and the benzene and cyanogen bromide were removed under reduced pressure. The residual oil was dissolved in dry benzene and treated with carbon. The mixture was filtered through sintered glass. The solvent was removed under reduced pressure (0.01 mm. Hg/24 hr.) to give a colorless oil, 3.04 Gm. (12.3 mmoles, 61.5%), $[\alpha]_D^{28}$ (benzene) +2.1° (c 3.04), exhibiting a band at 2208 cm.$^{-1}$ (film) (C≡N) in the infrared spectrum. There were no bands in the —OH or C=O region.

Anal.—Calcd. for $C_{14}H_{16}N_2O_2$: C, 68.8; H, 6.6. Found: C, 69.3; H, 6.2.

(±) - 1 - Cyano - 3 - benzoyl - 3 - hydroxypiperidine [(±)-VI]—To 2.44 Gm. (10 mmoles) of (+)-V in 40 ml. of methanol was added 10 ml. of 1 *N* hydrochloric acid. After standing for 24 hr., the methanol was removed under reduced pressure to give, on cooling, 1.87 Gm. (8.1 mmoles, 81%) of product which was optically inactive.[1] There were bands in the infrared spectrum (benzene solution) at 1665 (C=O), 2218 (C≡N), and 3285 cm.$^{-1}$ (OH). The spectrum was identical to that of an authentic sample prepared as previously described (5) melting point and mixed melting point of crystals from methanol, 131.5–132.5°.

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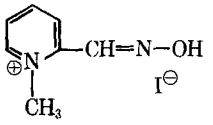
Synthesis of Aldoxime Analogs of Arecoline as Reactivators of Organophosphorus Inhibited Cholinesterase

By J. N. WELLS, J. N. DAVISSON, I. BOIME, D. R. HAUBRICH, and G. K. W. YIM

A series of arecoline-like aldoximes were synthesized and evaluated as potential reactivators of organophosphate-inhibited cholinesterase. These arecoline analogs were patterned after the potent quaternary oximes 2-PAM and TMB-4 and were synthesized by sodium borohydride reduction of the corresponding pyridinium aldoxime. Biological results show that although these aldoximes are less toxic than the quaternary aldoximes, they are much less effective as reactivators. They did exhibit weak muscarinic activity (1/60 that of arecoline) on dog blood pressure and guinea pig ileum.

THE EFFICIENT reactivating property of 2-pyridine aldoxime methiodide (2-PAM) has been attributed to the affinity of its quaternary nitrogen for the anionic site of cholinesterase (1). This affinity could cause the nucleophilic aldoxime oxyanion to be in a favorable position for reactivating phosphorylated cholinesterase.



2-PAM

The cholinergic agent arecoline contains a tertiary amine. Since the muscarinic activity of arecoline has been shown to be dependent on its protonated cationic nitrogen (2), the arecolinium ion might also possess a marked affinity for the anionic site of cholinesterase. Therefore, aldoxime analogs of arecoline, which should enter the CNS readily, might be expected to provide good reactivation of phosphorylated cholinesterase in the brain.

Received March 17, 1967, from the Medicinal Chemistry and Pharmacology Laboratories, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907

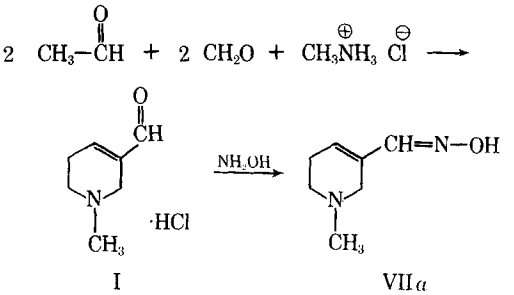
Accepted for publication June 5, 1967.

Presented to the Medicinal Chemistry Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

This investigation was supported in part by undergraduate research participation grant GE6181 from the National Science Foundation, Washington, D.C.

DISCUSSION

The authors have prepared a few 1,2,5,6-tetrahydropyridine aldoximes and report here the chemistry and pharmacological data. 1-Methyl-1,2,5,6-tetrahydropyridine-3-aldoxime (VII*a*) has been prepared by the reaction of formaldehyde, methylamine hydrochloride, and acetaldehyde to give 1-methyl-1,2,5,6 - tetrahydropyridine - 3 - carboxaldehyde (I) which was isolated as the oxime (3). (Scheme I.)

