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Nucleophilicity of Heteroaromatic Aldoximes Bearing an Aminoalkyl Side Chain[1]

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Rate constants are determined for the SN2 reaction in aqueous solution between diisopropyl phosphorofluoridate and various heterocyclic oximes bearing an aminoalkyl side chain. All measurements are based on F^- formation. The Brønsted plot relating basicity (pK_a) to nucleophilicity ($\log k_2$) indicates that the various oximes examined cannot be treated as a single class. A postive charge on the aminoalkyl side chain makes no contribution to nucleophilicity.

In a preceding paper[2] we reported that 4-hydroximinomethyl-**1**-[3′-(*N*,*N*-dimethylamino)-*n*-propyl]pyridinium chloride (**1**) is a powerful reactivator of acetylcholinesterase (AcChE) that had been inhibited with diisopropyl phosphorofluoridate (DFP). More recently Edery, *et al.*,[3] have also shown that **1** possesses excellent antidotal properties against organophosphate intoxication.

Structurally, **1** belongs to a series of *N*-alkylpyridinium aldoximes[2] (structure A, Table I), members of which display varying degrees of reactivity towards the phosphorylated enzyme. However, **1** is exceptional in being the first member bearing a tertiary rather than a quaternary amino group at the side chain and which is still endowed with a high order of reactivity *in vitro* as well as *in vivo*. This observation prompted a more detailed study of the nucleophilicity of **1** toward DFP as a convenient substrate, the reaction being a rational model for the second step of the reactivation process.[4] We have extended this study also to include analogs of **1** in both the pyridine and pyrimidine series (structure B, Table I). To this end, it was necessary to synthesize a number of new oximes.

Epstein, *et al.*,[5] observed that anions of hydroxybenzenes or hydrated aldehydes bearing cationic groups are more reactive toward isopropyl methylphosphonofluoridate in aqueous solution than corresponding hydroxybenzenes or hydrated aldehydes of the same proton basicity but devoid of cationic groups, whereas ketoximes and hydroxamic acids are much less sensitive to "charge" effects. The relevance of this finding to the present case will be discussed in the appropriate section.

Experimental Section

Materials.—Some of the oximes used are known compounds. These are: 2-hydroximinomethyl-1-*N*-methylpyridinium iodide[6] (**2**), mp 218–220°; 3-hydroximinomethyl-1-*N*-methylpyridinium iodide[6] (**3**), mp 148–152°; 4-hydroximinomethyl-1-*N*-methylpyridinium iodide[6] (**4**), mp 177–180°.

3-Hydroximinomethyl-1-*N*-[3′-(*N*,*N*-dimethylamino)-*n*-propyl]pyridinium chloride hydrochloride (**5**) was prepared according to the procedure described for **1**[2] except that the aldoxime used was 3-hydroximinomethyl pyridine (Aldrich).

4-Hydroximinomethyl-1-methyl-[1H,6H]-6-pyrimidone (**6**) was prepared according to the general instruction sgiven by Piantadosi, *et al.*[7] Na (1.1 g) was dissolved in EtOH (30 ml), then *N*-methylthiourea (4.5 g) and ethyl γ,γ-diethoxyacetoacetate[8] (10.0 g) were added, and the solution was refluxed for 2.5 hr. The solvent was evaporated under reduced pressure and the residual oil poured onto cold HCl (5%, 100 ml) and stirred for 15 min. The precipitate obtained (4 g) after being washed with H_2O and Et_2O was used immediately in the next step. Its spectrum (see Table II) agrees with the structure for **4-diethoxymethyl-1-methyl-2-thiono-[1H,2H,3H,6H]-6-pyrimidone** (**7**).[7] Compound **7**, desulfurized according to a known procedure,[9] gave **4-diethoxymethyl-1-methyl-[1H,6H]-6-pyrimidone** (**8**). The latter (**8**) (5.0 g) was treated with boiling H_2SO_4 (10%, 30 ml) containing $H_2NOH \cdot HCl$ (2.5 g). After 10 min, the solution was cooled and brought to pH 5 with dilute NaOH. The oxime **6** (3.5 g) was obtained as a precipitate.

