SYNTHESIS AND ANTICHOLINESTERASE ACTIVITY OF FLUORINE-CONTAINING α-AMINOPHOSPHOPHORYL COMPOUNDS

O. V. Korenchenko, Yu. Ya. Ivanov,
A. Yu. Aksinenko, V. B. Sokolov,
and I. V. Martynov

UDC 615.217.32.012.1.07

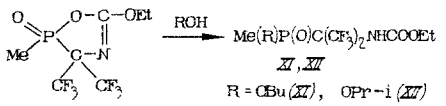
A considerable number of biologically active substances having low toxicity towards warm-blooded animals have been found amongst the fluorine-containing aminoalkylphosphoryl compounds. In particular, high antienzyme activity has been demonstrated by individual members of this group with regard to alanine racemase [7], proteinases [3, 8] and cholinesterases [2]. In the search for new biologically active substances we have synthesized for the first time several fluorine-containing α-aminoalkylphosphoryl compounds (I-XII).

Compounds I-X were obtained by reacting the appropriate hydrophosphoryl compounds with the carbonylimines of hexafluoroacetone.

$$RR^1P(O)H + (F_3C)_2C{=}NCOR^2 \rightarrow RR^1P(O)C(CF_3)_2NHC(O)R^2$$

I—X
R=OMe(I), OEt(II, IV), OPr(III), OPr-i(V), Ph(VI—X); R^1=OMe(I), OEt(II, IV), OPr(III), OPr-i(V), Ph(VI—X); R^2=OEt(I—III), OCH_2Ph(IV—VI), OPr(VII), OBu(VIII), OAm-i(IX), CF_3(X)

Compounds XI and XII were synthesized by reacting 1,4,2-oxazaphospholine with alcohols.



The biological activity of the fluorine-containing α-aminophosphoryl compounds was assessed from their effect on acetylcholinesterase (AChE, acetylcholine acetylhydrolase, EC 3.1.1.7) and butyrylcholinesterase (BuChE, acylcholine acylhydrolase, EC 3.1.1.8), and from their influence on neuromuscular conduction and their acute toxic effect on mice.

EXPERIMENTAL (CHEMICAL)

^1H, ^{19}F and ^{31}P NMR spectra were recorded on a Bruker CXP-200 instrument (Germany) in $CDCl_3$ solution, internal standard TMS.

O,O-Dimethyl-1-(ethoxycarbonyl)amino(perfluoro-1-methylethyl) Phosphonate (I). To a solution of 1.1 g (0.01 mole) of dimethylphosphate in 10 ml of ether was added a solution of 2.61 g (0.011 moles) of the ethoxycarbonylimine of hexafluoroacetone in 5 ml of ether. The ampule was heated for 12 h at 80°C. Then the ether was evaporated off and the residue was fractionated. Yield 2.0 g of I. Compounds II-V were obtained in a similar way.

O-Butyl-1-(ethoxycarbonyl)amino(perfluoro-1-methylethyl) Methylphosphinate (XI). To a solution of 2.99 g (0.01 mole) of 1,4,2-oxazaphospholine [4] in 15 ml of ether was added dropwise at 20°C a solution of 0.74 g (0.01 mole) of butanol in 5 ml of ether. After 1 h the ether was evaporated off and the residue was fractionated. Yield 2.89 g of XI. Compound XII was obtained in a similar way.

Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 6, pp. 21-23, June, 1992. Original article submitted July 25, 1991.

0091-150X/92/2606-0489$12.50

TABLE 1. Phyiscochemical Properties of Compounds I-XII

Compound	Yield, %	bp, °C/mm Hg mp, °C	n_D^{20}	Empirical formula
I	57,6	108/0,05	1,3992	$C_8H_{12}NO_5PF_6$
II	71,3	54—6*	—	$C_{10}H_{16}NO_5PF_6$
III	76,8	125—7/0,05	1,4058	$C_{12}H_{20}NO_5PF_6$
IV	44,2	52—4*	—	$C_{15}H_{18}NO_5PF_6$
V	50,7	69—71*	—	$C_{17}H_{22}NO_5PF_6$
VI	93,4	109—11	—	$C_{23}H_{18}NO_3PF_6$
VII	67,5	79—80	—	$C_{19}H_{18}NO_3PF_6$
VIII	83,4	89—90	—	$C_{20}H_{20}NO_3PF_6$
IX	87,5	97—8	—	$C_{21}H_{22}NO_3PF_6$
X	71,5	117—9	—	$C_{17}H_{11}NO_2PF_6$
XI	77,6	98—9/0,02	1,4101	$C_{11}H_{18}NO_4PF_6$
XII	69,4	103/0,05	1,4050	$C_{10}H_{16}NO_4PF_6$

*Recrystallized from hexane.

