ANTICHOLINESTERASE ACTIVITY OF O—CARBAMOYLATED ACYLHYDROXIMOYL CHLORIDES

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In a recent study [1] we described the synthesis of O-carbamoylated acylhydroximoyl chlorides and demonstrated their ability to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) on the example of eight compounds with various aliphatic radicals. This work presents the results of a study of the anticholinesterase activity of 16 new compounds of this class, with the formula

$$RR^1NC(O)ON{=}C(Cl)R^2,$$
I—XVI

where:

R=Ph(I—V), PhMe-*o*(VI—IX), PhMe-*m*(X—XIII), PhMe-*p*(XIV—XVI); R^1=H(I—IV, VI—XVI), Me(V); R^2= =Me(I, VI, X, XIV), Et(II, VII, XI, XV), Pr(III, VIII, XII), *i*-Pr(IV, V, IX, XIII, XVI).

The carbamates I-XVI were synthesized by the reaction of the corresponding chloroformylacylhydroximoyl chlorides with anilines.

$$ClOCON{=}CR^2Cl + 2RR^1NH \xrightarrow[-RR^1NH\cdot HCl]{} RR^1NOCON{=}CR^2Cl$$

EXPERIMENTAL (CHEMICAL)

The PMR spectra were recorded on a Bruker CXP-200 instrument with a working frequency of 200 MHz $(CD_3)_2CO$, internal standard TMS.

O-(N-Phenylcarbamoyl)acethydroximoyl Chloride (I). To a solution of 7.8 g (0.05 mole) O-(chloroformyl)acethydroximoyl chloride in 100 ml of ether we added 9.3 g (0.1 mole) of aniline dropwise with mixing at −10 to −15°C. The temperature of the reaction mixture was brought up to room temperature, the precipitate filtered off, washed with ether, and the filtrate evaporated. The residue was boiled in 100 ml of hexane; when the solution was cooled, white crystals precipitated. Yield 1 g (9.5%) of I, mp 122-124°C.

Compounds II-XVI were produced analogously from 0.05 mole of chloroanhydride and 0.1 mole of the corresponding amine. The composition and structure of I-XVI were demonstrated by the data of elementary analysis and the PMR-spectral characteristics (Table 1).

EXPERIMENTAL (BIOLOGICAL)

Preparations of AChE (acetylcholine acetylhydrolase, EC 3.1.1.7) from human erythrocytes and BuChE (acylcholine acylhydrolase, EC 3.1.1.8) from horse blood serum with specific activity 2.2 and 9.9 units/mg, respectively, produced by the Perm Scientific-Research Institute of Vaccines and Sera, were used. The kinetic measurements were performed by continuous potentiometric titration with a glass electrode in a pH-static system (pH 7.5) on a Radiometer RTS822 autotitrator (Denmark) at 25°C in a medium of 0.1 M KCl and 2 mM phosphate buffer. Acetylcholine bromide in concentrations of $1\cdot10^{-3}$ M and $1\cdot10^{-2}$ M was used as the substrate for AChE and BuChE, respectively. The volume of the sample was 10 ml, AChE concentration 0.04 mg/ml, BuChE 0.03 mg/ml, and oximes in the range of $1.5\cdot10^{-5}$ – $1\cdot10^{-7}$ M.

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TABLE 1. Physicochemical Properties and Data of PMR Spectra of Compounds I-XVI

Compound	Yield, %	Mp, °C	Gross-formula	PMR spectrum, ppm
I	9,5	122—124	$C_9H_9ClN_2O_2$	2,41s; 7,1—7,61m; 9,23 s
II	75,1	113—115	$C_{10}H_{11}ClN_2O_2$	1,23m; 2,68 q 7,1—7,6m 9,22 s
III	50,5	81—83	$C_{11}H_{13}ClN_2O_2$	0,98t; 1,72m, 2,64 t 7,1—7,62m; 9,18 s
IV	54,7	115—117	$C_{11}H_{13}ClN_2O_2$	1,27d; 3,0m; 7,1—7,61m 9,16 s
V	51,1	Oil	$C_{12}H_{15}ClN_2O_2$	1,20d; 2,94m; 3,35s; 7,28—7,61m
VI	31,4	73—75	$C_{10}H_{11}ClN_2O_2$	2,32s 2,44s; 7,17—7,60m; 8,6s
VII	38,2	50—51	$C_{11}H_{13}ClN_2O_2$	1,31t 2,37s; 2,78q 7,2—7,65m; 8,62s
VIII	71,4	61—63	$C_{12}H_{15}ClN_2O_2$	0,98t; 1,74m; 2.30 s; 2,64 t 7,1—7,62m, 8,6 s
IX	38,6	53—54	$C_{12}H_{15}ClN_2O_2$	1,31d 2,34s; 3,00m; 7,14—7,64m; 8,56s
X	15,4	73—75	$C_{10}H_{11}ClN_2O_2$	2,32s 2,41 s; 6,92—7,4m, 9,12s
XI	59,3	103—104	$C_{11}H_{13}ClN_2O_2$	1,32 t 2,37s; 2,76 q 6,99—7,47m; 9,15 s
XII	43,7	99—100	$C_{12}H_{15}ClN_2O_2$	1,00t; 1,75m; 2,34 s 2,64t; 6,92—7,42m, 9,1 s
XIII	73,0	103—105	$C_{12}H_{15}ClN_2O_2$	1,28d; 2,34s; 3,00 m; 6,93—7,44m 9,1 s
XIV	22,2	117—119	$C_{10}H_{11}ClN_2O_2$	2,33s; 2,45s; 7,11—7,60 m; 9,07s
XV	61,0	90—92	$C_{11}H_{13}ClN_2O_2$	1,25 t 2,32 s 2,70 q 7,15—7,48m; 9,1 s
XVI	49,5	122—124	$C_{12}H_{15}ClN_2O_2$	1,27d; 2,32s; 3,00 m 7,18—7,5 m 8,9 s

