samples in p.p.m. non-chloroform extractables	concn in samples in p.p.m. A	yield of A (%)
clover (S 100)	23. ix. 52	31	1·20	7·0	0·46	5·3
		36	0·37	2·9	0·17	4·9
clover (S 100)	31. x. 52	4·5	4·5	15	1·2	2·0
	and	10·5	23	28	1·9	3·8
	4. xi. 52	16·5	25	15	2·8	6·5
	(sprayed twice)	23·5	15	8·4	1·6	6·2
turnip foliage (Early Snowball)	28. v. 53	6	11	—	0·40	—
	and	15	6·6	—	0·25	—
	8. vi. 53	18	9·6	—	0·51	—
	(sprayed twice)	23	4·5	—	0·68	—

Concentrations are expressed as parts per million of the weight of foliage at harvest. The concentrations of degradation products are expressed as the concentrations of schradan containing the same quantity of ^{32}P. Two sets of plants were sprayed twice. The 3rd column shows time to sampling from midway between the applications.

It was shown that heptamethylpyrophosphoramide decomposed about twice as rapidly as schradan in outdoor-grown turnips in September.

Heptamethylpyrophosphoramide from oxygenated liver slices in vitro

Dilute aqueous solutions of radioactive schradan were incubated for 3 h with rat-liver slices in a stream of oxygen according to the method of Gardiner & Kilby (1952). Chromatograms of extracts showed the presence of heptamethylpyrophosphoramide.

An inhibitor from plants

Figure 4 shows a chromatogram of a plant extract in which assays of ^{32}P and anti-cholinesterase activity are superposed. The band of inhibitor starts somewhat before the band of heptamethylpyrophosphoramide, and this region is accompanied by no measurable ^{32}P, which indicates that it is a powerful inhibitor present in minute quantities.

The yield of inhibitor was found to be very variable. Its half-life in a macerate of turnip foliage is about 1 day at 25° C.

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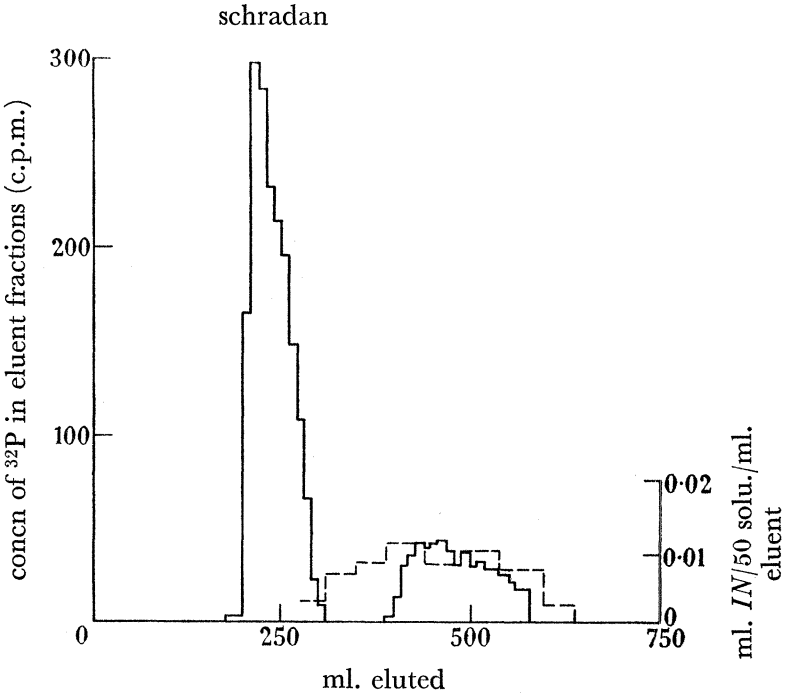

FIGURE 4. Simultaneous chromatograpms of ^{32}P (——) and anticholinesterase activity (– – –) in the chloroform extract of treated plants (clover). Eluent phase, 30 % $CHCl_3$/70 % C_2HCl_3, v/v; static phase, water.

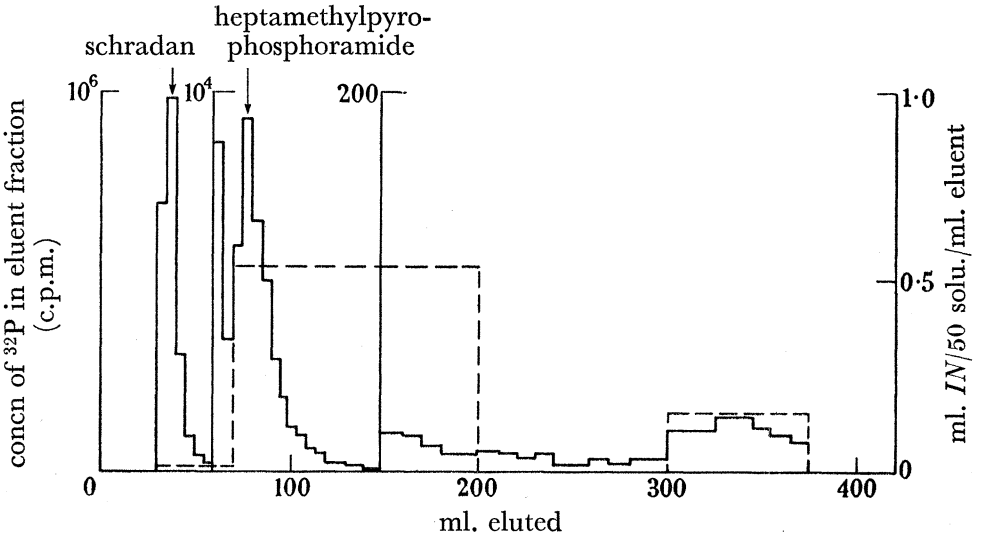

FIGURE 5. Simultaneous chromatograms of ^{32}P (——) and anticholinesterase activity (– – –) in the chloroform extract of the product of schradan treated with oxygenated liver slices. Eluent 30 % $CHCl_3$, 70 % C_2HCl_3.

Inhibitors from oxygenated liver slices

A similar chromatogram for an extract of oxygenated liver slices is shown in figure 5. A band of inhibitor is found in a similar position.

A second band of inhibitor is found eluting much later. In most experiments this second band was much smaller and considerable anticholinesterase activity was lost on the column.

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The chromatogram shown was obtained on an unusually fast-running column. It appears therefore that this inhibitor is exceptionally unstable.

Summary of experimental section

Aqueous hydrogen peroxide, oxygenated liver slices and several plant species all convert schradan to a compound or compounds not extractable by chloroform from water, to heptamethylpyrophosphoramide, and to an inhibitor not separable from it by partition chromatography on our columns. The constants given in table 3 seem to show that the inhibitors from all three sources are the same. The agreement is not excellent, but the differences are no greater than might be expected when handling materials difficult to separate and formed in such low yields that some results had to be obtained on solutions incompletely separated from natural products.