TABLE 2. IR and NMR Spectral Data for Compounds I-XII

Compound	IR spectrum, ν_{max}, cm^{-1}	PMR spectrum, δ, ppm. I, Hz	^{19}F, δ, ppm, I, Hz	^{31}P, δ, ppm
I	1740	1,29 t, 3,69 d, I_{P-H} 11, 4,18 q, 5,14 d, I_{P-H} 12	10,46 s	11,85 s
II	1750	1,2 t, 1,34 m, 4,10 q, 4,26 m, 5,80 d, I_{P-H} 12	11,20 s	9,74 s
III	1750	1,02 t, 1,30 t, 1,80 q, 4,20 m, 6,03 d, I_{P-H} 12	10,80 s	9,70 s
IV	1770	1,27 t, 4,23 q, 5,14 s, 7,40 br. s, 8,24 d, I_{P-H} 15	12,36 d, I_{F-F} 4,25	7,89 s
V	1745	1,40 m, 4,93 m, 5,16 s, 6,04 d, I_{P-H} 15,5, 7,35 br.s.	11,80 d, I_{F-F} 4,25	7,98 s
VI	1750	5,18 s, 7,20 d I_{P-H} 8,5, 7,38 s, 7,61 m, 8,03 m	14,43 s	32,77 s
VII	1750	1,00 t 1,72 m, 4,05 t, 6,93 d, I_{P-H} 11,7, 60 m, 8,02 m	14,38 s	32,40 s
VIII	1750	0,98 t, 1,42 m, 1,64 q, 4,08 t, 6,90 d, I_{P-H} 11,7, 60 m, 8,02 m	14,59 s	30,86 s
IX	1745	0,99 d, I_{H-H} 7,5 1,60 m, 1,72 m, 4,18 t, 7,06 d, I_{P-H} 11, 7, 56 m, 8,06 m	14,30 s	32,37 s
X	1765	7,66 m, 8,00 m, 8,84 d, I_{P-H} 7,5	1,81 s, 14,43 s	33,18 s
XI	1745	0,96 t, 1,30 t, 1,44 m, 1,70 m, 1,90 d, I_{P-H} 16, 4,22 m, 6,30 d, I_{P-H} 9	9,78 q, 13,97 q, I_{F-F} 9	42,77 s
XII	1745	1,16 d, I_{H-H} 4,5, 1,31 t, 1,78 d, I_{P-H} 15,5, 4,06 q, 4,78 m, 6,04 d, I_{P-H} 10	10,30 q, 14,20 q, I_{F-F} 9	42,10 s

1-(Benzylhydroxycarbonyl)amino(perfluoro-1-methylethyl) Diphenylphosphinoxide (VI). To a solution of 2.02 g (0.01 mole) of diphenylphosphonous acid in 100 ml of ether was added at 20°C a solution of 3.14 g (0.0105 moles) of the benzylhydroxycarbonylimine of hexafluoroacetone in 10 ml of ether. After 1 h the solvent was evaporated and the residue was recrystallized from hexane. Yield 4.82 g of VI. Compounds VII-X were obtained in a similar way.

The composition and structure of compounds I-XII was substantiated by elemental analysis, and IR and NMR spectral data (Tables 1 and 2).

