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Synthesis and Biological Activity of Conformationally Controlled 2-PAM Derivatives

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A series of conformationally controlled 2-PAM derivatives were prepared from 2-acetylpyridine and 2,3-pyrido[*b*]cycloalkenones in two steps and their reactivities towards parathion poisoned AChE were evaluated. The most planar 2,3-pyrido[*b*]cyclohexanone oxime methiodide showed an activity comparable to 2-PAM implying E-syn is that the most active comformation of 2-PAM in the biological system.

Key words: 2-PAM, Acetylcholine esterase(AChE), Parathion, 2,3-Pyrido[*b*]cyclohexanone oxime methiodide, 2,3-Pyrido[*b*]cycloheptanone oxime methiodide, 2,3-Pyrido[*b*]cyclooctanone oxime methiodide.

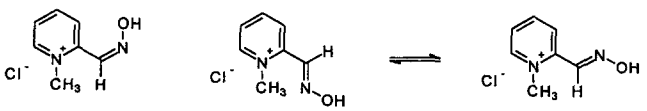
INTRODUCTION

A variety of pesticides have been introduced to control insects and diseases in plants, and thus, improve productivity in agriculture. The organophosphates are amongst the most useful, but unfortunately they are particularly toxic. This toxicity originates from the irreversible inhibition of acetylchlonesterase (AChE), which is a key enzyme that terminates the action of acetylcholine at the synaptic junction by hydrolysis, to cause an accumulation of acetylcholine. This in turn leads to poisoning with acetylcholine-like symptoms (Cocolas, 1982).

Reactivators of phosphorylated acetylcholinesterase (AChE) are of interest as antidotes for organophosphate poisoning from both the standpoint of accidental poisoning, of for example farm-workers, and as a result of deliberate action, for they have some use as nerve gasses. Reactivators studied so far include choline, hydroxylamines, hydroxamic acids, and oximes (Childs, *et al.*, 1955). Among them, 2-PAM (pralidoxime chloride) is the most effective agent and has been used clinically for decades (Wilson, *et al.*, 1955). Even though 2-PAM has been in use for this length of time as a reactivator it has some well recognized disadvantages, such as its inability to pass the blood-brain barrier and its lack of oral availability. Such ineffectiveness limits its usage and spurs medicinal chemists to design and synthesize new candidates (Wilson, 1958; Bodor, *et al.*, 1975; Kollmeyer, *et al.*, 1999).

As far as conformation is concerned, the 2-PAM molecule has E-anti (Jahng, 2000) isomer (**1c**) geometry in the crystal state (Caristrom, 1966). However, the most favored conformation in terms of maximizing its efficacy and enhancing its reaction with the phosphorylated AchE requires the E-syn isomer (**1b**) geometry. The two planar conformations (**1b** and **1c**) are separated by a relatively low energy barrier, which means that any of the many intermediate non-planar conformations may in fact be effective.

We describe herein the synthesis and biological activity of conformationally controlled pyridylketone oxime methiodides in which the length of the polymethylene bridge controls the dihedral angle between the oxime moiety and the pyridyl ring plane.



1a (Z-isomer) **1b** (E-syn) **1c** (E-anti)

MATERIALS AND METHODS

Experimental procedure

Melting points were determined using a Fischer-Jones melting point apparatus and are not corrected. Infrared (IR) spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. Nuclear magnetic resonance (NMR)

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spectra were obtained using a Bruker-250 spectrometer 250 or 300 MHz for ^{1}H NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS) or 2,2-dimethyl-2-silapentane-5-sulfonate (DDS). Chemicals and solvents were commercial reagent grade and used without further purification. Elemental analyses were performed on a Hewlett-Packard Model 185B elemental analyzer. The starting material **2a**, was obtained from the Aldrich Chemical Co., and **2b**, **2c**, and **2d** (Thummel, *et. al*, 1984, 1985) were prepared using either a previously reported method or a modification. *S*-Acetylthiocholine iodide, parathione, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from the Sigma Chemical Co..

