Non-linear Brønsted Correlations: Evidence for a Levelling Off in the Reactivity of Oximate Ions in Aqueous Solution

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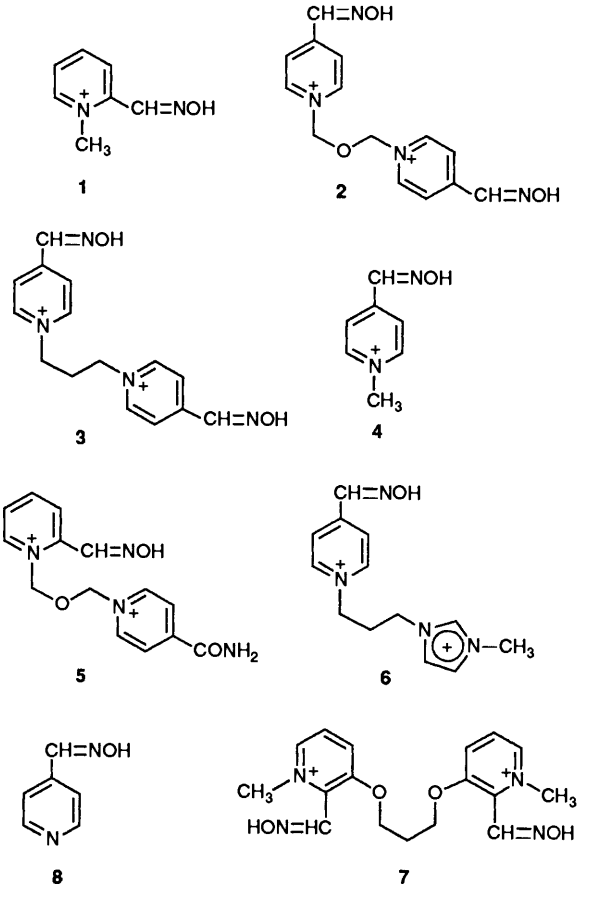
A kinetic study of the reactivity of a series of pyridinium carbaldoximate ions (pK_a = 7.13–9.02), which are all potential reactivators of acetylcholinesterase inhibited by organophosphorus poisons, towards *p*-nitrophenyl acetate in aqueous solution is reported. The corresponding Brønsted plot is non-linear, defining a plateau at pK_a 8 which fits very well the data previously obtained for a number of structurally different oximate ions. This phenomenon appears to be typical for the behaviour of these bases since Brønsted plots for reactions of oximate ions with other electrophiles show a similar levelling off. These observations support an explanation in terms of a requirement for desolvation of the oximate ion prior to nucleophilic attack which becomes more difficult with increasing basicity. Possible implications of the rapid levelling off in reactivity of oximate ions on their chemical ability to act as efficient reactivators are discussed.

In general, organophosphorus (OP) esters are powerful inhibitors of acetylcholinesterase (AChE), by phosphylation of a serine hydroxy group at the active site.[1–3],† Therapy of intoxication by these poisons is based on coadministration of anticholinergics and of so-called AChE reactivators. These must be capable of effecting a rapid displacement of the OP residue from the active site, thereby restoring the enzymatic activity.[4–14]

Pyridiniumcarbaldehyde oximes constitute the most important class of potential AChE reactivators.[5–8] As compounds having a positively charged moiety and an oximate functionality, they combine strong affinity for the inhibited enzyme, with pK_a values in the region of physiological pH, giving oximate ions which behave as typical α-nucleophiles and exhibit a high nucleophilic reactivity compared with common oxygen nucleophiles of similar basicities.[1,5,7–17] Although their cationic nature remains a disadvantage in terms of penetration of biological membranes,[7,9] the monooxime **1**, commonly known as 2-PAM (2-[(hydroxyimino)methyl]-1-methylpyridinium halide) and the dioximes **2** and **3**, commonly known as toxogonine (1,3-bis[4-(hydroxyimino)methyl]-1-pyridinio-2-oxapropane dichloride) and TMB-4 (1,3-bis[4-(hydroxyimino)-methyl]-1-pyridinio]propane dichloride), respectively, are the most efficient AChE reactivators currently used for clinical applications.[7,11,12]

