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Paraoxon and Parathion Hydrolysis by Aqueous Molybdenocene Dichloride (Cp_2MoCl_2): First Reported Pesticide Hydrolysis by an Organometallic Complex

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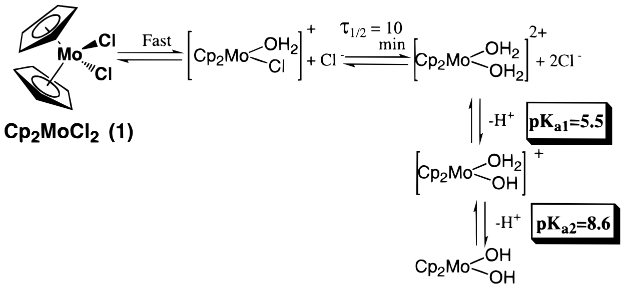
We report the first case of an organometallic complex that effectively hydrolyzes the organophosphate pesticides parathion and paraoxon. The complex is the water-soluble compound bis(η^5-cyclopentadienyl)molybdenum(IV) dichloride (**1**), which hydrolyzes parathion to produce ethanol and deethyl parathion in a biphasic reaction in D_2O. Rate accelerations were 130 and 10^5 at pH 7 and 3, respectively. Paraoxon is readily hydrolyzed by **1** to yield *p*-nitrophenol and diethyl phosphate with rate accelerations of 2300 and 27 at pH 7 and 3, respectively. Kinetic data for paraoxon hydrolysis by **1** are consistent with a process that involves intermolecular ($\Delta S^\ddagger = -49 \pm 10$ eu) hydroxide attack on the phosphate triester in which the aquated **1** serves as a coordinated Lewis acid that activates the organophosphate. Interestingly parathion hydrolysis by **1** occurs via nucleophilic attack at the α-carbon of the phosphorothioate pesticide that involves C−O bond cleavage. These parathion results represent one of the few cases of this type of unusual hydrolytic chemistry and the first case of an organometallic complex that accelerates organophosphate pesticide hydrolysis.

Introduction

Organophosphate triesters act as acetylcholine esterase inhibitors and are widely used as pesticides in the United States.[1] The thionate esters (P═S), which have a lower mammallian toxicity than the corresponding oxonate ester (P═O), are mainly used in agriculture.[2] The thionate ester is oxidized to the oxonate ester both inside and outside of the organism.[3] One of the principle means for degrading thionate ester pesticides in soil and water is hydrolysis by microorganisms.[4] To this date, there are a number of reports that use metal ions for degrading or hydrolyzing thionate pesticides. Ethyl parathion hydrolysis by Cu(II) was first reported in 1956,[5] and Hg(II)[6] was found to hydrolyze malathion, fenitrothion, methyl parathion, fention, and dichlorvos with 2 or 3 orders of magnitude increase. Clay minerals[7] and divalent metal ion-treated clays[8] also catalyze the hydrolysis of a variety of thionate ester pesticides. Surface-catalyzed hydrolyses of organophosphate pesticides by Al_2O_3, TiO_2, and FeOOH (goethite) have been reported.[9] Rhodium(III) and iridium(III) coordination complexes have also been studied as models for effecting phosphate triester hydrolysis.[10] However, to date there have been no reports of *organometallic* complexes that promote the hydrolysis of organophosphate pesticides.

Scheme 1



To that end, our group has been exploring the use of organometallic metallocenes to hydrolyze model phosphate esters. The compound bis(η^5-cyclopentadienyl)molybdenum(IV) dichloride (Cp_2MoCl_2: Cp = η^5-C_5H_5)[11] was chosen because of its water solubility and aqueous properties. Structurally, Cp_2MoCl_2 resembles a clamshell where both cyclopentadienyl ligands (the shell) flank a central tetrahedral Mo(IV) that is also coordinated to two chlorides. In water, the cyclopentadienyl rings remain bound to the tetrahedral Mo(IV) center even at neutral pH.[12] Meanwhile both chlorides rapidly hydrolyze off ($\tau_{1/2}$ ~20 min) to form aqueous species with pK_a values at 5.5 and 8.5 (Scheme 1).[13] These aqueous properties and the diamagnetic d^2 molybdenum center make this metallocene very amenable for NMR characterization.

