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ANTICHOLINESTERASE ACTIVITY AND RATE OF DECOMPOSITION OF SOME PHOSPHYLATED OXIMES

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Abstract—A method is described for the determination of the rate constants of decomposition and of acetylcholinesterase inhibition of phosphylated oximes. The method is based on measurements of the anticholinesterase activity of incubates of an organophosphate and an oxime in which the phosphylated oxime is formed *in situ*. Incubates of iso-propyl methylphosphonofluoridate (sarin), diethyl phosphorofluoridate and the corresponding *p*-nitrophenyl compounds with P2S (2-[(hydroxyimino)methyl]-1-methyl-pyridinium methanesulphonate) and with obidoxime (Toxogonin®) were investigated. The rather unstable phosphylated oximes turned out to be potent acetylcholinesterase inhibitors. Obidoxime phosphonylated by sarin was found to be the most powerful inhibitor having a rate constant of $1.7 \times 10^9\ M^{-1}\ min^{-1}$ (pH 7.5, 25°).

Some oximes can restore the activity of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) inhibited by organophosphates. The most potent reactivators have found use as antidotes in the treatment of organophosphate poisoning. The chemical basis of the reactivation is a nucleophilic attack on the phosphoryl residue of the inhibited enzyme (EP) by the oxime (OX) giving the free enzyme (E) and the phosphylated* oxime (POX). Already in 1955 Wilson [1] introducing the well-known reactivator 2-[(hydroxyimino)methyl]-1-methyl-pyridinium iodide (P2AM) noticed incomplete reactivation and suggested that the phosphylated oxime formed reinhibits the enzyme.

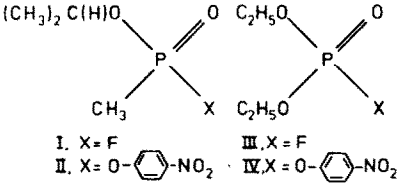
$$EP + OX \rightleftarrows E + POX \qquad (1)$$

Phosphylated oximes are also formed in the direct reaction of organophosphates and oximes. Although most of these compounds rapidly degrade in aqueous solution it has been suggested that they might have a harmful effect on the oxime-therapy of the poisoning by some organophosphates [2, 3].

To get a better insight into the role of phosphylated oximes in connection with reactivation and oxime-therapy, attempts have been made to synthesize these compounds. However, only a few of them could be synthesized [4–6]. The phosphylated oximes derived from potent antidotes, such as P2AM and trimedoxime, are too unstable to be isolated [4, 7, 8].

Indirect information on the stability and the anticholinesterase activity of phosphylated oximes has been obtained from studies of the inhibitory effects of organophosphates incubated with oximes; the phosphylated oximes are formed *in situ* in a direct reaction [4, 7–10]. The results suggest that some phosphylated oximes strongly inhibit acetylcholinesterase. However, no kinetic analysis of the reactions taking place has been given. The inhibitory potency of phosphylated oximes was also estimated from their effects on the course of the reactivation of phosphylated acetylcholinesterase when formed during the reaction [11, 12].

In the present work the inhibitory effects of incubation mixtures of organophosphates and oximes on acetylcholinesterase are determined after various time periods of incubation. Sarin (**I**), diethyl phosphorofluoridate (**III**) and the corresponding *p*-nitrophenyl compounds (**II** and **IV**) are studied together with the



well-known oximes 2-[(hydroxyimino)methyl]-1-methyl-pyridinium methanesulphonate (P2S) and obidoxime (1,1'-[oxybis(methylene)]bis[4-[(hydroxyimino)methyl]-pyridinium] dichloride, Toxogonin®). A detailed kinetic analysis of the reactions will be given. A method will be described to determine both the rate constant of inhibition and the rate constant of decomposition of the phosphylated oximes.

