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SYNTHESIS AND CHOLINE ESTERASE HYDROLYSIS OF O-ACYLATED ALKYLCHLOROFORMOXIMES

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The wide spectrum of their biological activity permits classifying oximes and their derivatives as a promising group of physiologically active compounds for practical utilization in human and veterinary medicine. Up to the present time, a fairly large number of O-substituted oximes containing various substituents in the oxime part of the molecule have been synthesized and their biological activity has been studied. At the same time, the question of the synthesis and biological activity of the simplest representatives structurally of these compounds, the O-substituted alkylchloroformoximes still remains unanswered, which, we believe, is due to absence of fairly reliable methods of synthesis, because of the low stability of the starting alkylchloroformoximes.

In the present work, we discuss a fundamentally new approach to the synthesis of O-substituted oximes, based on the use of available α-chloronitrosoalkanes, with which we were able to obtain various O-substituted alkylchloroformoximes. Their biological activity was tested: the toxicity and the action on the key enzyme of the parasympathic nervous system, acetylcholine esterase (ACE) and butyrylcholine esterase (BCE), the binding with which may cause loss of inhibitors at the pharmacokinetic stage of the bioresponse formation. The structure–activity relationship was analyzed for the series of O-acylated alkylchloroformoximes.

We believe that the biological activity of O-substituted alkylchloroformoximes is to a great extent determined by the acceptor properties of the oxime group, and therefore it seemed productive to evaluate the contribution of this group, taking as an example the O-acylated oximes of the general formula

$$RC(O)ON{=}C(Cl)R^1,$$

I—VII

where R = Me (I—III), Et (IV, V), Pr (VI), CH_2Cl (VII, VIII); R′ = Me (I, IV), Et (II), Pr (III, VII), *i*-Pr (V, VI, VIII).

Compounds I-VIII were obtained in a yield of 11-46% by reacting 1,1-dichloro-1-nitrosoalkanes with acid chlorides of the corresponding carboxylic acids in the presence of an equimolar amount of zinc dust.

$$O{=}N{-}CCl_2{-}R' + RCOCl \xrightarrow{Zn} \longrightarrow RC(O)ON{=}C(Cl)R'$$

EXPERIMENTAL (CHEMICAL)

The PMR spectra were recorded in $CDCl_3$ on a CXP-200 spectrometer ("Bruker", FGR) with a working frequency of 200 MHz, using TMS as internal standard.

O-Propylchloroformiminoacetate (III). A 15.6 g portion (0.1 mole) of 1,1-dichloro-1-nitrosobutane was added with stirring at 20°C to a suspension of 6.2 g (0.1 mole) of zinc

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TABLE 1. Physicochemical Properties of Compounds I-VIII

Compound	Yield, %	Bp, °C/mm Hg	n_D^{20}	Empirical formula
I	14,8	85—87/10	1,4405	$C_4H_6ClNO_2$
II	26,7	70—71/10	1,4522	$C_5H_8ClNO_2$
III	23,4	86/10	1,4464	$C_6H_{10}ClNO_2$
IV	46,8	75—6/7	1,4462	$C_5H_8ClNO_2$
V	28,2	88—90/7	1,4524	$C_7H_{12}ClNO_2$
VI	11,8	90—95/7	1,4508	$C_8H_{14}ClNO_2$
VII	23,1	78—79/2	1,4744	$C_6H_9Cl_2NO_2$
VIII	22,7	77—80/2	1,4734	$C_6H_9Cl_2NO_2$

TABLE 2. Data of IR and NMR Spectra of Compounds I-VIII

Compound	IR spectrum, ν_{max}, cm^{-1}		PMR spectrum, δ, ppm J_{H-H}, Hz
	C=N	C=O	
I	—	—	2,43 s, 2,23 s
II	—	—	2,56 q, J 7,1, 2,08 s, 1,13 t, J 7
III	—	—	2,41 t, J 7, 2,22 s, 1,76 m, 0,96 t, J 7
IV	1625	1725	2,52 m, 2,43 s, 1,24 t, J 7,1
V	1610	1785	3,05 m, 2,5 m, 1,23 m
VI	1615	1760	3,06 m, 2,45 t, J 7,2, 1,75 m, 1,27 d, J 7,3, 1,02 t, J 7
VII	1625	1800	4,26 s, 2,64 t, J 7,1, 1,74 m, 1,06 t, J 7,1
VIII	1620	1795	4,24 s, 3,66 m, 1,28 d, J 7,2

dust in 50 ml of ether and 7.85 g (0.1 mole) of acetyl chloride. The reaction mixture was stirred to disappearance of color, 50 ml of hexane was added, $ZnCl_2$ was separated, the filtrate was evaporated, and the residue was fractionated to yield 3.87 g (23.4%) of the desired end product, bp 86°C/10 mm t.p. Compounds I, II and IV-VIII were obtained in a similar way.

The composition and structure of compounds I-VIII were confirmed by the data of elemental analysis, IR and PMR spectral characteristics.