The Cp_2MoCl_2(aq) complex has been found to hydrolyze the activated phosphate esters *p*-nitrophenyl phosphate and bis(*p*-nitrophenyl phosphate) with rate accelerations as high as 10^7.[14] It was postulated that the mechanism proceeds through intramolecular attack on the phosphate by a Cp_2Mo-bound hydroxide.

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(1) Lacorte, S.; Largtiges, S. B.; Garrigues, P.; Barcelo, D. *Environ. Sci. Technol.* **1995**, *29*, 431−438.
(2) Hassail, K. A. *The Biochemistry and Uses of Pesticides*, 2nd ed.; VCH: Weinheim, 1990; pp 269−275.
(3) Eto, M. *Organophosphorus Pesticides: Organic and Biological Chemistry*; CRC Press: Boca Raton, FL, 1979; p 93.
(4) Li-Tse, O.; Sharma, A. *J. Agric. Food Chem.* **1989**, *37*, 1514−1519.
(5) Ketellaar, J. A. A.; Gersmann, H. R.; Beck, M. *Nature* **1956**, *177*, 392−393.
(6) Wan, H. B.; Wong, M. K.; Mok, C. Y. *Pest. Sci.* **1994**, *42*, 93−99.
(7) Saltzman, S.; Mingelgrin, U.; Yaron, B. *J. Agric. Food Chem.* **1976**, *24*, 739−743.
(8) El-Amamy, M. M.; Mill, T. *Clays Clay Miner.* **1984**, *32*, 67−73.
(9) Torrents, A.; Stone, A. T. *Soil Sci. Soc. Am. J.* **1994**, *58*, 738−745.
(10) Hendry, P.; Sargeson, A. M. *Aust. J. Chem.* **1986**, *39*, 1177−1186.
(11) Cooper, R. L.; Green, M. L. H. *J. Chem. Soc. (A)* **1967**, 1155−1158.
(12) Kuo, L. Y.; Sabat, M.; Kanatzidis, M. G.; Tipton, A. L.; Marks, T. J. *J. Am. Chem. Soc.* **1991**, *113*, 9027−9045
(13) Recently another pK_a at 2.2 was also found, but its identification was not known. Baxley, G. Ph.D. Thesis, University of Oregon, 1997.
(14) Kuo, L. Y.; Kuhn, S. *Inorg. Chem.* **1995**, *34*, 5341−5345.

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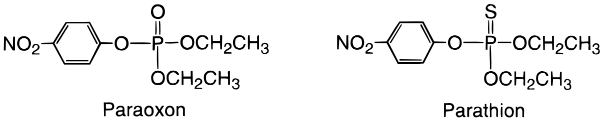
Scheme 2



More recently we have found that $Cp_2MoCl_2(aq)$ effectively hydrolyzes the inactivated phosphate diester, dimethyl phosphate, with a rate acceleration of 10^8, in which the Cp_2Mo-bound diester is attacked by a metallocene-coordinated water nucleophile.[15] Here, we report the first case of an organometallic complex that effectively accelerates the hydrolysis of two organophosphate pesticides, parathion and its oxygen analogue, paraoxon. Moreover, product identification and isotopic-labeling studies were done to show a rather novel pathway for parathion hydrolysis that involves nucleophilic attack at the α-carbon instead of the traditional phosphorus center.