TABLE 3. SOME PROPERTIES OF AN INHIBITOR FROM SCHRADAN, DETERMINED BY VARIOUS WORKERS

	source of inhibitor		
	H_2O_2	liver slices	plants
$CHCl_3/H_2O$ partition coefficient	*ca.* 1·4 (*a*)	*ca.* 1·4 (*a*) 1·6 (*b*) *ca.* 1·5 (*c*)	*ca.* 1·4 (*a*) 1·7 (*b*)
half-life in water at pH 7	37 h at 25° C (*a*)	*ca.* 1 day at 0° C (*d*)	*ca.* 1 day at 25° C (*e*)

(*a*) This work.
(*b*) Casida & Stahmann (1953).
(*c*) Aldridge, private communication (1952).
(*d*) Calculated from the fall in activity of the supernatant after a liver incubation, on storage for 1 day at 0° C, as given by Gardiner & Kilby (1952).
(*e*) This work, determined on the filtrate of a turnip macerate.

Some important properties of schradan and its derivatives in these systems are summarized in table 4.

TABLE 4. SOME PHYSICO-CHEMICAL PROPERTIES OF SCHRADAN AND ITS PRODUCTS

compound	schradan	heptamethyl pyrophosphoramide	first inhibitor	second inhibitor
systems in which present	H_2O_2 liver plants	H_2O_2 liver plants	H_2O_2 liver plants	liver
$CHCl_3/H_2O_2$ partition coefficient	7·1 (*g*)	1·43 (*a*)	1·5 (*abc*)	*ca.* 0·2 (*a*)
k (min)/[OH^-]	$4{\cdot}7 \times 10^{-3}$ (*d*)	$3{\cdot}1 \times 10^{-2}$ (*a*)	> 100 (*e*)	—
half-life in water at 25° C	years (*d*)	> weeks (*a*)	37 h (*a*)	hours (*a*)
IN/50 molar	7×10^{-2}	$3{\cdot}6 \times 10^{-4}$ (*a*)	$3{\cdot}5 \times 10^{-7}$ (*b*)	10^{-6} (*f*)

(*a*) This work.
(*b*) Casida & Stahmann (1953).
(*c*) Aldridge, private communication.
(*d*) Heath & Casapieri (1951).
(*e*) Gardiner & Kilby (1952).
(*f*) This work—the ^{32}P accompanying this band in chromatograms of liver slices is such that the maximum is 10^{-6} M. The band may contain other weaker inhibitors.
(*g*) Hartley, Heath, Hulme, Pound & Whittaker (1951).

Discussion

The three systems investigated all convert schradan partially into the same two chloroform-extractable compounds. Two are known to act by oxidation, so it is reasonable to

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suppose that plants also oxidize schradan, but this does not imply any detailed similarity between the biochemical systems which bring about the oxidation in mammalian liver and plants respectively.

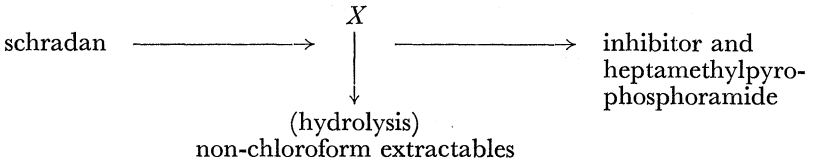
The results show that heptamethylpyrophosphoramide is a by-product. If the degradation scheme could be represented by

$$\text{schradan} \rightarrow \ldots \rightarrow \text{heptamethylpyrophosphoramide} \rightarrow \ldots,$$

the maximum yield of heptamethylpyrophosphoramide could be calculated from the ratio of its half-life in the plant to that of schradan. This ratio is about 0·5, which gives a maximum of 50 %, almost independent of the orders of the successive reactions chosen for the calculation. The yields found rarely exceed 5 % at any point in the degradation (see table 2). Most of the degradation does not, therefore, proceed via this compound. The same argument applied to the inhibitor suggests that this is a by-product also. Thus the ratio of the half-life of the inhibitor in turnip macerates to the half-life of schradan in plants is about 0·05, giving a theoretical yield of 5 %. The yields estimated from the anticholinesterase activity of plant extracts, as found both by Casida, Chapman & Allen (1952) and by ourselves, and the *IN*/50 given by Casida & Stahmann (1953) are 0·1 % or less. That some such low figure is correct may be concluded from the chromatogram in figure 4. The conclusion is not so certain in this instance, because the half-life of the anticholinesterase could not be found in plants, but only in a macerate. As most of the products in plants are compounds not extractable by chloroform, it may thus appear that schradan is hydrolyzed and oxidized simultaneously, and that direct hydrolysis is the more important reaction.

There are several reasons, however, why this should not be so. First, as discussed in the introduction, only if the initial attack on schradan and hexamethylphosphoramide is oxidative is it easy to explain why both are degraded at very similar rates in plants. Secondly, where the degradation in plants of the other organophosphorus compounds described in this paper leads to non-chloroform-extractable compounds, the rate of formation of such compounds is not very different from their rate of formation from schradan, but the hydrolysis rates of the parent compounds in water and alkaline solution range from that of schradan, the most stable, to a rate of 10^7 times faster. Finally, hydrogen peroxide also gives compounds inextractable by chloroform, showing that such compounds can be produced by an oxidizing agent. It is therefore assumed in the further discussion that the non-chloroform extractables are formed by the hydrolysis of metabolites, unstable in water, produced by the oxidation of schradan.

The argument thus leads to a reaction sequence:



in which the hydrolysis of *X* is about ten times as rapid as its conversion to inhibitor and heptamethylpyrophosphoramide.

An attempt will now be made to deduce the chemical constitution of *X* and the inhibitor.

