Reaction of Parathion and Malathion with Peroxytrifluoroacetic Acid, a Model System for the Mixed Function Oxidases†

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ABSTRACT: The products of the reaction of parathion with peroxytrifluoroacetic acid have been shown to be diethylphosphorothioic acid, paraoxon, and tetraethyl pyrophosphate. The major product of the reaction of peroxytrifluoroacetic acid with malathion has been shown to be dimethylphosphorothioic acid. Further studies have shown the reaction of parathion with peroxytrifluoroacetic acid is second order and that the energy of activation for the formation of paraoxon is approximately 6400 kcal/mole. Chemical mechanisms for the formation of paraoxon, diethylphosphorothioic acid, and dimethylphosphorothioic acid have been proposed.

A popular approach to the study of the mechanisms of enzymic-catalyzed reactions is the use of model or nonenzymic systems. Peroxytrifluoroacetic acid is a compound which has been used to study the mechanisms of the mixed function oxidase catalyzed hydroxylation of aromatic substrates (Guroff *et al.*, 1967; Jerina *et al.*, 1967, 1971; Ullrich *et al.*, 1968; Ullrich and Staudinger, 1969). The observation of intramolecular migration of aryl ring substituents (the NIH shift) during both enzymic and nonenzymic aryl hydroxylation using peroxytrifluoroacetic acid (Jerina *et al.*, 1971) verifies the usefulness of this compound as a model system for the mixed function oxidase catalyzed metabolism of aromatic compounds.

Another class of compounds metabolized by the mixed function oxidases is the phosphorothionate insecticides (Neal, 1967; Nakatsugawa and Dahm, 1967). In this study we report on the reactions of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothionate) and malathion (*O,O*-dimethyl *S*-(1,2-dicarbethoxy)ethyl phosphorodithionate), two widely used insecticides, with peroxytrifluoroacetic acid. The results of these studies indicate that the reaction of peroxytrifluoroacetic acid with the phosphorothionate insecticides is a suitable model system for the mixed function oxidase catalyzed metabolism of these compounds.

Materials and Methods

The procedure for the synthesis of [^{32}P]parathion was previously described (Neal, 1967). Standard diethyl phosphorothionate was prepared by hydrolyzing diethyl phosphorochloridothionate (K and K Laboratories, Inc., Plain View, N. Y.) in 50% ethanol which was 1 N with respect to sodium hydroxide. Paraoxon (*O,O*-diethyl *O*-*p*-nitrophenyl phosphate) was synthesized by coupling diethyl phosphorochloridate and the sodium salt of *p*-nitrophenol (Eastman Organic Chemicals, Rochester, N. Y.) in acetone as previously described (Neal, 1967). Tetraethyl pyrophosphate was prepared by hydrolysis of diethyl phosphorochloridate (Toy, 1948). The purity and identity of all compounds were determined by gas chromatography and gas chromatography–mass spectrometry. Dichloromethane and trifluoroacetic acid anhydride were obtained from Aldrich Chemical Company, Inc., Milwaukee, Wis. Hydrogen peroxide, 90%, was a gift from Du Pont Company, Wilmington, Del. Diazomethane was prepared by coupling Diazald (*N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, Aldrich Chemical Company, Inc., Milwaukee, Wis.) with Carbitol (diethylene glycol monoethyl ether, Fisher Scientific Company, Fair Lawn, N. J.) in sodium hydroxide according to the procedure described by Vogel (1966). Malathion, malaoxon (*O,O*-dimethyl *S*-(1,2-dicarbethoxy)ethyl phosphorothiolate) and potassium dimethyl phosphorothionate were a gift from American Cyanamid Company, Princeton, N. J. Solutions of peroxytrifluoroacetic acid were prepared as described by Jerina *et al.* (1971).

Identification of the Products of the Reaction of Parathion and Malathion with Peroxytrifluoroacetic Acid. Two methods were used to identify and quantitate the products of the reaction of parathion with peroxytrifluoroacetic acid. One method involved thin-layer chromatography of the ^{32}P-labeled reaction products. This method has been described previously (Neal, 1967).

