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Acetylcholinesterase Reactivators. Pyridyl and Anilyl Trifluoromethyl Ketoximes

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The synthesis of 2-, 3-, and 4-trifluoromethyl ketoxime isomers of pyridine and *N,N*-dimethylaniline is described. The *in vitro* testing on phosphorylated AchE revealed good reactivating potency for 3-pyridinium trifluoromethyl ketoxime methochloride (1Mb). The compounds were weak reactivators *in vivo*. In protective studies, **1a**, **1b**, and **1c** (2-, 3-, and 4-pyridyl trifluoromethyl ketoximes) were found to be quite active in protecting against several LD_{50} of either Paraoxon or Sarin.

Since the discovery of oximes[1] as potent reactivators of organophosphorus-inhibited acetylcholinesterase (AchE), 2-pyridyl aldoxime methiodide (2-PAM)[2] has been found to be be particularly effective, and most oximes assayed as potential reactivators have been modeled after this aldoxime. Many *N*-pyridinium derivatives of 2-PAM have thus been investigated.[3-6] Derivatives where the pyridine ring has been substituted or replaced by other N-heterocycles are also described as AchE reactivators.[5,7-9]

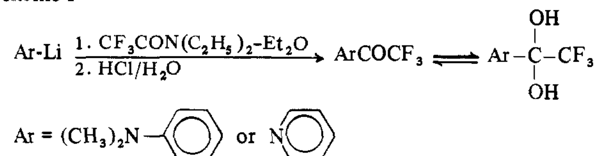
On the other hand, most oximes utilized or investigated as AchE reactivators are aldoximes, and only a few examples are known where the aldehydic hydrogen of 2-PAM has been substituted in order to obtain ketoxime analogs. The reactivating potencies of methyl[10] and phenyl[11] ketoxime derivatives of 2-PAM have been assessed. The phenyl ketoxime analogs although many times less active than the corresponding aldoximes are much better reactivators of organophosphorus-inhibited AchE than the methyl ketoxime analogs. While phenyl 3-pyridinium ketoxime methiodide is an excellent reactivator of methylsulfonylated AchE[12] most pyridinium aldoximes including 2-PAM are not. A closely related oxime, 2-pyridinium 1-acetophenone oxime methiodide shows one-sixth of the reactivating potency of 2-PAM with phosphorylated AchE.[13] Recently, 2-pyridyl 2-pyridinium ketoxime methyl bromide has been shown to be over 20 times as active as phenyl 2-pyridinium ketoxime methiodide.[14]

Since the substitution of the aldehydic hydrogen of 2-PAM analogs by an electron-rich group such as phenyl or pyridyl has led to active AChE reactivators, it appeared of interest to prepare some pyridinium and anilinium trifluoromethyl ketoximes (Table I, **1**, **1M**, **2**, **2M**). The rationale of the trifluoromethyl substituent was to obtain a pK_a value similar to that of 2-PAM while retaining an electron-rich but less bulky group in proximity of the oxime function.

The *in vitro* reactivating potencies of these new ketoximes have been evaluated with AchE inhibited by isopropylmethylphosphonofluoridate (Sarin), and correlations have been attempted with their structural and physical properties. Some *in vivo* data are also reported.

Chemistry. The new oximes were obtained in high yields (75% to quantitative) by standard techniques, *i. e.*, by refluxing the corresponding trifluoromethyl ketone or its *gem*-diol in a H_2O-MeOH solution with $NH_2OH \cdot HCl$ and sodium acetate. The trifluoromethyl ketones, some of which form very stable *gem*-diols, were prepared according to Scheme I as described previously.[15] The quaternization

Scheme I



of the basic nitrogen of the aryl moiety of the trifluoromethyl ketoximes was effected by refluxing with MeI in anhydrous EtOH or Me_2CO. The latter was found to be a better solvent giving cleaner reactions and better yields. The quaternary iodides were then converted to the chlorides by passing the former over an ion-exchange resin (IRA/400).

