SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF THIOESTERS OF CHLOROFLUORONITROACETIC ACID

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It was recently discovered that esters of chlorofluoronitroacetic acid showed high activity as irreversible inhibitors of cholinesterase (CE) [2, 3]. With the aim of exploring new inhibitors among this class of compounds, we synthesized thioesters of chlorofluoronitroacetic acid $O_2NCFClCOSR$ (Ia, b), R = Et (Ia) and Bu (Ib). The present work gives the results of a study of their anticholinesterase activity. They were compared in effectiveness with the oxygen analogs, the ethyl (IIa) and butyl (IIb) esters of chlorofluoronitroacetic acid.

EXPERIMENTAL (CHEMISTRY)

IR spectra were obtained with a Specord IR-75 spectrometer (GDR) on thin films, and ^1H NMR and ^{19}F NMR with a Bruker CXP-200 spectrometer (FRG), working at frequencies of 200 and 188 MHz with respect to TMS and CF_3COOH, respectively.

Butyl Chlorofluoronitrothioacetate (Ib). Chlorofluoronitroacetic acid (4.5 g, 0.029 mole) was added with stirring to 6 g of PCl_5 at 25°C, and the reaction mixture was distilled. The fraction with bp 97-102°C was treated with a small excess of butyl mercaptan. Vacuum distillation gave 3.65 g (55%) of Ib, bp 80°C (2 mm Hg), n_D^{20} 1.4540; d_4^{20} 1.384. Found, %: C 30.82; H 2.93; F 8.21; S 14.01, $C_6H_9ClFNO_3S$. Calculated, %: C 31.40; H 3.92; F 8.30; S 13.91. IR spectrum, ν, cm^{-1}: 1770 (C=O), 1600 (NO_2), 1040 (C–F). NMR spectrum, δ, ppm: 1.15 t (CH_3), 1.65 q (CH_2), 1.88 q (CH_2), 3.35 t (CH_2); F = 9.21 s.

Thioethyl ester (Ia) was prepared analogously. Yield 35%, bp 106°C (33 mm Hg), n_d^{20} 1.4663, d_4^{20} 1.397. Found, %: C 24.28; H 3.69; F 9.47; S 15.23. $C_4H_5ClFNO_3S$. Calculated, %: C 23.82; H 2.48; F 9.45; S 15.90. IR spectrum, ν, cm^{-1}: 1780 (C=O), 1590 (NO_2), 1025 (C–F). NMR spectrum, δ, ppm: 1.4 m (CH_3), 4.3 q (CH_2); F = 10.46 s.

Several of the physico-chemical constants for compounds Ia were published earlier [4] and agreed with the indicated values. The synthesis and physico-chemical constants of compounds IIa and b were published earlier [3].

EXPERIMENTAL (BIOLOGY)

The activity of compounds Ia, b on butylcholinesterase (BChE) was studied on horse blood serum (acylcholine acylhydrolase, EC 3.1.1.8) and acetylcholinesterase (AChE) from human erythrocytes (acetylcholine acetylhydrolase, EC 3.1.1.7). Both preparations were produced from Perm Scientific Research Institute vaccine and serum, with specific BChE and AChE activities of 9.6 and 2.2 E/mg, respectively. The irreversible bimolecular constants for inhibition (k_2) were found for AChE and BChE with compounds Ia and Ib at 25°C. In so far as the concentration of the inhibitor in the reaction medium significantly exceeded the concentration of the active center of the enzyme, k_2 was determined by an equation for a pseudomonomolecular reaction [5]. The potentiometric titration method was used and the experimental conditions described for IIa [2] were observed. The results were treated statistically [1].

The activities of Ia and Ib on neuromuscular conduction were tested in 10 experiments with phrenic-diaphragmatic preparations isolated from rats by the procedure of Bülbring [6]. A carbogenated physiological solution [7], pH 7.4 at 37°C, was used. An R-612 (USA) dynograph was used to measure the contraction of the muscle under an isometric regime compared to stimulation of the nerve by 0.1 msec supramaximal electrical impulses.