In the last few years, efforts have been made to design new reactivators which would show enhanced antidotal activity compared with **1–3**, including the consideration of non-positively charged reactivators which might be able to penetrate biological membranes more readily.[7,11,19–22] In this context, we have recently reported a study of the physicochemical and reactivating properties of a series of oximes, namely **9b–9e**, which are derived from the well-known 2-oxopropanal oxime **9a** (MINA)[14,19] by substitution of one of the methyl hydrogens by a sulphur substituent.[20] While none of these derivatives has proved to be superior to **1–3** in terms of overall therapeutic effectiveness, the physicochemical study of **9a–9e** has revealed an unexpected but very important feature regarding the ability of the corresponding oximate ions, $\mathbf{9a}^-$–$\mathbf{9e}^-$, to act as nucleophiles towards a model electrophile, namely *p*-nitrophenyl acetate (PNPA).[18,20] We found that the nucleophilicity of



1 **2** **3** **4** **5** **6** **8** **7**

$\mathbf{9a}^-$–$\mathbf{9e}^-$ levels off very rapidly, reaching a maximum value at pK_a 7.8–8. Interestingly, a comparison of our results with data

† For simplicity we use the term 'phosphylation' suggested by various authors to avoid distinction between OP poisoning by phosphorylation and phosphonylation.

$R-CH_2-CO-CH=NOH$

9a; R = H
b; R = SCH_3
c; R = $SOCH_3$
d; R = SO_2CH_3
e; $\overset{+}{S}(CH_3)_2$

previously reported for more basic but structurally different oximate ions has suggested that this saturation effect might be a characteristic of the $>C=NO^-$ functionality in aqueous solution.[18]

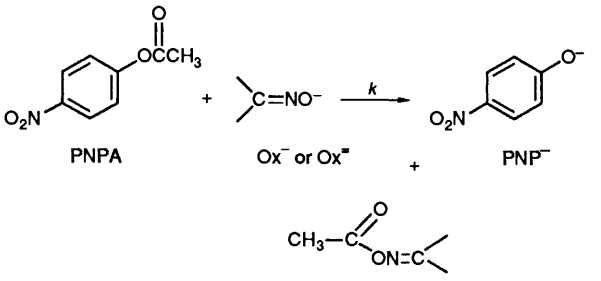
In view of the possible implications of the above discovery on the effective nucleophilic capability of oxime reactivators to restore the enzymatic activity we feel it necessary to extend our investigations to another homogeneous series of oxime compounds. We report a study of the reactivity of various pyridinium aldehyde oximes with PNPA under the same experimental conditions as those used for the **9a–9e** series. Besides **1–3**, we have studied the so-called 4-PAM (**4**) and HI-6 (**5**) and the two compounds **6** and **7** which were recently synthesized by some of us and referred to as CEB 1574 and R 665, respectively.[23,24] For the purpose of comparison, reported data for the neutral 4-[(hydroxyimino)methyl] pyridine **8** have also been considered. The results obtained confirm that most oximate ions of $pK_a > 8$–8.5 are subject to a levelling off of their reactivity, but they reveal that the new oximes **6** and **7** which both exhibit a remarkable antidotal activity against OP poisoning of AChE, have physicochemical behaviour consistent with efficient nucleophilic reactivity.

Results

Acidity of **1–8**.—The acidity of the monooximes **1**, **4**, **5**, **6** and **8** (pK_{a_1}) was measured at 25 °C by standard potentiometric methods using aqueous oximate buffer solutions with various [oximate]:[oxime] ratios.[20] The ionic strength I was kept constant at 0.1 mol dm^{-3} by adding KCl as required. The pK_{a1} and pK_{a2} values for the ionization of the two oxime groups of the dicationic dioximes **2**, **3** and **7** were too close to be independently determined in a similar way. For a reliable determination of these pK_as, potentiometric titrations of 0.01 mol dm^{-3} solutions of the dioximes by a 0.1 mol dm^{-3} NaOH solution were carried out at T = 25 °C and I = 0.1 mol dm^{-3}. Thus, the K_{a_1} and K_{a_2} values were derived from the titration data according to the Speakman equation.[25]

Table 1 summarizes all pK_a values derived for **1–8** at 25 °C and I = 0.1 mol dm^{-3}. Our results for **1–5** and **8**, are seen to be consistent with data previously obtained at various ionic strengths.[26–29]