MATERIALS AND METHODS

Two consecutive reactions proceed on incubation of an organophosphate (OP) with an oxime in aqueous solution [4, 7–10, 13]

$$OX + OP \xrightarrow[\text{leaving group}]{k_1} POX \xrightarrow{k_2} \text{decomposition products} \qquad (2)$$

It can be derived for scheme (2) that, if $[OX] \gg [OP]$,

$$[OP]_t = [OP]_0\, e^{-k_1[OX]t} \qquad (3)$$

A method will be described for the determination of k_1 by following the increase of the concentration of the leaving group (fluoride-ion or *p*-nitrophenolate-ion).

* "Phosphylated" covers phosphorylated, phosphonylated and phosphinylated (see R. F. Hudson and L. Keay, *J. chem. Soc.* 1859 (1960)).

After various time periods of incubation a sample of the incubate is added to acetylcholinesterase. Two irreversible inhibitors are present, the organophosphate and the phosphylated oxime. The rate constant of inhibition at time t of incubation ($k_{I,t}$) is given by

$$k_{I,t} = k_{OP}[OP]_t + k_{POX}[POX]_t \quad (4)$$

where k_{OP} and k_{POX} are the bimolecular rate constants of inhibition by the organophosphate and the phosphylated oxime, respectively. Equation (4) only holds if both the concentration of the organophosphate and the phosphylated oxime are in a large excess over the enzyme. Since the phosphylated oxime might be unstable, the inhibition experiment has to be carried out in a short period of time. A method will be described in which these requirements are largely met.

As follows from Eqns (3) and (4), the contribution of the phosphylated oxime to the inhibitory effect of the incubate is given by

$$k_{POX}[POX]_t = k_{I,t} - k_{OP}[OP]_0 e^{-k_1[OX]t} \quad (5)$$

From the experimental data of $k_{I,t}$, k_{OP} ($= k_{I,t}$ at $t = 0$) and k_1, values of $k_{POX}[POX]_t$ can be calculated.

For the two consecutive first-order reactions of (2) it can be derived [14] that

$$[POX]_t = \frac{[OP]_0 k_1[OX](e^{-k_1[OX]t} - e^{-k_2 t})}{k_2 - k_1[OX]} \quad (6)$$

and thus

$$k_{POX}[POX]_t = \frac{k_{POX}[OP]_0 k_1[OX](e^{-k_1[OX]t} - e^{-k_2 t})}{k_2 - k_1[OX]} \quad (7)$$

The two parameters k_{POX} and k_2 can be obtained by fitting Eqn (7) to a set of $k_{POX}[POX]_t$, t data by least-squares criteria. We used the method described by Kim [15], which is based on expansion of the function to be fitted in a Taylor series in which terms higher than first-order are neglected. In this method the best estimates of the parameters and their standard deviations are calculated by iteration starting from a trial set of estimates. The calculations were carried out with a computer (PDP 11/45)*.

The applicability of the method described is limited by the value of the ratio of $k_2/k_1[OX]$. If $k_2 \gg k_1[OX]$, $e^{-k_2 t}$ becomes rapidly negligible in Eqn (7) towards $e^{-k_1[OX]t}$ by increasing t. So, Eqn (7) reduces to

$$k_{POX}[POX]_t = \frac{k_{POX}[OP]_0 k_1[OX] e^{-k_1[OX]t}}{k_2} \quad (8)$$

If $k_2 \ll k_1[OX]$, $e^{-k_2 t}$ only slightly changes in a time period in which $e^{-k_1[OX]t}$ decreases considerably. At small values of t Eqn (7) becomes approximately equal to

$$k_{POX}[POX]_t = \frac{k_{POX}[OP]_0 k_1[OX](e^{-k_1[OX]t} - 1)}{-k_1[OX]} \quad (9)$$

On the basis of these two equations it is not possible to obtain k_2 and k_{POX} from $k_{POX}[POX]_t$, t data. Approximated values of k_{POX}/k_2 and of k_{POX} can only be calculated with Eqns (8) and (9), respectively.

*Computer program in BASIC is available on request.

Materials

Bovine erythrocyte acetylcholinesterase was obtained from Winthrop Laboratories Inc. and had a sp. act. of 25 nkat/mg protein at 25° in 0.5 mM phosphate buffer, pH 7.5, containing 3 mM acetylcholine perchlorate. P2S was purchased from Dr. F. Raschig, GmbH, Germany; obidoxime (Toxogonin®) was purchased from Merck, Darmstadt, Germany.

