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Structure–Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase V: Quaternary Salts of Hydroxyiminomethylimidazoles

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Abstract □ The methiodides of (*E*)-1-methyl-2-hydroxyiminomethylimidazole, (*E*)-1-benzyl-2-hydroxyiminomethylimidazole, and (*Z*)-1-methyl-5-hydroxyiminomethylimidazole were tested *in vitro* as reactivators of phosphorylated acetylcholinesterase. From the hydrolysis rate measurements, it was ascertained that only the compounds derived from 2-hydroxyiminomethylimidazole had an activity comparable with that of methiodide of 2-hydroxyiminomethylpyridine, whereas the methiodide of (*Z*)-1-methyl-5-hydroxyiminomethylimidazole was inactive. The structure–activity relationships of these reactivators are briefly discussed.

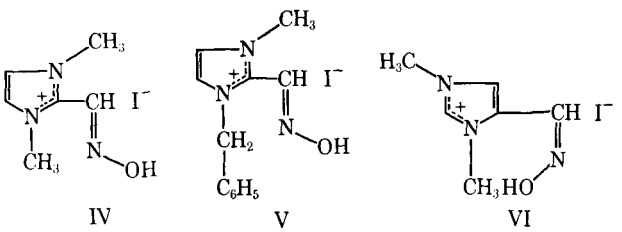
Keyphrases □ Acetylcholinesterase, organophosphorus inhibited—structure–activity relationships of reactivators (hydroxyiminomethylimidazole quaternary salts) □ Structure–activity relationships, quaternary salts of hydroxyiminomethylimidazoles—reactivators of organophosphorus-inhibited acetylcholinesterase □ Hydroxyiminomethylimidazoles, quaternary salts—structure–activity relationships

The quaternary salts of 2-hydroxyiminomethylpyridine and 4-hydroxyiminomethylpyridine are effective reactivators of organophosphorus-inhibited acetylcholinesterase (1, 2). On the basis of isosteric correlations, some authors studied the antidotic ability of quaternary salts of other heterocyclic aldoximes. Ashani *et al.* (3) described the synthesis and the pharmacological properties of 4-hydroxyiminomethyl-1-methylpyrimidinium iodide with *E*-configuration[1]; recently, Benschop *et al.* (5) studied the antidotic properties of some hydroxyiminomethyl-2-methylisothiazolium salts and those of related compounds. The tosylate of 3-hydroxyiminomethyl-2-methylisothiazolium restores the enzyme activity almost as rapidly and to the same extent as the isosteric 2-hydroxyiminomethyl-1-methylpyridinium methanesulfonate. (*Z*)-Isothiazole-5-carboxaldoxime reactivates the enzyme slowly but to a significant extent, whereas pyrazole-3(5)-carboxaldoxime, isoxazole-5-carboxaldoxime, and 5-hydroxyiminomethyl-2-methylisothiazolium tosylate are scarcely active.

In this note, quaternary salts derived from hydroxyiminomethylimidazoles (IV, V, and VI) were considered. The delocalization of the positive charge between the nitrogen atoms is a peculiar characteristic of these compounds, which can conceivably affect their ability to bind the enzyme anionic site.

In a previous study (6), the 2-hydroxyiminomethyl-1-hydroxyimidazole 3-oxide was prepared and found to be scarcely effective as a reactivator. The study of Compound IV has, therefore, the additional object of ascertaining the effect of the presence of a true quaternary ammonium group instead of an *N*-oxide.

For comparison with Compound V, the 2-hydroxyiminomethyl-1-benzylpyridinium iodide (VII) was prepared.



IV V VI

[1] The (*E*-*Z*) system of nomenclature for double-bond stereoisomers is used in this paper (4).

Table I—Hydroxyiminomethylimidazoles and Their Methiodides

Compound	R	R′	Derivative	Configuration	Melting Point	NMR Data (τ) H_2	H_4	H_5	H_a	OH	CH_3 (N − 1)	CH_3 (N − 3)	CH_2	C_6H_5	Formula	Analysis, % Calc.	Found
I	CH_3		2-CH=NOH[a]	*E*	—	—	2.30	2.04	1.04	−3.36	5.90	—	—	—	—	—	—
II	$CH_2C_6H_5$		2-CH=NOH[a]	*E*	—	—	2.78	2.48	1.74	−1.60	—	—	4.30	2.63	—	—	—
III*a*	CH_3		5-CH=NOH	*Z*	191–193°	0.60	0.86	—	0.47	−5.57	5.87	—	—	—	$C_5H_7N_3O$	C 47.98 H 5.64 N 33.58	47.66 5.56 33.36
III*b*	CH_3		5-CH=NOH	*E*	175–177°	0.63	1.36	—	0.06	−4.51	5.79	—	—	—	$C_5H_7N_3O$	C 47.98 H 5.64 N 33.58	48.30 5.48 33.58
IV	CH_3	CH_3	2-CH=NOH	*E*	223–225°	—	2.27	2.27	1.70	−2.46	6.00	6.00	—	—	$C_6H_{10}IN_3O$	C 26.93 H 4.04 N 15.73	27.09 4.11 15.95
V	$CH_2C_6H_5$	CH_3	2-CH=NOH	*E*	183–185°	—	—	2.19	1.60	−2.52	5.97	—	4.34	2.75	$C_{12}H_{14}IN_3O$	C 41.99 H 4.11 N 12.24	41.94 4.27 12.09
VI	CH_3	CH_3	5-CH=NOH	*Z*	185–187°	1.73	2.32	—	0.97	−2.13	5.95	6.02	—	—	$C_6H_{10}IN_3O$	C 26.93 H 4.04 N 15.73	27.18 4.25 15.58