Nucleophilicity of **1–8**.—Mono- and di-oximate ions (Ox^-, $Ox^=$) derived from **1–7** are yellow ions the absorption spectra of which strongly overlap with that of *p*-nitrophenoxide ion (PNP^-). Except in the cases of **1** and **4** and also for the neutral monooxime **8**, which gives a colourless oximate ion, this did not allow us to assess the reactivity of the various $>C=NO^-$ functionalities towards PNPA (Scheme 1) from simple experiments carried out in oximate buffers of high concentrations (10^{-3}–10^{-2} mol dm^{-3}) compared with that of PNPA (5×10^{-5} mol dm^{-3}).[18,20] Thus, we have studied the reactions by measuring the initial rates of appearance of PNP^- in series of experiments which were conducted with a large excess of PNPA (10^{-3} mol dm^{-3}) over the concentration of the various oximate ions. In each set of experiments, the pH was kept constant at 7.44, 7.74, 7.92, 10.04 or 10.34 (I = 0.10 mol dm^{-3}) by means of an external buffer [*N*-2-hydroxyethylpiperazine-*N*′-ethane-2-



Scheme 1

sulphonic acid (HEPES), 3-(cyclohexylamino)propanesulphonic acid (CAPS)] and the oxime concentration initially introduced in the solutions ($[Ox]_0$) was varied in the range 10^{-5}–2×10^{-4} mol dm^{-3}. This corresponded to initial concentrations in the reactive species Ox^- and $Ox^=$ which are given by eqn. (1) for the monooximes **1**, **4**, **5**, **6** and **8** and by eqns. (2) and (3) for the dioximes **2**, **3** and **7**. Under these experimental

$$[Ox^-]_0 = [Ox]_0 (1 + 10^{(pK_{a1} - pH)})^{-1} \quad (1)$$

$$[Ox^-]_0 = [Ox]_0 (1 + 10^{(pK_{a1} - pH)} + 10^{(pH - pK_{a2})})^{-1} \quad (2)$$

$$[Ox^=]_0 = [Ox]_0 (1 + 10^{(pK_{a2} - pH)} + 10^{(pK_{a1} + pK_{a2} - 2pH)})^{-1} \quad (3)$$

conditions, at pH = 7.44 and 7.74, the PNP^- ion resulting from Scheme 1 was partially converted into *p*-nitrophenol (pK_a = 7.05 at I = 0.1 mol dm^{-3})[30] which shows no appreciable absorption at the wavelength (400 nm) used for monitoring the kinetics. Accordingly, the initial rates of appearance of PNP^- were expected to obey eqn. (4) for **1**, **4**, **5**, **6** and **8** and eqn. (5) for **2**, **3** and **7**. In these equations, k_1 and k_2 represent the second-order rate constants for the decomposition of PNPA by mono- and di-oximate species, respectively, while k_0 represents the rate contributions of all possible competing pathways leading to PNP^- at the pH studied, namely the reactions of PNPA with water, hydroxide ion and the basic species of the HEPES buffer.

$$\left[\frac{d[PNP^-]}{dt}\right]_0 = \frac{k_0 + k_1[Ox^-]_0}{1 + 10^{7.05-pH}}[PNPA]_0 \quad (4)$$

$$\left[\frac{d[PNP^-]}{dt}\right]_0 = \frac{k_0 + k_1[Ox^-]_0 + k_2[Ox^=]_0}{1 + 10^{7.05-pH}}[PNPA]_0 \quad (5)$$

Details and examples of the treatment of initial rate data obtained at the various pH studied by combination of eqn. (4) with eqn. (1) and eqn. (5) with eqns. (2) and (3) are given in the Experimental section. This analysis leads to the second-order rate constants k_1 and k_2 which are listed in Table 1. Interestingly, the k_1 values thus obtained for **1**, **4** and **8** were perfectly consistent with those determined from experiments in oximate buffers. The values for **1** and **8** also compare well with previous determinations by Kenley *et al.* or Meyer and Viout.[9,31]

Discussion

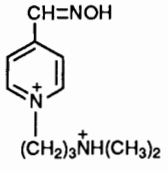
Acidity of **1–6**.—Table 1 shows that the pK_{a_1} values for ionization of the new pyridinium oximes **6** and **7** are equal to 8.05 and 7.33, respectively, thus fitting the requirements for a notable dissociation of the $>C=NOH$ functionality at pH 7.8, suitable for reactivation. If one corrects for a statistical factor of 2, the pK_{a1} value for **3** (pK_{a1}^{corr} = 8.09) is very similar to that for **6** (pK_{a1} = 8.05), both values being about 0.2 pK units lower

