Cycloplatinated aryl ketoximes as efficient biomimicking catalysts for hydrolysis of esters of phosphorothioic acid

G. M. Kazankov, V. S. Sergeeva, A. A. Borisenko, A. I. Zatsman, and A. D. Ryabov*

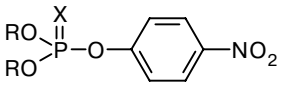
Department of Chemistry, M. V. Lomonosov Moscow State University, Leninskie gory, 119899 Moscow, Russian Federation
Fax: (095) 939 5417. E-mail: g_kazankov@hotmail.com

Cyclometallated aryl ketoximes are introduced as catalysts for hydrolysis of organophosphorus neurotoxins. Platinum-containing catalysts exhibit the highest activity and selectivity with respect to *O*-alkyl phosphorothioates (parathion, methyl parathion, coumaphos) and efficiently promote the hydrolysis of *S*-alkyl phosphorothioates and -dithioates (demeton-S, malathion) at the P—S bonds.

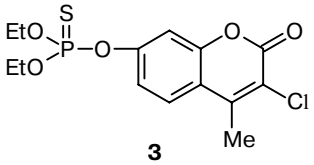
Key words: metal rings, neurotoxins; hydrolysis, catalysis, biomimicking, phosphorothioates, platinum and palladium complexes.

Study of metal complexes as models of the active sites of enzymes, in particular, of various phosphomono- and diesterases is one of the most vigorously developed lines of research in bioinorganic chemistry and biomimetics.[1—7]

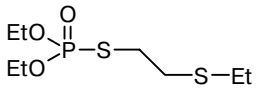
Hundreds of thousand tons of organophosphorus pesticides and toxic chemical agents corresponding to the class of *O*-alkyl phosphorothiates (parathion (**1**), methyl parathion (**2**), and coumaphos (**3**)) and *S*-alkyl phosphorothioates and -dithioates (demeton-S (**4**) and malathion (**5**)) have been produced during the last several decades. This fact has entailed grave environmental problems and calls for the development of methods for annihilation of these substances.[8—10]



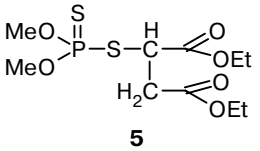
1: R = Et, X = S
2: R = Me, X = S
6: R = Et, X = O



3

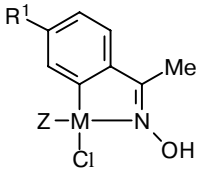


4

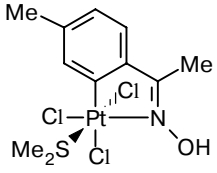


5

Enzymatic hydrolysis based on the use of organophosphate hydrolase (OPH), which is a metal-dependent phosphotriesterase, is among the most promising methods for this treatment.[11—14] On the basis of the mechanism of substrate transformation in the OPH active site,[15] in this work, we propose highly efficient metal complex catalysts **7—9** for the hydrolysis of sulfur-containing organophosphorus compounds prepared from cyclometallated complexes of aryl ketoximes. These catalysts provide nearly enzymatic rates of hydrolysis, possess high selectivity with respect to sulfur-containing esters, and they are much more stable than the enzyme. These complexes were chosen due to several reasons. Previously,[16—18] it has been shown that cyclometallated aryl ketoximes mimic metal-dependent esterases and accelerate hydrolysis of esters including amino acid esters with high regio- and stereoselectivity. Since Pt^{II} is a "soft" Pearson´s acid, it exhibits a high affinity toward a "soft" base, *i.e.*, the electron-donating sulfur atom.



7a—f, 8



9

7: M = Pt (**a—f**), Z = dmso (**a—e**), py (**f**),
R^1 = H (**a, f**), MeO (**b**), Me (**c**), F (**d**), Cl (**e**)
8: M = Pd, Z = py, R^1 = H

Study of the catalytic activity of these complexes with respect to substrates **1—6** is the object of the present study.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 10, pp. 1761—1765, October, 2001.