4-Hydroximinomethyl-1-[3′-(*N*,*N*-dimethylamino)-*n*-propyl]-[1H,6H]-6-pyrimidone (**9**) was prepared as follows: thiourea (3.8 g) and ethyl γ,γ-diethoxyacetoacetate (10 g)[8] were dissolved in NaOEt in EtOH (1*M*, 100 ml) and stirred at room temperature for 24 hr. The solution was then poured with cooling and stirring onto dilute H_2SO_4 (1 *N*, 200 ml). After 30 min the solution was brought to pH 4 and the precipitate obtained (4 g) was washed with H_2O and with Et_2O. Its spectrum agrees with the structure for **4-diethoxymethyl-2-thiono-[1H,2H,3H,6H]-6-pyrimidone** (**10**).[7] Compound **10** (5 g), desulfurized with Raney Ni as described earlier,[9] gave **4-diethoxymethyl-[1H,3H,6H]-6-pyrimidone** (**11**) (2.5 g).

Compound **11** was then converted into **9** as follows.[10] Its solution (2 g) in DMF (50 ml) was treated with NaH (0.8 g), then with anhyd 3-(*N*,*N*-dimethylamino)-*n*-propyl chloride (2 g) under N_2. The chloride had been previously freed from its HCl salt with excess aq NH_3 (28%) and extracted into Et_2O. The resulting solution was stirred for 1 hr at room temperature, then at 80° for 15 hr. The solvent was removed under reduced pressure (45–55° at 0.5 mm) and the residue refluxed in H_2SO_4 (10%, 20 ml) containing $H_2NOH \cdot HCl$ (2 g). The pH was adjusted to 8 and the solvent evaporated to dryness in a flash evaporator. The residual solid was extracted with EtOH and the dry extract (1.4 g) subjected again to continuous extraction with Me_2CO. Evaporation of the solvent gave pure **9**. The assignment of the site of alkylation at N_1 is borne out by the similarity of the spectra of **9** and **6**. Some physical constants of the new compounds and experimental data are given in Table II.

Diisopropyl phosphorofluoridate was prepared according to Chapman and Saunders[11] and purified by fractional distillation.

Dissociation Constants.—All measurements were made at a total ionic strength of 0.2 at 25°. The dissociation constants for **2, 3, 4,** and **6** were determined titrimetrically according to Albert and Serjeant.[12] In the case of **1, 5,** and **9** where two prototropic changes occur simultaneously, the macroscopic constants were

[1] This is part IV of a series: part III: Y. Ashani and S. Cohen *J. Med. Chem.*, **11**, 967 (1968).

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

(3) H. Edery, D. Soroker, and W. Kuhnberg, *Israel J. Med. Sci.* (in press).

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

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

(6) S. Ginsburg and I. B. Wilson, *ibid.*, **79**, 481 (1957).

(7) C. Piantadosi, V. G. Skulason, J. L. Irvin, J. M. Powell, and L. Hall, *J. Med. Chem.*, **7**, 337 (1964).

(8) T. B. Johnson and L. A. Mikeska, *J. Amer. Chem. Soc.*, **41**, 810(1919).

(9) A. Vincze and S. Cohen, *Israel J. Chem.*, **4**, 23 (1960).

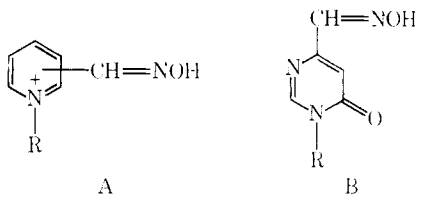
(10) A. P. Martinez and W. W. Lee, *J. Org. Chem.*, **30**, 317 (1965).

(11) N. B. Chapman and B. C. Saunders, *J. Chem. Soc.*, 1010 (1948).

(12) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co. Ltd., London, 1962.

Table I

Heterocyclic Aldoximes



A B

Compd	–CH=NOH	R	Compd	R
1	4	$-(CH_2)_3NMe$	6	Me
2	2	Me	9	$-(CH_2)_3NMe_2$
3	3	Me		
4	4	Me		
5	3	$-(CH_2)_3NMe_2$		

Rate measurements were made at a total ionic strength of 0.2 at 25° and are all based on rate of F^- formation. The rationale of this approach and experimental details have been presented in an earlier publication,[4] except that buffer concentration in the present work was 0.05 *M*.

Results and Discussion

The most salient feature of the oximes **1**, **5**, and **9** which embody the *N*,*N*-dimethylamino-*n*-propyl group is the occurrence in the molecule at neutral or near neutral pH of two prototropic processes which would certainly overlap, giving rise to four distinct species. This follows from the computation of the pK_a values, given in Table III. In the case of **1**, the situation may be represented by Scheme I.

