Pathways for the Hydrolysis of Phorate: Product Studies by ^{31}P NMR and GC-MS

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A new intramolecular mechanism is proposed for the hydrolysis of phorate. ^{31}P NMR was used to study the formation of P-containing products of phorate hydrolysis in situ. When hydrolysis was followed by ^{31}P NMR, a dominant P-containing product was found and was identified to be diethyl dithiophosphate using methylation and GC-MS. Combining the data from phorate hydrolysis at three different temperatures, thermodynamic parameters were calculated. The contributions of various possible pathways to phorate hydrolysis are discussed.

Keywords: *Phorate; diethyl dithiophosphate; ^{31}P NMR; intramolecular nucleophilic attack; activation entropy; activation enthalpy; GC-MS*

INTRODUCTION

Organophosphorus compounds are widely used as broad-spectrum pesticides, taking advantage of their inhibitory action on cholinesterase, a key enzyme involved in the metabolism of acetylcholine, one of the key neurotransmitters (Coats, 1993). In recent years, under mounting pressure from public and environmental health professionals, the U.S. EPA has decided to reassess the impact of organophosphorus pesticides on public health (Cooney, 1999). The data will be used to further regulate the application of organophosphorus pesticide, and may assist in determining if their use must ultimately be discontinued.

Phorate (**1**) (Figure 1) belongs to the dithioate subgroup organophosphorus pesticides, as does its structural cousin, disulfoton (**2**). It is a systemic pesticide that targets chewing and sucking pests on a variety of plants (Szeto and Brown, 1982). It can be oxidized to phorate oxon, phorate sulfoxide, phorate sulfone, etc. via either abiotic or biotic pathways (Eto, 1974; Chapman et al., 1982; Szeto et al., 1990). It can also undergo rapid hydrolysis at the pH of natural water systems (i.e., 5−9) (American Cyanamid Corp. data, 1990; Hong and Pehkonen, 1998). Two possible hydrolysis mechanisms were proposed earlier following the identification of several unusual, previously unreported hydrolysis products such as HCHO and diethyl disulfide (Hong and Pehkonen, 1998). Thermodynamic and kinetic studies are the two most important tools for the elucidation of reaction mechanisms in addition to product identification. Thermodynamic studies are very useful in estimating the relative contribution of each pathway and disproving certain pathways especially when multiple reaction pathways are present.

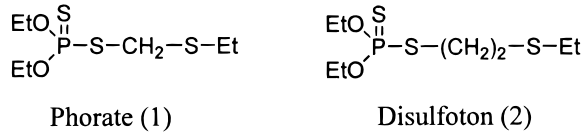

Figure 1. Structure of phorate and disulfoton.

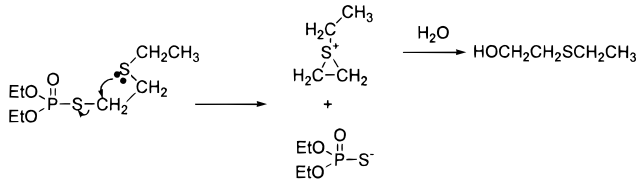

Figure 2. S$_N$*i* mechanism for demeton-S hydrolysis.

Nucleophilic attacks on the central phosphorus atom or on a side chain carbon atom are the main modes for hydrolysis of organophosphorus compounds including phorate. While an S$_N$2 mechanism usually dominates with a water molecule and/or hydroxide as the nucleophile (Schwarzenbach et al., 1993), the S$_N$*i* mechanism has also been proposed for several pesticides where internal nucleophiles are present, such as demeton-S (Schwarzenbach et al., 1993). In demeton-S, a sulfur atom can serve as an internal nucleophile and the hydrolysis pathway is relatively independent of pH (Figure 2). It is important to note that very little experimental evidence (e.g., product studies) exists to support an S$_N$*i* mechanism for the hydrolysis of organophosphorus pesticides (Muhlmann and Schrader, 1957; Schwarzenbach et al., 1993).