The reaction products of parathion with peroxytrifluoroacetic acid were also quantitated and identified by gas chromatography and gas chromatography–mass spectrometry. In this procedure the dichloromethane in the reaction mixture was evaporated under a stream of nitrogen and the residue was dissolved in methanol and allowed to react with diazomethane. The methylated and unmethylated products in the reaction mixture were then separated and quantitated by gas chromatography. These products were also analyzed for structure using an LKB 9000 gas chromatograph–mass spectrometer. Gas chromatography was performed on a 6 ft × 0.25 in. glass column packed with 3% OV-17, 60–80 mesh on Chromasorb Q (Applied Science Laboratories, Inc., State College, Pa.). Operating conditions for the gas chromatograph were as follows: carrier gas, He; flow rate 16 ml/min. The column was run isothermally with a column temperature of 200° for separation of unreacted parathion from paraoxon and tetraethyl pyrophosphate and at a column temperature of 125° for separation of the products of methylation of diethylphosphorothioic acid, namely, *O,O*-diethyl *O*-methyl phosphorothionate and *O,O*-diethyl *S*-methyl phosphorothiolate. Due to the distribution of charge over the sulfur–phosphorus–

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oxygen center of diethylphosphorothioic acid, methylation may occur at the oxygen or sulfur. The result is the formation of the *O*-methyl and *S*-methyl isomers. These isomers were distinguished by mass spectrometry. The retention times of parathion, paraoxon, and tetraethyl pyrophosphate and of *O,O*-diethyl *O*-methyl phosphorothionate and *O,O*-diethyl *S*-methyl phosphorothiolate using these conditions were 11.3, 10.5, 3.0, 4.5, and 8.0 min, respectively. The retention times and mass spectra of paraoxon, tetraethyl pyrophosphate, and methylated diethylphosphorothioic acid were compared with the appropriate standards.

The products resulting from the reaction of malathion with peroxytrifluoroacetic acid were also methylated using diazomethane in the manner described above. The reaction products were then separated, quantitated, and analyzed for structure using gas chromatography and gas chromatography–mass spectrometry. Operating conditions for the gas chromatograph for the separation of the methylated products of the reaction were the same as those described previously for separation of the methylated reaction products of parathion. Gas chromatography of the methylated reaction products of malathion with peroxytrifluoroacetic acid revealed two peaks with retention times of 2 and 4 min. The mass spectra of these compounds were identical with the mass spectra of *O,O,O*-trimethyl phosphorothionate and *O,O*-dimethyl *S*-methyl phosphorothiolate, the methylated products of dimethyl phosphorothioic acid. The reaction mixtures of malathion with peroxytrifluoroacetic acid were also analyzed for the presence of malaoxon and unreacted malathion by gas chromatography using the same conditions described previously for the separation of parathion, paraoxon, and tetraethyl pyrophosphate. The retention time of malathion and of malaxon using these conditions was 14 and 13 min, respectively.

Results

Products of the Reaction of Parathion with Peroxytrifluoroacetic Acid. [^{32}P]Parathion (0.34 μmole) was reacted with peroxytrifluoroacetic acid (0.68 μmole) in 2 ml of dichloromethane for 1 min at 0°. The reaction was terminated by adding 3.4 μmoles of unlabeled parathion to the reaction solution. An excess of parathion had been shown previously to rapidly use up unreacted peroxytrifluoroacetic acid and terminate the reaction. The reaction mixture was then subjected to autoradiography as previously described (Neal, 1967). These autoradiographs revealed three different products of the reaction. Two of these compounds were tentatively identified by their R_F values to be diethylphosphorothioic acid and paraoxon, compounds known to be products of the mixed function oxidase catalyzed metabolism of parathion by mammalian microsomes (Gage, 1953; O'Brien, 1959; Nakatsugawa and Dahm, 1967; Neal, 1967). Gas chromatography–mass spectrometry of the reaction mixture verified that paraoxon and diethylphosphorothioic acid were products of this reaction.

