Structure–Activity Relation in Organophosphorus-Inhibited Acetylcholinesterase Reactivators III: Methiodides of Hydroxyimino Derivatives of Pyridylethanes

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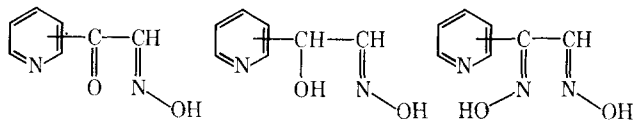
Abstract □ The methiodides derived from 2-hydroxyimino-1-pyridyl-ethan-1-ones, 2-hydroxyimino-1-pyridyl-1-hydroxyethanes, and 1,2-di(hydroxyimino)-1-pyridylethanes were tested as reactivators of phosphorylated acetylcholinesterase. The most active member, 2-hydroxyimino-1-(2′-pyridyl)-ethan-1-one, displays a rate of reactivation, in the case of tetraethyl pyrophosphate poisoning, slightly higher than that of the well-known reactivator 2-hydroxyiminomethyl-1-methyl-pyridinium iodide.

Keyphrases □ Acetylcholinesterase reactivators—structure–activity relationship □ Pyridylethane derivatives (methiodide salts)—synthesis □ Reactivators—organophosphorus-inhibited acetylcholinesterase □

It has recently been shown (1, 2) that methiodides of pyridine oximes, containing the ketoxime group on a position of the side chain one carbon atom distant from the ring, are able to reactivate organophosphorus-inhibited acetylcholinesterase. The compounds hitherto considered were 2-hydroxyimino derivatives of 1-pyridylethanes further substituted in position 2 by phenyl or amide groups; because these groups can shield the hydroxyimino group in relation to the active site of the enzyme, it was of interest to replace them with hydrogen.

Therefore, the methiodides derived from the three isomers of each of the following types of bases were synthesized: *anti*-2-hydroxyimino-1-pyridyl-ethan-1-ones (I), 2-hydroxyimino-1-pyridyl-1-hydroxyethanes (II), and *anti*-1,2-di(hydroxyimino)-1-pyridylethanes (III).



Ia, b, c
a = 2-pyridyl–

IIa, b, c
b = 3-pyridyl–

IIIa, b, c
c = 4-pyridyl–

Wilson *et al.* (3) have already examined the reactivating property of the methiodide salts of 1-(3′-pyridyl)-2-hydroxyimino-ethan-1-one and 1-(4′-pyridyl)-2-hydroxyimino-ethan-1-one, finding them less active than 2-hydroxyiminomethyl-1-methyl-pyridinium iodide (2-PAM). The picolinic isomer is a new compound, and its synthesis makes it possible to correlate the activities of the three isomeric methiodides (IV*a–c*) to their structure. It should be noted that Wilson *et al.* have proposed for Products I*b* and I*c* the configuration *syn* to the keto group; however, the authors find that I*b* and I*c*, like the new Compound I*a*, form complexes with copper and nickel salts and, by reaction with hydroxylamine, give the *anti*-1,2-di(hydroxyimino)-1-pyridylethanes. The *anti*-configuration should therefore be assigned to all the isomers I (4).

The methiodide salts of Compounds II and III were synthesized to ascertain how the biological activity varies with changing of the carbonyl adjacent to the ring.

EXPERIMENTAL[1]

***anti*-2-Hydroxyimino-1-(2′-pyridyl)-ethan-1-one (I*a*)**—To a stirred solution of 12 g. of isopentyl nitrite, cooled with ice, 2.3 g. of Na dissolved in 50 ml. of anhydrous EtOH was added dropwise. Stirring was continued for 12 hr.; 250 ml. of water was then added and the solution was extracted with ether. The water solution was acidified to pH 5–6 with dilute acetic acid and extracted with ethyl acetate; after evaporation of the solvent, the residual oil was chromatographed on silica gel, eluting with ethyl acetate. By evaporation of the first eluates, a product was obtained which was recrystallized from benzene; m.p. 113–115°.

Anal.—($C_7H_6N_2O_2$) C,H,N. $\lambda_{max.}^{EtOH}$ (log ε): 254 mμ (4.06).

The product gives colored precipitates with copper and nickel salts.

***anti*-1-Hydroxy-1-pyridyl-2-hydroxyiminoethanes (II*a–c*)**—To 0.3 g. of sodium borohydride in 4 ml. of water and 5 ml. of methanol, 1 g. of the corresponding α-hydroxyiminoketone in 20 ml. of methanol was added dropwise with stirring. After 2 hr. the solution was neutralized with dilute acetic acid and reduced under vacuum to one-fourth of its original volume. The solution was extracted with ethyl acetate, and the products were purified by chromatography on silica gel (ethyl acetate–methanol, 80:20) followed by crystallization from ethyl acetate–ethyl ether; m.p. II*a* 111–112°, II*b* 99–101°, and II*c* 97–99°.

Anal.—($C_7H_8N_2O_2$) C,H,N.

***anti*-1,2-Di(hydroxyimino)-1-pyridylethanes (III*a–c*)**—One gram of Compound I was refluxed for 2 hr. with 0.55 g. of hydroxylamine hydrochloride in 30 ml. of EtOH. The solution was then diluted with water, made basic with aqueous $NaHCO_3$, and extracted with ethyl acetate. The residue obtained after evaporation of the solvent was crystallized from EtOH; m.p. III*a* 143–145°, III*b* 173–175°, and III*c* 184–187°.