Experimental

^{1}H and ^{31}P NMR spectra were recorded on Varian VXR 400 and Bruker instruments operating at 400 and 300 MHz, respectively. Deuterated acetonitrile, chloroform, methanol, and water were used as solvents. Spectrophotometric studies were carried out on a Hitachi 150-20 spectrophotometer (Japan) equipped with a cell maintained at a constant temperature.

Paraoxon **6** (Sigma) was purified prior to use in the following way: 1 mL of the sample was dissolved in 250 mL of dichloromethane, the products of hydrolysis were extracted with water, the organic phase was separated, and $MgSO_4$ was added and filtered off. Dichloromethane was evaporated using a rotary evaporator. The purified paraoxon was dissolved in doubly distilled water (c $1 \cdot 10^{-2}$ mol L^{-1}).

Parathion **1** (Sigma), methyl parathion **2** (Fluka), coumaphos **3** (Fluka), demeton-S (**4**) (ChemService, USA), and malathion **5** (Fluka) and organic solvents, HPLC grade acetonitrile and dichloromethane (Reakhim), were used as received.

The cyclometallated complexes were synthesized by known procedures.[16,17] The kinetics of hydrolysis of esters **1—4** in the presence of compounds **7—9** was studied by spectrophotometry at 25 °C and an ionic strength of 0.01 M in sodium acetate and sodium 5,5′-diethyl barbiturate buffer solutions with a total concentration of $5 \cdot 10^{-3}$ mol L^{-1}.

General procedure of investigations. A solution of a complex in the appropriate buffer solution was prepared from an acetonitrile solution of the complex (c $1 \cdot 10^{-2}$ mol L^{-1}) in such a way that the concentration of the complex in the buffer solution was $5 \cdot 10^{-7}$—$5 \cdot 10^{-5}$ mol L^{-1}. An aliquot portion of an acetonitrile solution of an organophosphorus compound (c $1 \cdot 10^{-2}$ mol L^{-1}) was added to the buffered solution of the complex in such a way that the concentration of the organophosphorus compound in the reaction mixture was $1 \cdot 10^{-4}$ mol L^{-1}. The mixture was kept in the spectrophotometer cell at a constant temperature. The hydrolysis kinetics of substrates **1—4** was studied by monitoring the optical density of the solution at wavelengths corresponding to the hydrolysis products, namely, 4-nitrophenol (pK_a = 7.20, λ = 405 nm, ε = 17000 L mol^{-1} cm^{-1} for the deprotonated form, λ = 320 nm for the protonated form) or 7-chloro-8-hydroxybenzopyrone (λ = 384 nm, ε = 9000 L mol^{-1} cm^{-1}). The observed rate constants were determined either from analysis of full kinetic curves in the first-order reaction coordinates, $\ln(A_\infty/(A_\infty - A(t)) = k_{obs}t$, or using the Guggenheim[20] method. The rate constants determined by different methods were in satisfactory agreement with one another. The catalytic rate constants at a given pH were calculated as the slope ratio of the plot of k_{obs} *vs.* complex concentration using the least-squares method.

The kinetic isotope effect of the solvent (k_{H_2O}/k_{D_2O}) in the hydrolysis of **1** catalyzed by complex **7c** was found as the ratio of second-order rate constants in a borate buffer solution at 25 °C. The pH value for the buffer solution was calculated as pD = pH + 0.4, where pH is the reading of a pH-meter.

The activation parameters of the hydrolysis of **1** in the presence of complex **7a** were determined from the data obtained at 20—50 °C; the pH of the buffer solution was brought to 9.5 at each temperature. The resulting catalytic rate constants were analyzed in the $\ln k_2 - T^{-1}$ coordinates. The activation energy was found from the slope of this plot, while the activation enthalpy was calculated from the relation $\Delta H^{\neq} = E_a - RT$. The activation entropy was determined from the equation $k = k_B T/h \cdot \exp(-(\Delta H^{\neq} - T\Delta S^{\neq}/RT))$ at 25 °C, where k_B is the Boltzmann constant and h is the Planck constant.