N,N-Dimethyl 2-trifluoromethyl ketoxime could only be quaternized by prolonged heating (15 days) with MeI and gave only a poor yield (26.5%).

Determination of the pK_a. Effects of the CF_3 Group on the Oximino Function. The pK_a's of the trifluoromethyl ketoximes were measured by a spectrophotometric method[16] and are shown in juxtaposition to those of their aldoxime analogs in Table II. Dyatkin and coworkers[17] pre-

†Taken from the Ph.D. thesis of M. Saucier, University of Montreal, 1970.

Table I. Trifluoromethyl Ketoximes

$N\text{-}C_5H_4\text{-}C(CF_3)\text{=NOH}$ (**1**); $Me\text{-}N^+C_5H_4\text{-}C(CF_3)\text{=NOH}$ (**X-1M**); $Me_2N\text{-}C_6H_4\text{-}C(CF_3)\text{=NOH}$ (**2**); $Me_3N^+\text{-}C_6H_4\text{-}C(CF_3)\text{=NOH}$ (**X-2M**)

Compd	Position of oximino group	Reaction time, hr	Yield, %	Mp, °C Base	Mp, °C MeI	Mp, °C MeCl	Nmr abs (τ)[p] OH	Nmr abs (τ)[p] Me	Uv absorption λ_{max}, μ	Uv absorption ϵ_{max}, 10^3	Formula	Analyses
1a[a]	2	12	86	90.5–91.5[h]			−4.98		265[r]	4.6	$C_7H_5F_3N_2O$	C, H, N
1b[a]	3	5	quant	136–137[i]			−2.55		260[r]	5.35	$C_7H_5F_3N_2O$	C, H, N
1c[a]	4	24	82	149–150[j]			−3.05		262[r]	7.27	$C_7H_5F_3N_2O$	C, H, N
2a[a]	2	16	77	65.5–66[h]			−1.55				$C_{10}H_{11}F_3N_2O$	C, H, N
2b	3	12	quant	90–91[k]			−1.40		244, 238[s]	15, 13.5	$C_{10}H_{11}F_3N_2O$	C, H, N
2c	4	27	89	180–181[l]			−1.14		234[s]	19.5	$C_{10}H_{11}F_3N_2O$	C, H, N
Ph[b]		3	85	83–84[h]								
1Ma	2	120	90[c,d]		170	194–194.5[m]		5.87	305, 264[q]	2.28, 5.76	$C_8H_8ClF_3N_2O$	C, H, N, Cl
1Mb	3	120	93[c,d]		183–184	234–235[m]		5.80	285, 245, 262[q]	4.8, 7.0, 3.5	$C_8H_8ClF_3N_2O$	C, H, N, Cl
1Mc	4	72	87[c,e]		202–203	204–205[m]		5.75	338–267[q]	8.48, 8.10	$C_8H_8ClF_3N_2O$	C, H, N, Cl
2Ma	2	15 days	30[c,f]		167	173–174[n]		6.40–6.56	228[q]	5.7	$C_{11}H_{14}ClF_3N_2O$	C, H, N, Cl
2Mb	3	24	quant[c,e]		213–214	216.5–217[o]		6.34	228[q]	5.4	$C_{11}H_{14}ClF_3N_2O$	C, H, N, Cl
2Mc	4	85	60[c,g]		161–162	181.5–182[n]		6.55	230[q]	6.2	$C_{11}H_{14}ClF_3N_2O$	C, H, N, Cl

[a]Hydrates as starting material. [b]Phenyl trifluoromethyl ketoxime. [c]Methiodides. Reaction solvent. [d]Me_2CO. [e]EtOH. [f]MeI. [g]PhH. Recrystallized from [h]Ligroin (30–60°). [i]EtOH-H_2O. [j]$PhCH_3$. [k]*n*-Hexane. [l]PhH. [m]EtOH-Me_2CO. [n]MeOH-Et_2O. [o]Abs. EtOH. [p]15% Me_2CO solution with Me_4Si. [q]H_2O solution. [r]MeOH-H_2O 1% solution. [s]MeOH-H_2O 10% solution.

