

tively. Thus, the Schtraube syndrome was absent, which indicated a moderate analgetic action of no practical use.

The antimorphinic activity of the compounds tested was studied on a model of suppression of the analgetic effect of morphine [9]. The tests were carried out on rats, each weighing 110-120 g at a dose of the compounds of 10 mg/kg. No antimorphinic action was observed.

A sharp decrease in the pesticidal activity of compounds III was also revealed in varroasis of bees, compared with neorone.

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SYNTHESIS OF PHOSPHORUS-CONTAINING FORMHYDROXAMOYL HALIDES AND THEIR ANTICHOLINE ESTERASE PROPERTIES

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Hydroxamic acids and their derivatives have a wide spectrum of biological activity [1]. It was of interest to study the anticholine esterase properties of formhydroxamic acid, containing a dialkoxyphosphoryl group in its composition.

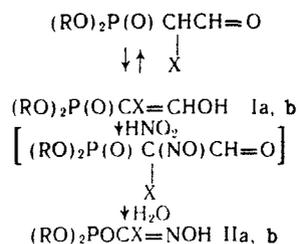
We have previously described a method for the preparation of (diisopropoxyphosphoryl)-formhydroxamoyl bromide (IIa) and chloride (IIb) by nitrosation of the corresponding (diisopropoxyphosphoryl)haloacetaldehydes (Ia, b) with nitrous acid in aqueous alcoholic medium [4].

In continuation of this work, we have improved the method for the preparation (dialkoxyphosphoryl)formhydroxamoyl halides II by eliminating the laborious extraction process and the time-consuming crystallization of the products, thus increasing the yield up to 90% or greater. The synthesis of the above compounds was carried out according to the following scheme:

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TABLE 1. Bimolecular Rate Constants of Inhibition of Choline Esterases

Compound	$k^{II}, M^{-1} \cdot \text{min}^{-1}$		LD ₅₀ , mg/kg
	ACE	BCE	
IIa	$5.79 \cdot 10^5$	$1.66 \cdot 10^7$	32,4 (25,5—41,2)
IIb	$7.92 \cdot 10^5$	$1.23 \cdot 10^7$	29,4 (19,1—45,2)



R = iso-C₃H₇; X = Br (Ia, IIa), Cl (IIb, IIb).

The nitrosation reaction of (dialkoxyphosphoryl)haloacetaldehydes I by nitrous acid was carried out in a hydrochloric acid medium at a temperature below 0°C. The compounds II obtained are sparingly soluble in an acid medium and precipitated in the course of the reaction, and were then separated and recrystallized.

The structure of the synthesized products II was confirmed by x-ray diffraction analysis, by ¹H, ³¹P NMR and IR spectroscopy, as well as by elemental analysis. In the IR spectra of the compounds obtained, absorption bands were observed in the regions of 3100-3200 cm⁻¹ (OH), 1601-1606 cm⁻¹ (C=N), 1228-1230 cm⁻¹ (P=O), 560-650 cm⁻¹ (C=Hal). In the PMR spectra of compounds IIa, b there are proton signals of the alkoxy groups of the phosphoryl fragment (δ_{CH_2} 1.40-1.42 ppm, δ_{CHO} 4.86-4.88 ppm) and a signal of the oxime group hydroxyl (δ_{NOH} 12.28-12.44 ppm).

EXPERIMENTAL (CHEMICAL)

The IR spectra were run in mineral oil on a UR-20 spectrophotometer and the PMR spectra on a "Tesla" BS-497 spectrometer (ν_0 100 MHz) in CDCl₃ solution. The ³¹P NMR spectra were run on a "Bruker WP-80" spectrometer. The chemical shifts of the protons were measured with reference to TMS and of phosphorus with reference to H₃PO₄ as external standard. The elemental analysis data corresponded to the calculated values.

(Diisopropoxyphosphoryl)formhydroxamoyl Bromide (IIa). A 25 g amount of ice was added to a solution of 7.18 g (0.025 mole) of (diisopropoxyphosphoryl)bromoacetaldehyde in 35 ml of a 36% hydrochloric acid, and then a solution of 2.07 g (0.03 mole) of sodium nitrite in 30 ml of water was added dropwise at -5-0°C with continuous stirring. After 20 min, the precipitate was separated, dissolved in carbon tetrachloride and precipitated from the solution with hexane. Yield, 6.5 g (90%) of colorless crystals, mp 61-62°C. C₇H₁₅BrNO₄P. $\delta_{31\text{P}}$ - 0.50 ppm (DMSO).

(Diisopropoxyphosphoryl)formhydroxyamoyl Chloride (IIb) was obtained in a similar way as compound IIa. Yield 95%. Mp 68-69°C. C₇H₁₅ClNO₄P. $\delta_{31\text{P}}$ - 1.00 ppm (DMSO).

EXPERIMENTAL (BIOLOGICAL)

The anticholine esterase activity was determined in experiments in vitro by a potentiometric method [2, 5] with pH adjusted to steady-state conditions on a "Radiometer RTS-822" autotitrator (Denmark) under standard conditions: 25°C; 0.1 M KCl, 1.33 mM phosphate buffer at pH 7.5; substrate - acetylcholine chloride.

A commercial preparation of acetylcholine esterase from human erythrocytes (ACE) and butyrylcholine esterase (BCE) from horse serum produced at the Perm Scientific Research Institute of Vaccines and Sera with a specific activity of 4.0 and 9.6 E per 1 ml of the protein were used.