The organophosphates were prepared in this laboratory according to methods known from literature. The *p*-nitrophenyl compounds, **II** and **IV**, were purified by short path destillation using a pot-still.

All other chemicals were commercial products of an analytical grade. Phenyl acetate was destilled before use.

Determination of the rate constants of formation of phosphylated oximes (k_1)

Fluoridates and oximes. Equimolar concentrations (0.1–0.2 mM) of organophosphate and oxime were incubated in 0.01 M veronal buffer, pH 7.5, at 25°. After various time periods samples were taken and diluted with the same volume of a citric acid buffer, pH 4.5, containing 0.02 M citric acid, 0.2 M sodium nitrate and 5.8 mM *N,N'*-1,2-cyclohexanediylbis[*N*-(carboxymethyl)-glycine] (Complexon IV®). The final pH of the mixtures was 4.8. No further conversion of the fluoridates appeared to take place at this pH. E.m.f. values in these mixtures were measured with a fluoride-ion-selective electrode (Orion) and a saturated potassium chloride–calomel electrode in connection with an Orion pH-meter. Fluoride-ion concentrations were obtained with a calibration curve made with sodium fluoride solutions in which the oxime was present in the same final concentrations. After correction of the fluoride-ion concentration for added 2 μM sodium fluoride and for 2-fold dilution a plot was made according to $1/([OP]_0 - [F^-]) = 1/[OP]_0 + k_1 t$. The value of k_1 was calculated as the slope by means of the method of least-squares. Determinations were performed in duplicate. Spontaneous hydrolysis of the fluoridates is negligible.

p-Nitrophenyl compounds and oximes. The reaction was started by addition of the *p*-nitrophenyl compound (final concentration 0.1–0.2 mM) to an oxime solution in 0.01 M veronal buffer, pH 7.5, 25°. The concentrations of the oximes which were used in an excess over the organophosphates, are given in Table 1 (see Results). The increase in absorbance of this solution at 25° was followed in a Beckman Acta III spectrophotometer at 420 or 430 nm in case of P2S or obidoxime, respectively. Values of the concentration of liberated *p*-nitrophenol were obtained with a calibration curve of the absorbances at 420 or 430 nm of *p*-nitrophenol solutions in 0.01 M veronal buffer, pH 7.5, containing the same final concentration of P2S or obidoxime. A plot of log ($[OP]_0 - [p\text{-nitrophenol}]_t$) vs t was made from which k_1 was calculated by means of the method of least-squares. Determinations were performed in duplicate. Spontaneous hydrolysis of the organophosphates was negligible at these conditions.

Determination of rate constants of inhibition ($k_{I,t}$ and k_{OP})

The inhibition of acetylcholinesterase was titrimetrically determined at 25° in the presence of phenyl acetate as a substrate. To 45 ml phenyl acetate solution (1–5 mM) in 0.1 M KCl were added 0.05 ml of 0.2 M phosphate buffer, pH 7.5 and 0.2 ml of an acetylcholinesterase solution in 4 mM phosphate buffer, pH 7.5, containing 1.9, 1.3, 4 or 0.88 mg enzyme/ml at substrate concentrations of 1, 2, 3 or 5 mM, respectively. A solution of 1 mg acetylcholinesterase/ml contains approximately 7.1 nM of active sites. The final enzyme concentrations were 30–120 pM. The substrate hydrolysis was followed during 30–60 sec at pH 7.5 in a nitrogen atmosphere with a Radiometer pH-stat equipment (pH-meter PHM64, titrator TTT60, recorder SBR2c and autoburette ABU12). As a titrant 0.01 N sodium hydroxide was used or 0.02 N sodium hydroxide in experiments with enzyme solution containing 4 mg enzyme/ml. Next, a sample (3 μl–1 ml) of the inhibitor solution was added and the alkali consumption was registered on the recorder and on punch tape. For this purpose the autoburette was equipped with an electronic counter B246d connected to a puncher. Commands for reading the electronic counter were obtained at a preselected time interval from a programmer as described previously [16]. In the present experiments readings were taken every 15 sec, or every 8 sec in case of samples taken from incubation mixtures of **I** or **II** and P2S. It has been demonstrated for the inhibition reaction in the presence of phenyl acetate as a substrate [16] that