Table 1 pK_a values and kinetic parameters for the reactions of mono- and di-oximate ions derived from **1–8** with PNPA in aqueous solution[a]

Parent oxime	No.	pK_{a1}	pK_{a2}	k_1/dm^3 mol^{-1} s^{-1}	k_2/dm^3 mol^{-1} s^{-1}
HI-6	**5**	7.13 7.28[b]		11.8	
R-665	**7**	7.33	9.02	34.5	63.3
Toxogonine	**2**	7.46 7.54,[b] 7.55[c]	8.17 8.12,[b] 8.20[c]	22.3, 20.74	37.2, 39.6
2-PAM	**1**	7.75 7.68,[b] 7.92,[d] 7.99,[e] 8[c]		25 16[d]	
TMB 4	**3**	7.79 7.78,[f] 7.99[c]	8.55 8.61,[f] 8.68[c]	32.1	48
CEB 1574	**6**	8.05		35	
4-PAM	**4**	8.27 8.55[c]		61	
Pyridine-4-carbaldehyde oxime	**8**	9.55 9.65[g]		65, 63[g]	

[a] T = 25 °C, I = 0.1 mol dm^{-3} KCl. [b] Ref. 26. [c] Ref. 27. [d] Ref. 29. [e] Ref. 9. [f] Ref. 18. [g] Ref. 31.

than that for the 4-(hydroxyimino) methyl analogue of **1**, *i.e.* **4** (pK_a = 8.27). This shows that interaction with the second positively charged pyridinium or pyrimidinium ring bonded to the n-propyl chain in **3** and **6** is still appreciable over the distance involved. This is most probably due to a field rather than to an inductive effect.[27] A similar situation was found in comparing the acidity of the >CH=NOH group of **4** with that of the analogue **10** with a protonated *N,N*-dimethylamino-n-propyl group.[27b] Based on the pK_{a1} values of **2** and **3**, it can also be seen that the replacement of the n-propyl chain by a 2-oxapropyl chain further increases the acidity by 0.3 pK units.



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In the same way that the acidity of the 2-CH=NOH group of **1** is greater than that of the 4-CH=NOH group of **4**, the acidity of the first 2-CH=NOH functionality of **7** is somewhat greater than that of the first 4-CH=NOH group of both **2** and **3**. In fact, the presence of an *ortho*-alkoxy-type substituent in **7** has no marked effect on the ionization of the first NOH functionality of this dioxime: the statistically corrected pK_{a1} value (pK_{a1}^{corr} = 7.63) is only slightly lower than that of the 2-PAM **1** (pK_{a1} = 7.75) and it seems reasonable to assume that this reflects mainly the field effect exerted by the second pyridinium ring. Comparison of the pK_{a2} values reveals more original behaviour. Contrasting with **2** and **3**, which exhibit essentially identical statistically corrected pK_{a1} and pK_{a2} values (pK_{a1}^{corr} = 7.76; pK_{a2}^{corr} = 7.87 for **2**; pK_{a1}^{corr} = 8.09; pK_{a2}^{corr} = 8.25 for **3**) indicating that the two oxime groups of these symmetrically structured compounds are identical as regards chemical behaviour, the pK_{a2}^{corr} for **7** is 1.1 unit higher than the corresponding pK_{a2}^{corr} value: pK_{a1}^{corr} = 7.63, pK_{a2}^{corr} = 8.72. Increased stabilization of the monooximate species Ox^- of **7** through intramolecular hydrogen bonding of the type –NOH···$^-$ON– does not appear to be a satisfactory explanation for this large ΔpK value. Such stabilization should notably decrease both pK_{a1} and k_1: a fact not borne out by the experimental results. We are therefore left with the idea that the two oxime groups of **7** might not be chemically identical. Indeed, preliminary X-ray experiments support this hypothesis, indicating that in the solid state of **7**, the two CH=NOH groups adopt a different spatial conformation with respect to the pyridinium ring to which they are bonded. Why this difference persists in solution is not understood at present.[32]