The third product of the reaction of parathion with peroxytrifluoroacetic acid is not seen as a product of the mixed function oxidase catalyzed metabolism of parathion. The reaction of [^{35}S]parathion with peroxytrifluoroacetic acid followed by thin-layer chromatography and radioautography indicated that the unknown compound did not contain sulfur. The unknown compound did not quench the fluorescence of silica gel containing a fluorescent compound, indicating that the product did not contain the *p*-nitrophenol group. Finally, the unknown compound was shown by mass spectrometry to have a molecular ion at *m/e* 290 and a mass spectra identical with that of standard tetraethyl pyrophosphate.

TABLE I: Comparison of the Rate of Formation of the Products Resulting from the Reaction of Parathion with Peroxytrifluoroacetc Acid.[a]

Reaction Product	Amount Formed[b] (μmole/min)
Paraoxon	0.110 ± 0.000
Tetraethyl pyrophosphate	0.027 ± 0.004
Diethylphosphorothioic acid	0.050 ± 0.003

[a] Parathion (0.400 μmole) was reacted with 0.800 μmole of peroxytrifluoroacetic acid in 2 ml of dichloromethane for 1 min at −10°. The reaction products were quantitated as described previously (Neal, 1967). [b] The values are a mean ± the standard deviation of the mean of three experiments.

Comparison of the Rate of Formation of Paraoxon, Tetraethyl Pyrophosphate, and Diethylphosphorothioic Acid during the Reaction of Parathion with Peroxytrifluoroacetic Acid. [^{32}P]Parathion (0.40 μmole) was allowed to react with peroxytrifluoroacetic acid (0.80 μmole) at −10° for 1 min. The reaction was terminated by adding 3.4 μmoles of unlabeled parathion, and the amounts of the products formed during the reaction were determined by thin-layer chromatography as described previously (Neal, 1967). A comparison of the mean rate of formation of the reaction products in three separate experiments is shown in Table I. On the average, 0.187 μmole of parathion was converted to 0.110 μmole of paraoxon, 0.027 μmole of tetraethyl pyrophosphate, and 0.050 μmole of diethylphosphorothioic acid in 1 min at −10°.

Determination of the Order of the Reaction of Parathion with Peroxytrifluoroacetic Acid. To determine the order of the reaction of parathion with peroxytrifluoroacetic acid, [^{32}P]-parathion (0.34 μmole) was added to each of three solutions of peroxytrifluoroacetic acid (0.68 μmole) in 2 ml of dichloromethane at 0°. The reactions were terminated at 1, 3, and 5 min by adding unlabeled parathion. The amount of [^{32}P]-parathion unreacted in each reaction mixture was quantitated (Neal, 1967) and a plot of the time (t) from the beginning of the reaction against the ratio of the time (t) to the amount of parathion (p) unreacted at time t (t/p) was made. The plot gave a line with a slope equal to 1. This indicated the reaction of parathion with peroxytrifluoroacetic acid was second order (Wilkinson, 1961).

Studies of the Stoichiometry of the Reaction of Parathion with Peroxytrifluoroacetic Acid. Carefully controlled experiments indicated only 54% of the parathion was converted into reaction products when 0.40 μmole of a freshly prepared solution of peroxytrifluoroacetic acid was allowed to react with 0.40 μmole of parathion. After storage of this solution of peroxytrifluoroacetic acid at 10° for 20 hr a reaction mixture containing equimolar parathion and peroxytrifluoroacetic acid converted only 18% of the parathion to products. Since more than half the parathion consistently reacted when equimolar quantities of the freshly prepared reagent and parathion were mixed, the stoichiometry of the reaction is thought to be 1:1. The lack of a stoichiometric yield of products is attributed to the instability of the peroxytrifluoroacetic acid reagent.