Experimental Methods

Proton and carbon NMR spectra were recorded with a Brucker QE-300 (300 and 75.5 MHz) spectrometer. Proton chemical shifts were referenced to $(CH_3)_4Si$ (TMS). The compounds Cp_2MoCl_2, paraoxon, and parathion were purchased from Strem Chemical Co. (Newburyport, MA) and Chem Service (West Chester, PA), respectively, and used as received. Deuterium oxide (99.9% D) and ^{18}O-water were purchased from Spectrum Chemical Co. (Gardena, CA) and Cambridge Isotope Lab. (Andover, MA),respectively. All other necessary reagents were purchased from Aldrich Chemcal Co. (Milwaukee, WI) and used as received.

All manipulations used standard Schlenk techniques under an argon atmosphere, and all aqueous solutions were thoroughly purged with prepurified argon prior to use. The pD measurements were done in D_2O using a Beckman Φ10 pH meter, and the reported pD values are uncorrected. Stock solutions of parathion and paraoxon (1 M) in acetone-d_6 were first made and were then added to $Cp_2MoCl_2(aq)$ under an argon atmosphere. The pD of $Cp_2MoCl_2(aq)$ was adjusted over a 6 h period before each study using concentrated HCl or NaOH; this ensured stability of the pD after complete dissolution of the Cp_2MoCl_2.

All kinetics studies were done under an argon atmosphere at temperatures ranging from 5 to 60 °C using thermostat-controlled water baths. Pseudo-first-order rate constants at pD 3 were obtained for the degradation of paraoxon by reacting 5 mM of the phosphate triester with 50 mM $Cp_2MoCl_2(aq)$. All reactions were followed to approximately 90% completion. Given the low solubility of parathion in water, we increased its concentration to 50 mM to obtain a clearer ^1H NMR spectra. Unlike the reactions involving paraoxon hydrolysis, the parathion hydrolysis studies were done in a biphasic aqueous solution.

Results and Discussion

Past studies on parathion and paraoxon (Scheme 2) hydrolysis by metal ions have shown that the predominant hydrolytic pathway proceeds with loss of *p*-nitrophenol.[16] Mechanistically, the process could be characterized as an associative process that involves attack of the hydroxide or water nucleophile onto the phosphorus center followed by expulsion of the *p*-nitrophenol leaving group. A metal ion can accelerate/catalyze this process by (1) Lewis acid activation of the electrophilic phosphorus center through coordination to the phosphate or thiophosphate, (2) coordination and delivery of the nucleophile (usually OH^-) to the phosphate center, and (3) coordination and activation of the leaving group.

It is conceivable that all three possible modes of rate acceleration could be in place simultaneously. Nucleophilic attack on the α-carbon of parathion/paraoxon can be postulated, but unequivocal evidence for this process has not been reported.

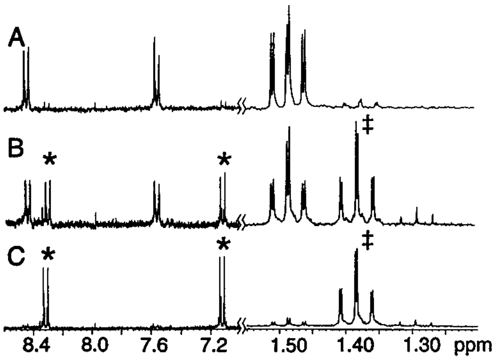


Figure 1. ^1H NMR spectra of 5 mM paraoxon and 50 mM Cp_2MoCl_2 at pD 3, 40 °C, at three time points. The asterisks (*) in the downfield region represents *p*-nitrophenol production, while the double dagger (‡ at 1.38 ppm) in the upfield region represents the methyl protons of diethyl phosphate. Spectra A, B, and C were taken after 15 min, 60 h, and 250 h, respectively.

Table 1. Observed Rate Constants of Paraoxon and Parathion Hydrolyses at 40 °C

	pD 3 (s^{-1})	pD 7 (s^{-1})
paraoxon blank	2.1×10^{-7}	2.5×10^{-7}
paraoxon + $Cp_2MoCl_2(aq)$	5.7×10^{-6}	5.8×10^{-4}
rate accleration for paraoxon hydrolysis	27	2300
parathion blank (50 mM)	*a*	3.5×10^{-7} [b]
parathion + $Cp_2MoCl_2(aq)$ 50 mM each	5.0×10^{-6}	4.5×10^{-5} [b]
rate accelertion for parathion hydrolysis	*a*	130

[a] Blank for parathion in water showed no product release even after three months. This may be due to the biphasic nature of the control reaction. [b] Initial rates. Solubility of parathion improved somewhat at pD 7 such that initial rates could be measured in water.