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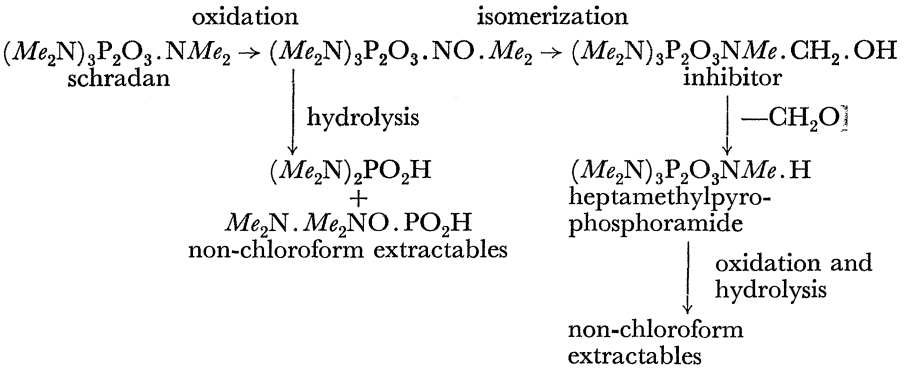
The degradation is brought about *in vitro* by hydrogen peroxide and also by peracetic acid (Tsuyuki, Stahmann & Casida 1955). These reagents are unlikely to attack any part of the molecule except the nitrogen atoms, and are known to give amine oxides readily. It is therefore reasonable to suppose that *X* is octamethylpyrophosphoramidic oxide, $(Me_2N)_3P_2O_3.NO.Me_2$, the compound first proposed as an intermediate in the degradation in plants by Heath *et al.* (1952*b*). The highly electrophilic nature of the amine oxide group is expected to open the neighbouring phosphorus atom to attack by nucleophilic agents such as water, so that it is reasonable to expect that this compound will be readily hydrolyzed. This suggests both a structure for the inhibitor and a mechanism for the formation of heptamethylpyrophosphoramide. Spencer & O'Brien (1953) have shown that the *N*-chloro derivatives of schradan isomerize rapidly to the chloromethyl derivatives. A similar isomerization of the amidic oxide would give heptamethylhydroxymethylpyrophosphoramide, which may be the inhibitor. Hydroxymethylamino compounds lose formaldehyde readily (Henry 1894); in this instance the product would be heptamethylpyrophosphoramide.

The complete reaction scheme proposed is thus:



Some indirect evidence can be obtained that the inhibitor has the structure assigned it.

Collander (1949) has shown that there is some correlation between changes of structure in organic compounds and changes of partition coefficients. Thus substituting methyl for hydrogen in any molecule increases its ether/water partition coefficient by a factor of 2 to 5. When the effect on the partition coefficient between chloroform and water of a given substitution in a series of organophosphorus compounds is studied (see the top part of table 5), it is found that a similar correlation holds. It will be noted that the substitution of *Me*— for H— produces a change of partition coefficient which is the same within a factor of about three, even though it is probable that there is considerable interaction between —NH groups and P=O groups in the phosphorus compounds for which there is no analogy in the carbon compounds. Perhaps the most important comparison in this series is between $R_2PO.OEt$ and $R_2PO.NMe_2$ and the exactly analogous carbon compounds.

At the bottom of the table it can be seen that the ratio inhibitor/heptamethylpyrophosphoramide is very similar indeed to the ratio $Et_2N.CH_2OH/Et_2NH$, but that the ratio inhibitor/schradan differs by a factor of 620 from the ratio Et_3NO/Et_3N. This is

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strong evidence for the hydroxymethylamide structure, although it is not of course conclusive. Collander finds differences of about 100-fold in three of his 200 comparisons, all of which are, however, in cases where some specific interaction can be postulated.

This leads to our postulating a formula for the inhibitor with which Casida *et al.* (1954) disagree. They have separated sufficient inhibitor to determine its infra-red spectrogram, and find a band at 1690 cm^{-1}, very close to the N—O stretching band in amine oxides. Thus they favour the amidic oxide structure. This evidence, however, is not conclusive

TABLE 5. PARTITION COEFFICIENTS IN THE SYSTEM CHLOROFORM/WATER OF ANALOGOUS COMPOUNDS

substitution	compounds	coeffi-cients	ratio of coeffi-cients	compounds	coeffi-cients	ratio of coeffi-cients
—*Me* for —H	$\frac{R_3P_2O_3NMe_2}{R_3P_2O_3NH.Me}$	$\frac{7{\cdot}1}{1{\cdot}43}$	5·0	$\frac{R_2PO.NMe_2}{R_2PO.NHMe}$	$\frac{6{\cdot}7}{0{\cdot}46}$	14·6
	$\frac{R_2PO.NH.Me}{R_2PO.NH_2}$	$\frac{0{\cdot}46}{0{\cdot}056}$	8·2	$\frac{Me_3N}{Me_2NH}$	$\frac{3{\cdot}6}{0{\cdot}80}$	4·5
	$\frac{Me_2NH}{MeNH_2}$	$\frac{0{\cdot}80}{0{\cdot}098}$	8·2	—	—	—
—O*Et* for —NMe_2	$\frac{R_2PO.OEt}{R_2PO.NMe_2}$	$\frac{60}{6{\cdot}7}$	9·0	$\frac{HCO.OEt}{HCO.NMe_2}$	$\frac{2{\cdot}35}{0{\cdot}056}$	42
	$\frac{RCO.OEt}{RCO.NMe_2}$	$\frac{98}{13{\cdot}8}$	7·1	—	—	—
—CH_2OH for —H	$\frac{\text{inhibitor}}{R_3P_2O_3NH.Me}$	$\frac{1{\cdot}5}{1{\cdot}43}$	1·05	$\frac{Et_2NCH_2.OH}{Et_2NH}$	$\frac{3{\cdot}7}{3{\cdot}9}$	0·95
≡NO for ≡N	$\frac{\text{inhibitor}}{R_4P_2O_3}$	$\frac{1{\cdot}5}{7{\cdot}1}$	0·21	$\frac{Et_3NO}{Et_3N}$	$\frac{0{\cdot}01}{29}$	0·00034

R =—NMe_2, Me =—CH_3, Et =—C_2H_5.

The constants for the aliphatic amines are taken from Seidell (1941), corrected for dissociation. Constants for ethyl formate and dimethylformamide were obtained on commercial samples redistilled, and the aqueous layers were analyzed by the methods of Hestrin (1949) and Bergmann (1952). Dimethyl-urethane and tetramethylurea were prepared by standard methods, and concentrations in the aqueous layers determined after partitioning by estimation of dimethylamine after prolonged acid hydrolysis in sealed tubes. Triethylamine oxide was prepared by the method of Dunstan & Golding (1899) and hydroxymethyldiethylamine by the method of Henry (1894). The coefficients for the phosphorus compounds were obtained on radioactive samples by counting the layers.

either. The modification in the structure of the schradan molecule which takes place when the inhibitor is formed is sufficient to increase its alkaline hydrolysis rate by a factor of 10^8. Consequently either an N—O or a CH_2OH system in the derivative of schradan is likely to be very different from the same groups in molecules such as trimethylamine oxide, where little interaction with neighbouring groups can take place; and the frequencies associated with these groups are expected to be correspondingly different. Thus the two lines of evidence are contradictory and indecisive.

The hydroxymethylamide structure, unlike the amidic oxide structure, enables us to account, in a simple way, for the proportions of the various metabolites produced in plants, and therefore seems to us the more probable.

