REACTIVATING EFFECT OF α,ω-BIS-(4-PYRIDINEALDOXIME)-2-TRANS-BUTENE DIBROMIDE ON ISOPROPYL-METHYLPHOSPHONYLATED ACETYLCHOLINESTERASE

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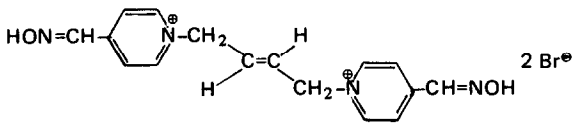
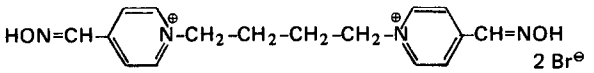
1. Introduction

It is known that some quaternary oximes are good reactivators of organophosphorus-inhibited acetylcholinesterase [1–3]. Reactivators containing pyridinium radicals are the most effective ones [3, 4]. The most active are those in which the oxime group is in the position 2 or 4 of the ring [1, 5].

In present work, the preparation of α,ω-bis-(4-pyridinealdoxime)-2-trans-butene dibromide (I) and its reactivating ability, with the use of isopropyl-methylphosphonylated bovine erythrocyte acetylcholinesterase (EC 3.1.1.7), are presented. Effect of compound I is compared with effect of α,ω-bis-(4-pyridinealdoxime)-butane dibromide (II).



I



II

2. Experimental

2.1. *Chemicals*

For preparation of α,ω-bis-(4-pyridinealdoxime)-2-trans-butene dibromide, 4-pyridinealdoxime* (5.12 g, 0.042 mole) was dissolved in dry dimethylformamide (30 ml) and mixed with a solution of 1,4-dibromo-2-trans-butene** (4.30 g, 0.08 mole) in dry dimethylformamide. A mixture was kept at room temperature for 24 hr and the solid product was collected by filtration. The product was recrystallized from an ethanol-water mixture as slight yellow plates (6.5 g, 71%); m.p. 255–257° (dec.), λ_{max} = 340 nm, $\epsilon = 4.85 \times 10^4$ in 0.1 N NaOH.

α,ω-Bis-(4-pyridinealdoxime)-butane dibromide was kindly supplied by Ing. M.Milojevič (Med. Acad., Belgrade, Yugoslavia) and had a melting point of 242–243° (reported m.p. 239–241° [6] and 254–256° [7]). λ_{max} = 339 nm, $\epsilon = 4.63 \times 10^4$ in 0.1 N NaOH.

All other chemicals were of reagent grade. Acetylcholinesterase used in this work was obtained from bovine erythrocytes [8] as a stable lyophilized powder, containing 280 mU./mg.

2.2. *Methods*

Determination of enzyme activity: Acetylcholinesterase activity was measured electrometrically with direct registration [9] at pH 8.0 and 25° with 5 mM acetylcholine iodide as substrate.

Determination of kinetic constants: The procedure used for determination of dissociation constant (K_R), the rate constant for breakdown of the intermediate complex (k_R), and bimolecular rate constant (k_r) were essentially the same as those described by Wang and Braid [4]. The bimolecular rate constants of reactivation in the presence of substrate (5 mM acetylcholine iodide) were measured according to the

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** Schuchardt, München

technique described by Kitz et al. [5]. Inhibition of acetylcholinesterase by isopropyl-methylphosphonofluoridate (1×10^{-9} M and 8×10^{-9} M, respectively, 30 min) and reactivation of phosphonylated enzyme by reactivators were carried out at pH 8.0 and 25°.

Spectrophotometrical measurements: UV absorbtion measurements were carried out with a manual Zeiss UR-2 spectrophotometer

The pK_a values of both oximes were obtained by potentiometric titration of their 1×10^{-2} M solutions in 0.1 N NaOH, as given by Speakmann [10].

