Unusual Course of the *p*-Nitrophenyl Phosphate Esters Cleavage by 3-Hydroxyiminoalkylpyridinium Salts in Micellar Solutions

Hana Kotoučová, Jiří Mazáč, Radek Cibulka, František Hampl,* and František Liška
Department of Organic Chemistry, Prague Institute of Chemical Technology, Technická 5, 166 28 Prague 6, Czech Republic

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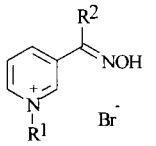
Two types of amphiphilic quaternary 3-pyridinium ketoximes with different position of the hydrophobic alkyl chain were synthesized and tested as hydrolytic micellar catalysts. A considerable positive deviation from the expected first-order curve was observed in the absorbance *vs* time plot when *p*-nitrophenyl diphenyl phosphate and *p*-nitrophenyl diethyl phosphate were hydrolyzed in micellar solutions of the prepared ketoximes under pseudo-first-order reaction conditions.

Pyridiniumcarbaldehyde oximes represent the most important class of the reactivators of phosphorylated or phosphonylated acetylcholinesterase (AChE).[1] Due to the high acidity of their hydroxyimino group, oximate anions are present in aqueous solutions in appreciable amount even at physiological pH. Oximate anions as alpha-nucleophiles exhibit higher reactivity towards phosphorus atom compared to oxygen nucleophiles of similar basicity.[2] This fact inspired many authors[3] to study amphiphilic quaternary aldoximes as potential hydrolytic micellar catalysts for destruction of neurotoxic organophosphorus compounds (chemical warfare as sarin, soman, VX etc. or pesticides as paraoxon, parathion etc.).

As a continuation of our previous work[3d] oriented on the structure-reactivity relationship in a series of isomeric amphiphilic quaternary pyridinium aldoximes we turned our attention to quaternary pyridinium ketoximes **1**. General formula **1** represents two different types of cationic surfactants, salts **1a** and **1b**, with "inversed" position of the hydrophobic alkyl chain. Ketoximes **1a** and **1b** were synthesized starting from nicotinic acid nitrile. Addition of the Grignard reagent R^2MgX and reaction of the arising ketones with hydroxylamine resulted in alkyl-3-pyridinyl-ketoximes which after quaternization with alkyl halides afforded the desired salts **1**. Bromide **1b** was prepared by the conversion[4] of the corresponding iodide, product of the quaternization with methyl iodide. All the prepared compounds gave correct elemental analyses and their structures were confirmed by ^1H NMR spectroscopy.[5]



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	R^1	R^2
1a	$C_{12}H_{25}$	CH_3
1b	CH_3	$C_{12}H_{25}$

Preliminary evaluation of the hydrolytic efficiency of the ketoximes **1a** and **1b** was carried out by measuring the rate constants k_{obs} of the model substrates cleavage under the conditions of the pseudo-first-order reaction. The following esters were employed in this kinetic screening: *p*-nitrophenyl picolinate (PNPP), *p*-nitrophenyl hexanoate (PNPH) and *p*-nitrophenyl diphenyl phosphate (PNPDPP), the last one as a model of neurotoxic organophosphorus compounds. The hydrolyses of both PNPP and PNPH were monitored by spectrophotometric determination of the released *p*-nitrophenoxide ion at λ = 400 nm and followed the first-order kinetics in all cases. The contribution of the uncatalyzed reaction (rate constant k_{OH^-} of the substrate hydrolysis at pH 7.2 in 0.05 M HEPES buffer) was taken into account although it was noticeable only below the critical micelle concentration (CMC). The concentration-rate profiles for the PNPH and PNPP hydrolyses are shown in Figure 1. CMC estimated from the plots depicted in the Figure 1 are approx. 3.5 x 10^{-3} M and 1 x 10^{-3} M for the ketoximes **1a** and **1b**, respectively.

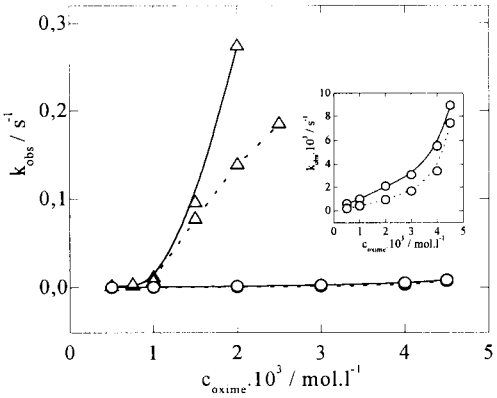


Figure 1. The concentration - rate profile for the PNPP (——) and the PNPH (-----) hydrolysis catalyzed by **1a** (O) and **1b** (Δ). Conditions: [substrate] = 2.0 x 10^{-5} M, t = 25.0 °C, pH 7.2 (HEPES buffer 0.05 M).