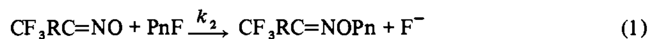
Table II. Rates of Hydrolysis of Sarin by the Trifluoromethyl Ketoximes at pH 7.6 and 30° (Aqueous KNO_3, 0.1 *M*)

Oximes[a]	pK_a	pK_a of corresponding aldoximes[b]	$t_{1/2}$, min	log *k* obsd	log *k* calcd[c]
1a	8.55	10.15	3.25	2.25	2.23
1b	8.51	10.34	2.6	2.26	2.16
1c	8.36	9.93	2.6	2.16	2.09
1Ma	5.95	7.82	55.0	−0.07	0.56
1Mb	7.25	9.01	34.0	0.32	1.40
1Mc	6.88	8.33			
2Mb	8.23		1.75	2.23	2.03
2Mc	8.36		1.5	2.43	2.11
2PAM		7.82	1.7	2.08	1.88

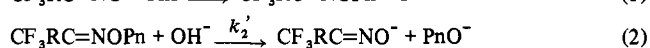
[a]Oximes **2a**, **2b**, and **2c** were not amenable to kinetics studies for reasons of solubility; their respective pK_a values are: 9.45, 9.30, and 9.65. Oxime **2Ma** which has a pK_a of 7.38 gives buffer properties to the reaction medium thus making the pH-Stat technique inadequate for kinetics. Oxime **1Mc** gives complex kinetics. [b]Taken from ref 19. [c]Calculated with the equation given by Epstein.[25] $\log k_2 = 0.642\ pK_a - 3.25$.

viously showed that the highly electronegative trifluoromethyl group greatly enhances the acidic properties of the oximino function. In Table II, it can be seen that the substitution of the aldehydic hydrogen of aldoximes by a CF_3 group increases the acidity of the oximino function by a constant factor (−1.70 ± 0.15 pK_a units). For the new trifluoromethyl ketoximes, the dependence of pK_a upon the sum of Hammett's σ substituents[18] was found to be a straight line expressed by eq 1 (Figure 1). This equation was obtained by a least-squares regression analysis of the variables, the scatter ($S_{1,2}$) being 0.17 and the *f* value 398. The σ values for the pyridyl and pyridinium radicals are the ones determined by Blanch.[19]

Nucleophilicity. Sarin was used as a convenient substrate to study the nucleophilic reactivity of the new oximes, the reaction being a rational model for the second step of the reactivation process.[20] The reaction of ketoximes with Sarin is limited to two steps.[21,22] In the initial one, the oximate ion is phosphonylated according to an SN2 mechanism with production of 1 mole of hydrofluoric acid.[23] In the second step, the phosphonylated oxime is susceptible to an SN2 attack by the hydroxyl ions to regenerate the oxime and produce a mole of isopropyl methylphosphonic acid.[21,22]



$$CF_3RC{=}NO + PnF \xrightarrow{k_2} CF_3RC{=}NOPn + F^- \quad (1)$$



$$CF_3RC{=}NOPn + OH^- \xrightarrow{k_2'} CF_3RC{=}NO^- + PnO^- \quad (2)$$

Where R represents the aromatic group of the new trifluoromethyl ketoximes, and Pn = $(i\text{-}PrO)CH_3P{=}O$.