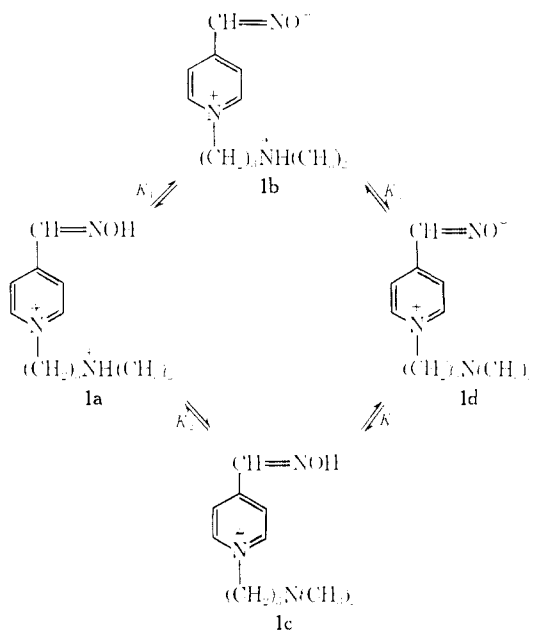
Table II

Physical and Chemical Data of Some New Heterocyclic Oximes

Compd	% yield[a]	Solvent of recrystn	Mp,[b] °C	R_f[c]	λ_{max} (mμ) 0.1 *N* HCl	0.1 *N* NaOH	pH 7.0	Anal.[d]
5	50	EtOH-*i*-PrOH	225–227		230, 250 (s), 280 (s)	247, 295	230, 250 (s), 280 (s)	$(C_{11}H_{19}Cl_2N_3O)$ C, H, Cl
6	68	EtOH	256–257	0.78[e] 0.54[g]	230, 280 (s)	255 (s), 298	232, 280 (s)	$(C_6H_9N_3O_2)$ C, H, N
7	36	*h*	122–125		220, 275	234, 262, 317	222, 272	*h*
8	60	*h*	135–140		223, 272	231, 272	225, 272	*h*
9	54	Me_2CO	173–176	0.74[f]	230, 280 (s)	260, 300	232, 280 (s)	$(C_{10}H_{16}N_4O_2)$ C, H, N
10	46	*h*	158		215, 273	260, 309	266, 310	*h*
11	57	*h*	119–120		225, 261	230, 270	223, 269	*h*

[a] Calculated for crude product. [b] Uncorrected, on Kofler microscopic hot stage. [c] On Whatman No. 1 filter paper, ascending. [d] Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values. [e] Cl: calcd, 25.4; found, 24.7. [f] Developed with H_2O satd with BuOH. [g] Developed with BuOH satd with H_2O. [h] Obtained as a precipitate and used without any further purification. [i] Developed with BuOH(40), PrOH (40), EtOH (10), 28% NH_3 (45), H_2O (15) by volume.

Scheme I



separated according to a previously published procedure.[4] The *microscopic* constants for these compounds were then determined spectrophotometrically according to Edsall, *et al.*[13] Measurements were made at 340 mμ for oximes **1** and **9** and 325 mμ for oxime **5**.

In all cases, pK_1 is appreciably lower than pK_3 showing that interaction with the positively charged N of the side chain is still appreciable over the distance involved. This is most probably due to a field rather than to an inductive effect.[13] Also, in the case of **1** and **5** proton abstraction from the trialkylamino group takes precedence over that from the oxime group ($pK_2 < pK_1$). However, the contrary is true for the pyrimidine derivative **9** ($pK_1 < pK_2$) where the vicinity of the carbonyl oxygen may contribute to the stability of the species **9b** by internal hydrogen bonding.

The validity of these results is corroborated by the close agreement between the values of pK_3 and the values of the corresponding analogs **3**, **4**, and **6** in which only a single dissociation, that of the oxime group, is possible. Also the values of the macroscopic constants *calculated* from the microscopic ones, are in good agreement with the macroscopic values determined by an independent procedure.

