being adjusted so that the temp of the liquid remained below 3°. After 7.5 hr, when nmr showed that nearly all of the starting material had reacted, the flow of Cl_2 was stopped and air was allowed to bubble through the soln for 30 min more to remove Cl_2 and HCl. Nmr analysis of the product showed about 20% of the desired material as well as about 80% of methyl 2,3-dichloro-2-methylpropionate and other unidentified materials. The mixt was distd using a 1.5-m fractionating column packed with glass helices. An 80-ml fraction, boiling at 62.5–64° (20 mm) and contg about 85% of **5**, was collected over a 5-hr period. Redistn of this fraction at 62–63° (20 mm) [lit.[5] 56–57° (10 mm)] gave 20 ml of pure **5** as well as 30 ml of product contaminated with 5% of methyl 2,3-dichloro-2-methylpropionate: nmr of **5** (neat), δ 3.55 (s, 3, OCH_3), 4.07 (d, 2, $J \simeq 1$ Hz, CH_2Cl), 5.72 (t, 1, $J \simeq 1$ Hz, vinyl H), 6.02 (s, 1, vinyl H) ppm.

[4-^{14}C]Itaconic Acid (7a). Unlabeled KCN (0.65 g) was dissolved in 2.5 ml of H_2O and 0.5 ml of this soln was added slowly to a stirred soln of 2.0 g of methyl 2-(chloromethyl)acrylate. A second 0.5-ml portion of the KCN soln was used to dissolve 6 mg (0.5 mCi; New England Nuclear) of [^{14}C]KCN. The resulting soln, followed by the remainder of the unlabeled KCN soln, was added to the reaction mixt, and stirring was continued at room temp. Alcohol was removed from the reaction mixt on the rotary evaporator, leaving a mixt of H_2O, an oil, and KCl, which was then added to 50 ml of Et_2O. The soln was dried (Na_2SO_4), filtered, and evapd yielding 1.6 g of crude **6a**, an oil; ir (neat), 2250 cm^{-1} (nitrile). Concd HCl (10 ml was added to the crude nitrile, and the stirred mixt was heated on the steam bath. After 1.5 hr, all of the oil had dissolved, and the soln was evapd nearly to dryness on the rotary evaporator. The residue was dissolved in 15 ml of water and the soln was passed through a column contg 20 g of Dowex 50-X8 (H^+ form) to remove ammonium ions. The column was washed with 150 ml of H_2O, and washings were combined and evapd on the rotary evaporator. The residue was dried overnight in a vacuum desiccator and then was triturated with 3 ml of Et_2O. The mixt was kept in the cold room for a few hours, and the insol crude itaconic acid was filtered off and washed with 1 ml of Et_2O; yield, 0.55 g (4.6×10^8 dpm). Unlabeled itaconic acid (0.1 g) was added to the Et_2O filtrate and the Et_2O was evapd. The solid residue was washed with 2 ml of Et_2O and was filtered off, yielding an addnl 78 mg (3.0×10^7 dpm) of **7a**. The 2 crops of crude crystals were combined: yield, 0.63 g; mp 147–157° (lit.[16] 162–165°); 4.9×10^8 dpm (44% from [^{14}C]KCN.

[4-^{14}C]Bromomesaconic Acid (4a).—Crude [4-^{14}C]itaconic acid (**7a**, 0.61 g) was dissolved in 5 ml of HOAc, and the soln was heated on a steam bath with stirring. A soln of 0.9 g of Br_2 in AcOH was added dropwise over a period of 30 min, and the mixt was stirred and heated an addnl 30 min. It was evapd to an oil on the rotary evaporator, and 10 ml of CCl_4 was added and evapd. The alternate addn and evapn of CCl_4 was repeated several times until the residue remained solid: yield of **8a**, 1.04 g; mp 164–167°. This material was heated at reflux with 10 ml of $(CF_3CO)_2O$ with exclusion of moisture. After 1 hr, the reaction mixt was cooled and evapd on a rotary evaporator. Traces of CF_3COOH were removed in a vacuum desiccator under high vacuum: yield of crude **9a** (white solid), 0.96 g; mp 51–55°. The crude anhydride **9a** (0.96 g) was dissolved in 20 ml of dry Et_2O. A soln of 0.39 g of dry Et_3N in 10 ml of dry Et_2O was added slowly to the anhydride soln with stirring and exclusion of moisture. The black soln was filtered to remove $Et_3N \cdot HBr$, and the Et_2O was evapd leaving a dark oil; yield of crude **10a**, 0.52 g (~27% from KCN). A portion (0.22 g) of the crude **10a** was dissolved in 2 hr, by stirring with 1.0 ml of H_2O. Excess H_2O was removed under vacuum on the rotary evaporator, and the oily residue was allowed to stand exposed to air overnight, during which time the oil solidified. The solid was washed with 2 ml of C_6H_6 and was filtered; yield, 0.2 g; mp *ca.* 160°. The crude product was dissolved in 0.2 ml of water, the soln was cooled in an ice bath, and then 0.3 ml of concd HCl was added. The white ppt which formed was filtered off, washed with a small amount of cold 6 *N* HCl, and dried under high vacuum: yield of pure [4-^{14}C]bromomesaconic acid, 0.1 g (~42% from bromomesaconic anhydride); mp 184–186°; sp act., 1.10×10^6 dpm/μmole.