The relative nucleophilic reactivity of the new oximes was thus obtained by a pH-Stat method utilizing the rate of acid production as a mean of measuring the rate of step one.[24] In order to employ this method it was necessary to demonstrate that the first step was the limiting one. This was accomplished by determination of the stoichiometry of acid production. The reaction of a tenfold excess of trifluoromethyl ketoxime with Sarin showed in all cases first-order kinetics and stopped after the production of 1 ± 5% mole of acid. Thus under the experimental conditions used, the phosphonylated oximes are stable for a period of time exceeding the half-time of the phosphonylation reaction. Oxime **1Mc** was the only exception. Its reaction with Sarin does not obey first-order kinetics and it shows an end point of 2.64 moles of acid which is in good agreement with the calculated value of 2.83 for the two steps, taking into account

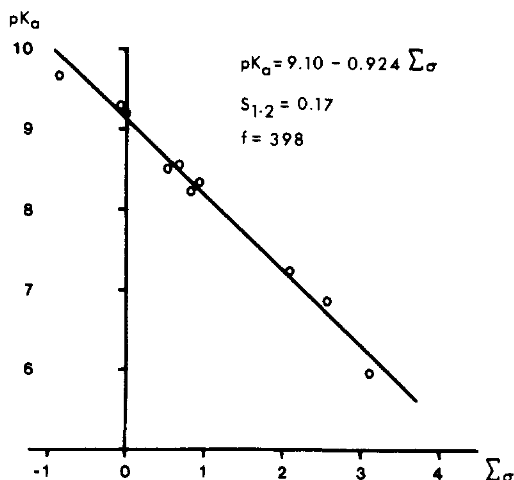


Figure 1. Correlation of Hammett's σ constants and pK_a of the trifluoromethyl ketoximes.

the ionization of **1Mc** at pH 7.6. Thus phosphonylated oxime **1Mc** is not stable and its rate of hydrolysis is of the same order of magnitude as its rate of formation giving a complex kinetics. Our standard oxime 2-PAM produced 1.82 moles of acid with first-order kinetics up to 85% of the end point.

The phosphonylation rates of the new oximes and that of 2-PAM are shown in Table II. It is seen that the nucleophilic character of the trifluoromethyl oximate anion is related to the pK_a of the corresponding oxime by the equation given by Epstein[25] for ketoximes reacted with Sarin under the same conditions. As observed by Ashani and Cohen,[26] deviations are greater when the quaternary nitrogen is part of the aromatic ring. Table II shows that the trifluoromethyl ketoximes with pK_a values ranging from 8 to 8.5 exhibit a nucleophilic reactivity comparable to that of 2-PAM.

Enzymology. The reactivating potency of the trifluoromethyl ketoximes was evaluated on bovine erythrocyte AchE inhibited with Sarin by a technique developed by J. J. Norman.‡

An aliquot of a stock solution of AchE is diluted and inhibited by Sarin. The excess of Sarin is removed by a continuous liquid-liquid extraction technique using *n*-hexane. Blanks were run with native enzyme, and no loss of activity could be detected following the extraction procedure. We also made sure that the excess of Sarin was completely eliminated by the extraction. For that purpose we used a technique described by Heilbronn[27] which consists in evaluating the esteratic activity of the native enzyme mixed with the inhibited enzyme after extraction. In all cases, no inhibition was observed. Thus after removal of the excess of Sarin, the per cent of reactivation of AchE was evaluated following an incubation period of 30 min at pH 7.4 (25°) with a $10^{-3}M$ solution of the oxime. The reactivation data given on Table III are in all cases relative to the reactivation potency observed with 2-PAM under the same experimental conditions, and are corrected for the percentage of inhibition by the respective oximes. A reactivation determination with 2-PAM was carried out before and after our experiments and a value of 78 ± 5% was accepted as the standard value.

Table III shows that the trifluoromethyl ketoxime **1Mb**

‡J. J. Norman, Defence Research Board of Canada in Ottawa, personal communication.