[a] See *Reference 7*.

CHEMISTRY

The already known 2-hydroxyiminomethyl-1-methylimidazole (I) and 2-hydroxyiminomethyl-1-benzylimidazole (II) (7) were prepared by reacting 1-methyl- and 1-benzylimidazole-2-carboxaldehyde (8) with $H_2NOH \cdot HCl$ in methanol; Compounds I and II [m.p. 170 and 168°, lit. (7) m.p. 176 and 170°, respectively] appear configurationally pure according to their NMR spectra. The *E*-configuration was assigned to the oximes I and II since they give copper chelates with cupric ions (3) and a red-brown color with ferrous salts (9). Attempts to isomerize (*E*)-I and (*E*)-II with concentrated aqueous HCl failed (10). Reaction of the 1-methylimidazole-5-carboxaldehyde with $H_2NOH \cdot HCl$ in ethanol led to a mixture of geometric isomers of the corresponding oxime. One of these isomers (m.p. 192°, III*a*) crystallizes readily as the hydrochloride in the reaction medium, whereas the other (m.p. 176°, III*b*) remains in solution. On comparing the NMR spectra of the two isomers (Table I), it was noted that the signal of the OH group and those of the ring protons *ortho* to the hydroxyiminomethyl group were downfield for the high melting isomer, whereas the reverse effect was found for the hydrogen bonded to the carbon atom of the hydroxyiminomethyl group. These chemical shift differences allow the assignment of the *Z*-configuration to the high melting isomer III*a* (12–15). The oximes I and II by reaction with methyl iodide in acetone at room temperature gave the respective quaternary salts IV and V (Table I).

The oxime (*Z*)-III*a*, heated at 60° with methyl iodide in methanol in a sealed vessel, was converted to the corresponding quaternary salt VI, whereas the oxime (*E*)-III*b* in similar experimental conditions gave a mixture of two quaternary salts in the ratio 9:11, as proved by the NMR spectrum.

BIOLOGICAL ASSAY

The quaternary salts obtained were subjected to biological assay, measuring the *in vitro* reactivating velocity of acetylcholinesterase from bovine erythrocytes inhibited by tetraethylpyrophosphate and diisopropylphosphonofluoridate, according to the pH-stat method described by Ashani *et al.* (3). The hydrolysis rate constants obtained were compared with those of 2-hydroxyiminomethyl-1-methylpyridinium iodide and 2-hydroxyiminomethyl-1-benzylpyridinium iodide. Since Compounds IV, V, and VI, as quaternary ammonium salts, could inhibit the enzyme's catalytic activity, this eventuality was verified by executing an assay of enzyme inhibition in the same experimental condition but without phosphorus ester. The results are shown in Table II.

RESULTS AND DISCUSSION

From the data obtained in the reactivation test, it can be seen that the derivatives of 2-hydroxyiminomethylimidazole are the only active compounds, whereas the (*Z*)-1-methyl-5-hydroxyiminomethylimidazole methiodide (VI) is inactive.

On acetylcholinesterase inhibited by diisopropylphosphonofluoridate, Compounds IV and V show a reactivating power that is, respectively, a half and a fourth of that of 2-hydroxyiminomethyl-1-methylpyridinium iodide.

Compound IV is twice more active than 2-hydroxyiminomethyl-1-hydroxyimidazole 3-oxide (6), in agreement with the hypothesis that the minor activity of the 1-hydroxyimidazole 3-oxide derivative depends on a partial neutralization of the positive charge on the nitrogen atom by the adjacent oxygen atom and, therefore, on a minor affinity with the anionic site of the enzyme.

The inactivity of Compound VI can be related to the fact that in the reactivator–phosphorylated enzyme complex the nucleophilic oximate group may be unsatisfactorily oriented for the reactivation. In the case of imidazole oximes, a large variation of activity does not occur by changing the poison used, whereas the pyridine oximes show a considerable reduction of activity when the enzyme is inhibited by diisopropylphosphonofluoridate instead of by tetraethylpyrophosphate. The substitution of a methyl with a benzyl group determines a remarkable improvement of the reactivating potency of IV in the poisoning by either tetraethylpyrophosphate or diisopropylphosphonofluoridate; this can depend on the presence of a receptorial area near to the enzyme anionic site which increases the bonding strength between reactivator and phosphorylated enzyme through van der Waals' and hydrophobic bonds.