The kinetics of hydrolysis of esters **4** and **5** in the presence of complexes **7—9** was studied by spectrophotometry. For this purpose, a solution of the Pt or Pd complex ($1 \cdot 10^{-5}$ to $5 \cdot 10^{-5}$ mol L^{-1}) in the corresponding buffer was prepared from an acetonitrile solution of the complex ($1 \cdot 10^{-2}$ mol L^{-1}). An aliquot portion of an organophosphorus compound in MeCN ($1 \cdot 10^{-2}$ mol L^{-1}) was added to the buffered solution of the complex in such a way that the organophosphate concentration in the buffer solution was $1 \cdot 10^{-6}$—$5 \cdot 10^{-5}$ mol L^{-1}. The mixture was kept in the thermostated spectrophotometer cell holder, the variation of the absorption spectrum of the reaction mixture with time was recorded, and the wavelength corresponding to the maximum change of absorption was determined. Then the kinetic data were obtained at this wavelength. The observed rate constants were determined from analysis of full kinetic curves, as described above.

Results and Discussion

Parathion (**1**) is hydrolyzed very slowly in aqueous solutions at neutral pH values (Table 1). The addition of catalytic amounts (10^{-6}—10^{-5} mol L^{-1}) of complexes **7—9** markedly accelerates the hydrolysis.

Analysis of the resulting full kinetic curves in the $\ln(A_\infty/(A_\infty - A(t)) - t$ coordinates, where A is the optical density, shows that the reaction obeys exactly the first order and proceeds to completion even with a 100-fold excess of the substrate, *i.e.*, the process is catalytic. This is also confirmed by the fact that the second-order rate constant, which determines the efficiency of catalysis, remains invariable in the range of parathion (**1**) concentrations of $5 \cdot 10^{-6}$—$1 \cdot 10^{-4}$ mol L^{-1}, *i.e.*, it does not depend on the substrate concentration. Hydrolysis of esters **2** and **3** in the presence of complexes **7—9** obeys the same kinetics. The observed first-order rate constants are described satisfactorily by the equation

$$k_{obs} = k_2/c_{cat}, \quad (1)$$

where c_{cat} is the catalyst concentration.

The kinetics of hydrolysis of substrates **1—4** was studied in the presence of complex **7a**, while that for substrate **1** was measured in the presence of each of

Table 1. Catalytic rate constants (k_2/mol L^{-1} s^{-1}) for the hydrolysis of esters **1—4** and **6** in the presence of complexes **7—9** at pH 8.5, 25 °C, 0.01 M $NaClO_4$

Complex	1	2	3	4	6
7a	310±17	175±7	141±5	22.4±2.2	10.2±0.1
7b	914±21	—	—	—	—
7c	773±29	—	—	24.4±2.8	—
7d	429±8	—	—	—	—
7e	452±17	—	—	27.2±2.6	—
7f	230±8	—	—		—
8	54±4	—	—	10.1±1.8	—
9	10.9±0.4	—	—	—	—
OH*	$(9.5\pm0.6) \cdot 10^{-5}$	$(2.6\pm0.4) \cdot 10^{-4}$	—	$6.67 \cdot 10^{-4}$	$7.5 \cdot 10^{-2}$

* The rate of alkaline hydrolysis.

complexes **7a—f**, **8**, and **9**. Equation (1) was found to hold in all cases. The second-order rate constants for hydrolysis of compounds **1—4** are presented in Table 1; the rate constants for alkaline hydrolysis are presented in the same Table for comparison. Comparison of the rate constants for alkaline and **7a**-catalyzed hydrolyses of parathion (**1**) demonstrates that the rate constant for the catalytic process exceeds the rate constant for the alkaline hydrolysis by a factor of 10^6—10^7.