Table III. Reactivation of AchE[a] Inhibited with Sarin by the Trifluoromethyl Ketoximes

Oxime, $1 \times 10^{-3} M$	pK_a	Reactivation[b] relative to 2-PAM	Relative reactivation[b] by the oximate ion	% inhibition by respective oxime, $1 \times 10^{-3} M$
2PAM	7.83	1[c]	1	34
1a	8.55	0.011	0.045	12.2
1b	8.51	0.011	0.041	11.7
1c	8.36	0.034	0.072	11.7
1Ma	5.95	0.035	0.010	19.7
1Mb	7.25	0.145	0.070	20.4
1Mc	6.88	0.066	0.024	16.6
2Ma	7.38	0.031	0.056	15.1
2Mb	8.23	None	None	81.7
2Mc	8.36	0.012	0.036	32.4

[a]After 30-min incubation at pH 7.4 (25°). [b]These values are corrected for the anticholinesterase activity of the respective oximes and are the mean value of 3 experiments. [c]2PAM regenerates 78% of the inhibited AchE.

Table IV. Phosphorylated AchE Reactivation. Structure-Activity Relationship in Pyridinium Oximes.

(Structure: pyridinium ring, N^+–CH_3, bearing R–C=NOH)

Position on the ring	R	pK_a	Relative potency of the oximate ion	Ref
2	H	7.82	1	
3	H	9.22	None	37
4	H	8.54	9×10^{-2}	37,
2	Me	9.05	1.5×10^{-4}	37, 10
3	Me	9.85	None	37, 10
4	Me	9.20	None	37, 10
2	Ph	8.70	8.9×10^{-3}	11
3	Ph		None	11
4	Ph	9.30	2.17×10^{-3}	11
4	2-Pyr	7.90	2×10^{-2}	14
2	CF_3	5.95	1.02×10^{-2}	
3	CF_3	7.25	7.00×10^{-2}	
4	CF_3	6.88	2.40×10^{-2}	

possesses the best reactivating potency of the new series being approximately 7 times less active than 2-PAM. The pyridinium trifluoromethyl ketoximes **1Mb** and **1Mc** are as good or even better reactivators than their corresponding aldoximes and most of the pyridiniums ketoximes listed in Table IV.

On the other hand, it is of interest to note that the reactivating potency of pyridyl derivatives **1a**, **1b**, and **1c**, when based on their oximate ion concentration, is comparable to that of their quaternary analogs calculated in the same manner.

In the anilinium series, although the pK_a of compounds **2Mb** and **2Mc** makes them as good or even better nucleophiles than 2-PAM, their reactivating potency is nil or poor. Compound **2Mb** even shows good anticholinesterase activity, which may be related to the fact that its structure is somewhat similar to that of the well-known anticholinesterase drug "Neostigmine."

Pharmacology. The only group of compounds that warranted pharmacological investigation, based on enzymatic studies, was that of the pyridine series **1**. All compounds showed negligible or no therapeutic (antidotal) activity against poisoning with Paraoxon or Sarin in mice. However, the protective activity of the oxime bases **1a**, **1b**, and **1c** against these same poisons is noteworthy. The data were compared

Table V. Protective Activity of the Trifluoromethyl Ketoximes against Organophosphate Poisons

Oxime[e] and Caramiphen (50 μmoles/kg[a])	Dose, μmoles/kg	% deaths in mice[d]				
		Paraoxon[b]				Sarin[c]
		$2LD_{50}$	$4LD_{50}$	$8LD_{50}$	$16LD_{50}$	$2LD_{50}$
1a	174	0	80	100		100
	348	0	60	100		100
1b	174	50	100			100
	348	0	30	100		70
1c	174	10	60	100		100
	348	0	10	70		50
1Ma	174	100				100
	348	90				100
	696	80				100
1Mb	174	100				100
	348	100				90
	696	70				90
1Mc	174	100				100
	348	100				90
	696	80				80
2-PAM·Cl⁻ alone	174	80	100			100
Caramiphen alone	50	100				
2-PAM·Cl⁻ Caramiphen	174 50	10	25	80	100	100

[a]See ref 38. [b]LD_{50}, 2.10 ± 0.09 μmoles/kg. [c]LD_{50}, 1.42 ± 0.08 μmoles/kg. [d]LD_{50} after 24 hr. [e]The oximes alone do not show any protective activity against Paraoxon or Sarin.