EXPERIMENTAL (BIOLOGICAL)

The hydrolysis was studied of compounds I-VIII by ACE, obtained from human erythrocytes (acetylcholine acetylhydrolase, EC 3.1.1.7) and BCE from equine blood serum, acylcholine (acylhydrolase, EC 3.1.1.8). The two enzymes were produced at the Perm' Scientific Research Institute of Vaccines and Sera. The specific activities of BCE and ACE were 156 and 2.2 (ChA)U/mg respectively. The rate of hydrolysis was measured at 25°C by potentiometric titration using a "Radiometer RTS 822" autotitrator (Denmark). The working solution contained $2 \cdot 10^{-3}$ M phosphate buffer at pH 7.5 for ACE and 7.8 for BCE, $2 \cdot 10^{-2}$ M KCl, 0.03-0.06 mg/ml of ACE or 0.016 mg/ml of BCE. The concentration of the substrates was $1 \cdot 10^{-5}$-$2 \cdot 10^{-3}$ and the volume of the sample was 10 ml. The Michaelis constant (K_M) and the maximal rate of hydrolysis (V) were determined graphically by the Lainuiwer and Berk method [3]. To calculate the activity of the catalytic center (a_c) according to Berry [4], the value of the active concentrations of the active centers was used, which was determined by titration of ACE with Gd-42 compound (O-ethyl-S-[β-ethylmercaptoethyl] methylthiophosphonate methylsulfomethylate), and BCE with diisopropyl fluorophosphate.

The acute toxicity was determined on white nonpedigreed male mice, weighing 20-30 g each. The compounds were dissolved in vegetable oil (acetylcholine in water) and were administered in a single intragastric dose. The animals were observed for 14 days. The LD_{50} was calculated on a NORD-10 computer, according to [5].

TABLE 3. Kinetic Parameters of Hydrolysis of O-Acylated Alkylchloroformoximes I-VIII by the Action of ACE and BCE and Their Acute Toxicity

Compound	ACE			BCE			Acute toxicity (LD_{50}) for mice enterally, mg/kg
	$K_M \cdot 10^{-4}$, M	$V \cdot 10^{-6}$, M·mg^{-1}·min^{-1}	$a_c \cdot 10^5$, min^{-1}	$K_M \cdot 10^{-4}$, M	$V \cdot 10^{-6}$, M·mg^{-1}·min^{-1}	$a_c \cdot 10^5$, min^{-1}	
I	11,0	9,1	6,1	13,0	11,2	1,8	79 (55—114)
II	3,5	4,6	3,1	4,9	6,0	1,0	148 (87—251)
III	2,8	4,5	3,1	3,5	8,4	1,3	146 (107—200)
IV	3,5	3,0	2,0	4,4	7,4	1,2	87 (65—115)
V	7,7	2,2	1,5	1,5	11,2	1,8	284 (241—335)
VI	0,3	0,3	0,2	3,2	9,8	1,6	381 (303—478)
VII	...	...	...	...	...	...	148 (124—177)
VIII	...	...	...	...	...	...	228 (197—263)
Acetylcholine iodide	1,3	2,2	1,5	5,4	3,8	0,6	>1500

Note. The kinetic parameters of VII and VIII were not determined because of low stability.

The kinetic investigations showed that compounds I-VIII may serve as substrates for ACE and BCE. The dependence of the rate of hydrolysis of I-VIII on their concentration is graphically represented by a bell-shaped curve in the case of ACE and by a hyperbola in the case of BCE. The kinetic parameters of the enzymatic hydrolysis of alkylchloroformoximes and acetylcholine iodide (for comparison) are given in Table 3. Comparison of the values of V and a_c shows that ACE hydrolyzes compound I with the highest rate, the latter containing methyl radicals in the acid and oxime parts of the molecule. However, its hydrolysis is characterized by the highest value of K_M. Increase in the length of the alkyl radicals leads to a decrease in the rate of hydrolysis. Compound VI containing propyl and isopropyl radicals, respectively, in the acid and oxime parts of the molecule, has the lowest rate of hydrolysis. It should be noted that the hydrolysis of VI has the lowest value of K_M, which indicates its highest affinity for the enzyme. This contradicts the known principle of "the higher the affinity, the more efficient the catalysis," and is probably explained by a nonproductive sorption of part of the substrate on the active surface of the enzyme. Because of the irregular sorption, the substrate is unable to undergo enzymatic hydrolysis [1, 2].

The BCE enzyme hydrolyzes compounds I and V at the highest rate. Compared with ACE, BCE is less sensitive to structural features of the substrate (see Table 3), which agrees with the literature data [2], according to which the selectivity of BCE is lower than that of ACE.

Compounds containing a chlorine atom in the acidic part of the molecule (VII and VIII) are very unstable even at pH 7.5, and therefore the kinetic parameters of their enzymatic hydrolysis were not determined, although they are also hydrolyzed by ACE and BCE. Compounds I-VI are much more stable at physiological values of pH and decompose appreciably only in more alkaline media (for example, the half-decomposition period of IV at pH 10.5 and at 25°C is 2.4 min).

The acute toxicity of I-VIII is independent of the kinetic parameters of their enzymatic hydrolysis. The LD_{50} is in the range of 79 (55-114)-381 (303-478) mg/kg (see Table 3).

Thus, we have shown that O-acylated alkylchloroformoximes are hydrolyzed by ACE and BCE. The results obtained can be used as a guide in the search for new physiologically active compounds having selective action on choline esterases.

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