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Review Article

Oxines Antagonistic to Inhibitors of Cholinesterase

Part II

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PHARMACEUTIC ASPECTS

Experiments with 2-PAM indicated that the iodide form of the oxime would be of little practical value because of its comparatively low solubility in water. The iodide is approximately 2% soluble in water at room temperature (144), while saturated aqueous solutions at 25° contain only 4.8%. In view of the fact that 2-PAM is effective in humans in doses of 20 or more mg./Kg., a volume of at least 30 ml. would be needed for a single injection. Such volumes are obviously impractical for intramuscular injection. There is an additional inherent disadvantage in the use of the iodide form. Large doses of 2-PAM can, and have, elicited symptoms of iodism (145).

In attempting to find more soluble salts of 2-formyl *N*-methyl pyridinium oxime (2FMPO), the nitrate was made by simply adding silver nitrate to 2-PAM (146). Fundamental principles of general chemistry tell us that this reaction should go to completion readily, with the formation of insoluble silver iodide and the nitrate of the oxime. The resulting nitrate was found to be 15 times more soluble than the iodide. Other salts were subsequently synthesized. Table III shows the water solubilities and the percentages of oxime in each salt. The chloride salt (2-PAMCl) on the basis of its excellent water solubility, its high oxime content per mole of compound and, most important, its physiological compatibility, was proposed as the oxime of choice. The methanesulfonate of 2-formyl *N*-methyl pyridinium oxime (2FMPOMS), has been championed by another group of investigators (147). The latter contains 56.6% oxime per mole of compound and is about as soluble as the chloride in water. Equivalent concentrations of various oxime salts reactivate inhibited eel cholinesterase at approximately the same rate; that is, there is little or no effect due to the anion moiety.

Aqueous solutions of the chloride can be autoclaved without significant breakdown, provided that a suitable pH is maintained. Unbuffered 2.5, 10, and 20% solutions of 2-PAMCl showed less than a 4% breakdown when autoclaved at 120° (15 pounds pressure) for 15 minutes. The decomposition of the oxime occurs *via* two pH-

TABLE III.—SOLUBILITY AND PER CENT OXIME MOIETY IN METHYL PYRIDINIUM ALDOXIME SALTS (145)

2-PAM Salt	Solubility, mg./ml. of Soln. at 25°	Percentage Oxime
Chloride	640	79.5
Nitrate	675	68.9
Dihydrogen phosphate	46	58.6
Hydrogen sulfate	640	58.6
Iodide	48	51.9
Fumarate	389	70.6
Acetate	...	69.9
Tartrate	565	64.9
Lactate	1000	60.6

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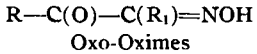
dependent mechanisms. At pH values below 4, the hydrogen ion catalyzes hydrolysis of the acid form of the oxime; various states of equilibrium between 2-PAM and its hydrolytic products, pyridine-2-carboxaldehyde methiodide and hydroxylamine, are established in accord with the pH and temperature of the reaction mixture. Above pH 4, the decomposition is due either to hydroxyl ion catalysis of breakdown of the acid form of the oxime or to noncatalytic, direct attack on the oximate ion. Hydroxyl ion attack at the methine hydrogen results in the removal of a proton and the formation of a carbanion as the rate-controlling step (134). Subsequent loss of hydroxide ion from the oximino nitrogen results in the formation of a triple bond—in this case the corresponding nitrile The nitrile, depending on whether there is direct hydroxide ion attack on the cyano grouping or addition to the pyridine ring, forms carbamidopyridinium or hydroxypyridinium ions. On further reaction with hydroxide ions, the latter forms a pseudo base which loses water to form *N*-methyl-α-pyridone. Ellin (123) showed the presence of 2-cyanopyridinium ion, cyanide ion, and *N*-methyl-pyridone in alkaline hydrolysates of 2-PAM. Kosower and Patton (148) conclusively showed that 2-carbamidopyridine methiodide formed when 2-cyanopyridine methiodide was placed in alkaline media. One would then expect the formation of this amide when 2-PAM is degraded in basic solution; its presence among the products of alkaline degradation of 2-PAM has been confirmed by paper chromatographic techniques.