with that of 2-PAM·Cl⁻ which is the generally accepted antidote against organophosphate poisons. The acute ip toxicities of the oximes in mice were as follows (compound, LD_{50} read after 24 hr, 95% confidence limits): **1a**, 0.79 (0.64-0.97) mmole/kg; **1b**, 1.58 (1.35-1.84) mmoles/kg; **1c**, 1.10 (0.87-1.40) mmoles/kg; **1Ma**, 2.29 (1.79-2.93) mmoles/kg; **1Mb**, 1.16 (1.07-1.22) mmoles/kg; **1Mc**, 0.94 (0.84-1.04) mmoles/kg; 2-PAM·Cl⁻, 0.90 mmole/kg.[28] It can be seen that the trifluoromethyl ketoximes of the pyridine series are less toxic than 2-PAM·Cl⁻ with one exception, that of compound **1a**. Table V shows that compounds **1a**, **1b**, **1c** in combination with caramiphen are active in protecting against Paraoxon in mice. The first two offer excellent protection against $2LD_{50}$ of the poison at 348 μmoles/kg but are somewhat weaker against $4LD_{50}$. Compound **1b** is better at this dosage. Compound **1c**, however, is an excellent protecting agent at a dose of 348 μmoles/kg surpassing in that respect the combination of 2-PAM·Cl⁻ and caramiphen at 175 μmoles/kg and 50 μmoles/kg, respectively. Moreover, compound **1c** in combination with caramiphen offers good protection against Sarin poisoning ($2LD_{50}$) whereas 2-PAM·Cl⁻ and caramiphen are totally ineffective in protecting against the more toxic poison. Hence, the basic trifluoromethyl ketoximes are good protective agents against both Paraoxon and Sarin intoxication but are poor antidotes against the same toxic agents *in vivo*. The quaternary salts of the same bases are almost inactive *in vivo* although compound **1Mb** is the best AchE reactivator of the new series.

Experimental Section

Melting points were detd by a capillary method in a Buchi apparatus and are uncor. The nmr spectra were obtained with a Jeol C-60H spectrometer using Me_2CO as a solvent for the bases and D_2O for the quaternary salts. The uv spectra were recorded by means of a Unicam SP-500 spectrophotometer in H_2O or MeOH-H_2O soln. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

Phenyl Trifluoromethyl Ketone. This compd was prepd by adding 17.1 g (0.15 mole) of trifluoroacetic acid to the Grignard reagent obtained from 70.65 g (0.45 mole) of bromobenzene and 10.94 g (0.45 g-atom) of Mg. The crude material was distd yielding 16 g (61%) of a product distg at 64-65° (30 mm), reported bp 152-153° (759 mm).[29]

Oximes. General Method. The trifluoromethyl ketone or its hydrate (0.04-0.1 mole) was dissolved in 150 ml of a MeOH-H_2O soln (50% v/v) containing 2 equiv of $NH_2OH \cdot HCl$ and 4 equiv of NaOAc. The mixt was refluxed for 3 to 27 hr. One half of the solvent mixt was evapd and the residue poured over cracked ice. The oxime was filtered, dried, and recrystd from an appropriate solvent. The data pertaining to the oxime bases are given in Table I.

Quaternary Methiodides. The oxime base (0.01 to 0.04 mole) was dissolved in a proper solvent and 2 equiv of MeI was added. The soln was placed in a pressure reaction bottle and heated at the solvent bp for 24 to 120 hr. The contents of the bottle were then cooled, the solvent was evapd, and the cryst product was washed repeatedly with anhydrous Et_2O.