The Brønsted Behaviour of **1–8**.—No satisfactory Brønsted line can be drawn on plotting the statistically corrected log k values for the reactions of the various mono- and di-oximate ions of **1–8** with PNPA *vs.* the corresponding statistically corrected pK_a values (Fig. 1). This result is not really unexpected in view of our recent observations that both the moiety bearing the >C=NO$^-$ functionality and the order of magnitude of the basicity are important factors governing the nucleophilicity of oximate ions.[18,20]. Thus, Fig. 1 shows the two different linear Brønsted plots of similar slope corresponding to a β_{nuc} value of *ca.* 0.7 analogous to that for the phenoxide reactions[35] that were defined for the reactions of moderately basic oximate ions of general structure **9** and **11** with PNPA.[11,18] However, a very important feature which emerges from Fig. 1 is that these plots start to curve at around pK_a 8 with the observed curvature fitting, apparently well, the data previously obtained for a number of structurally different and more basic oximate ions (pK_a 8–11). Since we have suggested that this levelling off in the reactivity is typical for the behaviour of all relatively basic oximate ions, it is interesting to find that the data obtained for **1–8** support this idea. While the reactivity of the less basic pyridiniumcarbaldoximate ions studied is slightly higher than that of similarly basic species in series **9** and **11**, it is clear that the

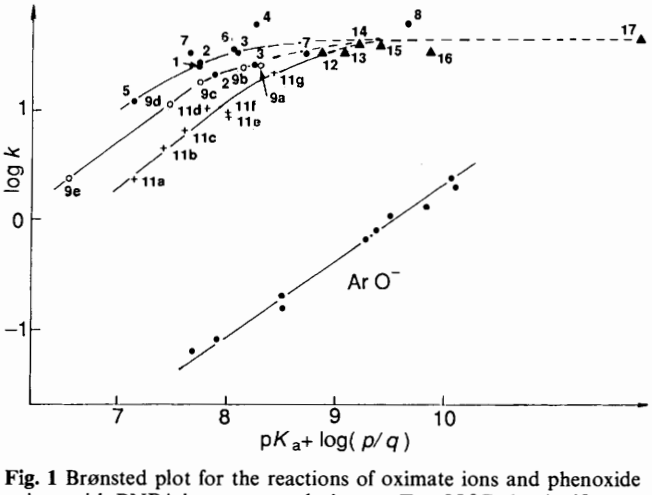


Fig. 1 Brønsted plot for the reactions of oximate ions and phenoxide anions with PNPA in aqueous solutions at T = 25 °C; the significance of numbers **1–11** is given in the text; **12** = $CF_3CH{=}NO^-$;[39] **13** = $ArC(CF_3){=}NO^-$;[39] **14** = salicylaldehyde oxime ion;[16] **15** = $CH_3COC(CH_3){=}NO^-$;[41] **16** = $CF_3C(CH_3){=}NO^-$;[39] **17** = $ArC(CH_3){=}NO^-$[39]

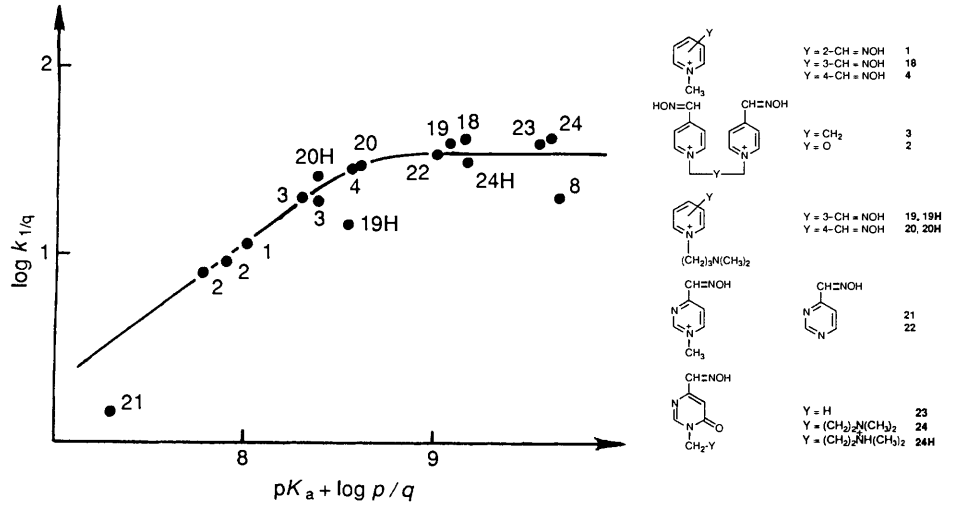
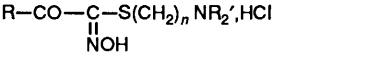