Preparation of oxime (General Method)

A mixture of 2-acetylpyridine and hydroxylamine · HCl in 10 mL of EtOH was refluxed for 2 h. The resulting solid was collected. Concentration of the filtrate afforded an additional crop, which was chromatographed on silica gel with EtOAc:CH_2Cl_2:hexane (3:14:3). The later fractions afforded the desired oxime.

2-Acetylpyridine oxime(3a)

cis-isomer: mp 122-123°C [lit.(Pinner, 1901) mp 121°C], Rf=0.20 (43%). Unreported spectral data are as follows: ^{1}H NMR(250 MHz) δ 11.51(s, OH), 8.5650(dd, *J*=4.8, 1.8 Hz, H6), 7.8462(t, 1H, *J*=8.7 Hz, H3), 7.78(td, 1H, *J*=8.7, 1.8 Hz, H4), 7.37(dd, 1H, *J*=8.6, 4.8 Hz, H5), 3.34 (s, 3H). *trans*-isomer: mp 116-117°C [lit. (Pinner, 1901) mp 121°C], Rf=0.17. (43%). ^{1}H NMR(250 MHz) δ 11.49 (s, OH), 8.5690(dd, *J*=4.8, 1.8 Hz, H6), 8.0725(t, 1H, *J* =8.7 Hz, H3), 7.74(td, 1H, *J*=8.7, 1.8 Hz, H4), 7.35(dd, 1H, *J*=8.6, 4.8 Hz, H5), 3.34(s, 3H).

2,3-Pyrido[*b*]cyclohexanone oxime(3b)

Only one set of resonances was observed 250 MHz ^{1}H NMR even though two isomers are possible. Yield: 95%. mp 180-182°C. ^{1}H NMR(250 MHz) δ 12.66(s, OH), 8.61 (dd, 1H, *J*=4.8, 1.8 Hz, H6), 8.40(dd, 1H, *J*=8.0, 1.8 Hz, H4), 7.87(dd, 1H, *J*=8.0, 4.8 Hz, H5), 2.93(t, 2H, *J* =6.7 Hz), 2.76(t, 2H, *J*=6.7 Hz), 1.85(qunitet, 2H, *J*=6.7 Hz).

2,3-Pyrido[*b*]cycloheptanone oxime(3c)

Only one set of resonances was observed 250 MHz ^{1}H NMR even though two isomers are possible. Yield: 92%. ^{1}H NMR(250 MHz) δ 12.74(s, OH), 8.72(dd, 1H, *J*= 4.8, 1.8 Hz, H6), 8.47(dd, 1H, *J*=8.0, 1.8 Hz, H4), 7.98(dd, 1H, *J*=8.0, 4.8 Hz, H5), 2.96(t, 2H, *J*=6.7 Hz), 2.76(t, 2H, *J*=6.7 Hz), 1.81(qunitet, 2H, *J*=6.7 Hz). 1.66 (quintet, 2H, *J*=6.7 Hz).

2,3-Pyrido[*b*]cyclooctanone oxime(3d)

Yield: 86%. Z-isomer (56%) mp 207-209°C. ^{1}H NMR (250 MHz) δ 12.74(s, OH), 8.57(dd, 1H, *J*=4.8, 1.8 Hz, H6), 8.38(dd, 1H, *J*=8.0, 1.8 Hz, H4), 7.85(dd, 1H, *J*= 8.0, 4.8 Hz, H5), 2.72-2.66(m, 2H), 2.57-2.53(m, 2H), 1.71-1.67(m, 2H), 1.56-1.53(m, 2H), 1.39-1.35(m, 2H). E-isomer(30%). mp 217-218°C. ^{1}H NMR(250 MHz), 12.74 (s, OH), 8.48(dd, 1H, *J*=4.8, 1.6 Hz, H6), 8.35(dd, 1H, *J*=8.0, 1.6 Hz, H4), 7.85(dd, 1H, *J*=8.0, 4.8 Hz, H5), 2.95-(2.90(m, 2H), 2.87-2.80(m, 2H), 1.71-1.67(m, 2H), 1.56-1.53(m, 2H), 1.39-1.35(m, 2H).