The presence of a second band of inhibitor in chromatograms of schradan incubated with liver slices may well be accounted for by further oxidation of heptamethylpyro-

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phosphoramide. It could, however, be the amidic oxide, for it partitions more in favour of water from chloroform than heptamethylpyrophosphoramide, though not by a large enough factor for this to be probable. Whichever is correct, the relatively rapid rate of attack on schradan by liver slices accounts for the observations of this inhibitor in liver extracts and our failure to observe it in extracts of plants.

Several workers, of whom Hartley (1951) was the first, have reported experiments on the oxidation of schradan by a neutral solution of potassium permanganate, and have attempted to relate their findings to the metabolites of schradan in plants and mammals. We have repeated this work, and have come to the conclusion that the sequence of reactions in a neutral solution of potassium permanganate differs considerably from the sequence in the systems described in this paper. Detailed consideration of these differences is therefore reserved for another publication.

Derivatives of tetramethylphosphorodiamidic acid in plants

The purpose of these experiments was to find whether plants decomposed compounds similar to schradan, but which do not possess an acid anhydride bond, and the derivatives of which are therefore stable to hydrolysis. The choice of compounds was limited by the need for high water solubility, as only water-soluble compounds are systemic. The following five compounds were therefore chosen and prepared by the methods given in the appendix. For clarity in exposition abbreviated names are also given.

systematic names	abbreviation
hexamethylphosphoramide	dimethylamide
pentamethylphosphoramide	monomethylamide
NN′ tetramethyl *N″*-*iso*propylphosphoramide	*iso*propylamide
NN′ tetramethyl *N″*-*n*-butylphosphoramide	butylamide
O-ethyl tetramethyldiamidophosphate	ethyl ester

Experimental section

A 0·4 % aqueous solution of each compound was applied to the foliage of turnips, var. Early Snowball. Samples were taken at intervals after treatment and filtered macerates of foliage analyzed by the method of exhaustive partitioning already described. The roots contained very little ^{32}P, so they were not analyzed. When the outer leaves, which contained most of the ^{32}P, began to die off, the experiments were stopped.

Each compound produced at least one well-defined derivative. The results are given in figures 6 to 10, and the partition coefficients used in calculating the results in table 6. Lines are drawn in figures 7 to 10 as a visual aid. Figure 6 is discussed later.

Discussion

In both table and figures the nomenclature 'derivative I' and 'derivative II' has been adopted, implying that the reaction scheme is

parent compound → derivative I → derivative II → non-extractables,

with no side-reactions or missing steps. The initial rates of increase in concentration of the second derivative and of the inextractable ^{32}P tend asymptotically to zero concentration as the time from treatment tends to zero. This is consistent with the scheme proposed

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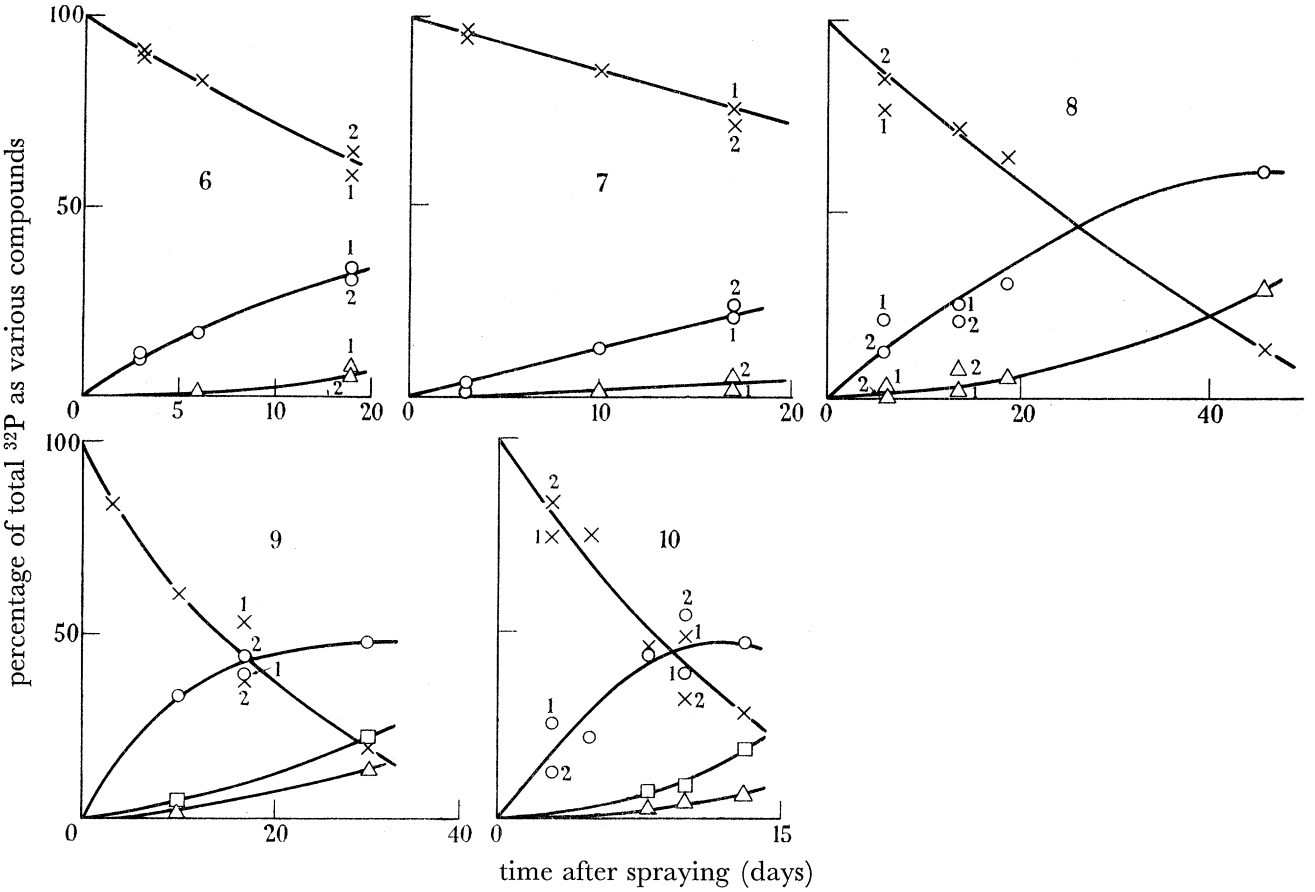
or alternatively with the formation of these compounds by an autocatalytic process. The simpler hypothesis seems more likely, and rate equations derived on it are in good agreement with the experimental results. Thus the curves in figure 6 were calculated assuming that both the formation and decomposition of derivative I follow first-order kinetics and that there are no side-reactions. In this instance no second derivative was



FIGURES 6 TO 10. The percentage of the total ^{32}P in turnip foliage as parent compounds and metabolites at various times after spraying with derivatives of tetramethylphosphorodiamidic acid. ×, parent compound; ○, chloroform extractable derivative I; □, chloroform extractable derivative II; △, compounds not extractable by chloroform.