At pH 8.0, the rate of the catalytic reaction in the presence of $1 \cdot 10^{-4}$ mol L^{-1} of complex **7b** is 10^9 times as high as the rate of hydrolysis of **1** without a catalyst. The catalytic effect attained exceeds substantially the values found previously for acceleration of hydrolysis of a series of 4-nitrophenyl phosphates in the presence of various transition metal complexes,[1—7,19] which are at most 10^4—10^6. Analysis of the rate constants determined, which are listed in Table 1, shows that substrate coordination to the softer Pt^{II} ion is more efficient for the catalysis of hydrolysis than the coordination to Pd^{II} and, similarly, the coordination of metal ion to the soft S atom of the P=S group is more favorable than the coordination to the "hard" O atom of the P=O group. The square planar Pt^{II} complexes (**7**) proved to be 30 times more active in the reaction in question than the octahedral Pt^{IV} complex (**9**). It also follows from Table 1 that cycloplatinated aryl ketoximes exhibit the highest catalytic activity when the aromatic ring of complex **7** contains electron-donating substituents (MeO in **7b** or Me in **7c**).

To elucidate the mechanism of hydrolysis of phosphorothioates in the presence of complexes **7—9**, the dependence of the catalytic constant for hydrolysis of ester **1** on the medium pH was studied. Figure 1 shows the dependence measured for complex **7a**. It can be seen from the Figure that the curve contains two horizontal sections at 8 < pH and pH < 7. The resulting pH profile containing two pH-independent sections indicates that two catalytically active forms of the complex related by an acid-base equilibrium are present in the solution.

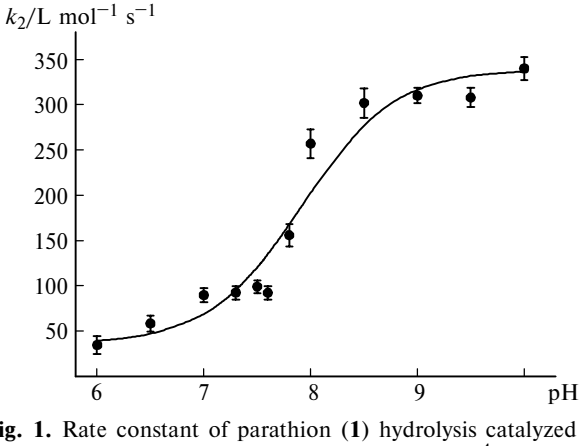


Fig. 1. Rate constant of parathion (**1**) hydrolysis catalyzed by complex **7a** (parathion concentration $1 \cdot 10^{-4}$ mol L^{-1}, μ = 0.01 mol L^{-1} $NaClO_4$, 25 °C) *vs.* pH.

$$k_2 = (k_{AH}[H^+] + k_A K_a)/([H^+] + K_a). \quad (2)$$

Fitting the experimental data to Eq. (2) made it possible to determine the k_{AH} and k_A constants corresponding to aqua and hydroxo forms of complexes: 40±20 and 340±20 M^{-1} s^{-1}, respectively.

The acid dissociation constant K_a for complex **7a** is $(1.2 \pm 0.5) \cdot 10^{-8}$ mol^{-1} L (pK_a 7.9). Previously,[16] it was shown that K_a refers to deprotonation of the hydroxy group of the oxime ligand.

The temperature dependence of the rate constant for parathion (**1**) hydrolysis in the presence of complex **7a** was used to calculate the activation enthalpy and entropy, 25.0±0.5 kJ mol^{-1} and −113±7 J (mol K)$^{-1}$, respectively. Since the first step of the process is very fast and cannot be detected by any experimental methods, it can be considered that the measured activation parameters refer to the rate-determining step of the reaction, namely, to the decomposition of the substrate—catalyst primary complex. The large negative entropy reflects, apparently, the necessity of spatial orientation of the reactant molecules for the intramolecular nucleophilic attack on the coordinated substrate by the oximate ion, *i.e.*, it attests to a highly ordered transition state in the bimolecular reaction in question.

The kinetic isotope effect k_{H_2O}/k_{D_2O} for the catalysis of hydrolysis of ester **1** by complex **7b** is 1.2±0.1. Therefore, it appears that no proton transfer takes place in the rate-determining step of the process, *i.e.*, the free OH^- ion does not participate in the reaction, and the nucleophilic attack occurs intramolecularly under the action of the coordinated oximate ion, *i.e.*, nucleophilic rather than specific basic catalysis is involved.