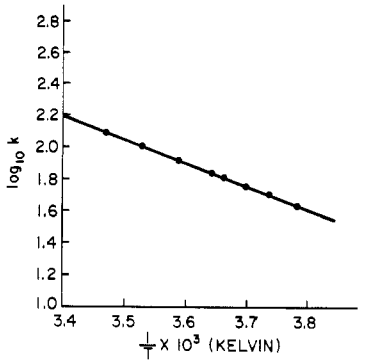

FIGURE 1: Arrhenius plot of the variation of the rate of formation of paraoxon with temperature in a reaction of parathion with peroxytrifluoroacetic acid. The constant k = rate of formation of paraoxon in nmoles/min.

Determination of the Energy of Activation for the Formation of Paraoxon from the Reaction of Parathion with Peroxytrifluoroacetic Acid. In this experiment the peroxytrifluoroacetic acid (0.68 μmole) was added to a solution of parathion (0.34 μmole) in 2 ml of Cl_2CH_2 and the mixture was allowed to react for 30 sec at −11°. The reaction was terminated by the addition of 3.40 μmoles of unlabeled parathion. The same reaction was also carried out at −8°, −5°, −2°, 0°, +5°, +10°, and +15°. When the reactions had been terminated, an aliquot from each reaction was spotted on thin layers of silica gel, and the amount of paraoxon formed was determined as described previously (Neal, 1967).

A plot of the logarithm of the rate of formation of paraoxon (log k) in nmoles/min *vs.* $1/T \times 10^3$ in degrees Kelvin is shown in Figure 1. From the slope of the line the energy of activation was calculated using the Arrhenius equation (Dixon and Webb, 1964). The energy of activation for the formation of paraoxon was calculated to be 6407 cal/mole.

A typical Arrhenius plot for the formation of diethylphosphorothioic acid and tetraethyl pyrophosphate could not be drawn. The rate of formation of these two products was unchanged or slightly decreased with increases in temperature. It is known from studies with the microsomal mixed function oxidase enzyme system that the metabolism of parathion to diethylphosphorothioic acid requires water (Ptashne *et al.*, 1971). The reactions of peroxytrifluoroacetic acid with parathion, however, are done in dichloromethane which has been distilled over calcium hydride. It was postulated, therefore, that water was the limiting factor in the formation of both diethylphosphorothioic acid and tetraethyl pyrophosphate and a typical increase in rate of formation of diethylphosphorothioic acid with increase in temperature could not be measured.

To examine if water was involved in the mechanism of formation of diethylphosphorothioic acid and perhaps tetraethyl pyrophosphate the following experiment was conducted. [^{32}P]Parathion was allowed to react for 30 sec at −5° with solutions of peroxytrifluoroacetic acid in 2 ml of dichloromethane that was (1) distilled over calcium hydride, (2) undistilled, or (3) saturated with water by shaking the dichloromethane in a separatory funnel with water and draining off the organic layer. The ^{32}P-labeled diethylphosphorothioic acid and tetraethyl pyrophosphate from each experiment were isolated and quantitated as described previously (Neal, 1967).

Table II shows the rate of formation of tetraethyl pyrophosphate and diethylphosphorothioic acid appear to be slightly increased when using undistilled CH_2Cl_2 or H_2O-saturated CH_2Cl_2 as compared to distilled CH_2Cl_2, suggesting that water may be involved in the mechanism of formation of these compounds.

TABLE II: Rate of Formation of Diethylphosphorothioic Acid and Tetraethyl Pyrophosphate from the Reaction of Parathion with Peroxytrifluoroacetic Acid in Dichloromethane Containing Various Amounts of Water.[a]

Solvent Used	Product Formed (μmole/0.5 min)	
	Tetraethyl Pyrophosphate	Diethyl Phosphorothionate
CH_2Cl_2 distilled	0.011	0.027
CH_2Cl_2 undistilled	0.013	0.035
CH_2Cl_2–H_2O saturated	0.016	0.034

[a] [^{32}P]Parathion (0.400 μmole) was reacted for 30 sec at −5° with solutions of peroxytrifluoroacetic acid (0.800 μmole) in 2 ml of dichloromethane that was (1) distilled over calcium hydride; (2) undistilled; or (3) saturated with water by shaking the dichloromethane with water in a separatory funnel and draining off the organic layer. The reactions were terminated by adding unlabeled parathion (3.4 μmoles), and the reaction products were quantitated as described previously (Neal, 1967).