Inhibitor Solutions for Enzyme Studies. A. Bromomesaconic Acid (4).—Stock solns of **4** have a half-life of about 15 min above pH 7. Weighed samples of **4** and Na_2HPO_4 were dissolved in H_2O, the pH was adjusted to the desired value, and final vol adjustments were made by adding H_2O. These operations were carried out as quickly as possible, and the stock solns were used immediately for inhibitor runs.

B. Bromocitraconic Acid (11).—Stock solns of **11**, have a half-life of about 5 hr at pH 7. Calculated amts of bromocitraconic anhydride (**10**) were hydrolyzed by stirring in H_2O for about 1 hr. Na_2HPO_4 was then added and the pH adjusted to the desired value. The addn of anhydride **10** directly to buffered solns leads to unknown side reactions.

Enzymes.—Pig heart fumarase was purchased from Calbiochem. Baker's yeast fumarase was prepared in partially pure form by the method of Cataldi and Stoppani.[17]

(16) R. L. Shriner, S. G. Ford, and L. J. Roll, "Organic Syntheses," Collected Vol. II, Wiley, New York, N. Y., 1943, p 368.

(17) M. A. Cataldi and A. O. M. Stoppani, *Biochim. Biophys. Acta*, **118**, 631 (1966).

Nucleophilicity of Some Reactivators of Phosphorylated Acetylcholinesterase[1]

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A rational approach is presented toward the establishment of a structure–activity relationship in a series of reactivators of DFP-inhibited AChE. The value of k_r, the first-order reactivation rate constant, at pH 7.4 is a function of both the nucleophilicity of the reactivator molecule and its basicity, reaching an optimum for compounds with a pK_a value in the range 7.6–8.0.

Heterocyclic oximes are recognized antidotes against intoxication with organophosphates.[2-4] In a series of studies[1,5,6] we have attempted to determine the structure–activity relationship of these compounds by correlating a relevant thermodynamic property, such as pK_a, with their nucleophilicity toward a common substrate, such as diisopropyl phosphorofluoridate (DFP). This is an arbitrary approach which dwells on the assumption that nucleophilicity toward this substrate reflects the same property toward phosphorylated acetylcholinesterase. The premise that all good reactivators of phosphorylated AChE are also good nucleophiles stems from a large volume of experimental information,[7]

(1) Part 5 of a series; part 4: Y. Ashani and S. Cohen, *J. Med. Chem.*, **13**, 471(1970).

(2) I. B. Wilson and S. Ginsburg, *Biochem. Pharmacol.*, **1**, 200(1956).

(3) W. K. Berry, D. R. Davies, and A. L. Green, *Brit. J. Pharmacol.*, **14**, 186 (1959).

(4) F. Hobbiger, D. G. O'Sullivan, and P. W. Sadler, *Nature (London)*, **182**, 1498 (1958).

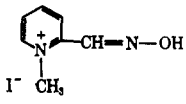
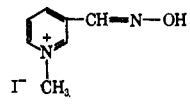
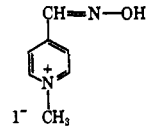
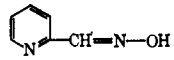
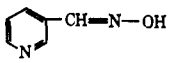
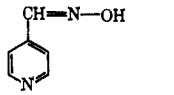
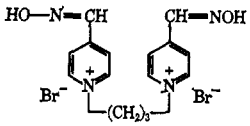
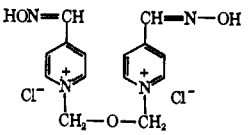
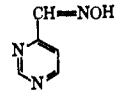
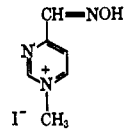
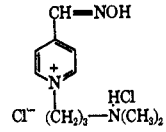
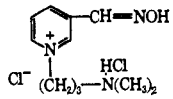
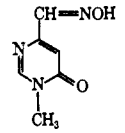
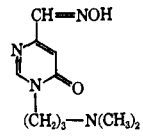
(5) Y. Ashani and S. Cohen, *Israel J. Chem.*, **5**, 59 (1967).

(6) Y. Ashani, N. Dinar, and S. Cohen, *J. Med. Chem.*, **11**, 967 (1968).

(7) F. Hobbiger in "Heffter-Heubners Handbuch der experimentellen Pharmakologie," Vol. 15, G. B. Koelle, Ed., Springer, Berlin, 1963, p 921.