The progress of chemical transformations of organophosphorus pesticides and their derivatives is monitored mainly by liquid or gas chromatography in combination with UV−vis or mass spectrometric detection. However, some of the derivatives are thermally labile or too polar for GC analysis. Phosphorus 31 NMR (^{31}P NMR) can be a very useful technique for the analysis of these types of compounds (Gurley and Ritchey, 1976; Lang and Martin, 1986; Lai et al., 1995). The disadvantage of ^{31}P NMR is its lower sensitivity compared to GC or LC. However, if concentration levels are high enough, the

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Table 1. NMR Data of Phorate Hydrolysis

time, h[a]	3 1/3	15 1/3	27 1/3	39 1/3	51 1/3	63 1/3	75 1/3	87 1/3	99 1/3	111 1/3	123 1/3	135 1/3	147 1/3	159 1/3
NMR signal[b] (phorate)	17.0	17.2	14.5	11.2	11.5	8.7	6.9	6.5	5.0	4.8	4.4	3.9	3.4	3.2
NMR signal (product[c])	N/A[d]	1.6	2.6	3.2	4.0	5.4	5.8	6.6	6.6	7.5	9.0	8.5	10.0	12.3

[a] Time between the onset of the experiment and the middle of the acquisition. [b] NMR spectra were printed; NMR peaks were cut and weighed to represent NMR signals (in mg). [c] Product refers to *O,O*-diethyl dithiophosphate. [d] Not distinguishable from the background noise.

technique is robust for kinetics studies because a reaction system can be continuously monitored in situ without being disturbed by sampling and various P-containing products can be identified without the need for isolation or derivatization.

In this work, ^{31}P NMR and GC-MS were used to further investigate phorate hydrolysis. The results from a series of kinetic, thermodynamic, and product studies together provide a plausible scenario for phorate hydrolysis under simulated natural water conditions.

EXPERIMENTAL METHODS

^{31}P NMR Analysis. The ^{31}P NMR analysis was performed at 161.98 MHz on a Bruker AMX 400 wide bore spectrometer equipped with a B-VT 1000 variable-temperature unit. A 30 mm nonspin phosphorus probe was used for the analysis. An external reference capillary of 1% (w/w) Na_2HPO_4 was used. This reference standard was calibrated against 85% phosphoric acid (Fisher Scientific, Pittsburgh, PA) and found to be 3.45 ppm downfield from phosphoric acid. A 45.0-μs, 90° pulse was used with a 0.5-s recycle delay time. Immediately after the onset of the experiment, 32000 16K spectra were acquired with a spectral window of 33 333.33 Hz (205.78 ppm) using the Bruker Kinet Experiment pulse sequence. The acquisition took about 6.67 h and was repeated every 12 h 13 more times. The temperature was maintained at 310.1 ± 0.1 K and the data were processed with a line broadening of 20 Hz.

Dimethyl sulfoxide, boric acid, NaOH, and Na_2HPO_4 were obtained from Fisher Scientific. Neat phorate (98%) was obtained from Chem Service (West Chester, PA). Deuterium oxide (Cambridge Isotope Laboratories, Inc., Andover, MA) was used for all aqueous solutions. A DMSO:D_2O (1:3 v/v) mixed solvent (containing 0.01 M boric acid, pH adjusted to ~9.0 with 0.1 M NaOH) was used to dissolve 385 μM (100 ppm) of phorate in a 30-PP-7 (30-mm outside diameter and 7-in. length) NMR sample tube, which was purchased from Wilmad Glass (Buena, NJ). A coaxial insert tube (520-3 from Wilmad Glass) was filled with 1% (w/w) aqueous solution of Na_2HPO_4 to serve as an external reference. One hundred milligrams per liter of diethyl dithiophosphate (Aldrich Chemical Co., Milwaukee, WI) was dissolved in DMSO:D_2O (1:3 v/v) mixed solvent and analyzed with the same ^{31}P NMR protocol.