Quaternary Methyl Chlorides. The iodide ion was exchanged for chloride on a column packed with amberlite IRA-400 resin which had been previously charged with 5% KCl. A MeOH soln of the methiodide (1 equiv of methiodide/10 equiv of charged resin) was passed slowly on the column. The eluent was evapd and the quaternary chloride recrystd from an appropriate solvent. The mp and other data pertaining to the quaternary salts are given in Table I.

pK_a Determination. The respective oximes were dissolved in aqueous buffers of acidic, basic, and intermediary pH values to obtain a final concn of the order of 10^{-4} *M*. The ionic strength was adjusted to 0.1 *M* with KCl, and the temp was maintained at 25° throughout the detn. For a soln of a given pH, the ratio [oxime]/[oximate ion] was calcd as follows by measuring the uv absorption of the oximate ion at a fixed wavelength in between 250 and 340 mμ: $[\text{oxime}]/[\text{oximate ion}] = (d' - d^{\circ})/(d^{\circ} - d)$ where d' = optical density of fully ionized oxime (pH > 12), d° = optical density at intermediary (pH), and d = optical density of nonionized oxime (pH < 1). The pK_a values were then obtained by introducing this ratio into the Henderson-Hasselbalch equation corrected for the ionic strength.[16] The brackets denote molar concn and μ is the ionic strength. $pK_a = \text{pH} + \log 10\ [\text{oxime}]/[\text{oximate ion}] + (0.509\mu 1/2)/(1 + \mu 1/2)$. The p$K_a$'s listed on Table II are the mean of four detn at different pH or wavelength and are believed to be accurate to 0.05 pK_a units.

Nucleophilicity. To 4.2 ml of a 0.1 *M* aqueous soln of KNO_3, containing 5×10^{-5} mole of oxime and equilibrated at pH 7.6 and 30°, was added 5×10^{-6} mole of Sarin. The reaction rates were then detd from the uptake of standard alkali by the reaction mixt maintained at pH 7.6 by means of an automatic titrator (Radiometer Copenhagen TTlc) according to Hackley.[30]

A sufficient excess of oxime was used in order to provide pseudo-first-order kinetics. Plots of log $(V/(V - Vx))$ *vs.* time were used to determine the pseudo-first-order rate consts k_1. The second-order rate consts k_2 were calcd from the k_1 values using the following equation,[23] $k_1 = k\,(\text{obsd}) - k\,(\text{solv}) = k_2 i[A] = (2.303/t)[\log V/(V - V_x)]$, where k (obsd) = obsd hydrolysis const, k (solv) = solvolysis const of Sarin, i = portion of ionized oxime at a given pH, A = molar concn of oxime, t = time in min, V = total vol of std alkali delivered, Vx = vol of std alkali delivered at time t. The rate consts given on Table II are thus corrected for the spontaneous hydrolysis of Sarin and are the results of two determinations

Enzymology. Stock Soln of AchE. The enzyme (1000 units) (AchE, Worthington biochemical) was dissolved in 15 ml of a 0.9% saline soln containing 0.1% of bovine cryst serum albumen (Brickman) and 0.2% of $MgCl_2 \cdot 6H_2O$.

Working soln of AchE is prepd by diln of 0.3 ml of the stock soln up to 20 ml with saline.

Inhibited AchE Soln. The inhibited enzyme soln of AchE is obtained by addn of 10 μl of an aqueous soln of Sarin (2 μl/100 ml) to 20 ml of the working soln thus giving a final concn of 7.8×10^{-8} *M* in Sarin. After a 45-min incubation period at 25°, the inhibited AchE soln is transferred into a liquid-liquid extractor (the section of the extractor containing the enzyme soln being kept in ice) and the excess of Sarin extd continuously with *n*-hexane (Fischer AR) for 2 hr.

Reactivation of Inhibited AchE. An aliquot of 0.5 ml of the inhibited enzyme soln and 0.5 ml of a 2×10^{-3} *M* soln of the respective oximes in saline (or in saline plus 1% of *p*-dioxane peroxide free if needed) were introduced into the reaction vessel of an automatic titrator (Copenhagen Radiometer TTlc) and the vol com-

pleted to 2 ml with saline. The pH was adjusted to 7.4 and the mixt was incubated under N_2 at 25° for 30 min. The soln was diluted to 5 ml, and the enzyme activity was detd by means of a pH-Stat procedure[31] using as a substrate Ach chloride at a final concn of 3.96 × 10^{-3} *M*. The esteratic activity of 0.5 ml of the working soln alone and in presence of the same concn of the respective oxime was detd by the same procedure.