Reactions of Malathion with Peroxytrifluoroacetic Acid. Malathion, like parathion, is a pesticide widely used for insect control. Both parathion and malathion are metabolized by the mixed function oxidases. The microsomal mixed function oxidase catalyzed metabolism of malathion probably results in two major products: malaoxon, the oxygen analog of malathion (O'Brien, 1957; Krueger and O'Brien, 1959); and dimethylphosphorothioic acid. The formation of dimethylphosphorothioic acid from malathion involves cleavage of a thio–phosphorus single bond. This reaction is analogous to the cleavage of the thio–phosphorus single bond of another phosphorodithionate dyfonate (*O*-ethyl *S*-phenyl ethylphosphonodithionate), a reaction which has been shown to be catalyzed by the mixed function oxidases (McBain *et al.*, 1971a). Since the major mixed function oxidase catalyzed metabolites of parathion (paraoxon and diethylphosphorothioic acid) were also products of the model system reaction, it was of interest to see if malathion reacted similarly with peroxytrifluoroacetic acid to form malaoxon and dimethylphosphorothioic acid.

Accordingly, malathion was allowed to react with peroxytrifluoroacetic acid in dichloromethane at −10° for 1 hr. These reaction mixtures were reduced to dryness, methylated, and analyzed by gas chromatography and gas chromatography–mass spectrometry. The results are shown in Table III. Methylated dimethyl phosphorothionate was identified as a product of this reaction by comparison of retention times and mass spectra of the two methylated products with those of standard methylated dimethyl phosphorothionate. Analysis of the reaction mixtures indicated that malaoxon was not formed during the reaction of malathion with peroxytrifluoroacetic acid under these conditions.

The sum of the dimethylphosphorothioic acid formed and the malathion recovered in each reaction did not equal the amount of malathion added. The nature of this unrecovered product is not known.

TABLE III: Product of the Reaction of Malathion with Peroxytrifluoroacetic Acid.[a]

Experiment No.	Initial Concentration of Malathion (μmoles)	Concentration of PTFA[b] (μmoles)	Dimethylphosphorothionate Formed (μmole)	Malathion Recovered (μmoles)
1	6.0	6.0	0.7	5.0
2	1.2	36.0	0.8	0.0

[a] Malathion was allowed to react with peroxytrifluoroacetic acid in 2 ml of dichloromethane at −10° for 1 hr. The dichloromethane was removed under a stream of nitrogen, and the residue dissolved in methanol and methylated as described in Materials and Methods. The methylated reaction mixture was then analyzed by gas chromatography and gas chromatography–mass spectrometry. [b] Peroxytrifluoroacetic acid.

Studies on the Reaction of Parathion, Paraoxon, and Malathion with Hydrogen Peroxide or Trifluoroacetic Acid Anhydride. It was of interest to determine if parathion, paraoxon, or malathion would react with either hydrogen peroxide or trifluoroacetic acid anhydride to form the products seen in the experiments with peroxytrifluoroacetic acid.

In these experiments, parathion and paraoxon were allowed to react separately with equimolar quantities of trifluoroacetic acid anhydride or 90% hydrogen peroxide in dichloromethane, and the reaction mixtures were analyzed by gas chromatography. Malathion was incubated with 30 molar equivalents of trifluoroacetic acid anhydride or 90% hydrogen peroxide in dichloromethane. Neither parathion, paraoxon, nor malathion reacted with trifluoroacetic acid anhydride or hydrogen peroxide alone. The results of these experiments indicated that the complete model system (peroxytrifluoroacetic) was required to convert parathion and malathion to the various products. Paraoxon was also allowed to react with a two molar excess of peroxytrifluoroacetic acid at 5° for 2 hr. Paraoxon does not react under these conditions to any measurable degree.