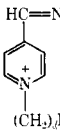
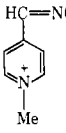
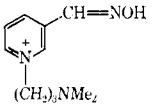
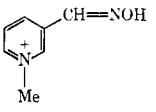
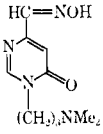
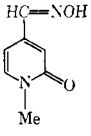
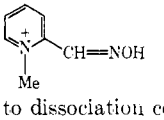
In view of these findings, it has been concluded that in all rate measurements of the nucleophilic displacement of F^- by an oximate of type **1**, **5**, or **9** one must necessarily reckon with the simultaneous participation in this reaction of at least two species, such as **1b** and **1d** (Scheme I):

$$\mathbf{1b} + DFP \xrightarrow{k_b} F^- + \text{products}$$

$$\mathbf{1d} + DFP \xrightarrow{k_d} F^- + \text{products}$$

(13) J. T. Edsall, R. B. Martin, and B. R. Hollingworth, *Proc. Natl. Acad. Sci. U. S.*, **44**, 505 (1958).

TABLE III
MICROSCOPIC AND MACROSCOPIC pK VALUES OF SOME OXIMES BEARING AN AMINOALKYL GROUP

Compd	Structure	Microscopic values[a] pK1	pK2	pK3	pK4	Macroscopic values obsd pK oxime	pK amine	calcd[b] pK oxime	pK amine
1		8.36	8.2	8.6	8.45	7.95	8.82	7.94	8.81
4				8.55					
5		8.55	8.32	9.07	8.84	8.17	9.29	8.12	9.27
3				9.15					
9		9.15	9.45	9.60	9.80	9.15	9.95	8.98	9.95
6				9.55					
2				8.00					

[a] Subscripts refer to dissociation constants bearing the same numbers in Scheme I. [b] Calcd according to J. T. Edsall, R. B. Martin, and B. R. Hollingworth, *Proc. Nat. Acad. Sci. U. S.*, **44**, 505 (1958).

The rate of appearance of F^- in solution at a given pH may then be expressed by the equation:

$$\frac{d[F^-]}{dt} = k_b[DFP][\mathbf{1b}] + k_d[DFP][\mathbf{1d}] \quad (1)$$

If total oxime concentration is $[Ox]_0$, fraction **1b** is β, and fraction **1d** is δ, then,

$$\beta = \frac{[H^+]K_1}{[H^+]^2 + K_2[H^+] + K_1[H^+] + K_2K_3} \quad (2)$$

$$\delta = \frac{K_2K_3}{[H^+]^2 + K_2[H^+] + K_1[H^+] + K_2K_3} \quad (3)$$

When the oxime is in large excess over DFP, then eq **1** becomes

$$\frac{d[F^-]}{dt} = [DFP]k_{obsd} \quad (4)$$

where

$$k_{obsd} = [Ox]_0[k_b\beta + k_d\delta] \quad (5)$$

By measuring k_{obsd} at least at two different pH values and assigning to β and δ their respective values from eq 2 and 3, then the set of simultaneous equations obtained may be solved for the two unknowns k_b and k_d.

By applying the same procedure to the reaction of DFP with **5** and with **9**, the rate constants for forms "**b**" and "**d**" of these compounds have been obtained. The results are given in Table IV.

TABLE IV
SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF VARIOUS OXIMATES WITH DFP[a]

Compd	Ionic species[b]	Moles^{-1} min^{-1}[c]
1	b	28.0
	d	31.0
4	d	28.5
5	b	14.0
	d	40.0
3	d	41.5
9	b	32.0
	d	42.0
6	d	40.0
2	d	11.0

[a] pH range, 7–9, oxime concentration 5.10^{-4}–5.10^{-3} *M*. [b] See Scheme I for explanation of symbols. [c] Standard deviation ± 10%.

Perhaps more revealing is the Brønsted plot for the various oximates relating their basicity (pK_a) to their reactivity (log k_2) (Figure **1**). All the oximes studied in the present work are classical "α-nucleophiles" and therefore according to the findings of Epstein, *et al.*,[5] they are not expected to respond to the "charge" effect. This requirement is strictly satisfied with only one exception (*e.g.*, where the positive charge is in the ring), wherever the location of charge is at the side chain. Thus, either in the pyridine series, **1b**, **1d**, **3d**, **4d**, and **5d**, or the pyrimidine series, **6d**, **9b**, and **9d**, the re-