We initially examined if the organometallic complex $Cp_2MoCl_2(aq)$, which upon dissolution forms a pH 3 green solution, could hydrolyze the water-soluble pesticide paraoxon in a homogeneous solution. Under anaerobic conditions ^1H NMR spectroscopy showed that the reaction of $Cp_2MoCl_2(aq)$ with paraoxon produced exclusively *p*-nitrophenol and diethyl phosphate at both pH 3 and 7 (Figure 1). Identification of these products was done by addition of authentic compounds.

In the interest of quantifying the rate acceleration of paraoxon hydrolysis by $Cp_2MoCl_2(aq)$, we used ^1H NMR to measure the pseudo-first-order rate constants of paraoxon hydrolysis under a variety of conditions in D_2O (Supporting Figure 1). The measured k_{obs} for paraoxon hydrolysis in water (40 °C) without $Cp_2MoCl_2(aq)$ at pD 7 and 3 are 2.5×10^{-7} and 2.1×10^{-7} s^{-1}, respectively (Table 1). These values are close to those measured by Smolen and co-workers[16] (3.0×10^{-8} s^{-1} at pH 7 and $5.3 \times 10^{-8} s^{-1}$ at pH 4), who used HPLC methods to quantify paraoxon hydrolysis at room temperature. The 15 °C temperature difference between these two studies may account for the 12−25-fold difference in observed rate constants in plain water.

When Cp_2MoCl_2 (50 mM) is added to paraoxon (5 mM), we see a 2300-fold and 27-fold rate acceleration of diethyl phosphate release at pD 7 and 3, respectively. The initial observed rates of diethyl phosphate were measured as a function of $Cp_2MoCl_2(aq)$ concentration and pD, and it was found that the hydrolytic process was first-order in $Cp_2MoCl_2(aq)$ and that there was a distinctive pD dependence in the pD 3−7 range (Supporting Figure 2). In addition, we also measured the temperature dependency of paraoxon hydrolysis by $Cp_2MoCl_2(aq)$ (Figure 2) and obtained a large negative entropy of

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(15) Kuo, L. Y.; Barnes, L. A. *Inorg. Chem.* **1999**, *38*, 814−817.
(16) Smolen, J. M.; Stone, A. *Environ. Sci. Technol.* **1997**, *31*, 1664−1673.

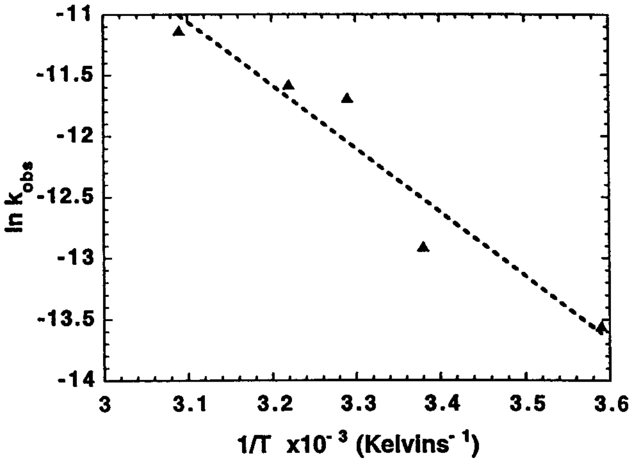
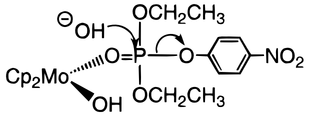


Figure 2. Temperature dependency for the reaction of paraoxon (5 mM) with Cp_2MoCl_2 (50 mM) at pD 3. ΔS of activation was −49 ± 10 eu, and $\Delta H^‡$ and E_a were 9.8 and 10 kcal/mol, respectively.