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

Compound, 5×10^{-3} M	pKa	Enzyme Inhibition, %	Inhibiting Group: Diethylphosphoryl $K_{obs.}$	Diethylphosphoryl Relative Rate Constant	Diisopropylphosphoryl $K_{obs.}$	Diisopropylphosphoryl Relative Rate Constant
2-Hydroxyiminomethyl-1-methylpyridinium iodide	7.9	41.3	1.0×10^{-2}	1	9.8×10^{-4}	1
VII	7.9	89.1	4.7×10^{-3}	0.46	8.1×10^{-4}	0.82
IV	8.3	35.3	3.4×10^{-4}	0.03	2.4×10^{-4}	0.24
V	8.3	67.0	1.1×10^{-3}	0.11	5.1×10^{-4}	0.52
VI	9.8	37.4	None	—	None	—

[a] $K_{obs.}$ is in min.$^{-1}$. [b] The pKa values were obtained by potentiometric titration.

EXPERIMENTAL[2]

(*Z*)-1-Methyl-5-hydroxyiminomethylimidazole (III*a*) and (*E*)-1-Methyl-5-hydroxyiminomethylimidazole (III*b*)—To a solution of 1.5 g. of 1-methylimidazole-5-carboxaldehyde (8) in 50 ml. of ethanol, 1.5 g. of $H_2NOH \cdot HCl$ was added; the solution was refluxed for 30 min. After cooling to room temperature, the product (III*a* hydrochloride) was filtered and recrystallized from methanol–water, m.p. 257–258°.

Anal.—Calc. for $C_5H_7N_3O \cdot HCl$: C, 37.26; H, 4.99; N, 26.00. Found: C, 37.42; H, 5.05; N, 25.88.

The hydrochloride was converted to the free base by treatment with dilute sodium carbonate solution; the product obtained (III*a*) was filtered off and recrystallized from methanol–water; $\lambda_{max.}^{EtOH}$: nm. ($\epsilon \times 10^{-3}$) 261 (18.6).

By evaporation of the ethanolic filtrate, a solid was obtained which was dissolved in water. The solution was neutralized with dilute sodium carbonate and the precipitate was filtered, washed with water, and recrystallized from ethanol (III*b*); $\lambda_{max.}^{EtOH}$: nm. ($\epsilon \times 10^{-3}$) 263 (14.8).

(*E*)-1-Methyl-2-hydroxyiminomethylimidazole Methiodide (IV)—To 1 g. of I (7) dissolved in 120 ml. of acetone, 1.2 ml. of CH_3I was added. After 2 days at room temperature, the precipitate was filtered, washed with acetone, and crystallized from methanol; $\lambda_{max.}^{EtOH}$: nm. ($\epsilon \times 10^{-3}$) 273 (9.6).

(*E*)-1-Benzyl-2-hydroxyiminomethylimidazole Methiodide (V)—This product was prepared from II (7) and CH_3I as IV. After standing 3 weeks at room temperature, the solvent was in part evaporated and the solid obtained was filtered and crystallized from isopropanol; $\lambda_{max.}^{EtOH}$: nm. ($\epsilon \times 10^{-3}$) 270 (7.7).

(*Z*)-1-Methyl-5-hydroxyiminomethylimidazole Methiodide (VI)—To 0.01 mole of III*a* dissolved in methanol, 0.015 mole of CH_3I was added; the solution was heated in a sealed vessel at 60° for 2 days. By evaporation of the solvent, a residue was obtained which was solidified with ethyl acetate, filtered, washed with ethyl acetate, and crystallized from methanol–ethyl acetate; $\lambda_{max.}^{EtOH}$: nm. ($\epsilon \times 10^{-3}$) 223 (15.0), 247 (sh) (9.6), 260 (sh) (6.9), 287 (3.1), and 293 (2.5).

(*E*)-2-Hydroxyiminomethyl-1-benzylpyridinium Iodide (VII)—To 1 g. of (*E*)-2-hydroxyiminomethylpyridine dissolved in 40 ml. of acetone, 2 g. of benzyl iodide was added. After 4 days at room temperature, the solid was filtered and crystallized from methanol–ethyl acetate, m.p. 202–203° dec.

Anal.—Calc. for $C_{13}H_{13}IN_2O$: C, 45.90; H, 3.85; N, 8.23. Found: C, 45.74; H, 4.02; N, 8.42.

[2] Melting points are uncorrected. UV spectra were recorded on a Unicam model SP800 spectrophotometer; the proton magnetic resonance spectra were measured on a JEOL INH-MH-60 spectrometer, using dimethyl sulfoxide-d_6 as a solvent and sodium 3-(trimethylsilyl)-propanesulfonic acid salt as an internal standard. The IR spectra are in accord with the proposed structure.

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