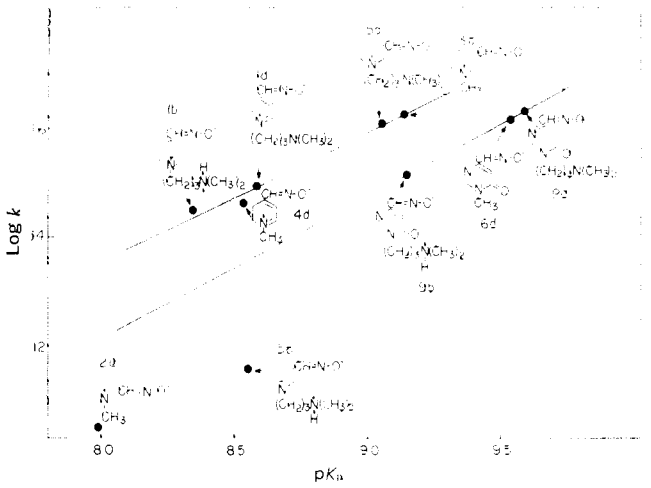


Figure 1.—Plot of log k *vs.* pK for the reaction of heteroaromatic oximate ions with DFP at 25°, μ = 0.2.

activity of the oximate is related to its pK_a, with but very slight deviations, by the expression:

$$\log k_2 = 0.23\ pK_a - 0.50 \text{ for the pyridine series}$$

$$\log k_2 = 0.25\ pK_a - 0.80 \text{ for the pyrimidine series}$$

The low values of the slopes (0.23 and 0.25) obtained in this study are not consistent with those reported by other authors using analogous systems.[14] A possible explanation for this difference could be due to the relatively weak bonds involved in the transition stages. This in turn would be reflected in the relative stability of DFP to nucleophilic attack.[15]

Were the charge on the ring nitrogen in the pyridine series also without effect on nucleophilicity because of the rigid classification of this group of compounds as "α-nucleophiles," then one would have expected members of both series to fall on the same plot. Actually the deviation, for example for compound **3d** (pK_a = 9.15), between the observed and expected value is log (k_2/k_c)[16] = 0.13. Considered in the light of the work of Epstein, *et al.*,[5] this value seems to be too low to be of practical significance. However, the present results do not justify such an assumption.

Deviations as low as log k_2/k_c = 0.1 may reflect some fundamental differences in the properties of the nucleophiles under consideration, such as charge localization or spatial conformation.

The position of **5b** and **2d** on the Brønsted plot is paradoxical and difficult to explain. Field effects are perhaps more operative in the case of **5** than in the case of either of its analogs **1** or **9**. Thus $\Delta\ pK_a$ for species **b** and **d** for **5** has a higher value (0.52) compared with the corresponding value for **1** (0.24) or **9** (0.40). Such effects may be of such consequences in **5b** that the spatial conformation of this species could be altered enough to justify, again, its inclusion in a separate class (log k_2/k_c for **5b** = −0.20).[16] The same may be said for **2d** in which the charged centers are even closer to each other (*e.g.*, log k_2/k_c = −0.17).[16]

The implications of the present findings to the reactivation process proper lead to a number of speculations which could be of practical importance. A charged N at the side chain of the reactivator molecule (*e.g.*, **1**) does not contribute to the nucleophilicity of the oximate ion *vs.* the phosphoryl group with respect to the uncharged species. Perhaps of greater consequence is the charge on the N which forms part of the heterocyclic system. Clearly, the pyridinium oximates, notwithstanding "conventional" drain of electrons by conjugative or other effects, are more nucleophilic than their "uncharged" pyrimidine analogs. This finding, which supports the principle underlying the approach of Epstein, *et al.*,[5] brings to mind the role of the pyridinium N in such compounds as TPN[17] and necessarily raises the question on the exact function of the charged nitrogen in reactivators of type A.

Acknowledgment.—The excellent technical assistance of Miss Judith Shragai is herewith acknowledged.

(14) For a comprehensive treatment of the subject, see R. F. Hudson, "Structure and Mechanism in Organo-Phosphorus Chemistry," Academic Press, London and New York, 1965, pp 99–102.

(15) J. Wolinski and K. Sawicki, *Ann. Soc. Chim. Polonorum*, **38**, 745 (1964).

(16) k_c is the calculated rate from the equation, log k_c = 0.25 pK_a − 0.80.

(17) P. Karlson "Introduction to Modern Biochemistry," Academic Press, London and New York, 1963, pp 94–98.