Hydrolysis Experiments. The setup for hydrolysis experiments of phorate was the same as previously described (Hong and Pehkonen, 1998). Three temperature ranges were chosen: 24.5−27.2 °C (room temperature), 11.4−14.0 °C (low temperature), 35.5−36.5 °C (high temperature). The temperature range reflects all the replicates, while the temperature for any given experiment varies no more than 2 °C. All experiments were homogeneous and carried out through at least three hydrolysis half-lives of phorate. Six to seven 10-mL samples were taken for each hydrolysis experiment. Each sample was immediately extracted with 2 mL of benzene containing 50 mg/L 4-chloro-3-methylphenol (internal standard for GC, obtained from Aldrich Chemical Co.) and stored at 4 °C prior to GC analysis. A HP 5890 Series II GC with an FID detector (Hewlett-Packard Co., Palo Alto, CA) was used for GC analysis. GC conditions were the following: a 30 m × 0.53-mm i.d. fused silica capillary column with 1.5-μm film thickness (DB-5, J&W Scientific, Folsom, CA) and a carrier gas of nitrogen (10 psi) were used; initial temperature was 105 °C for 2 min; temperature increased at 13 °C/min up to 215 °C for 3 min; injector port temperature was 250 °C; detector temperature was 300 °C.

Methylation and GC-MS. Diazomethane was synthesized by using a MNNG diazomethane-generation apparatus (Aldrich Chemical Co.). The manufacturer's protocol was followed. Samples for the methylation originated from phorate hydrolysis experiments (pH 8.5) at room temperature and the 7-day-long ^{31}P NMR experiment. Technical-grade diethyl dithiophosphate (**3**) (Aldrich Chemical Co.) was also methylated to serve as a GC-MS standard. The diethyl ether layers from the methylation experiments were used as the GC-MS samples. A HP 5890 Series II GC equipped with a HP 5970 MS detector (Hewlett-Packard Co.) was used for GC-MS analysis. GC-MS conditions were the following: 30-m × 0.25-mm i.d. fused silica capillary column with 0.25-μm film thickness (DB-5ms, J&W Scientific) and a carrier gas of helium (5.5 psi) were used; initial temperature was 40 °C; temperature increased at 8.5 °C/min up to 240 °C; injector port temperature was 225 °C; detector temperature was 250 °C.

RESULTS AND DISCUSSION

^{31}P NMR Analysis. The kinetics profile of phorate hydrolysis is shown in Figure 3 and the peak areas are summarized in Table 1. The reduction of the phorate peak (~90.35 ppm) with time fits well with pseudo-first-order kinetics. However, the increase of the peak area of the only significant product peak (~108.60 ppm) does not exactly satisfy the mass balance (Table 1). There are two possibilities: multiple pathways exist for phorate hydrolysis under the experimental condition, or the initial P-containing hydrolysis product can undergo further degradation. Based on an earlier study (Hong and Pehkonen, 1998), two possible P-containing products were expected: diethyl dithiophosphate and diethyl phosphorothioate. The P-containing product shown here in the NMR spectrum was later identified via methylation and GC-MS to be diethyl dithiophosphate. When diethyl dithiophosphate was dissolved in the same DMSO/D_2O mixed solvent and analyzed using the same ^{31}P NMR parameters, the same chemical shift (~108.6 ppm) was observed as compared to that of the dominant product peak in the hydrolysis kinetics study of phorate (see Figure 3). Hence the P−S bond was left intact during the hydrolysis under our experimental conditions. It is interesting to note that ^{31}P NMR results by Lai et al. (1995) show that catalytic hydrolysis by organophosphorus hydrolase does result in the cleavage of the P−S bond for five organophosphorus pesticides although the k_{cat} values for malathion and azinophosethyl, the two phosphorodithioates, are smaller than the k_{cat} of the other three organophosphorus pesticides. The k_{cat} value for malathion is especially small (more than 10^3 times smaller than those of acephate, demeton-S, and phosalone). This aspect will be discussed further in the Methylation and GC-MS section.