Fig. 2 Brønsted plot for the reactions of various pyridinium and pyrimidinium aldehyde oximes with diisopropyl phosphorotrifluoridate (DFP) in aqueous solution at T = 25 °C; data taken from ref. 27



11

11a; n = 2,	R = 4-$NO_2C_6H_4$	R′ = C_2H_5
b; n = 2,	R = CH_3	R′ = C_2H_5
c; n = 2,	R = C_6H_5	R′ = C_2H_5
d; n = 2,	R = 4-$CH_3OC_6H_4$	R′ = C_2H_5
e; n = 2,	R = 4-$CH_3OC_6H_4$	R′ = CH_3
f; n = 2,	R = 4-$CH_3OC_6H_4$	R′ = $(CH_3)_2CH$
g; n = 3,	R = 4-$CH_3OC_6H_4$	R′ = CH_3

reactivity of most basic pyridinium aldoximate ions tends to level off to about the same limit as all other basic oximate species, including the oximate ion derived from the neutral pyridinecarbaldehyde oxime **8**. However, the reactivity of the 4-PAM is somewhat higher than anticipated from the behaviour of all other oximes studied.

We have found that a reanalysis of data previously reported by Ashani and Cohen for the reactions of a large set of pyridinium and structurally related aldoximate ions with diisopropyl phosphorotrifluoridate (DFP) in aqueous solution provides further evidence that the oximate functionality is subject to a sort of saturation effect.[27] The statistically corrected Brønsted plot for these reactions is shown in Fig. 2. Obviously, the reactivity of the moderately basic oximate ions defines a linear Brønsted plot with a slope β_{nuc} = 0.75 which is close to that for the PNPA reactions discussed above. On the other hand, the more basic oximate functionalities define a clear limiting reactivity towards DFP. However, the curvature appears at $pK_a \approx 8.5$ for these reactions, as compared with pK_a = 8 for the PNPA reactions. This may indicate that the nature of the electrophile plays some role in determining the appearance of the levelling off.

Non-linear Brønsted correlations have been commonly observed in reactions of normal oxyanions like phenoxide and alkoxide ions with electrophiles like esters so that the presence of curvature in the Brønsted plots for oximate ions is not in itself a surprising phenomenon.[33–36] In previous cases, however, no significant curvature was detected at $pK_a < 10$ and this is confirmed by the linearity of the Brønsted plots drawn for phenoxide ions of $pK_a < 10$ in Fig. 1.[35,37] Accordingly, the finding that the reactivity of oximate ions tends to level off at $pK_a \approx 8$–8.5 is a significant result. We have recently discussed this phenomenon and suggested that it is the reflection of a stronger requirement for desolvation of oximate ions than of phenoxide ions before nucleophilic attack rather than of a change in the rate-determining step of Scheme 1.[18]

The rapid levelling off in the reactivity of oximate ions with increasing pK_a has several consequences. First, there is no doubt that it is responsible for the abnormally low β_{nuc} values (0.1–0.2) previously reported in many reactions of these derivatives.[38,39] Clearly, these puzzling values were the result of experiments carried out with a limited set of oximate ions that were too basic. Also, the reported decrease in the enhanced reactivity of oximate ions compared with normal oxyanions of similar basicity, *i.e.* the so-called α-effect, with increasing pK_a can readily be accounted for by the curvature observed in the corresponding Brønsted plots (see Fig. 1).[17] However, the most important consequence probably deals with the use of oximate ions as reactivators of phosphylated AChE.

An oxime reactivator (OxH) must restore the enzymatic activity at physiological pH where the concentration of the reactive species, *i.e.* the oximate ion Ox^-, is only a fraction of the total oxime concentration $[OxH]_0$, as given by eqn. (6).[7,9–12] Accordingly the observed rates for nucleophilic attack of Ox^- at this pH are referred to $[OxH]_0$ by means of eqn. (7) where k is the bimolecular rate constant which measures the intrinsic nucleophilic reactivity of the oximate ion and k_{eff} is the bimolecular rate constant that measures the effective nucleophilic capability of the oxime to reactivate inhibited AChE at pH 7.8.