FIGURE 6. Dimethylamide, $(Me_2N)_2PO.NMe_2$. ×, dimethylamide.

FIGURE 7. Monomethylamide, $(Me_2N)_2PO.NH.Me$. ×, monomethylamide. *Note.* The monomethylamide contained 7 % of dimethylamide. Allowance has been made for the dimethylamide in calculating the above results, but not for its derivatives, as these are indistinguishable from the monomethylamide and its derivatives.

FIGURE 8. *Iso*propylamide, $(Me_2N)_2PO.NH.iso\text{-}C_3H_7$. ×, *iso*propylamide. *Note.* The *iso*propylamide contained 15 % of dimethylamide, from which it is not easily separated during analysis. The results shown are for a mixture rich in *iso*propylamide.

FIGURE 9. Butylamide, $(Me_2N)_2PO.NH.n\text{-}C_4H_9$. ×, butylamide. *Note.* The butylamide contained 3 % of dimethylamide. This and its derivatives are easily separated from the butylamide and its derivatives during analysis. The results shown have been corrected.

FIGURE 10. Ethyl ester, $(Me_2N)_2PO.OEt$. ×, ethyl ester.

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extracted by the solvent used. It can be concluded therefore that none of the five compounds is directly hydrolyzed in plants, as hydrolysis products would be ions not extractable by the solvents used.

Certain modes of degradation can be ruled out. Thus the removal of *iso*propyl, butyl and ethyl groups from the *iso*propylamide, butylamide and ester respectively cannot have occurred. The removal of *iso*propyl and butyl radicals from the two amides would give *NNN'N'*-tetramethylphosphoramide, the chloroform/water partition coefficient of which is 0·056, and the removal of the ethyl group from the ester would give an ionizable compound. No compounds with these properties are produced.

TABLE 6. PARTITION COEFFICIENTS OF SOLVENT EXTRACTABLES

compound	solvent system	unchanged compound	derivative I	derivative II	pure unchanged compound at 18° C
dimethylamide	$\frac{CHCl_3}{H_2O}$	6·32, 5·72, 5·72, 6·2	0·40, 0·35, 0·40, 0·40	—	6·6 ± 0·1
monomethylamide	$\frac{CHCl_3}{H_2O}$	—	—	—	0·43 ± 0·01
	$\frac{CHCl_3}{N\text{-}NaOH}$	1·11, 1·10, 1·00	0·10, 0·10	—	1·04 ± 0·02
	$\frac{CHCl_3}{2N\text{-}NaOH}$	2·5, 2·5	0·25, 0·25	—	2·59 ± 0·07
*iso*propylamide	$\frac{CHCl_3}{H_2O}$	4·0, 4·0, 4·0	0·5, 0·5, 0·5	—	3·6
butylamide	$\frac{CHCl_3}{H_2O}$	21, 21	2·04, 2·47	0·316, 0·316	21·6 ± 0·4
	$\frac{C_2H_2Cl_4}{H_2O}$	11·3, 11·3	1·39, 1·22	less than 0·1	—
ethyl ester	$\frac{C_6H_6}{N\text{-}NaOH}$	0·71, 0·95	0·05, 0·152	0	0·81 ± 0·05
	$\frac{C_6H_6}{2N\text{-}NaOH}$	2·60, 2·60	0·163, 0·162	0	—
	$\frac{CHCl_3}{N\text{-}NaOH}$	—	5·0, 5·0	0·5, 0·5	> 50
	$\frac{CHCl_3}{2N\text{-}NaOH}$	—	11·0, 11·0	1·0, 1·0	> 50

The coefficients given are all those used in calculating the results given in the figures.

From table 6 it will be seen that the ratio of the partition coefficients of the parent compounds to those of their first derivatives is reasonably constant, and this is suggestive of the same molecular change in each case. By analogy with schradan, the derivative of the dimethylamide would be the monomethylamide, and this is confirmed by the agreement between the partition coefficients of the monomethylamide and the first derivative of the dimethylamide. Thus the degradation reactions are probably

$$=PO.NMe_2 \rightarrow =PO.NO.Me_2 \rightarrow =PO.NMe.CH_2OH \rightarrow =PO.NMe.H.$$

(It is likely that any related hydroxymethylmethylamide would be recorded as monomethylamide, by analogy with schradan.) It is equally consistent with the partition

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coefficient data that, in the degradation of the *iso*propylamide and butylamide, terminal methyl groups are removed, or that the butyl or *iso*propyl groups are hydroxylated in the 2, 3 or 4 positions. Such reactions appear improbable, but cannot be ruled out. If it is assumed that these groups are not attacked, there are two possibilities:

(i) only dimethylamido groups are attacked, or
(ii) primary amido groups are not attacked.

Some toxicity data on diamidic fluorides favours the first alternative. Thus in the two series of compounds: $(Me_2N)_2PO.F$, $Me_2N.Et_2NPO.F$ and $(Et_2N)_2PO.F$; and $(Me_2N)_2PO.F$, $Me_2N.O(CH_2CH_2)_2NPO.F$ and $(O(CH_2CH_2)_2N)_2PO.F$, the first two in each series are highly toxic to mammals and insects, the last in each are of very low toxicity. All are weak inhibitors, and must therefore be metabolized to exert toxic action. This indicates that dimethylamide groups are necessary for metabolism to take place.*

To sum up, it is certain that none of the five compounds is hydrolyzed, and that the butylamide and *iso*propylamide do not lose their butyl and *iso*propyl groups in one step. It is probable that the dimethylamide is demethylated in a similar way to schradan. There is a strong suggestion that in these compounds only the dimethylamide groups are attacked, the other groups being inert.

OO-DIETHYL (ETHYLTHIOETHYL) PHOSPHOROTHIOATES

Gardner & Heath (1953) have shown that the insecticide dimeton consists of a mixture of *OO*-diethyl *O*(ethylthioethyl) phosphorothionate ($(EtO)_2PS.OC_2H_4.SC_2H_5$) and *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate ($(EtO)_2PO.SC_2H_4.SC_2H_5$). Their paper gives methods of preparation, separation and analysis. These compounds are of a very different type from those already discussed, and this made a study of their decomposition in plants particularly interesting.