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TABLE 1. Anticholinesterase Effectiveness and Acute Toxicity of Thioesters Ia, Ib and Esters IIa and IIb of Chlorofluoronitroacetic Acid

Compound	k_2, $M^{-1} \cdot min^{-1}$		k_2, BChE / k_2, AChE	Mechanism for blockage of neuromuscular conduction	Toxicity, LD_{50}, for mice, internal, mg/kg
	BChE	AChE			
Ia	$(4,0\pm0,5)\cdot10^2$	$(0,15\pm0,02)\cdot10^2$	27	Nonanticholinesterase	170 (124—233)
Ib	$(1,0\pm0,2)\cdot10^3$	$(1,0\pm0,1)\cdot10^2$	10	Same	137 (113—168)
IIa	$(3,9\pm0,1)\cdot10^5$	$(1,9\pm0,1)\cdot10^4$	20	Anticholinesterase	86 (64—115)
IIb	$(5,1\pm0,5)\cdot10^5$	$(3,0\pm0,7)\cdot10^4$	17	Same	119 (101—139)

Note. The mechanism of blocking action of IIa and IIb and their anticholinesterase activity, and the values of k_2 were reported earlier [2, 3].

The acute toxicity of compounds Ia, b and IIa, b was determined on male white mice weighing 25-30 g. The materials were dissolved in vegetable oil and injected once into the stomach and the animals were observed for 14 days. The LD_{50}'s were calculated by the method of Litchfield and Wilcoxon [8].

A study of the kinetics of the interaction of Ia, b with BChE and AChE showed the inhibition of the enzymatic activity to possess an irreversible character. The values of k_2 are shown in Table 1. They are 2-3 times less than for IIa, b. In another series (7 experiments), the AChE (inhibited by compound Ia or Ib by 95-98%) was dialyzed for 16-18 hours through a permeable membrane at 5°C against a 500-fold excess of phosphate buffer (0.002 M, pH 7.5), containing 0.02 M KCl. An increase in the residual activity of AChE was not observed in this case. It did not increase in a period of several hours after analysis at 25°C, but began to increase even 1-1.5 min after addition of the active CE TMB-4 (10 μM). Similar activity was shown by TMB-4 against AChE inhibited by compound IIa [2, 3].

Millimolar concentrations of Ia, b induced neuro-muscular blockage by action on the phrenic-diaphragmatic preparation. The mechanism of this activity was nonanticholinesterase, since it did not occur, for example, by strengthening the blockage of a single impulse immediately after tetanization of the nerve (a characteristic sign of anticholinesterase blockage). Conduction was not reduced by TMB-4 blocked by Ia or Ib. The blockage produced by IIa, b, however, is of the anticholinesterase type and is reduced by TMB-4 [2]. In this manner, although Ia, b possess the property of inhibiting AChE, the disruption of the neuromuscular conduction is caused not by their activity on the synaptic AChE, but on some other formation.

Compounds Ia, b and IIa, b induced chronic-tonic convulsions and muscle weakness in mice. Of the four materials, three had LD_{50} greater than 100 mg/kg; IIa was more toxic (cf. Table 1).

Thus, the substitution of an atom of sulfur for an atom of oxygen in the chlorofluoronitroacetic acid esters leads to a sharp decrease in the anticholinesterase activity of the materials. The selectivity to BChE is preserved. As with the esters, the thioesters of chlorofluoronitroacetic acid interact with enzymes in a type of irreversible reaction with the formation of stable compounds. In both cases, TMB-4 reactivated the inhibited AChE. We are presently studying the mechanism of the retardation of CE by thioesters and esters.