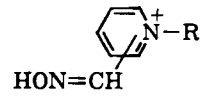
Because cyanide had been found to be a decomposition product of 2-PAM (101, 133), experiments were run to check for the presence of cyanide in aqueous solutions stored over long periods of time. Accelerated storage-stability studies established that the toxicity resulting from 10 and 20% concentrations of oxime was due only to the initial oxime concentration (145). From the established equations for the degradation of PAM, the stability of the oxime in aqueous solution at any pH and temperature may be predicted. Kinetic data show that a solution of 2-PAM, maintained at pH 4.36 at 25°, should retain half of its original concentration of oxime even after a period of 80 years.

Other oximes, such as the aforementioned TMB-4, show promise as antagonists of the toxic effects of organophosphorus compounds, but have not been investigated so extensively as 2-PAM. Experiments concerned with the

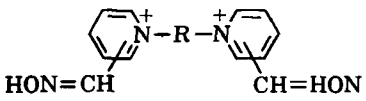
group have been found to possess activity as antibacterial, antifungal, antirickettsial, trypanocidal, tuberculostatic, anticonvulsant, centrally depressant, antispastic, insecticidal, or locally anesthetic agents, probably the most striking biological activity of this type of compound is the ability of some oximes to serve as reactivators of cholinesterase inhibited by certain organophosphorus and carbamate compounds and as antagonists of poisoning by these inhibitors of cholinesterases. The oximes that have been found to have value in these ways fall in general into one of three groups, all mentioned previously:



Oxo-Oximes
I



Monoquaternary Pyridine Aldoximes
II



Bisquaternary Pyridine Bisoximes
III

A few oximes antagonistic to organophosphorus compounds but having other ring structures, including one having the oxime group attached directly to a ring carbon, have been described. These compounds have not exceeded in activity the usual types of oximes.

TOXICITY OF OXIMES

The best known members of the oxo-oxime group (I) are MINA, DAM, and DINA; the best known of the group of monoquaternary pyridine aldoximes (II) are the chloride, iodide, and methanesulfonate salts of 2-formyl *N*-methylpyridinium oxime. Among the bisquaternary pyridine aldoximes (III), the best known is 1,3-bis-(4-formylpyridinium) propane bisoxime dibromide (B4FPBOBr$_2$ or TMB-4). Table IV gives toxicity information about a representative group of oximes. It is apparent that DINA is the most lethal of the group. O'Leary *et al.* (158) have examined the lethal effectiveness in the mouse of i.v. injection of mixtures of equal parts of pyridinium monoximes and bispyridinium bisoximes; in four cases (2FMPOI +

TABLE IV.—TOXICITIES OF SELECTED OXIMES

Oxime[a]	Species	Route	LD_{50}	Ref.
Oximinoacetamide	Mouse	i.p.	4200 mg./Kg.	(100)
MINA	Mouse	i.p.	150	(100)
	Rat (M)	i.p.	50	(150)
	Rat (F)	i.p.	74	(150)
DINA	Mouse	i.p.	20	(100)
DAM	Mouse	i.p.	51, 85, 900	(100, 151, 152)
2-Oximino-3-pentanone	Mouse	i.p.	350	(100)
2FMPOI(2-PAM)	Mouse	i.v.	140–178	(157, 158)
	Mouse	i.p.	136–260	(100, 150–154, 156, 159)
	Mouse	s.c.	290–340	(153, 159)
	Mouse	p.o.	1500–4000	(156, 160)
	Rat	i.p.	305	(150)
2FMPOCl	Mouse	i.v.	115	(158)
	Mouse	i.p.	205	(156)
	Mouse	p.o.	4100	(156)
	Rabbit	i.v.	95	(158)
2FMPOL	Mouse	i.v.	121	(158)
2FMPOMS(P2S)	Mouse	i.v.	118–122	(150)
	Mouse	i.p.	216	(150)
	Mouse	p.o.	3700	(161)
	Rat	i.v.	109	(150)
	Rat	i.p.	262	(150)
2FMPOMS(P2S)	Guinea pig	i.m.	305	(150)
	Rabbit	i.v.	147	(150)
	Monkey	i.m.	356	(150)
$4FPPOBr_2$	Mouse	i.p.	202	(154)
$B4FPBOBr_2$(TMB-4)	Mouse	i.v.	53–89	(157, 158)
	Mouse	i.p.	130	(154)
$B4FPBOCl_2$	Mouse	i.v.	57	(158)
	Rabbit	i.v.	44	(158)