$$\ln v_\tau = \ln v_0 - k'_{I,t}\tau \qquad (10)$$

where v_τ and v_0 are the velocity of substrate hydrolysis at time τ and at zero time of inhibition, respectively, and $k'_{I,t}$ the rate constant of inhibition measured in the presence of substrate which equals $k_{I,t}/(1 + [S]/K_m)$. The method of Steinberg and Mednick [17] was adopted for the calculation of v_τ according to

$$v_\tau = (P_{\tau+\beta} - P_{\tau-\beta})/\Delta\tau \qquad (11)$$

where $P_{\tau+\beta}$ and $P_{\tau-\beta}$ represent the amounts of alkali consumed at times $\tau + \beta$ and $\tau - \beta$, respectively, and $\Delta\tau = (\tau + \beta) - (\tau - \beta) = 2\beta$. The calculations were performed with a computer. The value of β was chosen as 15 sec. From the first 15 v_τ values $k'_{I,t}$ was calculated according to Eqn (10) by means of the method of least-squares. Deviations from first-order kinetics were observed in experiments with incubates of **I** and **II** with P2S after approximately 1.5 min of inhibition. Therefore, in these experiments β was chosen as 8 sec and $k'_{I,t}$ was calculated from the first 10 v_τ values. Sample volume and substrate concentration were so chosen that v_τ values were taken from the first 1–2 half-life times of the inhibition reaction. In this manner it is possible to obtain inhibition constants from experiments lasting only 3–4 min and in case of samples of incubates of **I** and **II** with P2S even 1.5 min. The value of $k'_{I,t}$ at zero time of incubation, k'_{OP} ($=k_{OP}/(1 + [S]/K_m)$), was measured in the same manner by addition of a sample of an organophosphate solution in 0.01 M veronal buffer, pH 7.5, to an enzyme–substrate solution containing the oxime in the same final concentration as used in the experiments with the corresponding incubates.

Determination of rate constants of decomposition and of acetylcholinesterase inhibition of phosphylated oxime (k_2 and k_{POX})

Organophosphate and oxime were incubated in 0.01 M veronal buffer, pH 7.5, at 25°. After various time periods of incubation samples were taken and added to a solution of phenyl acetate and acetylcholinesterase and inhibition was measured (see: *Determination of rate constants of inhibition*). All samples of one incubate were assayed for inhibition at the same concentrations of substrate, enzyme and titrant. From the values obtained for $k'_{I,t}$, k_1 and k'_{OP}, values of $k'_{POX}[POX]_t$ were calculated according to Eqn (5). Eqn (7) was fitted to these data yielding k_2 and k'_{POX}. The rate constants k'_{OP} and k'_{POX} were corrected with the factor $(1 + [S]/K_m)$ due to performing the inhibition experiments in the presence of a substrate: $k'_{OP} = k_{OP}/(1 + [S]/K_m)$ and $k'_{POX} = k_{POX}/(1 + [S]/K_m)$. The K_m value of phenyl acetate measured at the conditions described for the inhibition experiments was found to be 1.52 ± 0.05 mM (substrate concentrations: 1.0–5.0 mM).

RESULTS AND DISCUSSION

k_1 Values

The rate constants for the formation of phosphylated oximes (k_1) from oximes and organophosphates are given in Table 1. The rate of the reaction depends on the oxime-anion concentration. The rate constants

Table 1. Apparent bimolecular rate constants (k_1) for the formation of phosphylated oxime from sarin (**I**), *p*-nitrophenyl iso-propyl methylphosphonate (**II**), diethyl phosphorofluoridate (**III**) or paraoxon (**IV**) and P2S or obidoxime in 0.01 M veronal buffer (pH 7.5, 25°) and their standard deviations