Anal.—($C_7H_7N_3O_2$) C,H,N.

The *anti*-configuration of the dioximes was established on the basis of the red-orange colored complexes obtained with the nickel salts (5).

***N*-Methyl Pyridinium Iodides (Table I)**—The Products IV*a* and V*a* were obtained by heating the corresponding bases in anhydrous EtOH with methyl iodide at 60° for 48 hr. in sealed vessels; the residue obtained by evaporation of the solvent was washed with ethyl acetate and crystallized. The Products V*b*, VI*a*, VI*b*, and VI*c* were obtained in a similar manner, performing the reaction in acetone at room temperature; V*c* was obtained from II*c* in ether after 10 days of reaction. Products V*a* and V*c* were very hygroscopic.

All the quaternary salts were subjected to biological assay. The *in vitro* reactivating velocity of acetylcholinesterase inhibited by tetraethyl pyrophosphate (TEPP) or diisopropyl phosphorofluoridate (DFP) was measured according to the technique described

[1] Melting points are uncorrected. When no unusual spectral features were observed with the compounds described, no absorption peaks are reported. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Table I—1-Methylpyridinium Iodides

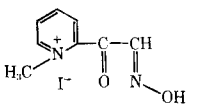
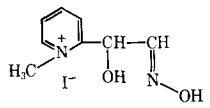
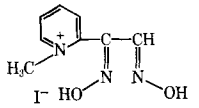
No.	Compd.	Crystn.	M.p.	Formula	Anal.
IV*a*		Me_2CO–MeOH	142–143°	$C_8H_9IN_2O_2$	C, H, N
V*a*		Me_2CO	102–104°	$C_8H_{11}IN_2O_2$	C, H, N
V*b*		MeOH–Et_2O	130–132°	$C_8H_{11}IN_2O_2$	C, H, N
V*c*		Me_2CO	115–117°	$C_8H_{11}IN_2O_2$	C, H, N
VI*a*		MeOH–EtOAc	183–185°	$C_8H_{10}IN_3O_2$	C, H, N
VI*b*		MeOH–EtOAc	156–158°	$C_8H_{10}IN_3O_2$	C, H, N
VI*c*		MeOH–EtOAc	178–180°	$C_8H_{10}IN_3O_2$	C, H, N

Table II—Reactivation of Inhibited Bovine Erythrocyte Acetylcholinesterase by Means of Oximes IV–VI (pH 7.4 and 25°)[a,b]

Oximes (Iodides), 5×10^{-3} *M*	pKa′	pKa″	Inhibiting Group: Diethyl phosphoryl $k_{obs.}$	Diethyl phosphoryl Relative Rate Constant	Diisopropyl phosphoryl $k_{obs.}$	Diisopropyl phosphoryl Relative Rate Constant
2-PAM	7.9		1.1×10^{-2}	1	1.7×10^{-3}	1
IV*a*	6.3		1.6×10^{-2}	1.47	8.3×10^{-4}	0.49
IV*b* (2)	7.2		1.3×10^{-2}	1.21	4.5×10^{-4}	0.26
IV*c* (2)	7.1		4.1×10^{-3}	0.38	8.1×10^{-4}	0.47
V*a*	9.8		None	—	None	—
V*b*	9.9		None	—	None	—
V*c*	9.8		None	—	None	—
VI*a*	7.3	9.8	2.6×10^{-3}	0.23	1.4×10^{-4}	0.08
VI*b*	8.1	9.3	9.2×10^{-4}	0.08	1.3×10^{-4}	0.075
VI*c*	7.5	8.7	1.8×10^{-3}	0.16	1.2×10^{-4}	0.07

[a] $k_{obs.}$ is in $min.^{-1}$. [b] pKa values were obtained by potentiometric titration and, for overlapping values, by application of the calculation method due to Noyes, as given by Albert and Serjeant (7).

by Ashani *et al.* (6). The results are shown in Table II, together with the values of pKa for the same compounds.

RESULTS AND DISCUSSION

The values of the hydrolysis rate in Table II indicate that pyridine derivatives, containing a hydroxyimino group in the β-position of the side chain, show reactivating ability. This is particularly evident in the series of Compounds IV. Among the three positional isomers the most active is the picolinic derivative, which, together with the nicotinic one, has an activity slightly higher than that of 2-PAM in the case of TEPP poisoning. The nearly equivalent activity for the two isomers is remarkable, in contrast to what is observed with the pyridine aldoxime methiodides where the nicotinic isomer is inactive (8). For DFP-poisoned acetylcholinesterase, the three isomers show nearly equivalent activity.

Structural modifications of the carbonyl group lead to a diminution of activity that can be ascribed to the different nucleophilicity of the hydroxyimino groups. This is particularly evident in the series of Compounds V; reduction of the carbonyl function to an alcoholic one results in a strong diminution of acidity, which is probably responsible for the inactivity of these compounds. The introduction of a second hydroxyimino group generally leads to less active compounds in comparison to the ketones.

From these results, compared with those discussed in the preceding notes (1, 2), it is possible to conclude that voluminous groups attached to the terminal carbon atom of the side chain in this type of compound decrease the ability to reactivate inhibited acetylcholinesterase; a shielding effect on the active site of the enzyme by the phenyl and arylamide groups present in the compounds described in preceding notes can then be postulated.

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