[a] **2FMPOI** = 2-formyl *N*-methylpyridinium oxime iodide (2-PAM); **2FMPOCl** = 2-formyl *N*-methylpyridinium oxime chloride; **2FMPOL** = 2-formyl *N*-methylpyridinium oxime lactate; **2FMPOMS** = 2-formyl *N*-methylpyridinium oxime methanesulfonate; **$4FPPOBr_2$** = 1-(4-formylpyridinium)-3-pyridinium propane oxime dibromide; **$B4FPBOBr_2$** = 1,3-bis(4-formylpyridinium)-propane bisoxime dibromide (TMB-4); **$B4FPBOCl_2$** = 1,3-bis(4-formylpyridinium)-propane bisoxime dichloride.

oximes was between those of the two components and close to the value calculated from the toxicities of the individual components. This last finding suggests that there is no potentiation of the toxicity of $B4FPBOBr_2$ (TMB-4) by either the iodide, the lactate, or the methanesulfonate of 2-formyl *N*-methylpyridinium oxime and none of that of $B4FPBOCl_2$ by the chloride of the monoxime.

EFFECTIVENESS OF OXIMES

Table V summarizes available information about the effectiveness of the same oximes in antagonizing the toxic effects of organophosphorus and carbamate inhibitors of cholinesterases. This table shows several things: (*a*) the oxime with which the greatest volume of work has been done is 2FMPOI (or 2-PAM), (*b*) the only poisoning of humans in which an oxime has been used fairly extensively is that by parathion, (*c*) pyridinium oximes (either mono or bis oximes) are more active than the oxo-oximes, and (*d*) although the oximes are effective antagonists to many of the inhibitors of cholinesterase, there

In the latter group, Sevin and Diazinon seem to be particularly likely to have their toxic effects enhanced by administration of oximes. This enhancement of toxicity probably occurs through the formation of stable phosphorylated (60, 231) or carbamylated derivatives of the oximes.

The only recourse available today as an aid to atropine in the treatment of severe poisonings by such compounds as Sevin and Diazinon is artificial ventilation. In carrying out this form of therapy, a first requirement is that the airway be rendered patent by removal of secretions and other occlusive material from the pharynx. The maintenance of a patent airway is aided by tilting backwards the head of the supine patient. With a patent airway, one must next insure that the method of artificial respiration being used actually produces effective pulmonary ventilation.

Some of the papers from which the data in Table V are derived contain other information of considerable importance for most effective employment of the oximes in therapy of poisonings by inhibitors of cholinesterase. One such piece

TABLE V.—ACTIVITIES OF OXIMES AND SALTS OF OXIMES AS ANTAGONISTS OF LETHAL AND OTHER EFFECTS OF ANTICHOLINESTERASE COMPOUNDS