Oxime	Oxime concn (mM)	Organophosphate	k_1 (M^{-1} min^{-1})
P2S	0.2	**I**	65.5 ± 0.7
	20	**II**	0.482 ± 0.002
	0.2	**III**	21.6 ± 1.4
	30	**IV**	0.058 ± 0.002
Obidoxime	0.1	**I**	224 ± 9
	16	**II**	0.87 ± 0.01
	0.15	**III**	64.8 ± 1.6
	16	**IV**	0.146 ± 0.001

Table 2. Bimolecular rate constants of acetylcholinesterase inhibition (k_{POX}) at pH 7.5 and 25° and rate constants of decomposition (k_2) of phosphylated oximes and their standard deviations obtained from inhibition experiments with incubation mixtures of sarin (**I**), *p*-nitrophenyl isopropyl methylphosphonate (**II**), diethyl phosphorofluoridate (**III**) or paraoxon (**IV**) and P2S or obidoxime in 0.01 M veronal buffer (pH 7.5, 25°). The rate constants of inhibition obtained for the parent organophosphates (k_{OP}) from three independent determinations are also given

Oxime	Organo-phosphate	Concn in incubation mixture: oxime (mM)	Concn in incubation mixture: organo-phosphate (mM)	Sample volume in inhibition assay (μl)	Substrate concn in inhibition assay (mM)	k_{OP} ($M^{-1}\,min^{-1}$)	k_{POX} ($M^{-1}\,min^{-1}$)	k_2 (min^{-1})
P2S	**I**	—	0.04	100	3	$(1.30 \pm 0.04) \times 10^7$		
		0.2	0.004	1000	3		$(6.2 \pm 0.4) \times 10^8$*	0.38 ± 0.03*
	II	—	0.4	120	3	$(7.5 \pm 0.2) \times 10^5$		
		20	0.6	20	3		$(6.4 \pm 0.3) \times 10^8$†	0.90 ± 0.06†
	III	—	0.6	100	1	$(3.8 \pm 0.1) \times 10^5$		
		0.2	0.04	1500	1		‡	‡
	IV	—	0.4	100	1	$(6.3 \pm 0.2) \times 10^5$		
		30	6	5	1		‡	‡
Obidoxime	**I**	—	0.008	400	5	$(1.24 \pm 0.05) \times 10^7$		
		0.1	0.004	20–100	5		$(1.7 \times 0.1) \times 10^9$	0.012 ± 0.005
		0.2	0.007	100–400	5		$(1.7 \pm 0.2) \times 10^9$	0.019 ± 0.014
	II	—	0.1	450	5	$(6.9 \pm 0.2) \times 10^5$		
		16	0.032	5	5		$(1.9 \pm 0.1) \times 10^9$	0.026 ± 0.004
	III	—	0.6	100	2	$(3.9 \pm 0.1) \times 10^5$		
		0.15	0.015	50	2		$(2.5 \pm 0.1) \times 10^8$	0.033 ± 0.0015
		0.2	0.015	50	2		$(2.2 \pm 0.1) \times 10^8$	0.028 ± 0.002
	IV	—	2	3	2	$(6.3 \pm 0.1) \times 10^5$		
		16	2	20	2		$(3.8 \pm 0.2) \times 10^8$*	0.21 ± 0.01*

* From mean $k'_{POX}[POX]_t$ values of two experiments.
† From mean $k'_{POX}[POX]_t$ values of three experiments.
‡ $k'_{POX}[POX]_t$ values were too small to calculate k_2 and k_{POX}.

given are not corrected for the partial dissociation of the oximes at pH 7.5. Nevertheless, they can be used for calculations of k_2 and k_{POX} which are determined at pH 7.5 as well.

Two oxime groups can be phosphylated in obidoxime. However, no deviations from first-order kinetics were observed for the reaction with **II** and **IV** probably due to the large excess used of obidoxime. Obidoxime reacted with equimolar concentrations of **I** and **III** up to approximately 40 per cent conversion of the fluoridates according to second-order kinetics, indicating that only one of the oxime groups is phosphylated.

k_2 And k_{POX} values

The conditions at which the determinations of k_2 and k_{POX} were performed, are summarized in Table 2. Samples were taken at incubation times up to 50–60 per cent conversion of the organophosphates. The oximes are in excess and the concentrations can be considered to be constant. From the observed kinetics at the determination of k_1 it is assumed that only one oxime group in obidoxime is phosphylated.