LITERATURE CITED

1. M. L. Belen'kii, Elements of Quantitative Tests of Pharmacological Effects [in Russian], 2nd edn., Leningrad (1963), pp. 16-32.
2. Yu. Ya. Ivanov, V. K. Brel', and I. V. Martynov, Dokl. Akad. Nauk SSSR, 279, 506-508 (1984).
3. Yu. Ya. Ivanov, V. K. Brel', L. V. Postnova, and I. V. Martynov, Khim.-farm. Zh., No. 8, 968-971 (1985).
4. I. V. Martynov, Yu. L. Kryglyak, and S. P. Makarov, Zh. Obshch. Khim., 33, 3384-3386 (1963).

5. V. A. Yakovlev, Kinetics of Enzyme Catalysis [in Russian], Moscow (1965), p. 113-125.
6. E. Bülbring, Br. J. Pharmacol., 1, 38-61 (1946).
7. A. W. Liley, J. Physiol. (London), 142, 650-666 (1956).
8. I. Litchfield and F. Wilcoxon, J. Phamacol. Exp. Ther., 96, 99-113 (1949).

QUANTITATIVE RELATIONSHIP BETWEEN ANTICONVULSANT ACTIVITY IN N-BENZHYDRYLAMIDES AND N-BENZHYDRYL-UREAS, THEIR STRUCTURES, AND ^{13}C NMR SPECTRA

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The benzhydryl group is found in numerous biologically active compounds [3, 4, 9]. In particular, N-benzhydrylurea is known to display quite high anticonvulsant activity [3, 4]. There have, however, been no literature reports on quantitative relationships between structure and activity in these compounds.

In order to establish quantitative relationships between anticonvulsant activity and the structures of compounds containing the common amide grouping $Ph_2CHNHCOR$, we have synthesized the N-benzhydrylamides (I-V) and N-benzhydrylureas (VI-XX), and measured their ^{13}C NMR chemical shifts and anticonvulsant activity as expressed by the anticorazole index.

$Ph_2CHNHCOR$ $RC_6H_4CH(Ph)NHCONH_2$

I—VI VII—XX

R=H (I), Me (II), Et (III), Pr (IV), *i*-Pr (V), NH_2 (VI). o-F (VII), m-F (VIII), p-F (IX), o-Cl (X), m-Cl (XI), p-Cl (XII), o-Br (XIII), m-Br (XIV), p-Br (XV), o-I (XVI), m-I (XVII), o-Me (XVIII), m-Me (XIX), p-Me (XX).

The synthesis of benzhydrylformamide (I) was effected by a modified Leuckart reaction from benzophenone and formic acid, using the more readily available urea in place of the more usual formamide [5]. Compounds (II-V) were obtained by acylating benzyhydrylamine with the appropriate acid chlorides. The N-benzhydrylureas were obtained by standard methods from the appropriate benzhydrylamines and urea [1]. The constants of the products (I-XX) are given in Tables 1 and 2.

The anticonvulsant activity of (I-VI) is shown in Table 1, from which it will be seen that the activity of the N-benzhydrylamides decreases with increasing length and branching of the alkyl radical R. A linear relationship is evident between the value of the exponent of the anticorazole index for compounds (I-V) and the Charton steric constant ν [6] [Fig. 1, equation (1)].

$$e^A = 13.01 - 14.28\,\nu;\ r = 0.980,\ S_0 = 0.24. \quad (1)$$

The introduction of other steric constants for the alkyl substituents (E_S, E_S^0 [6]) gives a somewhat less satisfactory correlation. N-benzhydrylurea (VI) is the most active compound in the series (I-VI). Although there is at present no strictly unified scale of steric constants for various types of functional groups, and it is not possible from the steric constants available to make comparisons between the amino-group and alkyl radicals, it is nevertheless clear that (VI) basically does not comply with the relationship (1), since the van der Waals or covalent radii of the NH_2 group are at the very least greater than those of hydrogen. The much higher than expected activity of the urea (VI) as compared with amides (I-V) suggests that there is no similarity between the mechanisms of anticonvulsant activity of the benzhydrylureas (VI-XX) and the amides (I-V).

Since the high activity of the benzhydrylurea (VI) suggests the possible practical value of this type of compound as an anticonvulsant drug, we have examined the influence of

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