OO-*diethyl* O(*ethylthioethyl*) *phosphorothionate*

Experimental

Turnips were sprayed with a dilute emulsion of the radioactive compound. Filtered extracts of samples taken 8 days later were subjected to exhaustive partitioning using in turn as extracting solvents *iso*-octane, benzene and chloroform. It was shown that at least three solvent extractable derivatives are formed. No unchanged parent compound was extracted, and only very small quantities of non-chloroform extractables were left at the end of the procedure.

No further work on this compound was then carried out, as it was found that the toxicity of the insecticide in question was almost entirely due to the other isomer.

OO-*diethyl* S(*ethylthioethyl*) *phosphorothiolate*

The experiments on this compound were similar to those on schradan and are presented below in the same way.

* The low toxicity of tetraethylphosphorodiamidic fluoride has been reported by Topley (1950) and Schrader (1951); the toxicity of tetramethylphosphorodiamidic fluoride by Schrader (1948) and numerous other workers. The toxicities of the remaining compounds were determined in these laboratories.

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Oxidation with hydrogen peroxide

The reaction between *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate and an equimolar quantity of hydrogen peroxide yields *OO*-diethyl *S*(ethylsulphinylethyl) phosphorothiolate, $(EtO)_2PO.SC_2H_4SO.C_2H_5$, almost quantitatively (see B.P. 33119/52).

The product was dissolved in water and purified by extracting the aqueous solution twice with *iso*-octane to remove unchanged parent compound. The required product was then extracted into chloroform solution, the solvent removed *in vacuo* and the residue subjected to molecular distillation at 100° C. Finally, the product was chromatographed (eluent benzene, standing phase water).

The constitution of the product was proved by comparing its properties with those of a synthetic sample (see table 7).

TABLE 7. SOME PROPERTIES OF *OO*-DIETHYL *S*(ETHYLSULPHINYLETHYL) PHOSPHOROTHIOLATE PREPARED IN DIFFERENT WAYS

	method of preparation		
	by synthesis	by H_2O_2	by plants
partition coefficients at high dilution:			
Sorensen buffer, pH 7·15/benzene at 18° C	5·82 ± 0·10	5·83 ± 0·10	5·73 ± 0·10
Sorensen buffer, pH 7·15/trichloroethylene at 18° C	2·25 ± 0·05	2·19 ± 0·05	2·16 ± 0·05
chloroform/water at 18° C	—	38 ± 3	41·6 ± 2
hydrolysis constant in 0·01 N-NaOH at 25·4° C k (min)/(OH^-)	3·34 ± 0·06	3·31 ± 0·07	3·25 ± 0·25

A second compound can be produced by boiling *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate, or, better, its sulphinyl derivative, with an equal volume of 100 vol. hydrogen peroxide for several minutes. An aqueous solution of the product was extracted with *iso*-octane as above and then with three equal volumes of benzene. The benzene extracts were bulked and washed with three equal volumes of water. By this process 99·5 % of the sulphinyl compound is extracted from the benzene solution, but only 50 % of the required product. By evaporating the benzene and distilling the residue in a molecular still, the remaining 50 % is obtained pure. The yield of this second compound before purification never exceeded 50 %, the other products being non-extractable from water. Some properties are given in table 8. The structure of this compound is not known.

TABLE 8. SOME PROPERTIES OF A SECOND DERIVATIVE

	method of preparation	
	by H_2O_2	by plants
partition coefficients:		
benzene/water at 10° C	3·79 ± 0·10	3·76 ± 0·10
iso-octane/water	*ca.* 0·01	*ca.* 0·01
hydrolysis constant in 0·01 N-NaOH at 18° C k (min)/(OH^-)	88 ± 10	74 ± 10

The metabolites formed in plants

Plants were treated with a dilute aqueous solution of the thiolate and several days later were extracted with chloroform in the usual way. Aqueous solutions prepared from these extracts were partitioned exhaustively with *iso*-octane and benzene. The presence of two

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derivatives was demonstrated. A 10 kg sample of treated stinging nettles and a 30 kg sample of treated sugar-beet leaves were extracted similarly, and the two derivatives separated by exhaustive partitioning followed by partition chromatography (eluent benzene, standing phase water). From tables 7 and 8 it will be seen that these two derivatives are identical with those formed by hydrogen peroxide oxidation.

A concentrate of the aqueous residue of the plant material after chloroform extraction was further extracted with secondary butanol. This extract exhibited considerable anticholinesterase activity, and killed a mouse with signs typical of anticholinesterase poisoning. This is evidence for the formation of a third, very water-soluble derivative. It was not possible, however, to separate it from plant material, and little is known of it.

The concentrations of the various products at different times after treatment were found in lettuce seedlings and stinging nettles. Analyses were performed by exhaustive partitioning, which is a sensitive method for compounds of such well-differentiated partition properties. The results are given in table 9.

TABLE 9. THE CONCENTRATIONS OF *OO*-DIETHYL *S*(ETHYLTHIOETHYL) PHOSPHOROTHIOLATE AND ITS DERIVATIVES IN TREATED PLANTS

		per cent. total ^{32}P in various fractions				
crop	days after treatment	parent	1st derivative	2nd derivative	non-chloroform extractables	total ^{32}P as p.p.m. parent*
lettuce seedlings	6	1	52	37	11	14
	14	1	11	53	36	10
	20	1	0	38	62	3
stinging nettles	3	2	76	12	10	144
	4	2	73	15	10	107
	6	1·5	73	11	15	30
	8	2	*x*†	*x*/3	—	—
	13	1	38	25	36	75

* Concentration expressed as parts per million of the weight of the sample as harvested.
† Only the percentage parent compound and the ratio 1st derivative/2nd derivative are known for this sample.

Summary of experimental results

Both *OO*-diethyl *O*(ethylthioethyl) phosphorothionate and *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate are converted rapidly in plants to non-ionic compounds. The latter compound is oxidized to *OO*-diethyl *S*(ethylsulphinylethyl) phosphorothiolate and to a further derivative of unknown constitution. Both may be prepared from it by the action of hydrogen peroxide. Some properties of the thiolate isomer and its derivatives are given in table 10.

Discussion

The derivatives in plants of the thiolate have been shown to be oxidation products and as the $—C_2H_4SC_2H_5$ system, which appears to be the first attacked, is the same in both molecules, it is likely that those of the thionate are also oxidation products. Thus these systemic compounds, which are very different from the dimethylamido compounds described in previous sections, are also degraded in plants by oxidation. In these instances

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the parent compounds disappear rapidly and the derivatives are the more stable. Consequently most of the ^{32}P introduced is found as derivatives. This rapid degradation of the parent compounds was also demonstrated by Metcalfe, March, Fukuto & Maxon (1954), but they did not demonstrate the existence of two derivatives from the thiolate.