TABLE I

pK_a VALUES OF SOME HETEROCYCLIC OXIMES[a] AND SECOND-ORDER RATE CONSTANTS OF THE SN2 REACTION OF THE CORRESPONDING OXIMATES WITH DFP IN DILUTE AQUEOUS SOLUTION

No.	Structure	pK_a	$k(\pm 10\%)$, mole^{-1} min^{-1}[b]
1		8.00 ± 0.03	11.0
2		9.15 ± 0.03	41.5
3		8.55 ± 0.03	28.5
4		9.85 ± 0.05	17.7
5		9.95 ± 0.05	13.5
6		9.65 ± 0.05	19.5
7		7.99 ± 0.02	19.6
		8.68 ± 0.02	32.0
8		7.55 ± 0.03	7.90
		8.20 ± 0.03	18.06
9		9.00 ± 0.05	31.5
10		7.30 ± 0.03	1.5
11		8.36[c]	28.0
		8.60[d]	31.0
12		8.55[c]	14.0
		9.07[d]	40.0
13		9.55 ± 0.05	40.0
14		9.15[c]	32.0
		9.60[d]	42.0

[a] The oximes were prepared according to the following references: compds **1–6**, S. Ginsburg and I. B. Wilson, *J. Amer. Chem. Soc.*, **79**, 481 (1957); compd **7**, W. K. Berry and D. G. O'Sullivan, *Nature (London)*, **182**, 1498 (1958); compd **8**, A. Luttringhaus and I. Hagedorn, *Arzneim.-Forsch.*, **14**, 1 (1964); compd **9**, **10**, Y. Ashani, H. Edery, J. Zahavy, W. Kuhnberg, and S. Cohen, *Israel J. Chem.*, **3**, 133 (1965); compd **11**, Y. Ashani and S. Cohen, *Israel J. Chem.*, **5**, 59 (1967); compds **12–14**, Y. Ashani, and S. Cohen, *J. Med. Chem.*, **13**, 471 (1970). [b] The second-order rate constant, k, refers to the oximate anion and not to its conjugate acid. [c] Species b. [d] Species d.

and use of model reactions with an organophosphate ester as substrate has been found both expedient and indicative.[8] Yet, the *relative* importance of nucleophilicity in the overall reactivation process has remained, so far, poorly understood.

We wish to present now a comparison of the activity of a number of heterocyclic oximes toward DFP on the one hand, and DFP-inhibited AChE (acetylcholine hydrolyase, E.C.3.1.1.7) on the other, then refer these properties, wherever possible, to some common denominator, such as pK_a.

Experimental Section

All the oximes used have been described earlier and are given in Table I. pK_a measurements were made by potentiometric titration at ionic strength 0.2 (KCl) and 25°.[1,6] Rate determinations are based on F^- formation, in accordance with the general equation

$$RCH{=}NO^- + FP(O)(O\text{-}i\text{-}Pr)_2 \xrightarrow{k} F^- + RCH{=}NOP(O)(O\text{-}i\text{-}Pr)_2 \quad (1)$$

Under these conditions, spontaneous hydrolysis of DFP is insignificant and may be neglected. The rational of this approach and the experimental details have been presented earlier.[1,6] For our present purpose, log k, the second-order rate constant, is taken as a measure of the nucleophilicity of the oxime. In all cases it has been shown that the reaction is first order with respect to either reactant, the anion or DFP, the rate equations being

$$k_{obsd} = \ln \frac{[F^-]_\infty}{[F^-]_\infty - [F^-]_t} \times \frac{1}{t} \quad (2)$$

$$k = \frac{k_{obsd}}{[Ox]_0 \alpha} \quad (3)$$

where $[F^-]_\infty$ and $[F^-]_t$ are the concentration of F^- ion at the end of the reaction and time t, respectively, and α is the fraction of oxime present as anion.

Reactivation studies were carried out by the method of Davies and Green[9] which is a modification of the electrometric procedure of Michel.[10] Bovine erythrocytes AChE (Sigma) with a specific activity of 2–4 μmoles/mg were used as the enzyme source. Inhibition of the enzyme was carried out by adding 0.1 ml of a freshly prepared solution of DFP in water ($\sim 5.10^{-5}$ M) to 6 ml of an enzyme solution (1 mg/ml) in phosphate-veronal buffer

(8) G. Steinberg, C. N. Lieske, R. Boldt, J. C. Goan, and H. E. Podall, *J. Med. Chem.*, **13**, 435 (1970).

(9) D. R. Davies and A. L. Green, *Biochem. J.*, **63**, 529 (1956).

(10) H. O. Michel, *J. Lab. Clin. Med.*, **34**, 1564 (1949).

(sodium veronal, 0.01 M-KH_2PO_4, 0.002 M-KCl, 0.3 M), followed by incubation at 25° for 1 hr.

Reactivation was started at t_0 by the addition of 6 ml of a solution of an oxime at the desired concentration in phosphate–veronal buffer. Subsequently and at regular intervals of time, the restored enzyme activity was determined by the addition of 15 ml of a solution of acetylcholine perchlorate, 0.01 M in phosphate–veronal buffer, to 2 ml of the reactivated enzyme solution, then reading ΔpH throughout 1 hr at 25°. Excess substrate inhibits further reactivation.

Two sets of experiments run under identical conditions confirmed that spontaneous reactivation of inhibited enzyme or partial inhibition of free enzyme by the oximes tested was negligible.

Results

A. Nucleophilicity toward DFP.—Treatment of k_{obsd} for monovalent oximes possessing a single dissociable group (**1–6**, **9**, **10**) is straightforward. As an example, the plot of k_{obsd} *vs.* oximate ion concentration for compounds **1**, **2**, and **3** is given in Figure 1; k was derived from eq **3**.