Scheme 3



activation of −49 ± 10 eu.These data are consistent with a mechanism that involves an intermolecular attack of a hydroxide nucleophile onto the phosphorus center of paraoxon followed by release of *p*-nitrophenol.

The bimolecular nature of this mechanism is further supported with its rate acceleration in comparison with other known metal-promoted phosphate hydrolytic processes. The rate acceleration for Cp_2MoCl_2-promoted hydrolysis (pD 7) is only 6-fold larger than that measured for the bimolecular hydrolysis of a metal-coordinated trimethyl phosphate.[10] In the latter case, a free hydroxide nucleophile attacks the phosphorus center of a pentaammine(trimethyl phosphate)iridium(III) complex to yield a rate acceleration of only 400. Chin has proposed that a 10^2 rate acceleration would be observed for metal-promoted intermolecular hydrolytic processes, in which the metal serves as a Lewis acid to activate the phosphate center.[17] On the other hand, an intramolecular process where the nucleophile is bound to the metal would yield a substantial rate acceleration of $>10^7$.[17] Our $\sim10^3$ measured rate acceleration and the large negative entropy of activation is consistent with a bimolecular process that uses the Cp_2Mo moiety as a Lewis acid to activate the phosphate triester center for nucleophilic attack by OH^- (Scheme 3). However, an intramolecular pathway with a constricted transition state that yields a large negative $\Delta S^‡$ cannot be ruled out.

The unexpected result obtained in this study was found in the reaction of parathion with Cp_2MoCl_2(aq). While the difference between paraoxon and parathion is subtle, it leads to solubility differences[18] that made thorough quantitative analyses of the parathion reaction with ^1H NMR spectroscopy onerous. The poor aqueous solubility of parathion could be improved with the addition of methanol (5−20%), but the Cp_2MoCl_2 complex is poorly soluble in such a mixture. Furthermore, reactions of Cp_2MoCl_2(aq) with alcohols have been documented,[19] and therefore methanolic addition to improve parathion solubility in water would lead to problematic side reactions with the metallocene.

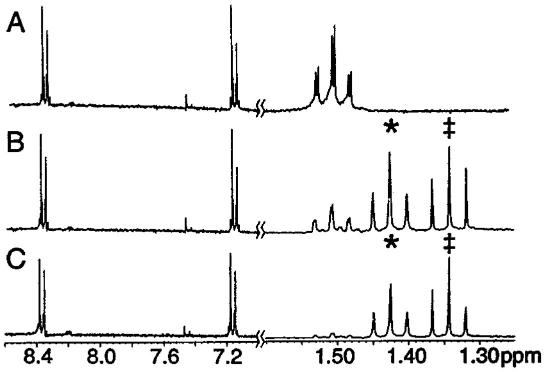


Figure 3. ^1H NMR spectra of 50 mM parathion and 50 mM Cp_2MoCl_2 at pD 3, 40 °C, at three time points. The downfield region represents the unchanged *p*-nitrophenol signals, while the upfield region represents methyl protons. The triplets at 1.51, 1.43 (*), and 1.34 (‡) ppm represent parathion, ethanol (authentic addition), and deethyl parathion, respectively. Spectra A, B, and C were take after 15 min, 60 h, and 250 h, respectively.

In a typical reaction, equimolar concentrations (50 mM) of parathion and Cp_2MoCl_2(aq) were reacted together at 40 °C (pD 3) under anaerobic conditions. Despite the appearance of small droplets, a plot of the amount of parathion versus time gave a straight line for the first few time points to yield initial rates. We found that the biphasic reaction of parathion (50 mM) and Cp_2MoCl_2 (50 mM) in D_2O (40 °C) alone yielded ethanol as the primary product (Figure 3).