EXPERIMENTAL (BIOLOGICAL)

Domestically produced human erythrocyte AChE and horse blood serum BuChE, having specific activity of 2.2 units/mg and 9.9 units/mg respectively, were used in the tests. Inhibition of these enzymes by compounds I-XII was investigated at 25°C in a medium comprising 0.1 M KCl and 0.002 M phosphate buffer. The initial enzyme activity (V_0) and residual activity (V_t) after incubation with a specific concentration of inhibitor [I] for t min were determined by the continuous potentiometric titration method with a glass electrode at constant pH (7.5) using a Radiometer RTS 822 automatic titration apparatus (Denmark). Acetylcholinebromide was used as the substrate in concentration of $1 \cdot 10^{-3}$ M in tests with AChE and in $1 \cdot 10^{-2}$ M concentration for BuChE. The irreversible inhibition rate constant (k_{II}) was calculated from the graphs of $\log V_0/V_t$ as a function of t from the equation derived in [5]:

$$K_{II} = \frac{1}{t[I]} \cdot 2{,}3 \lg \frac{V_0}{V_t}.$$

The effect of XI and XII on neuromuscular conduction was studied using a diaphragmatic specimen separated from a rat by the Bulbring method [6] under the same conditions as outlined in [1].

TABLE 3. Anticholinesterase Activity of Fluorine-Containing α-Aminophosphoryl Compounds I-XII

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

Acute toxicity for compounds IV-VI, VIII, IX, and XI was tested on male white mice weighing 20-30 g. The substances were suspended (or emulsified) in a 2% aqueous starch solution and introduced in a single dose into the stomach. The observation period lasted 14 days and the LD_{50} values were calculated with the aid of a NORD-10 computer using the technique described in [9].

The inhibiting activity of compounds I-XII depends on the structure of the radicals (Table 3). For example, when the R and R^1 methoxyl groups on the phosphorus atom (compound I) are replaced by ethoxyl groups (compound II), activity towards the two enzymes is reduced, while in the case of the analog with propoxyl substituents (compound III), an increase is seen. With isopropyl substituents a sharp decrease in activity is recorded (comparing IV and V), while phenyl substituents produce a more favorable effect than ethoxyl ones comparing VI and IV). Compounds XI and XII exhibit markedly higher activity in terms of AChE inhibition than the other compounds (k_{II} of $7.0 \cdot 10^6$ and $1.0 \cdot 10^6$ $M^{-1} \times min^{-1}$ respectively).

A relationship between activity and the structure of the carbamate substituent can be seen from the example of compounds VI-X. As R^2 grows in size from propoxyl to isoamyloxyl (compounds VII-IX), inhibition activity towards both enzymes shows a decrease. Inhibitor VI, containing the benzyl substituent, has almost the same activity as the analog with the propoxyl substituent (VII).

In response to nerve stimulation by infrequent (0.1 Hz) single impulses, compounds XI and XII in concentration of $1 \cdot 10^{-4}$ M intensified contractions of the isolated diaphragm and fasciculation, and produced a negative reaction to tetanization and a sharp increase in sensitivity to the blocking action of acetylcholine. TMP-4 ($1 \cdot 10^{-5}$ M) improved the capacity of the muscle to hold back tetanus. All these signs point to the fact that compounds XI and XII exert a blocking action via an anticholinesterase mechanism.

The LD_{50} of compound XI was 75 (58-97) mg/kg. No LD_{50} values were found for the other compounds as the dose–effect relationship did not conform to any particular pattern. For example, when the doses of V and IX were increased from 200 to 900 mg/kg, there was no rise in the number of deaths. The toxic effect of compound VI was of a paradoxical nature: more animals died (50%) from a 150 mg/kg dose than from a 730 mg/kg dose (17%). Compounds IV and VIII in a dosage of 1000 mg/kg caused death in only a third of the animals.

The results obtained may be used in the pursuit of new anticholinesterase substances that are suitable for application in medicine and the economy in general.

LITERATURE CITED

1. Ya. Yu. Ivanov, V. K. Brel', and I. V. Martynov, Dokl. Akad. Nauk., 279, 506-508.
2. V. V. Kuusk, I. V. Morozova, R. S. Agabekyan, et al., Bioorg. Khim., 16, No. 7, 1500-1508 (1990).
3. E. V. Parfenova, D. G. Popov, A. A. Novikov, et al., Bioorg. Khim., 15, No. 7, 987-990 (1989).
4. V. B. Sokolov, O. V. Koronchenko, A. Yu. Aksinenko, and I. V. Martynov, Izv. Ross. Akad. Nauk, Ser. Khim., No. 5, 1213 (1989).