Oxime	Anti-ChE Compound[a]	Test Object[b]	Antagonism[c]	Ref.
Oximinoacetamide	Sarin	m, r	+	(100)
MINA	Sarin	m, r, gp	+	(59, 100, 102, 120, 144, 163–167)
MINA	Sarin	direct reaction	+	(120, 167)
MINA	Tabun	r	+	(168)
MINA	DFP	r, c	+	(59, 163, 166, 169)
MINA	DFP	rb eye	+	(167, 173, 174)
MINA	TEPP	m; r	−; +	(175; 59, 163, 166)
MINA	DMNP	r	+	(166)
MINA	OMPA; Parathion	m	−; +	(175)
MINA	CH_3-Parathion	m	0	(175)
MINA	Diazinon	m	−	(175)
MINA	EPN, Malathion	m	0	(175)
MINA	Dipterex	m	±	(175)
MINA	Physostigmine	rb eye	+	(171)
DINA	Sarin	m; r	±; +	(100; 59, 163)
DINA	DFP, TEPP	r	+	(59, 163)
DAM	Sarin	m, r, gp, rb, mk; man	+; ±	(59, 100, 102, 120, 144, 158, 164, 165, 171, 172; 218–220)
DAM	Sarin	direct reaction	+	(120)
DAM	Tabun	r	+	(168)
DAM	DFP	r, c	+	(59, 165, 169)
DAM	DFP	rb or human eye	+	(170, 174)
DAM	TEPP	m, r	+	(59, 118, 127, 165, 175)
DAM	OMPA	m, man	±	(175, 218–220)
DAM	Paraoxon; Parathion	m	0	(118; 175)
DAM	CH_3-Parathion	m	−	(175)
DAM	Diazinon; EPN	m	−; ±	(175)
DAM	Malathion; Dimefox	m	−; 0	(175; 118)
DAM	Dipterex	m	±	(175)
DAM	Physostigmine	human eye	+	(170)
DAM	Neostigmine	c; man	+; ±	(123; 218–220)
DAM	Bisneostigmine	man	+	(218–220)
DAM	Bispyridostigmine	man	±	(218–220)
DAM	Ambenonium	man	+	(218–220)
2-Oximino-3-pentanone	Sarin	m, r	+	(100)
2-Oximino-3-pentanone	DFP	rb eye	+	(174)
2FMPOI	Sarin	m, r	±	(100)
2FMPOI	Sarin	m, r, rb, c, d, man	+	(59, 102, 120, 124, 125, 144, 152, 158, 163, 176–181, 182, 218–220)
2FMPOI	Sarin	direct reaction	±	(60, 120)
2FMPOI	Soman	m, rb	0	(181, 182)
2FMPOI	Tabun	m, r, c, d	+	(124, 176)
2FMPOI	DFP	m, r, gp, c, man	+	(59, 81, 82, 116, 117, 154, 155, 163, 178, 183–185)
2FMPOI	DFP	m, rb or human eye	+	(116, 168)
2FMPOI	DFP	direct reaction	+	(185)
2FMPOI	TEPP	m, r, c	+	(59, 117, 123, 127, 154, 160, 163, 175, 176, 187)
2FMPOI	TEPP	direct reaction	+	(186)
2FMPOI	OMPA	m	0; +	(81, 82, 125; 155, 175)
2FMPOI	OMPA	man	±	(218–220)
2FMPOI	Paraoxon	m, r, gp, rb, c	+	(81, 82, 116–118, 121, 122, 151, 155, 178, 184, 188, 189)
2FMPOI	Paraoxon	m eye	+	(116)
2FMPOI	Paraoxon	direct reaction	+	(186)
2FMPOI	Parathion	r	0	(176, 188)
2FMPOI	Parathion	m, r, gp, rb, c, d, h, man	+	(122, 129–132, 151, 153, 175, 176, 178, 184, 189–209)
2FMPOI	CH_3-Parathion	m, r	+	(175, 191, 193, 210)
2FMPOI	Demeton	m, r	+	(191, 211, 213)
2FMPOI	CH_3-Demeton	m, rb	+	(190, 191)
2FMPOI	Isosystox	r	+	(161)
2FMPOI	Phenkapton	m, r	+	(191, 193)