Confirmation that the triplet at 1.43 ppm was indeed ethanol was done with authentic addition of the alcohol to the reaction mixture. The downfield region showed no change throughout the course of the parathion hydrolysis, which substantiates ethanol was the principle product formed in this hydrolytic process.

To further discern whether the ethanol results from C−O versus P−O bond cleavage of parathion, we carried out an isotopic labeling experiment with ^{18}O-labeled water. The parathion + Cp_2MoCl_2 reaction took place in a 1:1 mixture of $^{18}OH_2/^{16}OD_2$ solution, and the α-carbon of the produced ethanol was observed with ^{13}C NMR spectroscopy after the reaction had reached 95% completion. If ethanol production was the result of P−O bond cleavage, then the nucleophile attacked the phosphorus atom. The result would be an oxygen atom attached to the α-carbon (of ethanol) that comes exclusively from a parathion molecule that is ~100% ^{16}O. However, C−O bond cleavage comes from nucleophilic attack on the α-carbon of the ethoxide that results in an α-carbon bearing a 1:1 mixture of ^{16}O and ^{18}O. The ^{13}C NMR spectrum of the α-carbon of ethanol would then have an isotopic chemical shift resulting in two carbon signals (60 ppm) separated by 0.05 ppm.[20]

Control studies with ethanol in ^{18}O-enriched water have previously shown that under the conditions of this study (pH 4 and 60 °C) no oxygen exchange with the water takes place at the α-carbon of ethanol.[21] Moreover, a control reaction of just Cp_2MoCl_2(aq) with ethanol also shows no incorporation of ^{18}O-water onto the α-carbon. It can be seen from Figure 4 that the

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(17) Kim, J. H.; Chin, J. *J. Am. Chem. Soc.* **1992**, *114*, 9792−9799.

(18) Windholz, M., Ed. *The Merck Index*, 10th ed.; Merck & Co. Inc.: Rahway, NJ, 1983.

(19) Balzarek, C.; Tyler, D. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 2406−2408.

(20) Gould, S. J.; Kirchmeier, M. J.; LaFever, R. E. *J. Am. Chem. Soc.* **1996**, *118*, 7663−7666.

(21) Dunbar, J. *Z. Phys. Chem.* **1982**, *130*, 247−250.

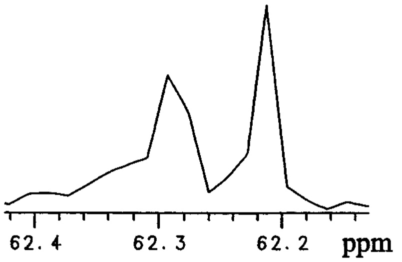
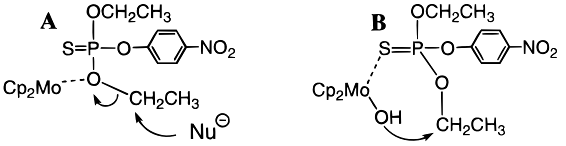


Figure 4. ^{13}C spectrum of the α-carbon region (60 ppm) of the ethanol product for the reaction of aqueous Cp_2MoCl_2 with parathion (pD 3, 60 °C) in a 1:1 mixture of $^{16}OD_2$ and $^{18}OH_2$.

Scheme 4



signal for the α-carbon of the ethanol leaving group is indeed split by 0.08 ppm. This suggests that the oxygen of the ethanol originated from the 1:1 $^{16}OD_2/^{18}OH_2$ solvent mixture, which indicates a C−O bond cleavage process.