Discussion

The reaction of parathion with peroxytrifluoroacetic acid proceeds with the formation of paraoxon and diethylphosphorothioic acid as the major products. These same products are formed when parathion is incubated with mammalian liver microsomes (Neal, 1967; Nakatsugawa and Dahm, 1967). The formation of diethylphosphorothioic acid by both the enzymic and nonenzymic reaction requires the participation of water. In addition, paraoxon is not a substrate for the enzymic reaction nor a reactant with peroxytrifluoroacetic acid. Therefore the reaction of parathion with peroxytrifluoroacetic acid appears to be a valid model for the mixed function oxidase catalyzed metabolism of this compound.

It has been postulated that peroxytrifluoroacetic acid functions *via* an oxygen atom transfer mechanism (Henbest, 1965; Jerina *et al.*, 1971). A singlet oxygen atom, or "oxene" (electronically similar to a carbene or nitrene) which has six paired electrons, or a closely related enzyme-bound oxenoid species has also been proposed to be the form of oxygen which is

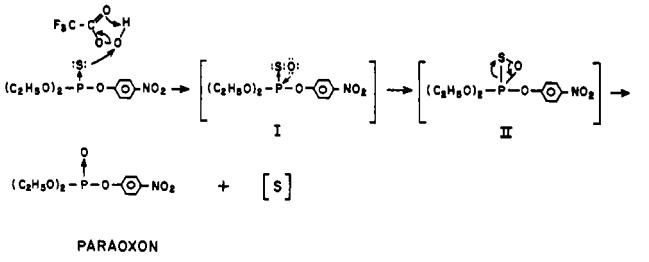

FIGURE 2: A proposed mechanism for the formation of paraoxon from the reaction of parathion with peroxytrifluoroacetic acid.

transferred in mixed-function oxidase catalyzed reactions (Hamilton, 1964; Ullrich and Staudinger, 1966; Jerina *et al.*, 1970). Independent studies of the mixed-function oxidase catalyzed metabolism of parathion (Ptashne *et al.*, 1971) and dyfonate (McBain *et al.* 1971a) using oxygen-18 have led to the proposal of similar chemical mechanisms for the metabolism of these two sulfur-containing organophosphates. These proposed mechanisms involve the addition of oxygen to form a cyclic phosphorus–oxygen–sulfur intermediate which can rearrange to lose the thiono sulfur or be subject to nucleophilic attack by water to lose *p*-nitrophenol in the case of parathion and thiophenol in the case of dyfonate. Similar mechanisms are proposed in Figures 2 and3 for the formation of paraoxon and diethylphosphorothioic acid when peroxytrifluoroacetic acid reacts with parathion.

In Figure 2 we have shown the initial reaction to be a direct transfer of an oxygen atom from peroxytrifluoroacetic acid to an unbonded electron pair on the sulfur. Direct oxygen transfer appears to be the favored reaction in aromatic hydroxylation by this reagent (Jerina *et al.*, 1971). The next step is a cyclization of intermediate I to form intermediate II. Intermediate II then rearranges to release atomic sulfur and form paraoxon. In the enzymatic reaction most of the sulfur released becomes covalently bound to macromolecules (Nakatsugawa and Dahm, 1967). The fate of the sulfur in the model reaction is unknown.

The proposed mechanism for the reaction of parathion with peroxytrifluoroacetic acid to form diethylphosphorothioic acid, a reaction which appears to involve water, is shown in Figure 3. As in the mechanisms proposed for the mixed-function oxidase catalyzed metabolism of the sulfur-

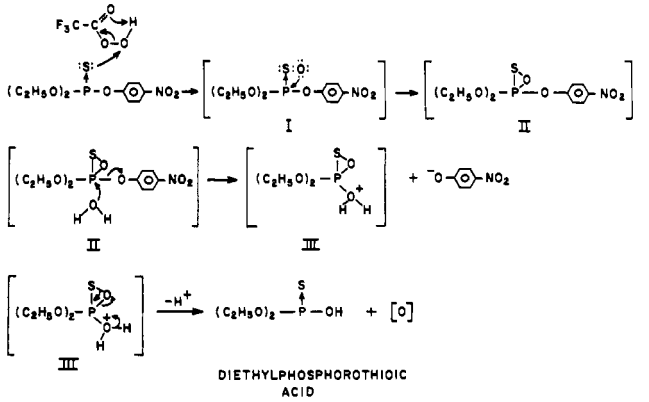

FIGURE 3: A proposed mechanism for the formation of diethylphosphorothioic acid from the reaction of parathion with peroxytrifluoroacetic acid.