Hydrolysis Experiments. Hydrolysis rate constants (k_{obs}) are listed in Table 2. Apparently, k_{obs} of phorate hydrolysis increases with temperature at both pH 5.7 and 8.5. The activation enthalpy, $\Delta H^{\ddagger}$, was determined by plotting $\ln(k_{obs}/T)$ vs $1/T$ (Figure 4) and measuring the slope (eq 1), while the activation entropy, $\Delta S^{\ddagger}$, was calculated from eq 2 (Carey and Sundberg, 1984):

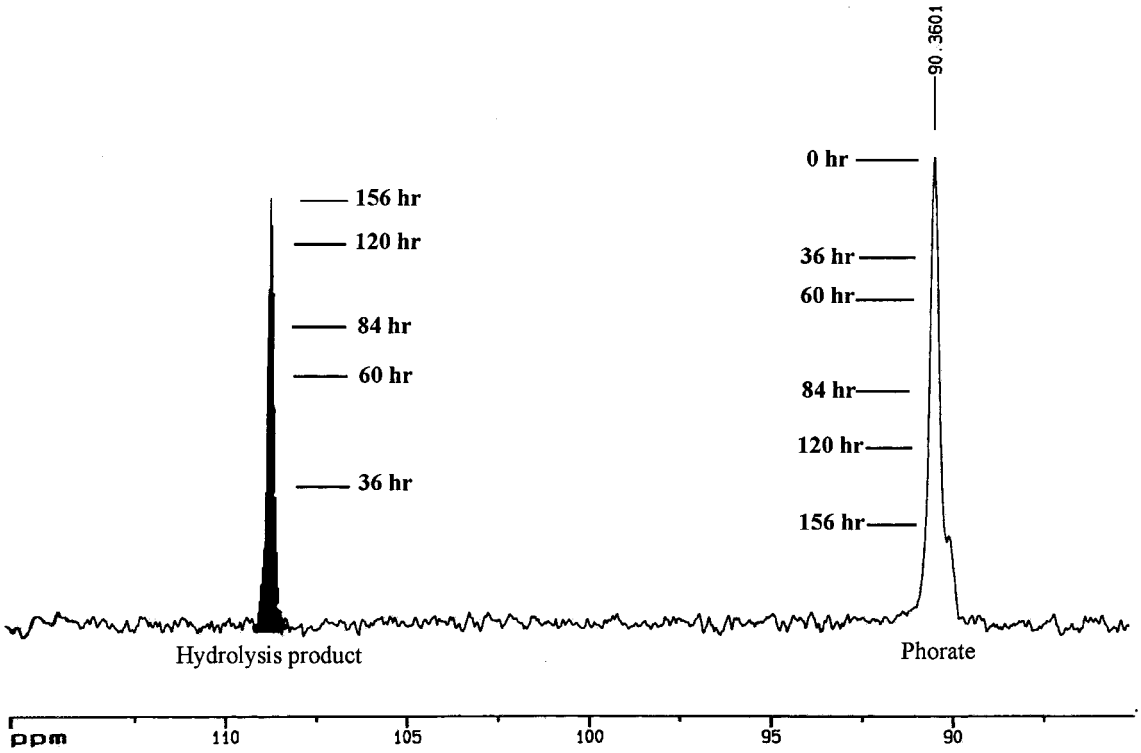

Figure 3. ^{31}P NMR spectrum of phorate hydrolytic kinetics.