$$[Ox^-] = [OxH]_0 (1 + 10^{pK_a - 7.8})^{-1} \quad (6)$$

$$k_{obs} = k[Ox^-] = \frac{k[OxH]_0}{1 + 10^{pK_a - 7.8}} = k_{eff}[OxH]_0 \quad (7)$$

Because of the levelling off observed in the intrinsic nucleophilicity of oximate ions, there is no chemical way of compensating for the lowest degree of ionization of oximes with $pK_a > 8$–8.5 (the $1 + 10^{pK_a - 7.8}$ term increases with increasing pK_a) and these will necessarily exhibit lower k_{eff} values than oximes with pK_as in the range 7–8. Notwithstanding the fact that there are other factors determining the reactivation potency, in particular the affinity for the inhibited enzyme, physicochemical studies suggest that oximes that are too weakly acidic cannot be effective reactivators. In fact all known efficient reactivators, *e.g.* **1–3** have pK_a values in the range 7.5–8. Significantly, the new oximes **6** and **7** which both exhibit remarkable antidotal activity against OP poisoning of AChE, (**6** should shortly be available for clinical application) also have a pK_{a1} value suitable for effective nucleophilic reactivity.

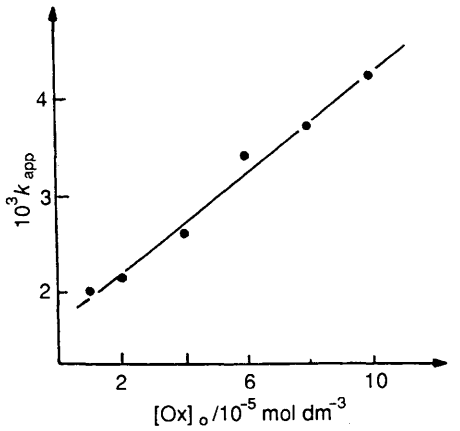


Fig. 3 Effect of the total oxime concentration on the apparent rate constant k_{app} for the reaction of PNPA with the mono-oxime **6** at pH = 7.92 and T = 25 °C in aqueous solution

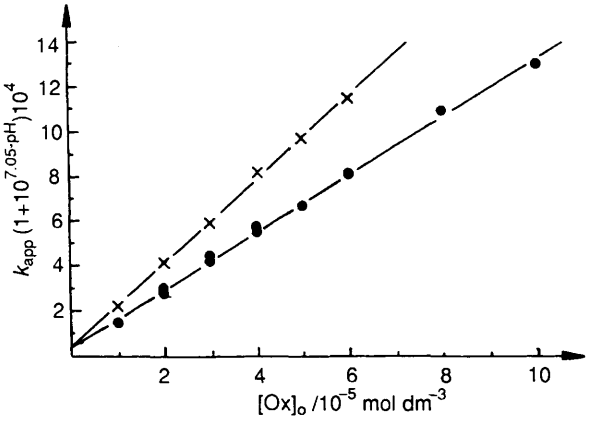


Fig. 4 Effect of the total oxime concentration on the apparent rate constant k_{app} for the reaction of PNPA with toxogonine **2** at pH = 7.44 (●) and 7.74 (×) and T = 25 °C in aqueous solution

Experimental

Materials.—The oximes **1**, **2**, **3**, **5** and **8** were provided by the *Centre d'Etudes du Bouchet* and were recrystallized prior to use. 4-[(Hydroxyimino)methyl]-1-methylpyridinium iodide **4** was prepared by methylation of **8** according to a standard procedure.[40] The oximes **6** and **7** were prepared as recently described.[23,24]

Acidity Measurements.—The acidity constants of **1**–**8** were measured by potentiometry at 25 °C and I = 0.1 mol dm^{-3} KCl, using an electronic pH meter (Tacussel Isis 20000). The pK_{a1} values for ionization of the NOH group of the mono-oximes (**1**, **4**, **5**, **6**, **8**) were determined from buffer solutions with [oximate]:[oxime] ratios equal to 1:2, 1:1 and 2:1; these solutions were prepared so that the molarity of the oximate species was equal to 0.01 mol dm^{-3}. Under these experimental conditions, the pK_{a1} values at I = 0.1 mol dm^{-3} were simply obtained from the measured pH values of the buffers by means of eqn. (8).