Preparation of oxime methiodide

A mixture of oxime and a twofold excess of methyl iodide was refluxed in CH_3NO_2 or nitrobenzene for 8 h. Crystalline solid was collected from the reaction mixture.

2-Acetylpyridine oxime methiodide(4a)

Yield: 8%. mp 160-161°C [lit.(Engler & Rosumoff, 1891) mp 161°C]. Unreported spectral data are as follows: ^{1}H NMR(250 MHz) δ 11.98(s, OH), 8.65(dd, 1H, *J*=5.0, 1.8 Hz, H6), 8.12(t, 1H, *J*=8.0 Hz, H4), 8.01(d, 1H, *J*=8.0 Hz, H3), 7.59(dd, 1H, *J*=8.0, 4.8 Hz, H5), 4.44(s, 3H), 3.34(s, 3H).

2,3-Pyrido[*b*]cyclohexanone oxime methiodide(4b)

Yield: 14%. ^{1}H NMR(250 MHz) δ 11.86(s, OH), 8.90 (d, 1H, *J*=5.9 Hz, H6), 8.59(d, 1H, *J*=8.1 Hz, H4), 8.08 (dd, 1H, *J*=8.1, 5.9 Hz, H5), 4.53(s, 3H), 3.25(t, 2H, *J*= 6.0 Hz), 2.91(t, 2H, *J*=6.0 Hz), 2.23(quintet, 2H, *J*=6.0 Hz). Anal. Calc. for $C_{10}H_{13}N_2OI$. C, 39.49; H, 4.31; N, 9.21. Found: C, 39.48; H, 4.28; N, 9.19.

2,3-Pyrido[*b*]cycloheptanone oxime methiodide(4c)

Yield: 18%. ^{1}H NMR(250 MHz) δ 12.79(s, OH), 8.97 (d, 1H, *J*=6.0 Hz, H6), 8.67(d, 1H, *J*=8.2 Hz, H4), 8.10 (dd, 1H, *J*=8.0, 6.0 Hz, H5), 4.55(s, 3H), 3.29(t, 2H, *J*= 6.7 Hz), 2.96(t, 2H, *J*=6.7 Hz), 2.28(qunitet, 2H, *J*=6.7 Hz). 2.16(quintet, 2H, *J*=6.7 Hz). Anal. Calc. for $C_{11}H_{15}N_2OIH_2O$. C, 39.30; H, 5.10; N, 8.33. Found: C, 39.32; H, 5.08; N, 8.30.

2,3-Pyrido[*b*]cyclooctanone oxime methiodide(4d)

Yield: 16%. ^{1}H NMR(250 MHz) δ 12.75(s, OH), 8.87 (d, 1H, *J*=6.1 Hz, H6), 8.68(d, 1H, *J*=8.0 Hz, H4), 8.10 (dd, 1H, *J*=8.0, 6.1 Hz, H5), 3.27-3.24(m, 2H), 3.02-2.95(m, 2H), 2.27-2.24(m, 2H), 2.15-2.10(m, 2H), 1.79-1.70(m, 2H). Anal. Calc. for $C_{12}H_{17}N_2OI$. C, 43.39; H, 5.16; N, 8.43. Found: C, 43.41; H, 5.18; N, 8.42.

Biological assay

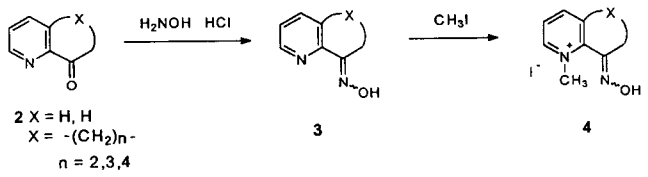
The previously described method (Ellman, *et al.*, 1961) was employed to evaluate the reactivating activity of the

compounds prepared. Membrane bound human AChE was obtained by lysing and washing human erythrocytes. The *in vitro* reactivating activity was determined by spectrometically measuring the inhibition of acetylthiocholine iodide hydrolysis using the Ellman assay.