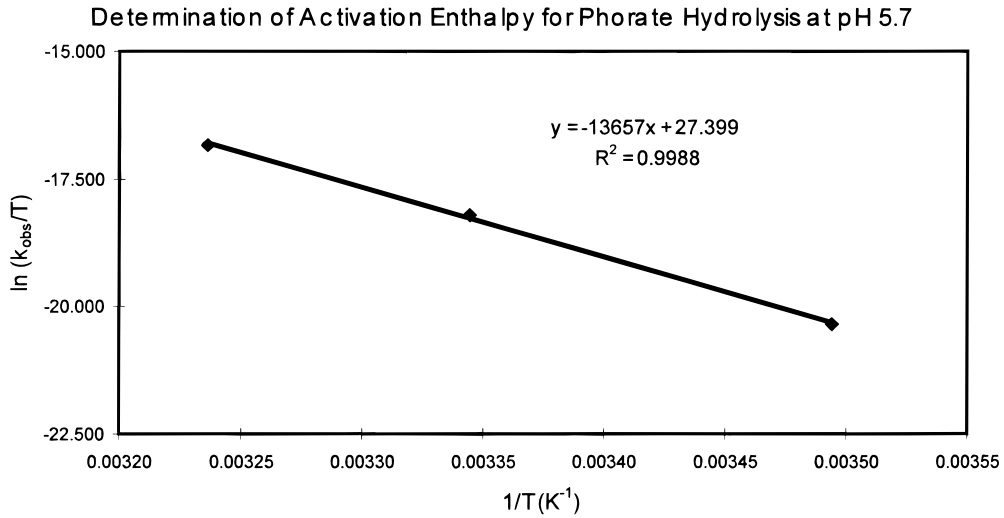

Figure 4. Determination of activation enthalpy for phorate hydrolysis.

Table 2. k_{obs} (in s^{-1}) for Homogeneous Hydrolysis of Phorate

	11.4−14.0 °C	24.5−27.2 °C	35.5−36.5 °C
pH 5.7	$4.17 \times 10^{-7} \pm 3.93 \times 10^{-8}$	$3.71 \times 10^{-6} \pm 3.69 \times 10^{-7}$	$1.50 \times 10^{-5} \pm 5.70 \times 10^{-7}$
pH 8.5	$3.75 \times 10^{-7} \pm 1.96 \times 10^{-8}$	$3.15 \times 10^{-6} \pm 5.03 \times 10^{-7}$	$1.47 \times 10^{-5} \pm 2.67 \times 10^{-6}$

$$\ln(k_{obs}/T) = -\Delta H^{\ddagger}/RT + \text{constant} \quad (1)$$

$$\Delta S^{\ddagger} = (\Delta H^{\ddagger}/T) + R \ln[hk_{obs}/(k\kappa T)] \quad (2)$$

where h is the Planck's constant, k is the Boltzmann's constant, κ is the transmission coefficient (∼1), and k_{obs} is the observed hydrolysis rate constant in s^{-1}. The Arrhenius activation energy, E_a, can be obtained from

$$E_a = \Delta H^{\ddagger} + RT \quad (3)$$

The obtained activation parameters for homogeneous hydrolysis of phorate are listed in Table 3. Activation entropy reflects the loss or gain of degrees of freedom between the starting compounds and the transition state (i.e., the activated complex). However, because multiple pathways may simultaneously contribute to the hydrolysis of phorate, the magnitude and the sign of $\Delta S^{\ddagger}$ can be used to evaluate the relative significance of the individual pathways. The negative $\Delta S^{\ddagger}$ for phorate hydrolysis at pH 8.5 suggests that pathways other than the common S_N2 (Schwarzenbach et al., 1993) play an important role in phorate hydrolysis at that pH.

Methylation and GC-MS. The one dominant product peak (∼108.60 ppm) from ^{31}P NMR data suggests a dominant P-containing hydrolysis product. When the

Table 3. Activation Parameters for Homogeneous Hydrolysis of Phorate and Disulfoton

pesticide	pH	$\Delta H^{\ddagger}$, kJ/mol	$\Delta G^{\ddagger}$, kJ/mol	$\Delta S^{\ddagger}$, J/mol K	E_a, kJ/mol
phorate	5.7	120	130	39	120
	8.5	104	103	−2.8	110
disulfoton[a]	5.7	65	78	−150	68
	8.5	95	80	−54	98

[a] Data from Dannenberg and Pehkonen, 1998.