The data presented above suggest the following mechanism for the catalysis of hydrolysis of phosphorothioates by cycloplatinated and cyclopalladated aryl ketoximes. The substrate can be coordinated to the metal ion through the S atom upon substitution of one ligand in the coordination sphere. The formation of this complex decreases the electrophilicity of the attacked phosphorus center of the substrate. This is followed by intramolecular attack on the phosphorus atom by the coordinated oximate ion.

Compounds **4** and **5** are barely hydrolyzed in water and are exceptionally stable even against enzymatic hydrolysis. The k_{cat}/K_m ratio (k_{cat}, K_m are parameters of the Michaelis—Menthen equation for a successive two-step reaction mechanism) for hydrolysis of paraoxon, demeton-S, and malathion in the presence of OPH are $1.27 \cdot 10^8$, $1.6 \cdot 10^3$, and 0.8 mol^{-1} L s^{-1}, respectively, whereas the constants of alkaline hydrolysis differ by only 2 or 3 orders of magnitude.[12,14] In addition, demeton-S exerts a substantial inhibitory effect on OPH.

Hydrolysis of esters **4** and **5** in the presence of cyclometallated aryl ketoximes was studied by ^{31}P NMR spectroscopy and by spectrophotometry. Analysis of the ^{31}P NMR spectra recorded before and after incubation of ester **5** in an aqueous solution with pH 7.0 for 48 h at 25 °C in the absence of a complex shows that the initial compound is responsible for a singlet at δ 32, and no changes take place in the spectrum during this period. The spectrum of demeton-S recorded 15 min after the addition of complex **7d** to the reaction mixture exhibits a signal with a chemical shift of 0.6 ppm, typical of diethyl hydrogen phosphate, formed upon hydrolysis of ester **5**. This indicates unambiguously that the P—S bond is cleaved in the presence of the Pt^{II} complex. Integration of the spectrum shows that the amount of reacted ester **5** is equivalent to the amount of complex **7d** introduced in the system, *i.e.*, the complex acts as a promoter rather than as a catalyst. In all probability, this is due to the formation of a stable complex of Pt^{II} with the chelating thiol $HS(CH_2)_2SEt$ liberated during the reaction.

The kinetics of hydrolysis of demeton-S (**4**) in the presence of metallacyclic compounds **7** and **8** was studied by UV spectroscopy. Figure 2 shows a time variation of the spectrum of the reaction mixture containing ester **4** and complex **7b**. Analysis of the variation of the absorption of the reaction mixture at 325 nm for complexes **8** and **9** and demeton-S : complex ratios of 1 : 1 to 1 : 10 shows that the reaction has the exactly first order with respect to both reactants.

Study of the dependence of the second-order rate constant ($k_2 = k_{obs}/c_{complex}$) on the pH showed that the values of these constants for complexes **7** and **8** in a pH range of 6.8—12.0 do not depend on pH within the experimental error. In addition, the rate constants for complexes **7a—f**, containing substituents in the benzene ring, are close and, as can be seen from Table 1, they lie in the range of 20—27 mol L^{-1} s^{-1}. As shown above, the cleavage of the ester bond in the hydrolysis of esters **1—4** depends on the pH and also on the presence of substituents in the aromatic ring of aryl ketoxime. This suggests that the variations observed in the UV spectrum of the reaction mixture are due to the complex formation between the ester and cyclometallated aryl ketoxime, and, apparently, it is complexation that is the rate-determining step of hydrolysis of ester **5** promoted by complexes **7** and **8**, while the subsequent rupture of the P—S bond is fast.