The symmetrical quaternary oximes of the pyridine series, **7** and **8** undergo two anionic dissociations which overlap at the pH range 7–8. Therefore, each of these compounds occurs as two distinct species which participate simultaneously, but at different rates, in the SN2 reaction with DFP. The pK_a and second-order rate constant for each species has been derived by an elaborate procedure described in detail in an earlier publication.[6] It is remarkable that **8** is a stronger acid than **7**. In both cases, the dianion is only twice as active as the monoanion in terms of k (Table I) and since the compounds are symmetrical, it is inferred that the increase in activity of the dianion by a factor of 2 is simply due to the same increase in the concentration of the reactive species.

The monovalent oximes bearing an aminoalkyl side chain, **11**, **12**, and **14** offer a special case because each of them could give rise to two distinct nucleophilic species under the experimental conditions used. Schematically, these species are $(Me)_2NXCH{=}NO^-$ and $(Me)_2N^+HXCH{=}NO^-$, heretofore and in Table I, denoted as d and b, respectively. A comprehensive treatment of this situation has been given in an earlier paper.[1] The pK_a and k values are given in Table I for the species b and d.

The Brønsted plot relating basicity (pK_a) to nucleophilicity (log k) for the oximes **1–14** is given in Figure 2. The general relationship, calculated by the least-squares method, is given by eq 4 with a correlation factor,

$$\log k = 0.278\text{p}K_a - 1.155 \qquad (4)$$

$r = 0.61$.

The high confidence intervals, however, as calculated from eq 4, do not favor the assumption that all the oximes considered may indeed be treated as a homogenous class. The opposite approach, advocated in an earlier publication in this series,[1] would be to classify all compounds into *four* different groups, in accordance with the magnitude of their deviation, γ, from eq 4, γ being defined as log k measured/log k expected where k measured is the *measured* second-order rate constant and k expected is the *calculated* rate from eq 4. The results, shown in Table II, imply that the closely related quaternary pyridinium aldoximes such as **1** and **3** are members of different groups; also the presence of compound **10**, a quaternary pyrimidinium aldoxime,

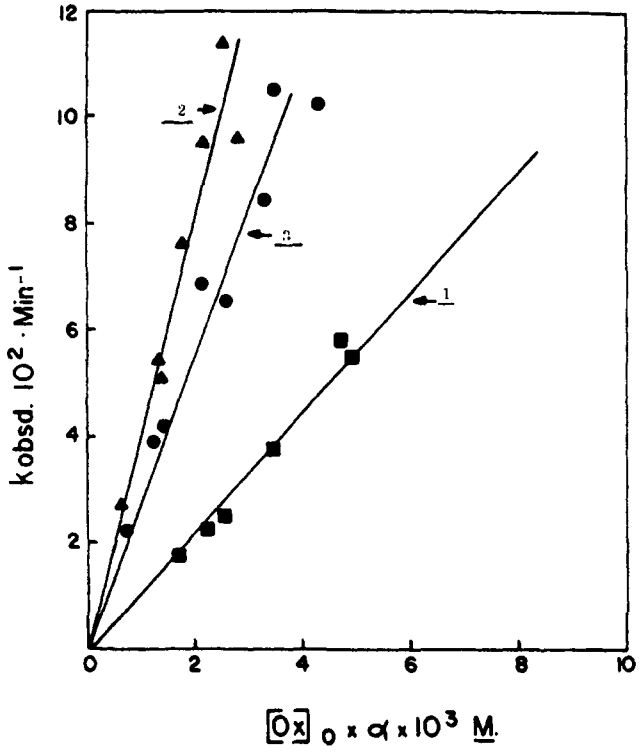


Figure 1.—Plot of k_{obsd} *vs.* oximate anion concentration for the reaction of monovalent oximes with DFP: t, 25°; μ, 0.2.

TABLE II
CLASSIFICATION OF HETEROCYCLIC OXIMES INTO HOMOLOGOUS GROUPS FROM EQUATION 4 (SEE TEXT)

Compd	Group No.	Deviation value	Brønsted equation for the group
3, 2, 7, 11b, 11d, 12d	I	$\gamma > 0.2$	$\log k_1 = 0.269\text{p}K_a - 0.836$; $r = 0.986$
9, 13, 14b, 14d	II	$0.1 < \gamma < 0.2$	$\log k_1 = 0.215\text{p}K_a - 0.452$; $r = 0.984$
1, 8, 12b	III	$-0.05 < \gamma < 0.1$	$\log k_1 = 0.247\text{p}K_a - 0.955$; $r = 0.989$
4, 5, 6, 10	IV	$\gamma < -0.05$	$\log k_1 = 0.407\text{p}K_a - 2.779$; $r = 0.975$

in group 4 is beyond any plausible explanation. In addition, the low values of the slopes in the respective Brønsted equations are not consistent with results reported for analogous systems.[11,12]

We have now abandoned this approach in favor of the more logical presentation of initially grouping the compounds according to some common structural parameter, such as charge on ring nitrogen. Accordingly, if only the data for quaternary oximes were used now for the calculation of the Brønsted equation, one would obtain the relationship

$$\log k = 0.619\ (\pm 0.105)\text{p}K_a - 3.950\ (\pm 0.900) \qquad (5)$$

with a correlation factor, $r = 0.82$. This is shown graphically in Figure 3. The confidence intervals of eq 5 are shown in Figure 4. It may be seen that almost all compounds are included within these intervals with the exception of **4–6**.