3. Results and discussion

The kinetics of the reactivation process may be represented by the scheme:

$$EI + R \underset{}{\overset{K_R}{\rightleftharpoons}} EIR \xrightarrow{k_R} E + P$$

where EI is the inhibited enzyme, R is the reactivator, E is the reactivated enzyme, EIR is the intermediate complex, and P is the product. K_R and k_R are the dissociation constants and the rate constants for breakdown of the intermediate complex, respectively. These constants may be calculated from equation [3, 4]:

$$\frac{1}{k_{app}} = \frac{1}{k_R} + \frac{K_R}{k_R [R]},$$

where

$$k_{app} = \frac{2.303}{t} \log \frac{a_0 - a_r}{a_0 - a_i}$$

and a_0 represents the activity of normal enzyme, a_r the activity of reactivated enzyme, and a_i the activity of inhibited enzyme.

By plotting of $1/k_{app}$ vs. $1/[R]$ we obtained a straight line from which both K_R and k_R can be calculated. When $[R] \ll K_R$, k_R/K_R can be set equal to a bimolecular rate constant (k_r).

This type of plot for both compounds I and II is shown in fig. 1. The kinetic constants obtained from this plot are given in table 1. From the values of both bimolecular rate constants it is clear that compound I is about a seven to ten times better reactivator than compound II. This cannot be explained by different pK_a values of these compounds: $p(K_a)_1 = 7.95$ and $p(K_a)_2 = 8.88$ for compound I and $p(K_a)_1 = 7.95$ and $p(K_a)_2 = 8.72$ for compound II; reported $p(K_a)_1 = 7.9$ [7] for compound II and bimolecular rate constants measured in the absence of substrate recounted on anion concentration of reactivator at pH 8.0 are 1.58×10^4 M^{-1} min^{-1} and 2.30×10^3 M^{-1} min^{-1}, respectively.

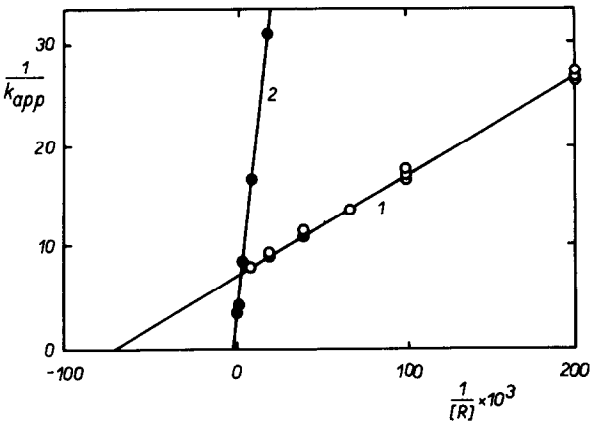

Fig. 1. The reciprocal plot of apparent velocity constants of reactivation k_{app} vs. the concentration of reactivator [R]. The intercept on the ordinate gives $1/k_R$ and the intercept on the abscissa gives $1/K_R$. Bimolecular rate constants k_r were calculated from the slope of the lines. Both lines were drawn according to the method of the least squares.
1: compound I, 2: compound II.

The results reported in this work indicate that compound I is a very good reactivator of isopropyl-methylphosphonylated acetylcholinesterase. The reactivating ability of this compound is comparable with the reactivating ability of Toxogonine ® (bis-(4-pyridinealdoximemthyl)-ether dichloride, $K_r = 9.50 \times 10^3$ M^{-1} min^{-1} [11]). Compound I is a 3.3 times better reactivator than TMB-4 (α,ω-bis-(4-pyridinealdoxime)-propane dibromide, $k_r = 3.29 \times 10^3$ M^{-1} min^{-1} [11]) and 43 times better than 2-PAM (2-pyridinealdoxime methiodide, $k_r = 2.50 \times 10^2$ M^{-1} min^{-1} [11]).

A strong reactivating effect of compound I can be

Table 1
Kinetic constants of reactivation.

Compound	k_R	K_R	k_r[a]	k_r[b]
	min^{-1}	10^{-5} M	10^3 M^{-1} min^{-1}	10^3 M^{-1} min^{-1}
I	0.143 ± 0.018	1.40 ± 0.16	10.20 ± 0.80	9.70 ± 0.85
II	0.360 ± 0.022	22.80 ± 3.62	1.58 ± 0.36	0.96 ± 0.18

[a] Measured in the absence of substrate.
[b] Measured in the presence of substrate (5 mM acetylcholine).

explained by a very good complementarity [12] of the molecule of the reactivator to enzymatic active site of phosphonylated acetylcholinesterase.

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