The build-up of non-chloroform extractables is slow, indicating that hydrolysis does not occur at an early stage in the degradation. The presence of over 50 % of the total ^{32}P as the second derivative shows that this is not hydrolyzed readily, although its hydrolysis rate in alkaline solution is about 10^7 greater than that of schradan. Thus these results support the hypothesis that the initial means of degradation of schradan in plants is not hydrolysis.

TABLE 10. SOME PROPERTIES OF *OO*-DIETHYL *S*(ETHYLTHIOETHYL) PHOSPHOROTHIOLATE AND ITS DERIVATIVES IN THE PLANT

properties	parent	derivative I*	derivative II	derivative III
solubility in water	0·20 %	miscible	miscible	—
partition coefficients				
iso-octane/water	15 (*a*)	< 0·01	0·01	< 0·01
benzene/Sorensen buffer pH 7·15	v. large	0·172	3·78	< 0·01
chloroform/water	v. large	41	v. large	< 0·01
sec-butanol/water	v. large	v. large	v. large	*ca.* 0·3
hydrolysis constant in 0·01 N-NaOH, k (min)/(OH^-)	0·81 (*a*)	3·33	80	—
IN/50 (human plasma cholinesterase)	3×10^{-6} M	2×10^{-6} M	4×10^{-7} M†	$< 10^{-5}$ M†

(*a*) Gardner & Heath (1952).

* *OO*-diethyl *S*(ethylsulphinylethyl) phosphorothiolate.

† The molar concentrations of the unidentified derivatives are given in terms of the parent *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate.

GENERAL SUMMARY AND DISCUSSION

It has been proved that *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate is attacked in plants by an oxidative mechanism, and with little less certainty that schradan is also oxidized. From the similarity of the products to those of the above compounds it was concluded that various amides and esters of tetramethylphosphorodiamidic acid, and *OO*-diethyl *O*(ethylthioethyl) phosphorothionate are also oxidized. It appears therefore most probable that in general phosphorus systemic compounds are degraded initially by oxidation, until the derivatives produced are sufficiently unstable in water to be decomposed by aqueous hydrolysis, i.e. plants oxidize such compounds, but do not catalyze their hydrolysis. It is interesting to note that in three instances oxidation by plants can be simulated by oxidation with hydrogen peroxide *in vitro*.

The mechanism of oxidation of schradan is discussed in detail in the section on that compound. It appears to consist of the following steps:

$$PNMe_2 \rightarrow PNO.Me_2 \rightarrow PNMe.CH_2OH \rightarrow PNH.Me \rightarrow \text{further oxidation products.}$$

The first step leads to a compound very unstable in water, so that the yields from the second and subsequent reactions are very small.

The derivatives of tetramethylphosphorodiamidic acid studied appear to undergo the same sequence of reactions, except that, as the amidic oxides produced are not readily hydrolyzed, high yields of non-ionic derivatives are produced. It was also shown that

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the degradation of these compounds proceeded most probably by the demethylation of dimethylamido groups, the other groups taking no direct part in the degradation.

As we found no evidence that plants catalyze the hydrolysis of organophosphorus compounds except by general acid-base catalysis by the salts present in plant fluids, it is not surprising that the instability of systemic insecticides in plants is not related to the ease with which they are hydrolyzed.

We would like to thank Dr G. S. Hartley for his constant advice and encouragement during the preparation of this paper; Dr E. F. Edson for allowing us to use some toxicity data; Mr D. J. Walbridge for his work on developing and running the chromatographing machine and the determination of hydrolysis rates; Mrs M. V. Llewellyn and Mr R. W. Lloyd for technical assistance; and the directors of Fisons Pest Control Limited for permission to publish.

Appendix. The preparation of the compounds

Preparations performed repeatedly are described in general terms. Whenever a preparation was performed once only actual quantities used are given.

All phosphorus compounds were made radioactive. Phosphorus trichloride, phosphorus oxychloride and thiophosphoryl chloride were purchased from the Radiochemical Centre, Amersham, Bucks, with an activity on receipt of about 1 mc ^{32}P/g.

These compounds were first converted into a number of intermediates.

Preparation of intermediates

Tetramethylphosphorodiamidic chloride was prepared by the method of Hartley *et al.* (1951) from phosphorus oxychloride, and purified by fractionation: b.p. 118° C at 18 mm.

Dimethylphosphoroamidic dichloride was prepared by heating 20 g of tetramethylphosphorodiamidic chloride with 17·1 g of phosphorus oxychloride (slight excess over equimolar) at 140° C for 1 h. The product was fractionated using a short Vigreaux column: b.p. 68 to 74° C at 10 mm, yield 26 g.

Trimethylphosphorodiamidic chloride was prepared by the reaction

$$2MeNH_2 + Me_2NPO.Cl_2 \rightarrow MeNH.Me_2NPO.Cl + MeNH_3.Cl.$$

9·7 g (theoretical) monomethylamine in 50 ml. chloroform was run into 25·4 g of dimethylphosphoroamidic dichloride in 250 ml. of chloroform with brisk stirring. The temperature rose rapidly to 40° C, at which it was maintained for 2 h. The product was cooled, transferred to a separating funnel, shaken with 100 ml. of water, and the bottom layer run immediately into a vacuum flash-evaporator to concentrate and dry it. The product was fractionated: b.p. 106 to 108° C at 1·0 mm; yield 7 g. Found: Cl, 22·3 %; N, 17·2 %; theor. Cl, 22·7 %; N, 17·9 %.

The slight deficiency of nitrogen is probably to be accounted for by some contamination with unchanged dimethylphosphoroamidic dichloride.

Sodium tetramethylphosphorodiamidate was prepared by running 72·3 g of tetramethylphosphorodiamidic chloride into 34·5 g of sodium hydroxide dissolved in 170 ml. of water at 55 to 80° C, concentrating slightly on the water-bath and leaving to crystallize.

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The product consisted of crystalline plates, $(Me_2N)_2PO_2Na.6H_2O$, yield 57·2 g. This was dehydrated in a vacuum desiccator at 1 mm. over phosphorus pentoxide for 3 days.

Diethyl phosphorochloridate. The reactions are given by Kosolapoff (1950). About 2 to 3 % excess over 3 mol. of ethanol was run into each mol. of phosphorus trichloride in 10 % solution in dry carbon tetrachloride. The temperature was kept at 20 to 30° C by cooling. At the end of the addition the product was allowed to stand for 30 min at room temperature. Chlorine gas was then passed into the reaction mixture, the temperature being maintained below 30° C, and the reaction was complete when no temperature rise occurred on removing the water bath. Dry nitrogen was then bubbled through briskly for about 2 h to remove HCl, the solution concentrated, and the product fractionated: b.p. 83 to 84° C at 11 mm; yield, about 90 %.