B. Reactivation Studies.—In the reactivation of phosphorylated AChE with an oxime the net result is removal of the phosphoryl residue from the active

(11) A. L. Green, G. Sainsbury, B. Saville, and M. Stanfield, *J. Chem. Soc.*, 1583 (1958).
(12) J. H. Smith, *ibid.*, 521 (1943).

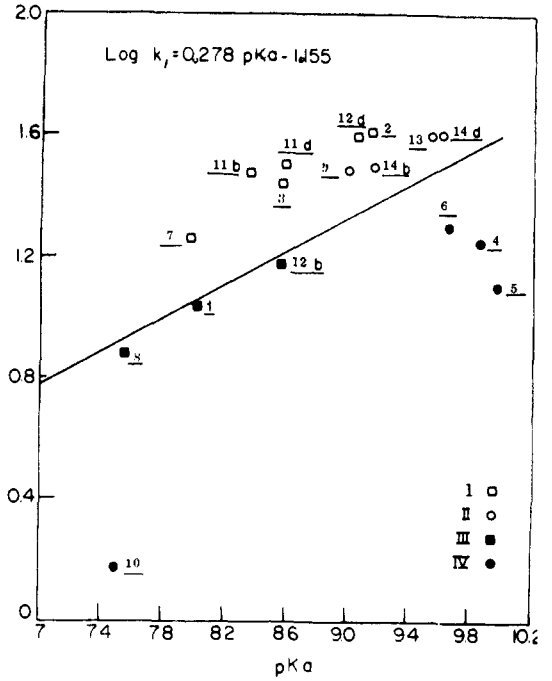


Figure 2.—Brønsted plot calculated from data for all oximes examined: t, 25°; μ, 0.2; for explanation of the different groups see Table II.

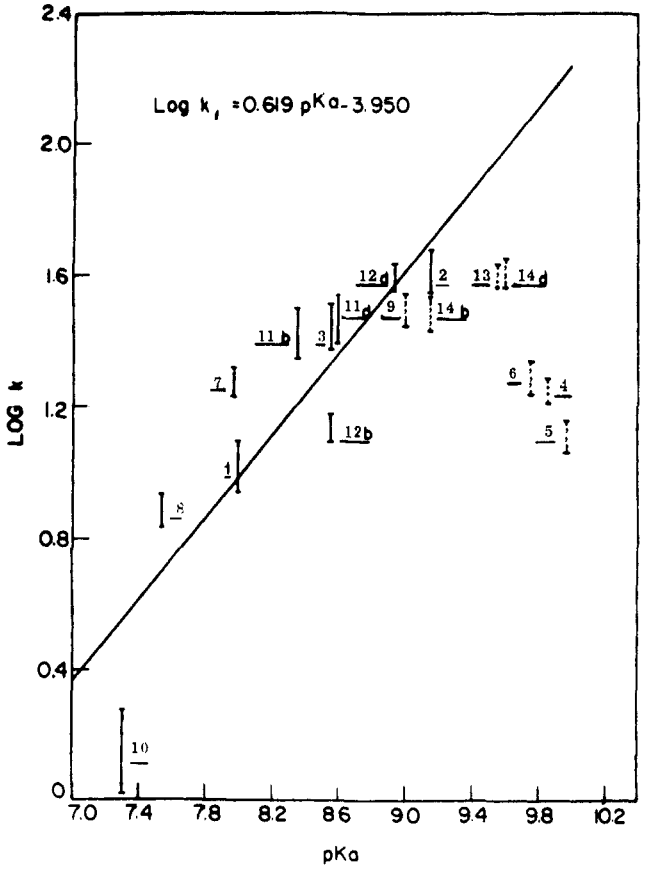


Figure 3.—Brønsted plot calculated from data for quaternary oximes (solid lines).

center of the enzyme. The reaction is most probably related to the SN2 attack of oximate ion on the phosphorus atom of DFP resulting in the liberation of fluoride ion, but is also more complex because of more rigid steric requirements imposed by the enzyme molecule.