Oxime	Anti-CHE Compound[a]	Test Object[b]	Antagonism[c]	Ref.
2FMPOI	CH_3-Diazinon	m	0	(191)
2FMPOI	Guthion	r	−	(211)
2FMPOI	C_2H_5-Guthion	r	−	(211)
2FMPOI	EPN	m, man	+	(175)
2FMPOI	Malathion	m; ct; man	−; +; ±	(175; 212; 198)
2FMPOI	Morphothion; Dimethoate	r	±; 0	(211; 193, 211)
2FMPOI	DBD; Thimet	m; r	+	(160; 211)
2FMPOI	Endothion	m, rb	+	(190)
2FMPOI	D600	direct reaction	+	(186)
2FMPOI	I, II, III	m	+	(214)
2FMPOI	Phospholine	m, rb, c	+	(170, 216)
2FMPOI	Phospholine	rb or human eye	+	(170, 215)
2FMPOI	Phosdrin	r, man	+	(211, 217)
2FMPOI	$R_0$3-0340, $R_0$3-0351, $R_0$3-0422	m	+	(117, 154)
2FMPOI	$R_0$3-0340, $R_0$3-0422	direct reaction	+	(186)
2FMPOI	Dimefox	m; r	+; 0	(118; 193, 211)
2FMPOI	Phosphamidon	m, r	+	(211, 221, 222)
2FMPOI	Dipterex	m	+	(175)
2FMPOI	Physostigmine	gp	+	(184)
2FMPOI	Physostigmine	rb or human eye	+	(170)
2FMPOI	Neostigmine	m; man	0; +	(154, 217; 218–220)
2FMPOI	Bisneostigmine	man	+	(218–220)
2FMPOI	Mestinon	m	±	(217)
2FMPOI	Bispyridostigmine	man	±	(218–220)
2FMPOI	Dimetilan	m; r	+; ±	(191, 223; 211)
2FMPOI	Isolan	m; r	±; +	(223; 211)
2FMPOI	Sevin	r	−	(204, 211)
2FMPOI	Humorsol	m	±	(217)
2FMPOI	Pyramat; Pyrolan	m	+; ±	(223)
2FMPOI	G23091; Dimetan	m	+; 0	(223)
2FMPOI	Ambenonium	m; man	0; +	(216; 218–220)
2FMPOI	Edrophonium	m	0	(216)
2FMPOCl	Sarin, Tabun	rb	+	(158)
2FMPOL	Sarin	rb, d	+	(158, 225)
2FMPOMS	Sarin	m, r, gp, rb, d	+	(150, 158, 161, 164, 225, 226)
2FMPOMS	Tabun	m, r, gp, rb	+	(158, 161, 164)
2FMPOMS	DFP	m, r, gp, rb	+	(161)
2FMPOMS	DFP	rb eye	+	(174)
2FMPOMS	TEPP	m, r, gp, rb	+	(150, 161)
2FMPOMS	OMPA	m, r, gp, rb	+	(161)
2FMPOMS	Paraoxon	m, r, gp, rb	+	(161)
2FMPOMS	Demeton	r	+	(211)
2FMPOMS	Isosystox	m, r, gp, rb	+	(161)
2FMPOMS	Vinylphos	m, r, gp, rb	+	(161)
2FMPOMS	Guthion, C_2H_5-Guthion	r	+	(211)
2FMPOMS	Morphothion; Thimet	r	0; +	(211)
2FMPOMS	Phospholine	rb eye	+	(174)
2FMPOMS	Amiton	m, r, gp, rb	+	(161, 226)
2FMPOMS	S-l, Ch-l	r	+	(225)
2FMPOMS	Phosdrin, Phosphamidon	r	+	(211)
2FMPOMS	Physostigmine	m, r, gp, rb	+	(162)
2FMPOMS	Dimetilan	r	±	(211)
2FMPOMS	Isolan; Sevin	r	+; −	(211)
2FMPOMS	Humorsol	rb eye	+	(174)
4FPPOBr$_2$	Sarin, DFP	m	+	(154, 187)
4FPPOBr$_2$	TEPP, $R_0$3-0340	m	+	(154, 187)
B4FPBOBr$_2$	Sarin	m, r, rb, c, d	+	(89, 158, 164, 172, 177, 182, 187, 228)
B4FPBOBr$_2$	Soman	m, r	±	(182)
B4FPBOBr$_2$	Tabun; DFP	r; m	+	(168; 154, 172, 228)
B4FPBOBr$_2$	DFP	rb or human eye	+	(170)
B4FPBOBr$_2$	TEPP; OMPA	m	+; 0	(86, 127, 154, 187; 229)
B4FPBOBr$_2$	Paraoxon	m	+	(214)
B4FPBOBr$_2$	Parathion, CH_3-Parathion	r	+	(193)
B4FPBOBr$_2$	Armin, Phenkaptone	m, r	+	(193, 229)
B4FPBOBr$_2$	Diazinon; Dimethoate	r	±; 0	(193)
B4FPBOBr$_2$	I, II, III	m	+	(214)
B4FPBOBr$_2$	Phospholine, $R_0$3-0340	m	+	(154, 215)
B4FPBOBr$_2$	Dimefox; Phosphamidon	m	0; +	(193, 229; 221, 229)
[illegible]	[illegible]	[illegible]	+	(229)

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