On the other hand, considerable positive deviation from the hypothetical first-order curve was observed in the absorbance *vs* time dependence at λ = 400 nm when the PNPDPP was hydrolyzed in micellar solutions of ketoximes **1a** and **1b**, as shown in the Figures 2 and 3. In all cases, the final absorbance corresponded with the theoretical amount of the *p*-nitrophenol liberated from the totally hydrolyzed substrate, as was verified by independent experiments. The same type of the anomalous absorbance *vs* time dependence exhibited also the hydrolysis of *p*-nitrophenyl diethyl phosphate (paraoxon). We observed this effect only in micellar systems. The hydrolysis of PNPDPP catalyzed by non-micellizing homologue N-methyl-3-(1-hydroxyiminoethyl)pyridinium iodide **1c** followed the first-order kinetics in all cases. According to our best knowledge, no similar phenomenon has been reported in the hitherto published studies dealing with the hydrolyses of organophosphates in

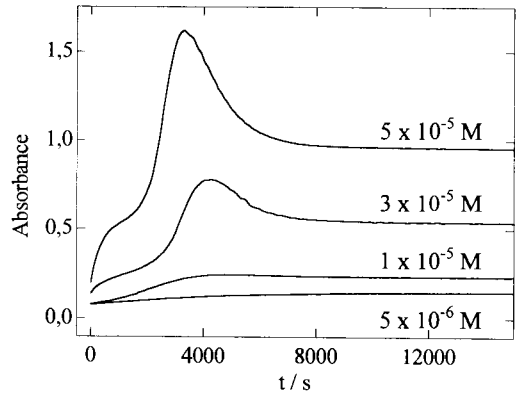


Figure 2. Absorbance vs time dependence for the PNPDPP hydrolysis catalyzed by **1a** at different substrate concentrations. Conditions: [**1a**] = 3.5 x 10^{-5} M, t = 25.0 °C, pH 7.2 (HEPES buffer 0.05 M).

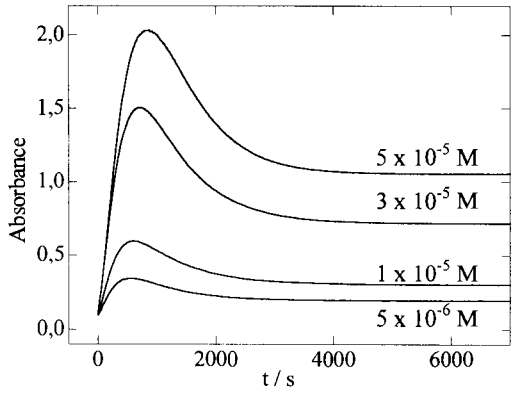


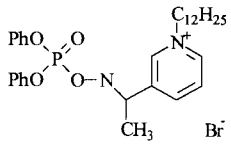
Figure 3. Absorbance vs time dependence for the PNPDPP hydrolysis catalyzed by **1b** at different substrate concentrations. Conditions: [**1b**] = 2.0 x 10^{-5} M, t = 25.0 °C, pH 7.2 (HEPES buffer 0.05 M).

micellar solutions of functional surfactants even among the structurally related compounds as 3-pyridiniumaldoximes[3a,3c,3d] and 2- and 4-pyridiniumketoximes.[7]

The observed effect seems to be most likely a superposition of the PNPDPP hydrolysis (following the first-order kinetics) and the origin and decay of a light-absorbing short-living species formed from PNPDPP in micelles of salts **1a** and **1b**. Only one new band with the same absorption maximum at λ = 400 nm as the *p*-nitrophenoxide anion appeared and disappeared during the hydrolyses of PNPDPP and paraoxon.

The hypothesis that the phosphorylated ketoximes resulting from the nucleophilic displacement of the *p*-nitrophenoxy group in the substrates by oximate anion might be the substance of the unusual absorbance *vs* time dependence was contradicted by the synthesis of one of the supposed phosphorylated intermediates of the hydrolysis. Compound **2** prepared[8] by phosphorylation of ketoxime **1a** did not exhibit any light absorption at λ = 400 nm.

Another hypothesis represents the formation of some kind of C-T complex of the phosphate in micelles of **1a** and **1b**. This assumption is in accord with the dependence of the maximum absorbance on the concentration of the substrate. The investigation of this interesting phenomenon is in progress.



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4 The conversion was performed by anion exchange on Amberlite IRA 400 (OH^- form) followed by neutralization with hydrobromic acid.

5 **1a**: m. p. 118 - 120 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.86 (3H, t, *J* = 6.9 Hz), 1.33 (18H, bs), 2.05 (2H, m), 2.22 (3H, s), 4.92 (2H, t, *J* = 7.2 Hz), 8.16 (1H, m), 8.63 (1H, d, *J* = 7.9 Hz), 9.19 (1H, d, *J* = 5.1 Hz), 9.40 (1H, s), 11.12 (1H, s). **1b**: m. p. 106 - 108 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.87 (3H, t, *J* = 6.5), 1.24 (18H, bs), 1.46 (2H, m), 2.81 (2H, t, *J* = 6.5), 4.64 (3H, s), 8.11 (1H, dd, *J* = 8.0, *J* = 6.2), 8.54 (1H, d, *J* = 8.2), 9.06 (1H, d, *J* = 5.9), 9.24 (1H, s), 9.93 (1H, s). **1c**: m.p. 220 - 223 °C (ref.[6] 213 - 214 °C), ^{1}H NMR (400 MHz, DMSO d-6): δ = 2.25 (3H, s), 4.41 (3H, s), 8.13 (1H, dd, *J* = 8.2, *J* = 6.0), 8.76 (1H, d, *J* = 8.3), 8.97 (1H, d, *J* = 5.9), 9.20 (1H, s), 12.10 (1H, s).

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8 Ketoxime **1a** was phosphorylated by diphenyl chlorophosphate in dichloromethane in the presence of triethylamine.