containing organophosphates (Ptashne *et al.*, 1971; McBain *et al.*, 1971a), it is attractive to propose that the formation of paraoxon and diethylphosphorothioic acid proceed through the same cyclic intermediate. The support for this concept comes from the results of the oxygen-18 experiments with the enzymatic system and the fact that paraoxon is not a substrate for the mixed-function oxidase enzymes nor does it react to any measurable degree with peroxytrifluoroacetic acid. Thus the thiono sulfur appears to be the site of addition of oxygen to form paraoxon and diethylphosphorothioic acid in both the enzymatic and model reactions. In the case of diethylphosphorothioic acid formation the common intermediate II is subject to nucleophilic attack by water in a reaction leading to the release of *p*-nitrophenol. Although the attack of water on intermediate II is shown as a concerted reaction, in previous work it has not been possible to differentiate whether hydrolysis of phosphorus triesters occurs in a reaction in which the leaving group is being expelled at the same time the substituting group is entering or a two-step process in which the intermediate decomposes so rapidly it does not equilibrate with the solvent (Cox and Ramsay, 1964). The positively charged intermediate III rearranges to lose a proton and an oxygen atom, forming diethylphosphorothioic acid. By substituting malathion for parathion in Figure 3, the same mechanism may be proposed for the formation of dimethylphosphorothioic acid from malathion. The fate of the oxygen atom released in the formation of diethylphosphorothioic acid and dimethylphosphorothioic acid in the proposed mechanism is unknown.

Malaoxon, the oxygen analog of malathion, is not formed during the reaction of malathion with peroxytrifluoroacetic acid, suggesting that the diethyl α-mercaptosuccinate group and not sulfur is the preferred leaving group. This is in contrast to the reaction of parathion with peroxytrifluoroacetic acid in which the sulfur is more readily lost than the *p*-nitrophenol group leading to the formation of paraoxon at a faster rate than diethylphosphorothioic acid. The fact that paraoxon is formed but malaoxon is not may be due to the difference in bond strengths between the P–S bond of malathion (P–S–diethyl succinate) and the P–O bond of parathion (P–O–nitrobenzene). The bond strength in the diatomic molecule P–S has a value of 120 kcal/mole while the diatomic molecule P–O has a bond energy of 149 kcal/mole (Gaydon, 1968). Thus, the P–S bond of malathion is probably weaker than the P–O bond of parathion. This could explain why dimethyl phosphorothionate is the preferred reaction product of malathion while paraoxon is the preferred reaction product of parathion. An alternative explanation is the sulfur of the thioester bond of malathion is oxidized to the corresponding sulfoxide by peroxytrifluoroacetic acid, a reaction which would facilitate the loss of the α-substituted diethyl succinate group.

Tetraethyl pyrophosphate was formed during the reaction of parathion with peroxytrifluoroacetic acid. This compound does not accumulate as a product of the mixed-function oxidase catalyzed metabolism of parathion. The results of unpublished experiments performed in our laboratory indicate that parathion and not paraoxon is the starting material for formation of tetraethyl pyrophosphate. As indicated earlier, water may also be involved in the reaction. It has not been possible to propose a simple mechanism for the formation of this product starting with parathion.

Added in Proof

Following the submission of this manuscript, a report on the products of the reaction of dyfonate with *m*-chloroperbenzoic acid appeared in the literature (McBain *et al.*, 1971b). These workers have isolated an unstable oxidation product of dyfonate, the chemical and physical properties of which are consistent with the cyclic intermediate II (or some resonance form of it) shown in Figures 2 and 3. These workers have not detected a similar oxidation product of parathion (personal communication). The reason may be that the equivalent intermediate of parathion is too unstable.

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