TABLE 2. Anticholinesterase Activity of O-Carbamoylated Acylhydroximoyl Chlorides I-XVI

Compound	K_{II}, M^{-1} min^{-1}	
	AChE	BuChE
I	$2,4 \cdot 10^4$	$1,8 \cdot 10^6$
II	$1,0 \cdot 10^5$	$2,4 \cdot 10^6$
III	$1,3 \cdot 10^5$	$2,9 \cdot 10^6$
IV	$6,9 \cdot 10^5$	$4,0 \cdot 10^6$
V	$2,8 \cdot 10^5$	$6,0 \cdot 10^5$
VI	$3,0 \cdot 10^4$	$3,2 \cdot 10^6$
VII	$1,7 \cdot 10^5$	$6,8 \cdot 10^6$
VIII	$2,6 \cdot 10^5$	$7,7 \cdot 10^6$
IX	$6,9 \cdot 10^5$	$8,9 \cdot 10^6$
X	$1,2 \cdot 10^5$	$2,0 \cdot 10^6$
XI	$2,8 \cdot 10^5$	$2,3 \cdot 10^6$
XII	$1,8 \cdot 10^5$	$3,7 \cdot 10^6$
XIII	$7,5 \cdot 10^5$	$5,1 \cdot 10^6$
XVI	$1,0 \cdot 10^5$	$1,9 \cdot 10^6$
XV	$2,5 \cdot 10^5$	$3,1 \cdot 10^6$
XVI	$1,0 \cdot 10^6$	$6,0 \cdot 10^6$

The apparent bimolecular rate constant of inhibition (k_{II}) was calculated according to the formula for a pseudomonomolecular reaction [2].

The acute toxicity was determined on male white mice weighing 20-30 g. Substances dissolved in sunflower seed oil were introduced into the stomach in a single dose; the animals were under observation for 14 days, and LD_{50} was calculated on a NORD-10 computer according to [3].

The values of k_{II} are presented in Table 2. All the compounds have high anticholinesterase activity with respect to both enzymes and exhibit selectivity for BuChE, which is most pronounced for I (its k_{II} for BuChE is 192 times as high as for AChE).

The inhibitory activity with respect to AChE is somewhat increased (by a factor of 2-5) when the Me group is introduced into the benzene ring. Under these conditions compounds containing a methyl substituent in the meta- and para-positions are somewhat more active than those with a methyl substituent in the ortho-position. The effectiveness with respect to BuChE is also increased a little in this case (by a factor of 1.8-2.8), but when the Me group is in the ortho-position.

Lengthening R^2 from Me to Pr leads to a several-fold increase in the activity with respect to inhibition both of AChE and of BuChE, but replacement of Pr by i-Pr makes it even higher (see Table 2). Replacement of a hydrogen atom at the nitrogen atom by Me leads to a decrease in the activity with respect to inhibition of AChE (by a factor of 2.5) and BuChE (by a factor of 6.7) (compare compounds IV and V).

The toxicity was determined for compounds II, IV, V, VIII, XI, XIII, XV, and XVI. For VIII and XVI the dose—lethal outcome relationship was sigmoid; the values of LD_{50} were 510 (267-969) mg/kg and 405 (231-710) mg/kg, respectively. As for the remaining compounds, their effect was paradoxical within a definite dose range: With increasing doses the number of animals that died did not increase but decreased. It was characteristic that not all the animals died even with relatively high doses (400-799 mg/kg). And yet, k_{II} for AChE for these compounds is 10^5-10^6 $M^{-1}\cdot min^{-1}$. Many other inhibitors with such high constants cause death of mice in substantially lower doses. Thus, LD_{50} of O-(N,N-dimethylcarbamoyl)isobutyrylhydroximoyl chloride with such a high k_{II} for AChE ($1.4\cdot10^5$ $M^{-1}\cdot min^{-1}$) is 32 (25-41) mg/kg [1]. And it differs from V only in that it contains a phenyl substituent instead of methyl at the nitrogen atom. We are now determining the cause of the relatively low toxicity of the compounds indicated above.

Thus, O-carbamoylated acylhydroximoyl chlorides containing aryl substituents in the amide portion of the molecule have high anticholinesterase activity and comparatively low acute toxicity. The results obtained can be used in the targeted search for anticholinesterase substances suitable for use in the national economy and medicine.

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MACROHETEROCYCLES.

IV. SYNTHESIS AND ANALOGESIC ACTIVITY OF CROWN ETHERS CONTAINING LEU-ENKEPHALIN AND THYROLIBERIN GROUPS

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It is difficult to make use of the ability of crown ethers and cryptands to directly influence excitable structures in the body [1, 2, 6] unless organotropic compounds of this type can be obtained. Of the methods usually employed for the directed transport of drugs, we selected the vector type as being closest in modeling the natural humoral control mechanisms.

There have been a few reports in the literature of 'vector' macrocyclic compounds for which biological test results are available. For example, addition of the formyl derivative of benzo-15-crown-5 to 1-hydroxymorphine resulted in a decrease in analgesic activity as compared with codeine, while maintaining affinity for opioid receptors [10]. These workers were inclined to attribute the decreased activity to the biological properties of the crown ether component (an example of negative modulation). It has also been reported [11] that the use of macrocycles results in a considerable

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