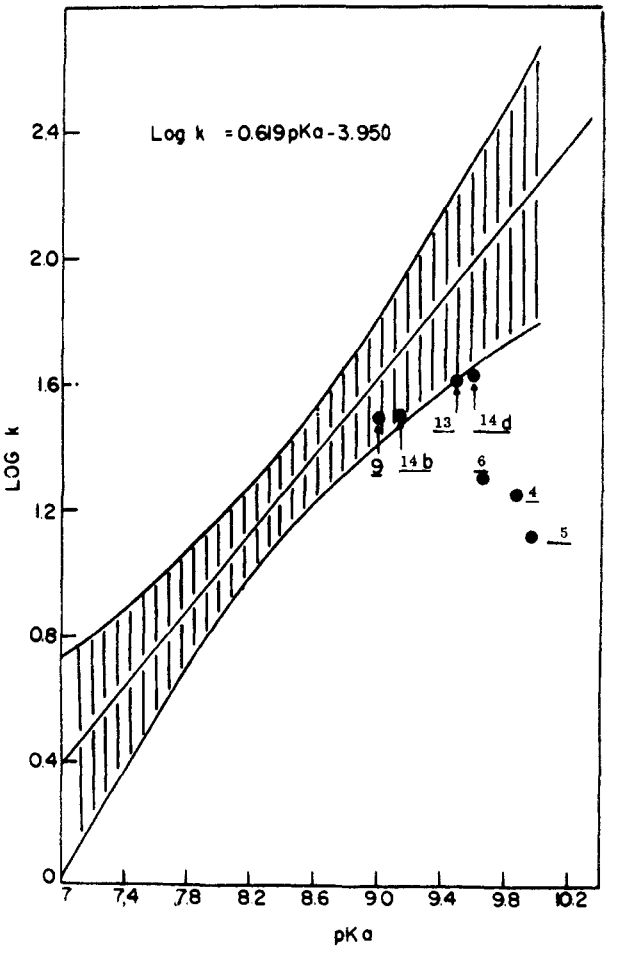


Figure 4.—Confidence intervals calculated for the equation, log $k = 0.619pK_a - 3.950$.

In general, the major processes that lead to reactivation may be represented as follows

$$E_i + Ox \overset{K}{\rightleftharpoons} E_i\text{-}Ox \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E_f + \text{phosphoryl-}Ox \xrightarrow{k_2} E_f + \text{products} \quad (6)$$

where E_i and E_f are, respectively, the inhibited and free enzyme, Ox is the reactivating oxime, and E_i–Ox the reactivator–inhibited enzyme complex. Under conditions where $k_1 \gg k_{-1}$, k_2 becomes almost equal to k_1 and the sequential reaction 6 may be further simplified to

$$E_i + Ox \overset{K}{\rightleftharpoons} E_i\text{-}Ox \xrightarrow{k_r} E_f + \text{products} \quad (7)$$

where k_r is defined as the rate constant of reactivation.

The distinction between affinity of the reactivator molecule toward E_i (K) and rate of displacement of the phosphoryl group (k_r) from the enzyme surface was first drawn by Green and Smith[13] who proposed the use of equation 8, where k_{obsd} is the measured reactivation

$$\frac{1}{k_{obsd}} = \frac{K}{k_r[Ox]} + \frac{1}{k_r} \quad (8)$$

rate. In practice, the backward phosphorylation of the enzyme by the phosphorylated oxime, a powerful inhibitor in itself,[14] could be neglected only if values of the *initial* reactivation rate be considered, *i.e.*, at t_0. Ac-

(13) A. L. Green and H. J. Smith, *Biochem. J.*, **68**, 28 (1958); **68**, 32 (1958).

(14) J. C. Lamb, G. M. Steinberg, S. Solomon, and B. E. Hackley, *Biochemistry*, **4**, 1475 (1965).

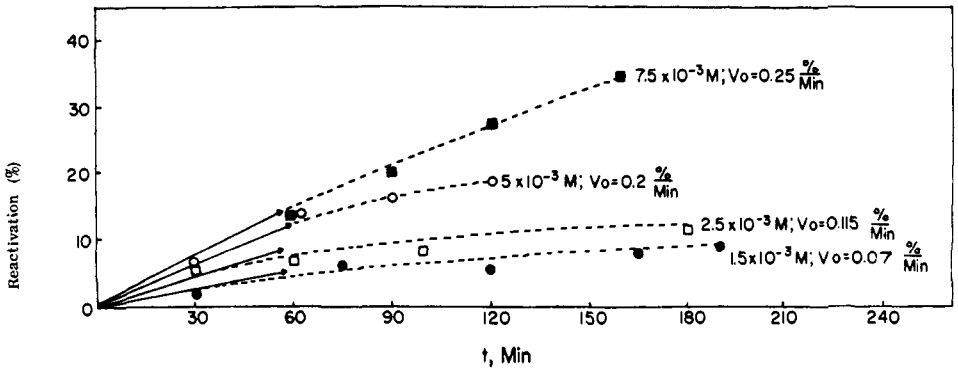


Figure 5.—Plot of per cent reactivation *vs.* time in the reaction between DFP-inhibited AChE and oxime **11**.

TABLE III

SOME KINETIC PARAMETERS OF THE REACTIVATION OF DFP-INHIBITED AChE WITH VARIOUS OXIMES

Oxime	Oxime concn range, *M*	*K*, moles/l. Absolute value	*K*, moles/l. Relative value	k_r, min^{-1} Absolute value	k_r, min^{-1} Relative value	V_0 (per cent of reactivation per minute) at [Ox] = 1.10^{-3} *M* Absolute value	V_0 (per cent of reactivation per minute) at [Ox] = 1.10^{-3} *M* Relative value
1	$1\text{–}5 \times 10^{-3}$	5.45×10^{-3}	4.85	3.56×10^{-3}	18.5	0.55	5.5
3	$1\text{–}10 \times 10^{-3}$	1.03×10^{-3}	1	1.96×10^{-3}	1	0.10	1
5	$0.5\text{–}2 \times 10^{-4}$	1.52×10^{-3}	1.47	1.42×10^{-1}	72	5.5	55
8	$1\text{–}5 \times 10^{-4}$	1.47×10^{-3}	1.42	1.25×10^{-1}	64	5.00	50
10	$1.5\text{–}7.5 \times 10^{-3}$	2.6×10^{-2}	25.2	7.15×10^{-3}	3.70	0.026	0.26
11	$1.5\text{–}7.5 \times 10^{-3}$	2.0×10^{-2}	19.5	1.0×10^{-2}	5.1	0.05	0.5