The results of the parathion reaction with $Cp_2MoCl_2(aq)$ unequivocally show that ethanol is produced. This is unusual in that most past studies on parathion hydrolysis by metals have resulted in *p*-nitrophenol as the leaving group, due to its higher acidity (pK_a 7.2) relative to ethanol (pK_a 15). Moreover, our isotopic labeling studies clearly show that this ethanol production results from nucleophilic attack on the α-carbon of the parathion ethoxide. Nucleophilic attack at the α-carbon of an ethoxide has been proposed for the hydrolysis of disulfoton.[22] In this case however, it was the leaving group of the first hydrolytic reaction that subsequently attacks the α-carbon. The *β*-ethyl (mercaptothio)ether nucleophile, which was generated initially in the first hydrolytic reaction with OH^- (pH 8.5), attacks the α-carbon of diethyl thiophosphoric acid to generate the isolated 1,2-bis(ethylthio)ethane.

What is especially perplexing is the dramatic shift in product formation between paraoxon and parathion when the only difference is a nonbridging oxygen for the former versus sulfur for latter. Paraoxon hydrolysis by $Cp_2MoCl_2(aq)$ produces the traditional *p*-nitrophenol leaving group through nucleophilic attack at the Cp_2Mo-coordinated phosphate. However, parathion hydrolysis under identical conditions produces ethanol through nucleophilic attack at the α-carbon. It is conceivable the oxophilic Mo(IV) center binds the ethoxide oxygen of parathion when the α-ethyl carbon is attacked by a nucleophile. This putative coordination mode uses the Cp_2Mo moiety to accelerate the hydrolytic process by stabilizing the oxoanion leaving group (Scheme 4A), which has been seen in a number of metal-mediated biological processes.[23]

Alternatively, one can also speculate Cp_2Mo coordination to the polarizable sulfur atom. This is followed by intramolecular attack of the Cp_2Mo-hydroxide on the α-ethyl carbon to form a six-membered-ring transition state (Scheme 4B). Both the intermolecular and intramolecular pathway would yield an ^{18}O atom on the departing ethanol from the solvent.

Unfortunately, the poor solubility of parathion in water alone made temperature dependence studies problematic, and thus it is uncertain whether this nucleophilic attack on the α-carbon is an intra- or intermolecular process. Parathion's poor water solubility also complicated our attempts to measure a rate acceleration for Cp_2MoCl_2 hydrolysis due to the biphasic nature of the control reaction in D_2O (pD 3) that yielded little or no product release.

Past studies with metal oxide surfaces (TiO_2)[9] have shown indirect evidence for alcohol release in methyl parathion. This was deduced by looking at the amount of parathion lost and comparing it with the relative amount of *p*-nitrophenol in the product mixture. Differences between the two measurements were attributed to phosphate mono- and diesters that could be formed from release of methanol. Our studies show unequivocal evidence for alcohol liberation as well as data that suggest the Cp_2Mo moiety effects nucleophilic attack at the α-carbon of the parathion pesticide.

Conclusion

We have found the first case of an organometallic complex that promotes the hydrolysis of the well-known organophosphate pesticides paraoxon and parathion. The organometallic compound Cp_2MoCl_2 is a water-soluble metallocene that has previously been shown to promote the hydrolysis of phosphate mono- and diesters. The rate accelerations for triester hydrolysis are in the 10^2-10^3 range at pD 7 for both the paraoxon and parathion pesticides. Analyses of the kinetic data for paraoxon hydrolysis by Cp_2MoCl_2 is consistent with a model that involves Cp_2Mo-phosphate coordination followed by attack of a hydroxide nucleophile onto the phosphate triester that yields a more constricted transition state. The thionate ester parathion is also hydrolyzed by Cp_2MoCl_2, but the nucleophile attacks the α-carbon of the ethoxide to liberate ethanol and deethyl parathion. This finding clearly opens a new pathway for metal-mediated hydrolysis of thionate pesticides that deserves more critical investigation.

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Supporting Information Available: Two supporting figures are available free of charge via the Internet at http://pubs.acs.org.

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(22) Dannernberg, A.; Pehkonen, S. O. *J. Agric. Food Chem.* **1998**, *46*, 325−334.

(23) Piccirilli, J. A.; Vyle, J. S.; Caruthers, M. H.; Cech, T. R. *Nature* **1993**, *361*, 85−88.