The procedure is based on the assumption that the concentrations of the organophosphates and phosphylated oximes, do not change during the inhibition reaction (Eqn (4)). This condition was largely fulfilled by choosing the concentrations of organophosphate, phosphylated oxime, enzyme and substrate in the inhibition assay in such a way that the ratio of the concentration of the organophosphate as well as of the phosphylated oxime to that of the enzyme amounted to at least fifteen. The phosphylated oxime concentration was calculated by means of Eqn (6) after determination of k_2.

The determination of k_2 and k_{POX} was carried out in such a way that the concentrations of the oximes in the inhibition assay were as low as possible in order to slow down a simultaneous reactivation of the inhibited enzyme by the oximes. No influence of the oximes on the inhibition reaction was observed.

Incubation of the organophosphates with obidoxime causes a large increase of the anticholinesterase activity ($k'_{I,t}$) as shown in Fig. 1. An increase of anticholinesterase activity, although to a somewhat less extent, was also found for **I** and **II** on incubation with P2S. The inhibitory action of **III** and **IV** was not increased by incubation with this oxime (Fig. 2).

Plots of $k'_{POX}[POX]_t$ values vs t are given in Figs 3 and 4. By fitting Eqn (7) to these data the two parameters of the equation, k_2 and k'_{POX}, were obtained. Values of k_2 and k_{POX} are summarized in Table 2.

A very small contribution of the phosphorylated oxime to the anticholinesterase activity of the incubates of **III** and **IV** with P2S was observed, from which k_2 and k_{POX} could not be calculated. From the results it is not possible to establish whether this small contribution is due to a rapid decomposition or to a weak anticholinesterase activity of the phosphorylated oxime.

Phosphylated oximes are reported to have a high

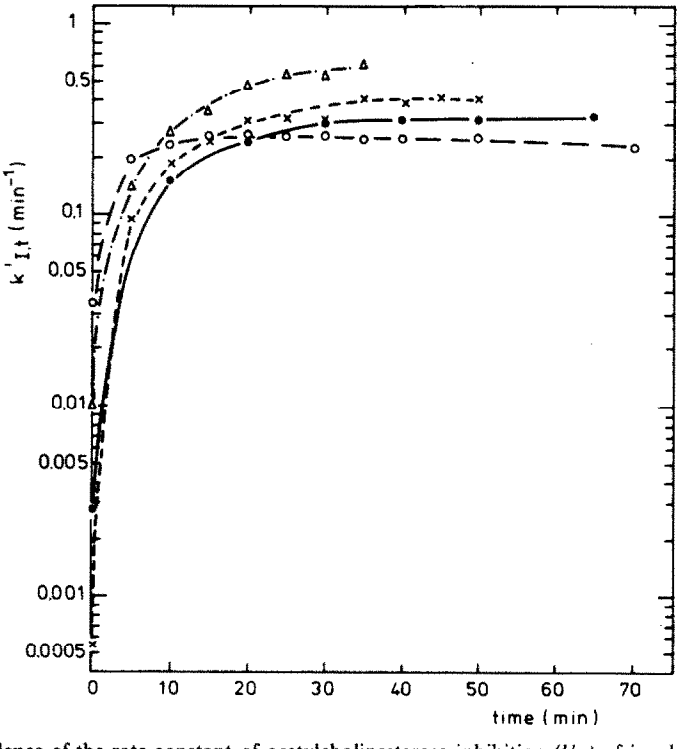


Fig. 1. Dependence of the rate constant of acetylcholinesterase inhibition ($k'_{I,t}$) of incubates of I (sarin, +0.1 mM obidoxime, △), **II** (*p*-nitrophenyl isopropyl methylphosphonate, ×), **III** (diethyl phosphorofluoridate, ●) and **IV** (paraoxon, ○) with obidoxime on incubation time. The rate constants of the incubates of I assayed with different sample volumes are adjusted to those of 40 μl samples. The $k'_{I,t}$ values at zero time of incubation are calculated as k'_{OP} values multiplied by the concentration of the organophosphate used in the inhibition assay of the incubates. For experimental details see Table 2 and text.