cordingly, the following modification of eq 8 has been used to calculate K and k_r

$$\frac{100}{V_0} = \frac{K}{k_r[\mathrm{Ox}]} + \frac{1}{k_r} \qquad (9)$$

where V_0 = velocity of reactivation at t_0, expressed as per cent of reactivated enzyme per minute. A typical plot of per cent reactivation *vs.* time for the oxime **11** is given in Figure 5. The graphic solution of eq 9 for the two oximes **1** and **11** is given in Figure 6. Table III summarizes the results for all the oximes that exhibited appreciable reactivation properties. All the remaining compounds considered in this study were ineffective as reactivators of DFP-inhibited AChE.

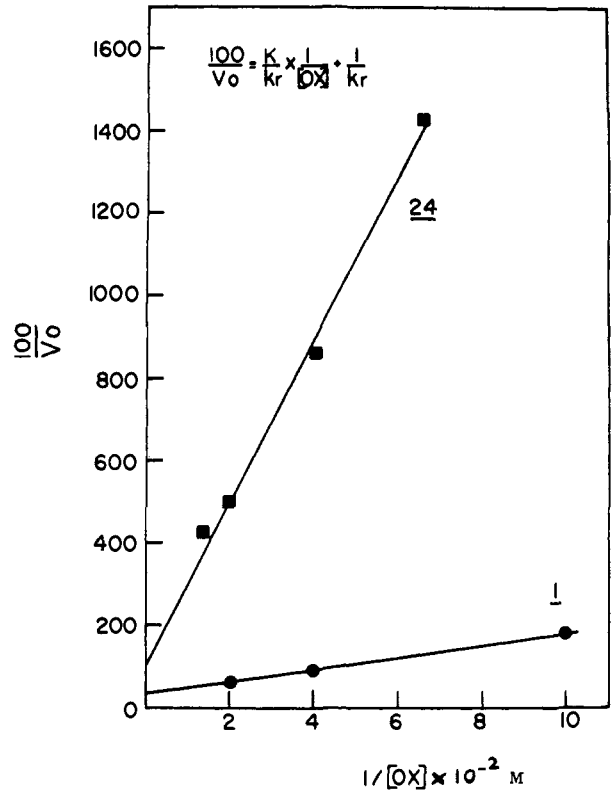


Figure 6.—Plot of $100/V_0$ *vs.* $1/[\mathrm{Ox}]$ in the reactivation of DFP-inhibited AChE with oximes **1** and **24**.

Discussion

In the interpretation of the present results one must remember that the molecular structure of DFP imposes an appreciable steric hindrance in the immediate vicinity of the phosphorus atom. This effect must also find expression in k, the second-order rate constant of the SN2 reaction between DFP and an oximate, in addition to the nucleophilicity of the latter. For example, such an effect could account for the discrepancy in response to "charge effect"[15] between **4** on the one hand, and **5** and **6** on the other, as these are quaternized to **1**, **2**, and **3**, respectively. Compd **1–3** become more reactive toward DFP than their tertiary analogs, although **1** exhibits a reduction in reactivity compared to **4**, most probably on account of *additional* steric hindrance due to the introduction of a methyl group adjacent to the reactive oximate center.

Steric requirements become even more stringent in the case of DFP-inhibited AChE, and this is reflected in the low number of oximes capable of reactivating the enzyme. The paucity of data from reactivation studies poses a serious limitation to any conclusion that may be reached at this time; yet, we feel that certain views may be expressed in the interest of future research.

The affinity constant, K, gives a measure of the concentration of the reactivator molecule at the site of

(15) J. Epstein, P. L. Cannon, H. O. Michel, B. E. Hackley, and W. A. Mosher, *J. Amer. Chem. Soc.*, **89**, 2937 (1967).