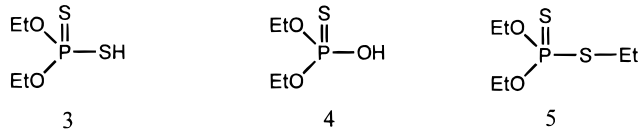

Figure 5. Structure of selected phorate hydrolysis products.

hydrolysis mixture was methylated following the 7-day ^{31}P NMR experiment, GC-MS identified the product as diethyl methyl dithiophosphate, which is the logical methylation product of *O*,*O*-diethyl dithiophosphate (**3**) (Figure 5). It is well-known that phosphorus has a strong tendency to bond with oxygen and that the P−O bond is about 40% stronger than the P−S bond (Lide and Frederikse, 1997), yet diethyl dithiophosphate seems to be rather stable in the mixed solvent applied here. One possibility is that the hydrolysis of diethyl dithiophosphate (**3**) to diethyl phosphorothionate (**4**) may not be kinetically favored. The other possibility is that DMSO interferes with the hydrolysis of diethyl dithiophosphate. While DMSO was found to slow the hydrolysis of phorate at pH 9.0 by 60% at room temperature (that is the reason for the elevated temperature in the ^{31}P NMR experiment), its polar and inert nature and its low nucleophilicity makes it an unlikely culprit for the absence of the thermodynamically favored P−S to P−O transition. The identification of diethyl dithiophosphate provides further support for the hydrolysis mechanism proposed earlier by Hong and Pehkonen (1998).

There is another possible P-containing product, *O*,*O*,*S*-triethyl dithiophosphate (**5**) (Figure 5). This compound was tentatively identified from several hydrolysis mixtures of phorate at pH 8.5 (at room temperature), accounting for approximately 10% of the initial phorate concentration based on relative GC peak areas. Using the Wiley 275L searchable library of mass spectra, a 90% match between the experimentally obtained mass spectrum and the library mass spectrum of *O*,*O*,*S*-triethyl dithiophosphate (**5**) resulted from the search. Unfortunately, the pure compound is not commercially available, which makes a more conclusive identification difficult. The mechanism leading to this compound is not so straightforward by just examining the structure of phorate (**1**). After extensive consultation and insightful input from several colleagues, two intriguing mechanisms (Figure 6) can be proposed to account for this never-reported product. All mechanisms proposed for phorate hydrolysis so far are also summarized in Figure 6. The S_N2 pathway in Figure 6 is supported by the identification of diethyl dithiophosphate, HCHO, and diethyl disulfide (Hong and Pehkonen, 1998). A thermodynamically stable six-membered ring transition state (Figure 6) builds a strong case for the intramolecular nucleophilic attack pathway (S_Ni). In the meantime, the activation entropy data from phorate hydrolysis at pH 5.7 and 8.5 seem to support the S_N1 pathway (Figure 6) for the production of *O*,*O*,*S*-triethyl dithiophosphate (**5**). Both S_N2 and S_Ni pathways would lower