$$pK_{a1} = pH - \log\frac{[oximate]}{[oxime]} \quad (8)$$

pK_{a1} and pK_{a2} for ionization of the dicationic dioximes (**2**, **3**, **7**) were determined by potentiometric titration of 5 × 10^{-3} or 10^{-2} mol dm^{-3} solutions of these derivatives (at I = 0.1 mol dm^{-3}) by a 0.1 mol dm^{-3} sodium hydroxide solution containing 0.05 mol dm^{-3} KCl. The presence of KCl in the added NaOH solutions served to minimize the initial decrease in I caused by the predominance of an $AH_2^{++} \rightleftharpoons AH^{\pm+}$ exchange in the first part of the titration. Since such compensation was no longer required for the $AH^{\pm+} \rightleftharpoons A^{\pm\pm}$ exchange, the presence of KCl in the NaOH solution caused a slight increase in I during the second part of the titration. However, this effect was then attenuated by the dilution effect so that $I \approx 0.1$ mol dm^{-3}. The K_{a1} and K_{a2} values were calculated from a least-squares treatment of the pH data obtained after addition of 0.5–1.5 equiv. of base by means of eqn. (9). In the corresponding pH range, the h term in eqn. (9) was simply given by eqn. (10) where [NaOH] and $[Ox]_0$ represent the added NaOH concentration and the total oxime concentration, respectively.[25]

$$10^{-pH}\frac{1-h}{2-h}K_{a1} + K_{a1}K_{a2} = \frac{10^{-2pH}h}{2-h} \quad (9)$$

$$h = \frac{[NaOH]}{[Ox]_0} \quad (10)$$

Kinetic Measurements.—The initial rates of the reactions shown in Scheme 1 were measured at 25 °C in a Durrum stopped-flow spectrophotometer under the experimental conditions described in the Results section. From these data, the second-order rate constants k_1 and k_2 for reaction of the various mono- and di-oximate species (Ox^-, $Ox^=$) were obtained as follows.

In the case of the monooximes (**1**, **4**, **5**, **6**, **8**), combination of the rate eqn. (4) with eqn. (1) leads to eqn. (11) which predicts a linear dependence of the apparent rate constant k_{app} upon the total oxime concentration $[Ox]_0$ at a given pH. As shown in Fig. 3 which refers to the reaction of **6** with PNPA at pH 7.74, the experimental results agreed with this expectation. This allowed an easy determination of k_1 for the various oximes, with a good agreement between the values obtained from the experiments conducted at pH 7.44, 7.74 and 10.04. With the exception of **8**, all of the monooximes were almost completely ionized at pH 10.04, allowing a simple derivation of k_1 from the simplified eqn. (12).

$$k_{app} = \frac{1}{[PNPA]_0}\left(\frac{d[PNP^-]}{dt}\right)_0 = \frac{k_0 + k_1(1 + 10^{pKa1-pH})^{-1}[Ox]_0}{1 + 10^{7.05-pH}} \quad (11)$$

$$k_{app} = \frac{1}{[PNPA]_0}\left(\frac{d[PNP^-]}{dt}\right)_0 = k_0 + k_1[Ox]_0 \quad (12)$$

In the case of the dioximes (**2**, **3**, **7**), combination of the rate equation (5) with eqns. (2) and (3) leads, after some rearrangement, to eqn. (13) where the A, B and C terms are defined by eqns. (14), (15) and (16), respectively. In accord with eqn. (13), a linear dependence of k_{app} upon the total oxime concentration was observed at each of the pH studied (7.44, 7.74, 10.04 and 10.34). Combination of the pH-dependent slopes afforded the k_1 and k_2 values listed in Table 1. For experiments carried out with **2** and **3** at pH 10.04 and 10.34, eqn. (13) reduces in a first approximation to the simplified form of eqn. (17).

$$k_{app} = \frac{1}{[PNPA]_0}\left(\frac{d[PNP^-]}{dt}\right)_0 = \frac{k_0 + \frac{k_1B = k_2C}{A}[Ox]_0}{1 + 10^{7.05-pH}} \quad (13)$$

$$A = 1 + 10^{pH - pK_{a1}} + 10^{2pH - pK_{a1} - pK_{a2}} \quad (14)$$

$$B = 10^{pH - pK_{a1}} \quad (15)$$

$$C = 10^{2pH - pK_{a1} - pK_{a2}} \quad (16)$$

$$k_{app} = \frac{1}{[PNPA]_0}\left(\frac{d[PNP^-]}{dt}\right) = k_0 + k_0[Ox]_0 \quad (17)$$

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