The inhibition kinetics of the enzymes was studied under pseudomolecular conditions ($[I]_0 \gg [E]_0$) by determining the residual activity of the enzyme after incubation with the inhibitor [5]; the time of incubation was from 15 sec to 4 min. The antienzymatic activity was evaluated from the values of the bimolecular rate constants of inhibition of the enzyme.

The pseudomonomolecular inhibition rate constants were determined from the dependence

$$\lg V_t = \lg V_0 + k' t / 2.3,$$

where V_0 is the rate of enzymatic hydrolysis of the substrate in the absence of the inhibitor; V_t is the rate of hydrolysis of the substrate after the reaction of the enzyme with the inhibitor during a time t .

The bimolecular inhibition rate constants k^{II} were found from the dependence

$$k' = k^{II} [I]_0 + b,$$

where $[I]_0$ is the concentration of the inhibitor in the reaction mixture, while b allows for the spontaneous inactivation of the enzyme, were calculated by the linear regression method.

The acute toxicity of the compounds was determined in tests on white nonpedigree mice, each weighing 18-24 g. The compounds were dissolved in acetone and were administered intraperitoneally in a volume of not more than 50 μ liter. The control animals received acetone. The observation period was 24 h. The LD_{50} values were determined by calculation on a NORD-10 computer according to [7].

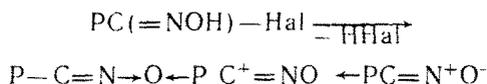
The study of the reaction of acid bromide IIa and acid chloride IIb with ACE from human erythrocytes and BCE from horse serum showed that these compounds irreversibly inhibit the two enzymes - the degree of inhibition increases proportionally with the time of incubation of the enzyme with the inhibitor. The bimolecular inhibition rate constants of choline esterases determined from the kinetic data are listed in Table 1.

As can be seen from Table 1, the compounds studied are highly inhibiting with respect to ACE, where the inhibition constants have an order of magnitude of 10^5 , and particularly with respect to BCE, where the order of magnitude of the constants is 10^7 .

The LD_{50} values of compounds IIa and IIb obtained in the tests on mice indicate that they have fairly high toxicity, which correlates with their high anticholine esterase activity.

The high anticholine esterase activity in experiments in vitro, as well as the high acute toxicity of the compounds studied, which are not from the point of view of their structure anticholine esterase agents, i.e., do not contain the leaving group X usual for organophosphoric choline esterases inhibitors $[R_1R_2P(O)X]$ [3], indicate the possibility of formation of this inhibitor directly in the reaction mixture and(or) in the organism.

We showed that on heating or by the action of bases (Et_3N , $NaHCO_3$) on (diisopropoxyphosphoryl)formhydroxamoyl halides, highly reactive and relatively unstable phosphorylnitrile oxides are formed, the structure of which was confirmed by IR and ^{31}P NMR spectroscopy.



The formation of similar structures was shown previously for nonphosphorylated analogs of these compounds [6, 8].

It is thus possible to assume that the high anticholine esterase activity of (diisopropoxyphosphoryl)formhydroxamoyl halides studied is due to the formation of active phosphorylnitrile oxides in aqueous solution.

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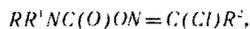
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SYNTHESIS OF O-CARBAMOYLATED ALKYLCHLOROFORMOXIMES AND THEIR ANTICHOLINE
ESTERASE ACTIVITY

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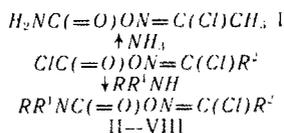
UDC 615.355:577.152.311].012.1.07

In continuation of our investigations on the synthesis of biologically active compounds in the series of O-substituted alkylchloroformoximes [4], which can be considered as novel effective pesticidal preparations [3], in the present article we discuss the synthesis of O-carbamoylated alkylchloroformoximes and their biological activity. Since by using O-acylated alkylchloroformoximes as an example, we have previously found [4] the alkylchloroformoxime group to have high acceptor properties, it was logical to take advantage of this for the preparation of compounds with anticholine esterase activity. We have indeed accomplished this in the synthesis of the previously unknown O-carbamoylated alkylchloroformoximes of the general formula



I-VIII
where $R=H(I-III), Me(IV-VI), Et(VII, VIII); R^1=H(I),$
 $Me(II-VI), Et(VII, VIII); R^2=Me(I, IV), Et(II, VIII),$
 $Pr(III, V, VII), i-Pr(VI).$

Compounds I-VIII were obtained by amidolysis of the corresponding O-chloroformylhydroxi-moyl chlorides with ammonia, primary and secondary amines in a yield of 44-79%:



The biological activity of O-carbamoylated alkylchloroformoximes was evaluated from the action on acetylcholine esterase (ACE, the acetylhydrolase of acetyl choline, CE 3.1.1.7) and butyryl choline esterase (BCE, the acylhydrolase of acylcholines, CE 3.1.1.8), and also from the influence on the nerve-muscle conductivity, and from the acute toxic action on mice.

EXPERIMENTAL (CHEMICAL)

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

O-Carbamoylacethydroximoyl Chloride (I). A 1.7 g portion (0.1 mole) of ammonia was bubbled through a stirred solution of 7.8 g (0.05 mole) of O-(chloroformyl) acethydroximoyl chloride in 100 ml of ether at a temperature from -10 to -15°C. The precipitate formed was filtered off and boiled in 100 ml of benzene; on cooling the benzene solution, crystals precipitated. Yield, 4.2 g (61%) of colorless crystals of I, mp 107-109°C.

Compounds II-VIII were obtained in a similar way from 0.05 mole of acid chloride and 0.1 mole of the corresponding amine. The composition and structure of I-VIII were established by the elemental analysis data corresponding to the calculated values and the PMR spectral characteristics (Table 1).

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