RESULTS AND DISCUSSION

Chemistry

The synthesis of desired compounds was straight forward as is shown. The prerequisite ketones **2**, prepared by known procedure, were converted to their oxime derivatives **3** in quantitative yields. Two oxime isomers are possible **3a** and **3d** which were separated by column chromatography to afford the *Z*- and *E*-isomers, respectively, and assigned by NMR. Although two isomers are possibly present in both **3b** and **3c**, only one set of proton resonances was observed by 250 MHz ^{1}H NMR, respectively. Efforts to identify two possible isomers by GC and HPLC have not been successful as yet. The oximes **3** were, then, methylated using methyl iodide to afford the desired compounds **4** in 8-16% yield. Such low yields are a general phenomena observed in the quaterization of pyridine aldoximes (Ginsburg, *et al.*, 1957; Profft, *et al.*, 1957). Although nitromethane has been found to be the solvent of choice for quarterizing pyridine aldoximes that are difficult to alkylate, the solubility of oximes (**3**) in nitromethane was too poor to allow us to increase yields. Interestingly, the *Z*-isomer did not undergo methylation even in a high boiling solvent such as nitromethane in a sealed tube, presumably due to steric hindrance caused by the hydroxyl group in the oxime moiety. The bridge of **3d** and **4d** are rigid at room temperature in the NMR time scale and showed as a doublet of a doublet of a doublet (J_{gem}=13.6 Hz, J_{vic} =6.7 Hz, J_{vic}=2.3 Hz) for the methylene protons adjacent to the pyridine nucleus and oxime moiety. Attempts to separate the two enantiomers have not been successful as yet.



Biological activity

The irreversible inactivation of AChE by organophosphates leads to the production of phosphorylated AChE which can be reactivated by an oxime. The reactivating ability of the compounds prepared was evaluated by a previously described method and the results compared to the the activity of 2-PAM, these are summarized in Table I.

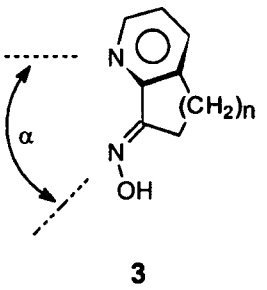
The reactivating activity is closely related to the dihedral angle between the oxime moiety and pyridyl ring plane.

Table I. Biological activity of the oximes prepared

Compounds	% Reactivation[a)]	Relative Potency
3a	53 ± 2	0.53
3b	92 ± 3	0.92
3c	36 ± 3	0.36
3d	6 ± 2	0.06
2-PAM	100	1.0

a) Averaged value of the five different experiments

Table II. Dihedral angle between the oxime moiety and the pyridine ring



3

n	α (°)[a]
1	0
2	15(20)
3	30(35)
4	52(56)

[a] Values are obtained from the Dreiding Model and values in parenthesis are calculated from a Serena Software PC Model

The dihedral angles were estimated using the Dreiding Model and a PC Modeling Program to show 15(20)° for **3b**, 30(35)° for **3c**, and 52(56)° for **3d** as shown in Table II. The most planar **3b** is also the most active in terms of reactivating phosphorylated AChE whose potency is comparable to 2-PAM. Activity decreased in regular fashion with increasing dihedral angle. This results implies that the planar E-syn form is the most favorable conformation in terms of allowing the antidote to maximize its interaction with phosphrylated AChE.

In conclusion, conformationally controlled pyridyl ketones were converted to their oxime methiodides and their reactivating activities were evaluated. Their activity was found to decrease on increasing the dihedral angle between the oxime plane and the quaternary pyridinium salt. Such a result implies that the most active conformation of 2-PAM is a conformation close to E-syn.

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