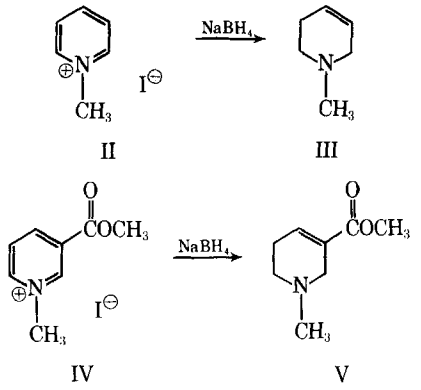


Scheme I

This reaction is quite laborious and gave poor yields of impure VII*a*. Thus, a more general method was sought which would be applicable to 1-alkyl-1,2,5,6-tetrahydro-3- and 4-aldoximes.

Potassium borohydride has been shown to reduce *N*-methylpyridinium iodide (II) to 1-methyl-1,2,3,6-tetrahydropyridine (III) (4). Similarly, the reduction of methyl nicotinate methiodide (IV) with

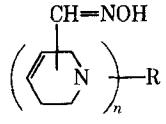
sodium borohydride has been shown to give arecoline (V) (5). The conditions of these reactions seemed applicable to these systems. (Scheme II.)



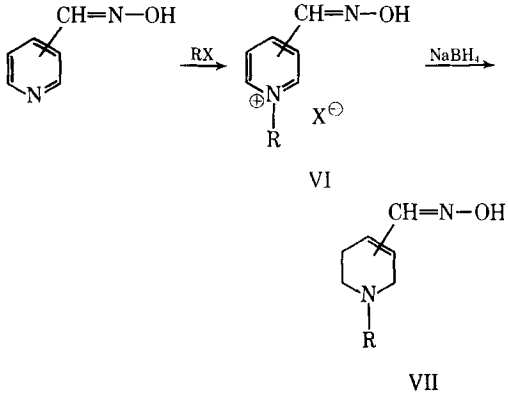
Scheme II

The quaternary pyridine aldoximes (VI) were prepared by reacting the pyridine aldoxime with an appropriate alkyl halide. Reduction of the resulting pyridinium aldoxime with sodium borohydride in methanol or ethanol gave the 1-substituted 1,2,5,6-tetrahydropyridine-3- or 4-aldoxime (VII) in 50–74% yield (Table I). (Scheme III.)

TABLE I—1-SUBSTITUTED-1,2,5,6-TETRAHYDROPYRIDINE-3- OR 4-ALDOXIMES



	Position of Aldoxime	*n*	R	% Yield from Reduction
VII*a*	3	1	CH_3	59
VII*b*	4	1	CH_3	50
VII*c*	4	2	$(CH_2)_3$	74



Scheme III

Elemental analysis of these compounds (VII) indicated that the tetrahydropyridine system had been formed but the position of the double bond had to be established.

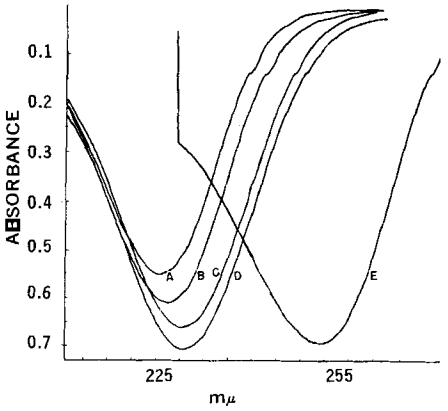


Fig. 1—Ultraviolet absorption spectrum of tetrahydropyridine aldoximes. Key: A, *VII*b *in 0.05* N *HCl;* B, *VII*a *in 0.05* N *HCl;* C, *VII*b *in water;* D, *VII*a *in water;* E, *VII*b *in base (pH 11.5). Bausch & Lomb model 505 spectrophotometer.*

Infrared and NMR spectra were consistent with the Δ-3 system but were not stringent proof. Another point of confusion arose from the fact that Mannich reported the melting point of VII*a* hydrochloride to be 240–242° (3), while the product (VII*a* hydrochloride) from the sodium borohydride reduction melted at 261.5–262°.