Toxicology. Male albino mice (Swiss) weighing 18-22 g were utilized. The basic oximes were dissolved in 75% v/v DMSO-saline and administered ip; the soluble quaternary salts of the oximes were dissolved in saline and also given ip. Solns were prepd extemporaneously and the final diln was adjusted for a vol of injection of 0.1 ml/20 g for all tests including the protection studies. Paraoxon and Sarin were administered sc as 10% v/v EtOH-H_2O solns.[32] The LD_{50} values were detd on 4 groups of 10 animals each and calcd according to the method of Litchfield and Wilcoxon.[33]

Pharmacology. The protective studies were accomplished on 40 groups of 10 animals each for the oxime bases 1a, 1b, and 1c, and 37 groups of 10 animals each for the quaternary salts 1Ma, 1Mb, and 1Mc. Caramphen and/or oxime are given ip at time zero followed in 15 min by Paraoxon or Sarin sc (dorsally) in multiples of their LD_{50}.

Discussion

In 1958, Wilson and coworkers[10] described essential criteria for reactivation of phosphorylated AchE by oximes. These were a property of the nucleophilic reactivity of an oxime which was more or less a complement for the active site of the enzyme. Thus the high potency of 2-PAM was attributed to the efficacy of its quaternary nitrogen atom to bind at the anionic site of the phosphorylated AchE in such a way that its nucleophilic oximino function is oriented toward the phorphorus atom of the inhibitor.[34]

At physiological pH, the value of the reactivation rate constant is a function of both the nucleophilicity of the reactivator molecule and its basicity, reaching an optimum for compounds with pK_a value in the range 7.6-8.0.[35]

It is difficult to evaluate the importance of the molecular complementation brought about by the quaternary nitrogen atom relative to the nucleophilicity and the basicity of the reactivator because, in most instances, the inductive effect of the quaternary nitrogen atom of the reactivator largely influences both its nucleophilicity and basicity.

In our new series, the inductive effect of the CF_3 group is sufficient to bring the pK_a value of the oximes in the range for maximal reactivation properties. So we have nonquaternary oximes which have a better nucleophilic reactivity and a better pK_a value than their quaternary analogs to promote reactivation. Moreover, the nucleophilic character of their oximate ion is also better than their quaternary analogs and comparable to that of 2-PAM (see Table II). If we examine the the results in Table III, we see that the relative reactivation potency of the nonquaternary oximes **1a**, **1b**, **1c**, when based on the oximate ion concentration is comparable to that of their quaternary analogs **1Ma**, **1Mb**, **1Mc**, and even better than most of the pyridinium analogs of Table IV.

It would thus appear that when the quaternary nitrogen atom is not needed for its inductive effect on the oximino function, its presence in the reactivator molecule is not of critical importance to promote reactivation. This is the case of oxooximes particularly of monoisonitroso acetone (MINA) which is as good or even a better reactivator than 2-PAM although it does not have a quaternary nitrogen atom in its molecule.[36]

We can see that our results are in good agreement with the statement of Ashani and Cohen.[35] The oximes with the most nucleophilic reactivity in a series are not necessarily the best reactivators, but rather those with a pK_a value in the range of 7.6-8. In fact, oxime **1Mb**, the poorest nucleophile of the series exhibits a reactivation potency equal to that of the best nucleophile of the series, oxime **1c**.

In conclusion, we can say that among the new trifluoromethyl ketoximes of Table II as well as their analogs of Table IV, the ones with a pK_a value closest to 7.6-8.0 are the best reactivators regardless of the nucleophilic properties of their oximate ion or the presence of a quaternary nitrogen atom in their moleucle.

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