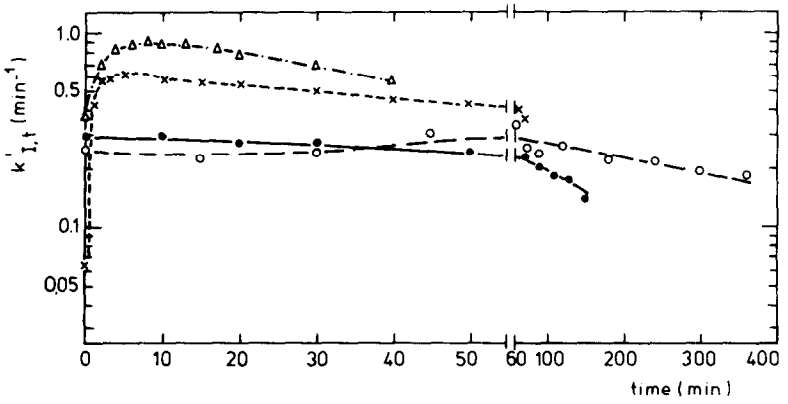


Fig. 2. Dependence of the rate constant of acetylcholinesterase inhibition ($k'_{I,t}$) of incubates of **I** (sarin, △), **II** (*p*-nitrophenyl iso-propyl methylphosphonate, ×), **III** (diethyl phosphorofluoridate, ●) and **IV** (paraoxon, ○) with P2S on incubation time. The $k'_{I,t}$ values at zero time of incubation are calculated as k'_{OP} values multiplied by the concentration of the organophosphate used in the inhibition assay of the incubates. For experimental details see Table 2 and text.

anticholinesterase activity [4, 7–12]. In agreement with this the phosphylated oximes investigated in this paper turn out to be very potent acetylcholinesterase inhibitors. The rate constant found for obidoxime phosphonylated by **I** is one of the largest values reported in literature for acetylcholinesterase inhibition [17].

The k_{POX} values obtained from experiments with the *p*-nitrophenyl compounds and the corresponding fluoridates forming the same phosphylated oxime, differ only slightly, as should be expected, except for the values of obidoxime phosphorylated by **III** or by **IV**. The k_2 values, however, differ considerably. Since the oximes react far more slowly with the *p*-nitrophenyl compounds than with the corresponding fluoridates (see Table 1), a relatively high oxime concentration was used in the incubates of the *p*-nitrophenyl compounds. It is known that oximate-ions can catalyze the decomposition of phosphylated oximes [13, 18]. Therefore, it seems reasonable that catalysis by 16–20 mM P2S or obidoxime may contribute considerably to the velocity of the decomposition of the phosphylated oximes.

Blanch [13] determined the bimolecular rate constants for the hydroxide-ion-catalyzed and the oximate-ion-catalyzed decomposition of P2S phosphonylated by **I** by following the acid production in incubates of the organophosphate and the oxime. The k_2 value obtained with the present method for this phosphonylated oxime and that determined in the presence of 20 mM P2S are in close correspondence to the values which can be calculated for pH 7.5 from