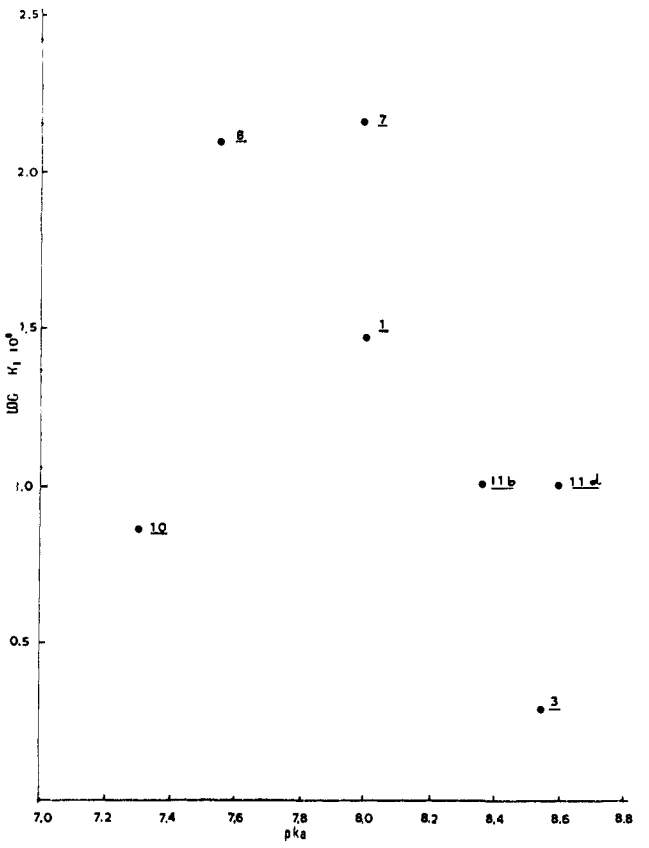


Figure 7.—Relationship between k_r and pK_a for a number of reactivators of DFP-inhibited AChE.

reaction on the enzyme (the lower the value of K, the higher the affinity to the enzyme).[16] No distinction is made here between oximate anion or its conjugate acid. The first-order rate constant, k_r, on the other hand, is independent of affinity and, therefore, should be a function of the basicity or nucleophilicity of the reactivator molecule in a series, provided steric parameters are kept equal for all members of the series. A plot of log k_r against pK_a values for the oximes **1**, **3**, **7**, **8**, **10**, and **11** reveals that the most reactive members are not necessarily the most nucleophilic ones, but rather those having a pK_a value in the range 7.6–8.0 (Figure 7). It must be noted that for the symmetrical oximes **7** and **8**, only the first pK_a's ought be considered since it is highly unlikely that both oximate groups on a single molecule have simultaneous access to the phosphoryl residue in the enzyme active site. As for the oxime **11**, the pK_a values of both forms b and d are given, although it is believed that there should be a preponderance of the former form at pH 7.4 [macroscopic pK_a of the amino group = 8.82[1]] either because of extensive protonation or donation of the nitrogen lone pair of electrons through another process.

In this context, application of the equation of Epstein, *et al.*,[17] relating reactivity, pK_a, and pH should be revealing. $K_a = [H^+](1 - \alpha/\alpha)$, if $[H^+] = 4.10^{-8}$ and $\alpha = 0.619$ (± 0.105) from eq 5, then $K_a = 2.46 \times 10^{-8}$ or pK_a 7.6.

In other words, a member of a series possessing a pK_a of 7.6 should also exhibit the highest k_r value at pH 7.4. At this state of knowledge, these conditions are in fair agreement with the experimental results.

(16) For an excellent treatment of the case of chymotrypsin, see B. F. Erlanger, "Proceedings of the Conference on Structure and Reactions of DFP Sensitive Enzymes," E. Heilbronn, Ed., Swedish Research Institute of National Defence, Stockholm, 1967, p 143.

(17) J. Epstein, H. O. Michel, and W. A. Mosher, *J. Theor. Biol.*, **19**, 320 (1968).

Notes

Comparative Pharmacology of 5-Hydroxytryptamine and Its Benzofuran, Benzo[*b*]thiophen, and Indene Isosteres

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The proposal by Vane[1] that 5-hydroxytryptamine (5-HT) attaches to its receptor site by a 2-point interaction involving the 5-OH group and the terminal NH_2 has received indirect support from experiments with tryptamine isosteres. It has been concluded that replacement of the indolic imino moiety of tryptamines by CH_2 or by S does not alter appreciably either the contractile activity of tryptamines upon the rat stomach fundus preparation[2,3] or their central activity in rabbits as measured by quantitative electroencephalography,[4] and it is apparent that the indolic imino group does not participate significantly in interactions with peripheral or central tryptamine receptors. Nevertheless, it is equally apparent that there exist distinct 5-HT and tryptamine receptors;[3,5] for example, phenoxybenzamine blocks the response of the rat stomach fundus to 5-HT, but is much less effective against tryptamines and their isosteres, which can presumably occupy the phenoxybenzamine-resistant tryptamine (PRT) re-

(1) J. R. Vane, *Brit. J. Pharmacol.*, **14**, 87 (1959).

(2) J. C. Winter, P. K. Gessner, and D. D. Godse, *J. Med. Chem.*, **10**, 856 (1967).

(3) J. C. Winter and P. K. Gessner, *J. Pharmacol. Exp. Ther.*, **162**, 286 (1968).

(4) E. Campaigne, E. S. Neiss, C. C. Pfeiffer, and R. A. Beck, *J. Med. Chem.*, **11**, 1049 (1968).

(5) For reviews, see (a) S. Garattini and L. Valzelli, "Serotonin," Elsevier, Amsterdam, 1965; (b) L. B. Kier, "Fundamental Concepts in Drug-Receptor Interactions," J. F. Danielli, J. F. Moran, and D. J. Triggle, Ed., Academic Press, London, 1970, pp 15–45.