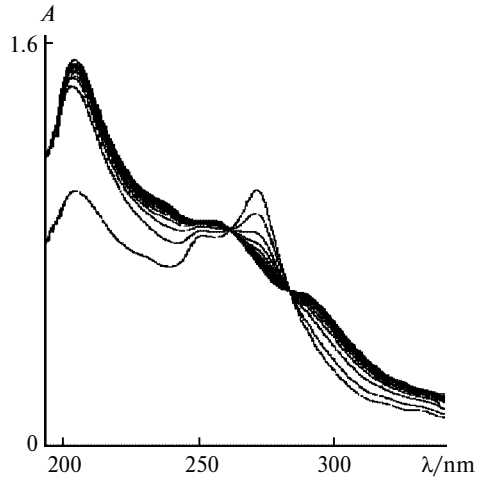


Fig. 2. UV spectrum of demeton-S (**4**) in the presence of complex **7b**; **[4]** = **[7b]** = $2 \cdot 10^{-5}$ mol L^{-1}, $5 \cdot 10^{-3}$ *M* $Na_2B_4O_7$—NaOH, pH 9.25, 25 °C, the range of recording the spectra is 1 min.

The results obtained suggest the following mechanism of reaction between cyclometallated aryl ketoximes and **5**: the ester is coordinated to the metal ion of complexes **7—8** through the sulfur atom attached to phosphorus. This is followed by the nucleophilic attack by the coordinated oximate ion, resulting in the cleavage of the P—S bond and abstraction of the diethyl hydrogen phosphate. The next step is, apparently, complexation between the catalyst and the chelating ligand. The formation of the chelate complex with demeton-S might also occur at the first, rate-determining step of the reaction, which is followed by cleavage of the P—S bond.

Malathion (**5**) is exceptionally stable against both alkaline and enzymatic hydrolysis. Investigation of an aqueous solution of malathion (pH 9.25, a 0.05 *M* $Na_2B_4O_7$—NaOH buffer) by ^{31}P NMR spectroscopy

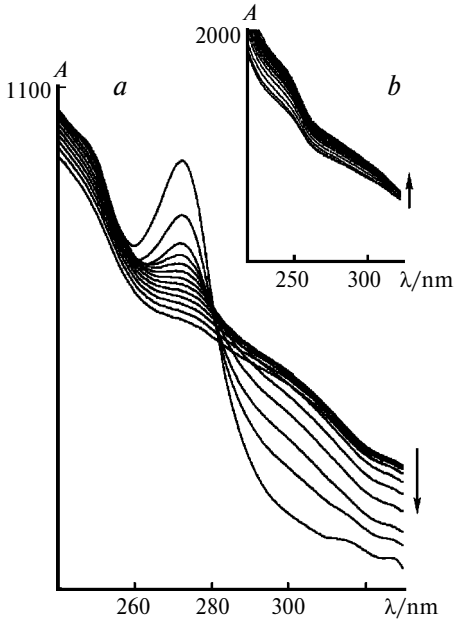


Fig. 3. UV spectrum of the reaction mixture containing malathion and complex **7d**: (*a*) the first and second steps of the reaction, the range of recording the spectra is 1 min, (*b*) the third step of reaction, the range of recording the spectra is 15 min. The arrows mark the directions of variation of the spectra in the second and third steps of reaction; **[5]** = $2 \cdot 10^{-5}$ mol L^{-1}, **[7d]** = $2 \cdot 10^{-5}$ mol L^{-1}, buffer, $5 \cdot 10^{-3}$ mol L^{-1} $Na_2B_4O_7$—NaOH, pH 9.25, 20 °C.

demonstrated that dimethyl hydrogen phosphorothioate is not formed at 25 °C even after 120 days. The NMR spectrum is a singlet with δ 96.3, corresponding to the initial compound **5**. After the addition of complex **7b** to a solution of malathion, organophosphate is hydrolyzed in 22 h in a concentration equivalent to the concentration of the complex added. At 50 °C, the reaction proceeds completely over a period of less than 3 h. The appearance of a signal with a chemical shift of 61.8 ppm attests to the formation of dimethyl hydrogen phosphorothioate and, hence, to the cleavage of the P—S ester bond. Thus, cyclometallated aryl ketoximes are able to increase the rate of malathion hydrolysis by several orders of magnitude; their activity in this reaction is dozens times as high as that of OPH. The time variation of the absorption spectrum of **5** in the presence of complex **7d** (Fig. 3) was also studied. Analysis of these data indicates that hydrolysis of malathion in the presence of Pt^{II} complexes occurs in, at least, three steps, whose nature is currently under study.