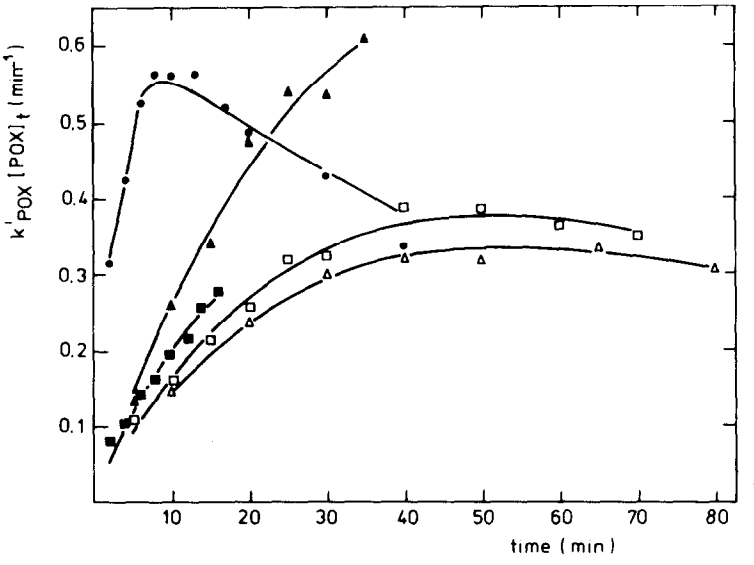


Fig. 3. Dependence of the contribution of phosphylated oxime to the anticholinesterase activity ($k'_{POX}[POX]_t$) of incubates of **I** (sarin) with P2S (●) and with obidoxime (0.1 mM, ▲; 0.2 mM, ■) and of **III** (diethyl phosphorofluoridate) with obidoxime (0.15 mM, △; 0.2 mM, □) on incubation time. The rate constants of incubates of **I** with 0.1 mM and 0.2 mM obidoxime assayed with different sample volumes are adjusted to those of 40 and 100 μl, respectively. The lines represent curves of $k'_{POX}[POX]_t$ calculated according to Eqn (7) with k_2 and k'_{POX} obtained in these experiments. For experimental details see Table 2 and text.

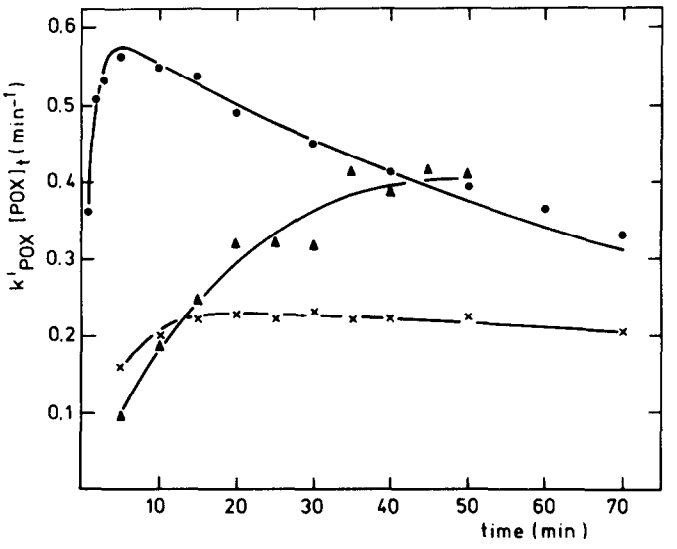


Fig. 4. Dependence of the contribution of phosphylated oxime to the anticholinesterase activity ($k'_{POX}[POX]_t$) of incubates of **II** (*p*-nitrophenyl iso-propyl methylphosphonate) with P2S (●) and obidoxime (▲) and of **IV** (paraoxon) with obidoxime (×) on incubation time. The lines represent curves of $k'_{POX}[POX]_t$ calculated according to Eqn (7) with k_2 and k'_{POX} obtained in these experiments. For experimental details see Table 2 and text.

the rate constants reported by Blanch: 0.36 min^{-1} and 0.36 + 0.48 = 0.84 min^{-1} (pK_a of P2S = 7.9). The higher stability of obidoxime phosphonylated by **I** toward that of the corresponding compound derived from P2S is in correspondence with the general findings concerning the stability of phosphylated pyridinium-4-aldoximes and pyridinium-2-aldoximes [4, 8].

Knowledge of k_{POX} and k_2 may contribute to a better understanding of the extent to which phosphylated oximes interfere in oxime-therapy of organophosphate poisoning. As a first step, the rate constants reported in this paper are being used to estimate the influence of the phosphylated oxime being formed during reactivation of phosphylated acetylcholinesterase on the process of the reactivation.

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