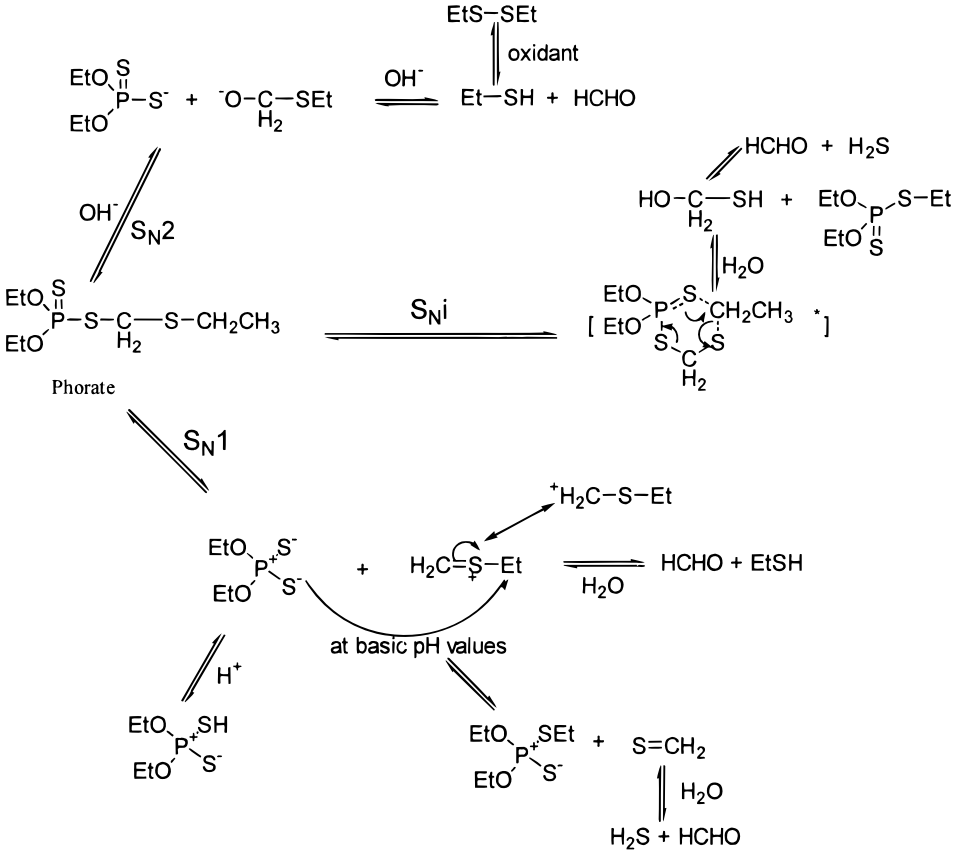

Figure 6. Summary of phorate hydrolysis pathways.

the entropy of the system in going from the reactant ground state to the transition state; in fact, the loss of translational freedom in the S_N2 pathway may lead to an entropy loss of as much as 100 J/K-mol (Isaacs, 1995), while the entropy loss in closing rings of six members or less (S_Ni) would be smaller than that of the S_N2 pathway (March, 1992). For phorate hydrolysis, the proposed S_N1 pathway might reduce or increase the entropy of the system, depending on the rate-determining step: at acidic pH, the protonation step should be much faster than the formation of the carbocation and the $\Delta S^{\ddagger}$ contribution should be positive (Figure 6); at basic pH, the $\Delta S^{\ddagger}$ contribution would be negative if the second step is the rate-determining step (Figure 6). The positive $\Delta S^{\ddagger}$ at pH 5.7 and the negative $\Delta S^{\ddagger}$ at pH 8.5 for phorate hydrolysis suggest a large S_N1 pathway at pH 5.7. By way of comparison, the $\Delta S^{\ddagger}$ for disulfoton hydrolysis (Table 3) is negative at both pH 5.7 and 8.5. Apparently, no stable carbocation intermediate forms in disulfoton hydrolysis, precluding an S_N1 pathway. In fact, the S_N1 pathway is rarely suggested for the hydrolysis of (thio)phosphate esters (Schwarzenbach et al., 1993).

Finally, the only structural difference between phorate (**1**) and disulfoton (**2**) is an additional $-CH_2-$ between the two sulfur atoms in the side chain in the latter (Figure 1). However, a similar intramolecular nucleophilic attack in disulfoton would result in an intermediate with a less stable seven-membered ring, and *O*,*O*,*S*-triethyl dithiophosphate (**5**) was not observed in the hydrolysis of disulfoton (Dannenberg and Pehkonen, 1998).

CONCLUSIONS

^{31}P NMR analysis of phorate hydrolysis at pH 8.5 suggested one dominant reaction pathway. The disappearance of the pesticide peak fits extremely well with the appearance of the dominant product peak. The dominant product was later identified via methylation and GC-MS as diethyl dithiophosphate.

An interesting intramolecular nucleophilic pathway was proposed for phorate hydrolysis based upon the product studies using GC-MS. The six-membered ring within the transition state makes the hypothetical pathway plausible. Additionally, this pathway is independent of pH and, if dominant, should result in the degradation of phorate even in the absence of external nucleophiles such as OH^- and H_2O.

Activation parameters were determined for homogeneous hydrolysis of phorate. Activation enthalpy, activation free energy, and activation energy of phorate hydrolysis are all similar to those of other structurally related organophosphorus pesticides (Dannenberg and Pehkonen, 1998).

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