The ultraviolet spectrum of compound VII*b* in water showed $\lambda_{max.}$ 226 mμ; ϵ = 17,700. The $\lambda_{max.}$ 226 mμ is consistent with that expected of an α, β-unsaturated aldoxime (6). The molar absorptivity clearly indicates conjugation. Since very little shift was noted when the spectrum was obtained from an acidic solution of any of the compounds (VII*a* or *b*), conjugation with the amino nitrogen may be ruled out in all cases (see Fig. 1). The slight shift to shorter wavelengths in acidic solution must have been due to suppression of ionization of the oxime hydroxyl. The U.V. spectrum of VII*a* in water gave $\lambda_{max.}$ 226 mμ; ϵ = 17,100. Again a conjugated oxime is indicated. Of the two possibilities for location of the double bond, only the 3–4 position should give the same $\lambda_{max.}$ as compound VII*b*. If the double bond of VII*a* were in the 2–3 position one would also see a contribution from the amine electrons which would give rise to absorption at a longer wavelength.

The results of the pharmacological evaluation of compounds VII*a*, *b*, and *c* are summarized in Table II. Only 6–20% reactivation of the TEPP inhibited erythrocyte cholinesterase was obtained with these aldoximes, whereas 60–75% reactivation was observed with even lower concentrations of 2-PAM. In agreement with this weak *in vitro* activity was the lack of protection afforded by the tetrahydropyridine aldoximes against TEPP toxicity in mice. In contrast, 2-PAM afforded 80% protection, and all six mice injected with 20 mg./Kg. TMB-4 survived the TEPP treatment. Only one mouse survived of 22 control mice that were injected with the 1 mg./Kg. dose of TEPP.

Compound VII*a* showed definite muscarinic activity. It caused contractions of the isolated

TABLE II—PHARMACOLOGICAL DATA ON TETRAHYDROPYRIDINE ALDOXIMES STUDIED

Compd.	Reactivation of Phosphoryl-ChE *In Vitro*[a]	LD_{50} in Mice,[b] mg./Kg. i.p.	Effect on TEPP Toxicity in Mice[b,c] (1 mg./Kg. TEPP S.Q.)	Ionization Constants[d] Ring Nitrogen pKa	Ionization Constants[d] Aldoxime pKa	% Ionized at pH 7.4 Ring Nitrogen	% Ionized at pH 7.4 Aldoxime
2 PAM	76.2% at $1.5 \times 10^{-4}M$	...	10/12 survived at 50 mg./Kg.	...	7.77 ± .03	100%	30%
VII*a*	19.5% at $3.0 \times 10^{-3}M$	274 (254–295)	0/12 survived at 100 mg./Kg.	8.04 ± .10	10.84 ± .14	83%	0.03%
VII*b*	6.6% at $3.0 \times 10^{-3}M$	275 (237–319)	0/12 survived at 100 mg./Kg.	8.15 ± .11	10.91 ± .07	85%	0.03%
VII*c*	11.0% at $3.0 \times 10^{-4}M$	78 (62–97)	0/12 survived at 35 mg./Kg.	...	...	...	...
VIII	18.6% at $3.0 \times 10^{-4}M$	91 (72–114)	0/12 survived at 50 mg./Kg.		10.11 ± .02	100%	0.02%
Arecoline	...	...	...	7.78 ± .10	...	70%	...

[a] Bovine erythrocyte ChE, inhibited with $10^{-7}M$ TEPP, was incubated with the aldoximes for 55 min. ChE activity was measured manometrically (Umbreit *et al.*, 1964). [b] Swiss albino mice weighing 25–35 Gm. were used. [c] Aldoximes were given 6–8 min. prior to TEPP; TEPP killed 21 of 22 control mice. [d] Dissociation constants were determined potentiometrically by titration of 0.01 *M* solutions of the compounds with 0.1 *N* KOH according to the method of Albert and Serjeant (1962).

guinea pig ileum, gave depressor responses in the anesthetized cat, and induced salivation, urination, and defecation in mice. These actions were readily antagonized by atropine. When atropine was combined with the aldoxime pretreatment, the mice were still not protected from the lethal effects of TEPP. Thus, it is unlikely that beneficial effects of possible reactivation induced by the aldoximes were masked by the toxic muscarinic effects of the aldoximes.