5. V. A. Yakovlev, Kinetics of Enzyme Catalysis [in Russian], Moscow (1965), p. 1213.
6. E. Bulbring, Br. J. Pharmacol., 1, 38-61 (1946).
7. G. A. Flynn, D. W. Beight, E. H. W. Bohme, and B. W. Metcalf, Tetrahedron Lett., 26, No. 3, 285-288 (1985).
8. L. A. Lamden and P. A. Bartlett, Biochem. Biophys. Res. Commun., 112, No. 3, 1075-1090 (1983).
9. I. Litchfield and F. Wilcoxon, Pharmacol. Exp. Ther., 96, 99-113 (1949).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-ARYL-2-OXA-5-AZA-5R^1-6-OXOCYCLOOCTANO[6,7-b]INDOLES

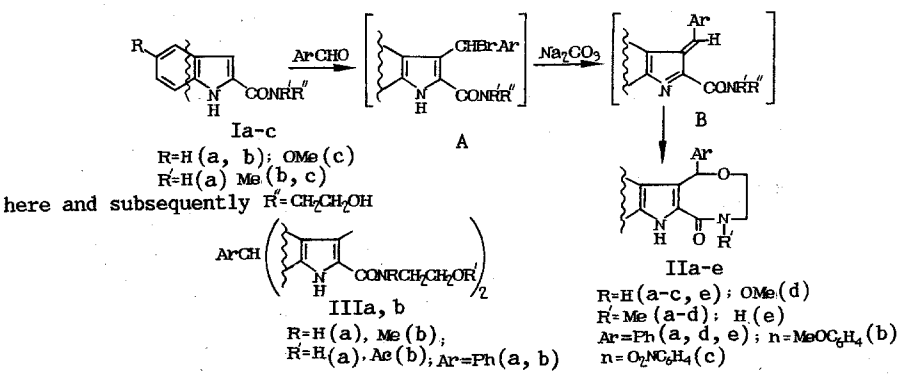
M. I. Vlasova, N. A. Kogan,
E. E. Lesiovskaya, and L. V. Pastushenkov

UDC 615.277.3:547.336.2].012.1.07

Acid condensation of aromatic aldehydes with amides of indole-2-carboxylic acid (ICA) has previously yielded the dihydropyrrolo[3,4-b]indoles [1]; the analogous reaction with ICA thioamides produced the dihydrothieno[3,4-b]indoles [2], and with ICA hydrazides the dihydropyridazino[4,5-b]indoles were synthesized [3]. The preparation of medium and macroheterocyclic rings condensed with indole is a complex problem and the Fischer reaction has fundamental limitations in this regard.

In the present work readily-available ICA β-ethoxyamides and aromatic aldehydes were used as the starting reagents to synthesize in one stage (with 50-60% yield) the eight-membered heterocyclic ring 2-oxa-5-azacyclooctane, condensed with indole on the "b" side. Compounds of this series were shown to have antihypoxic properties and to display antiarrhythmic activity.

The ICA β-ethoxyamides used initially (Ia-c) (Table 1) were obtained in 65-70% yield by treating ICA esters with ethanolamine and N-methylethanolamine in the absence of solvent at 100-105°C. Condensation of ICA β-ethoxyamides with aromatic aldehydes was carried out in AcOH saturated with HBr. Derivatives of 3-(α-bromobenzyl)indole (A), which could not be separated in an anlytically pure form, were formed as the intermediate. On standing for a prolonged period these substances were transformed into diindolylphenylmethanes (III), which prevented intramolecular cyclization. In view of this, the reaction mass was immediately neutralized after the rapid formation of bromide A by adding crystalline anhydrous soda or Et_3N. After the free HBr had been neutralized, the intermediate A was dehydrobrominated, an indolidene derivative (B) being formed as a result. This was borne out by the appearance of a yellow-green color, which is typical of these substances [4]. The disappearance of the color pointed to addition of the ethanolamide fragment hydroxyl group at the double bond with the resulting formation of structure II.



Leningrad Institute of Pharmaceutical Chemistry. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 26, No. 6, pp. 23-26, June, 1992. Original article submitted April 9, 1991.

 0091-150X/92/2606-0492$12.50