OO-*diethylphosphorothionic chloride*. Two mol. of sodium ethylate in 10 % solution in ethanol was run into each mol. of thiophosphoryl chloride in 30 % solution in benzene. The temperature was maintained below 20° C by cooling. The product was left overnight, and concentrated *in vacuo* to remove most of the alcohol. Benzene was then added, and the remainder of the alcohol removed *in vacuo*. The benzene solution was washed with water, to remove salt, concentrated, and the product fractionated: b.p. 70 to 72·5° C at 5 mm; yield, 80 to 90 %.

Sodium OO-*diethylphosphorothioate* was prepared by hydrolyzing *OO*-diethylphosphorothionic chloride in the theoretical quantity of sodium hydroxide in aqueous solution. Most of the water was removed *in vacuo*, absolute ethanol added, and the mixture further concentrated to remove most of the remaining water. The product was filtered to free it from sodium chloride, and the alcohol removed *in vacuo*.

Preparation of final products

The syntheses of the final products are now described in the order in which the compounds appear in the text.

Schradan (octamethylpyrophosphoramide) was prepared by the method of Hartley *et al.* (1951). A treatment with N-sodium hydroxide for 15 min at 100° C hydrolyzed the major impurities. The schradan was then extracted with chloroform, the solvent was distilled off and the product fractionated: b.p. 110° C at 0·1 mm, m.p. 30° C. Analyzed by the method of Hartley *et al.* (1951), but estimating phosphorus by ^{32}P assay instead of dimethylamine by titration, the purity was shown to be better than 99·8 % in most instances.

Heptamethylpyrophosphoramide was prepared by the reaction

$$(Me_2N)_2PO_2.Na + MeNH.Me_2NPO.Cl \rightarrow (Me_2N)_2PO.OPO.NMe_2NH.Me + NaCl.$$

This reaction is unambiguous except for the possibility of some disproportionation.

5·7 g of trimethylphosphorodiamidic chloride were maintained at 100° C for 5 h with 7·7 g of sodium tetramethylphosphorodiamidate. To decompose any unchanged amidic chloride the product was allowed to stand 1 h in M/2-phosphate buffer, pH 6·7. It was then extracted three times with chloroform, the chloroform solution concentrated, and the product purified by chromatography on the partition cascade separator (static phase, chloroform; eluent phase, water). Yield 67 %.

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The purity was checked by chromatography in a column (eluent phase 30 % chloroform/70 % trichloroethylene, v/v). Only one sharp band was found.

Hexamethylphosphoramide was prepared by letting tetramethylphosphorodiamidic chloride react with a considerable excess of dimethylamine in dry chloroform at 40° C for 2 to 3 h. The chloroform solution containing the product was shaken with aqueous sodium hydroxide to remove unchanged amidic chloride, concentrated, and the hexamethylphosphoramide fractionated: b.p. 121° C. at 15 mm.

Pentamethylphosphoramide, NN′ *tetramethyl* N″-iso*propylphosphoramide and* NN′ *tetramethyl* N″-n-*butylphosphoramide* were prepared by the reaction of tetramethylphosphorodiamidic chloride with a large excess of the appropriate primary amine in dry chloroform at 30 to 40° C for 24 h. The products were purified with dilute aqueous sodium hydroxide solution as above, and the solvent removed *in vacuo*. These compounds were made on too small a scale to be fractionated. The purity was determined by exhaustive partitioning. The sample of pentamethylphosphoramide used for the experiments with plants contained 7 % hexamethylphosphoramide, the isopropylamide about 15 % of hexamethylphosphoramide, and the *n*-butylamide less than 3 % hexamethylphosphoramide. (The formation of these impurities is probably attributable to moisture in the amines used in the preparation. Moisture leads to hydrolysis of tetramethylphosphorodiamidic chloride, and the liberation of some dimethylamine, which is more reactive than the primary amines used.)

O-*ethyl tetramethylphosphorodiamidate* was prepared by allowing tetramethylphosphorodiamidic chloride to react with sodium ethylate in alcohol under reflux for 1 h. The alcoholic solution was diluted with water, extracted with chloroform, and the chloroform solution concentrated. Water was added, the remaining chloroform removed *in vacuo*, the aqueous solution made normal in sodium hydroxide, and extracted twice with an equal volume of benzene. This extracts the product, but very little hexamethylphosphoramide. The benzene layers were then concentrated, water added, and the remaining benzene removed *in vacuo*. The product was analyzed by exhaustive partitioning, and was at least 98 % pure.

OO-*diethyl* O(*ethylthioethyl*) *phosphorothionate and* OO-*diethyl* S(*ethylthioethyl*) *phosphorothiolate* were prepared by the method of Gardner & Heath (1953), and shown to be pure compounds by the partition chromatographic methods described in that paper.

OO-*diethyl* S(*ethylsulphinylethyl*) *phosphorothiolate* was prepared by the reaction

$$(EtO)_2P(OS)Na + BrC_2H_4.SO.C_2H_5 \rightarrow (EtO)_2PO.SC_2H_4SO.C_2H_5.$$

This reaction also yields the thionate isomer.

Bromethylthioethane was prepared from hydroxyethylthioethane, bromine and red phosphorus and was oxidized with hydrogen peroxide in glacial acetic acid. The product, which was presumed to be bromoethylsulphinylethane was allowed to react with sodium *OO*-diethylphosphorothionate in absolute alcohol for $2\frac{1}{2}$ h under reflux. The alcohol was evaporated *in vacuo*, benzene added and the evaporation continued to remove the alcohol. Chloroform was added and the evaporation continued to remove the benzene. The chloroform solution was then freed of sodium bromide by washing with water, and the chloroform solution concentrated. Partition chromatography on the cascade separator, using

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trichloroethylene as the static phase and water as the eluent phase, gave two bands of radioactivity.

The more water soluble compound was *OO*-diethyl *S*(ethylsulphinylethyl) phosphorothiolate, as it is identical with the product produced in nearly quantitative yield by the oxidation, with an equimolar quantity of hydrogen peroxide, of *OO*-diethyl *S*(ethylthioethyl) phosphorothiolate (see table 7, main text). The product of this reaction must be a sulphoxide, as only one atom of oxygen is used per molecule of thiolate, and the reaction proceeds, though slowly, in very dilute aqueous solution. It must therefore be either $(EtO)_2PO.SC_2H_4SO.C_2H_5$ or $(EtO)_2PO.SO.C_2H_4SC_2H_5$. But the compound has been prepared above by the condensation of an oxidation product of bromoethylthioethane with the thioate, and in the bromo compound only one sulphur is present. Therefore the structure must be $(EtO)_2PO.SC_2H_4SO.C_2H_5$.

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