The ionization data offer a feasible explanation for the low activity of the tetrahydropyridine aldoximes. The pKa values of compounds VII*a* and VII*b* (8.04 and 8.15, respectively) were close to the dissociation constant of the basic ring nitrogen of arecoline, and were therefore attributed to the dissociation of the basic ring nitrogens. Similarly, the pKa values of compounds VII*a* and VII*b* (10.84 and 10.91, respectively) were close to the dissociation constant of the aldoxime group of compound VIII (the methiodide of VII*a*) and were attributed to the dissociation of the aldoxime groups. The data in the last column indicate that, as predicted, the ring nitrogen of the tetrahydropyridine aldoximes would be sufficiently protonated at physiological pH to form the cationic head which could be attracted to the anionic site of the phosphorylated enzyme. The aldoxime groups of compounds VII*a* and VII*b* were almost completely unionized, as contrasted to the 30% ionization of the aldoxime group of 2-PAM. Thus, the weak reactivating activity of the tetrahydropyridine aldoximes can be attributed to insufficient ionization of the oxime group to the active oximate form. The importance of the steric configurations of 2-PAM and the nonaromatic aldoximes could not be ascertained from the present study.

EXPERIMENTAL

1 - Methyl - 1,2,5,6 - tetrahydropyridine - 4-aldoxime (VIIb)—To a solution of pyridine-4-aldoxime methiodide (7) (10.6 Gm., 0.04 mole) in 200 ml. of methanol was added 6.0 Gm. (0.16 mole) of sodium borohydride in small portions. The reaction was stirred continuously and the temperature was maintained between 25–30°. The solution was stirred for an additional 0.5 hr. at room temperature after the addition was completed.

The solution was concentrated *in vacuo* to 50 ml. and diluted with 200 ml. of water. The aqueous solution was saturated with Na_2CO_3 and extracted with ether. The ether solution was dried over K_2CO_3 and then removed *in vacuo* to give 2.8 Gm. (50% yield) of white crystalline solid, m.p. 156–158°.

The hydrochloride was prepared and recrystallized from ethanol, m.p. 241–242°.

Anal.—Calcd. for $C_7H_{12}N_2O \cdot HCl$: C, 47.59; H, 7.42. Found: C, 47.43; H, 7.48.

1 - Methyl - 1,2,5,6 - tetrahydropyridine - 3-aldoxime (VIIa)—Pyridine-3-aldoxime methiodide (7) (8.6 Gm., 0.03 mole) was reduced with 4.7 Gm. (0.12 mole) of $NaBH_4$ using the same conditions as for VII*b* except that ethanol (200 ml.) was used as solvent. Using the work-up described for VII*b*, 2.5 Gm. (59% yield) of VII*a* was obtained. The hydrochloride was prepared and recrystallized from ethanol, m.p. 261.5–262°. [Lit. (3) m.p. 240–242°.]

Anal.—Calcd. for $C_7H_{12}N_2O \cdot HCl$: C, 47.59; H, 7.42. Found: C, 47.73; H, 7.26.

The methiodide (VIII) was prepared by reaction of VII*a* with methyl iodide in acetone, m.p. 181–182°.

1,3 - Bis(4 - formyl - 1,2,5,6 - tetrahydropyridyl)-propane Dioxime (VIIc)—To a solution of 7.0 Gm. (0.02 mole) of 1,3-bis(4-formylpyridinium bromide)-propane dioxime (8), in 200 ml. of methanol, was added portionwise 4.8 Gm. (0.13 mole) of sodium borohydride. A precipitate formed as the reaction proceeded. The solid was collected and dissolved in 20 ml. of 6 *N* hydrochloric acid. Ethanol (100 ml.) was added. It was necessary then to add a small quantity of ether to initiate crystallization. After recrystallization from ethanol, 2.6 Gm. (77% yield) of white crystalline solid was obtained, m.p. 246–247° dec.

Anal.—Calcd. for $C_{15}H_{24}N_4O_2 \cdot HCl$: C, 49.32; H, 7.12. Found: C, 49.08; H, 7.25.

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