Thus, cycloplatinated and cyclopalladated aryl ketoximes, which are easy to synthesize, can serve as highly efficient catalysts for hydrolysis of sulfur-containing pesticides stable to degradation, such as parathion and coumaphos. The catalytic effect of these complexes is comparable with the enzymatic activity of OPH. In addition, these complexes are effective promoters of hydrolysis of thiol esters **4** and **5**; their activity in these reactions markedly exceeds the activity of organophasphatehydrolase.

The authors wish to thank Prof. S. D. Varfolomeyev for participation in the discussion of the results.

This work was financially supported by the INTAS (grant 97-0166).

References

1. R. G. Clewley, H. Slebocka-Tilk, and R. S. Brown, *Inorg. Chem. Acta*, 1989, **157**, 233.
2. M. A. De Rosch and W. C. Trogler, *Inorg. Chem.*, 1990, **29**, 2409.
3. J. Chin and X. Zou, *J. Am. Chem. Soc.*, 1988, **110**, 223.
4. P. Hendry and A. M. Sargeson, *J. Am. Chem. Soc.*, 1989, **111**, 2521.
5. W. H. Chapman and R. J. Breslow, *J. Am. Chem. Soc.*, 1995, **117**, 5462.
6. N. H. Williams, A.-M. Lebuis, and J. Chin, *J. Am. Chem. Soc.*, 1999, **121**, 3341.
7. N. H. Williams, W. Cheung, and J. Chin, *J. Am. Chem. Soc.*, 1998, **120**, 8079.
8. M. A. Gallo and N. J. Lawryk, *Organic Phosphorous Pesticides. The Handbook of Pesticide Toxicology*, Acad. Press, San Diego, **CA**, 1991.
9. S. M. Somani, R. P. Solana, and S. N. Dube, *Chemical Warfare Agents*, Acad. Press, San Diego, 1992, 67.
10. J. K. Grimsley, V. K. Rastogi, and J. R. Wild, *Biological Detoxification of Organophosphorus Neurotoxins, Vol. 2*, Technomic Publishing Company, Inc, Lancaster PA, 1998.
11. D. P. Dumas, S. R. Caldwell, J. R. Wild, and F. M. Raushel, *J. Biol. Chem.*, 1989, **264**, 19659.
12. K. Lai, N. J. Stolowich, and J. R. Wild, *Arch. Biochem. Biophys.*, 1995, **318**, 59.
13. J. E. Kolakowski, J. J. De Frank, S. P. Harvey, L. L. Szafraniec, W. T. Beaudry, K. Lai, and J. R. Wild, *Biocatal. Biotransform.*, 1997, **15**, 297.
14. B. Di Sioudi, J. K. Grimsley, K. Lai, and J. R. Wild, *Biochemistry*, 1999, **38**, 2866.
15. V. S. Sergeeva, E. N. Efremenko, G. M. Kazankov, and S. D. Varfolomeyev, *J. Mol. Cat. B, Enz.*, 2000, **10**, 571.
16. A. K. Yatsimirsky, G. M. Kazankov, and A. D. Ryabov, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1295.
17. A. D. Ryabov, G. M. Kazankov, S. A. Kurzeev, P. V. Samuleev, and V. A. Polyakov, *Inorg. Chim. Acta*, 1998, **280**, 57.
18. S. A. Kurzeev, G. M. Kazankov, and A. D. Ryabov, *Inorg. Chim. Acta*, 2000, **305**, 1.
19. J. H. Mendez, R. C. Martinez, and J. S. Martin, *Anal. Chem.*, 1986, **58**, 1969.
20. I. V. Berezin and A. A. Klesov, *Prakticheskii Kurs Khimicheskoi i Fermentativnoi Kinetiki* [*A Practical Course of Chemical and Enzymatic Kinetics*], Izd-vo MGU, Moscow, 1976 (in Russian).

Received March 5, 20001
in revised form May 18, 2001