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Polyfluoroalkoxy Phosphonic and Phosphinic Acid Derivatives: II.[1] Reversible Esterase Inhibitors

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Abstract—Polyfluoroalkyl esters of phosphoric, alkylphosphonic and 1-hydroxypolyfluoroalkylphosphonic acids exhibited significant antiesterase activity against various esterases of animal and microbial origin. Moreover, with some compounds reversible inhibition of enzymes was observed due to the specific influence of hydrophobic fragments of the target products.

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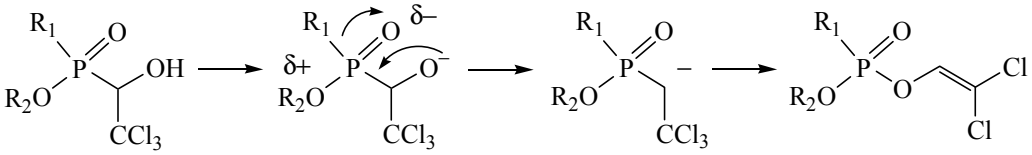
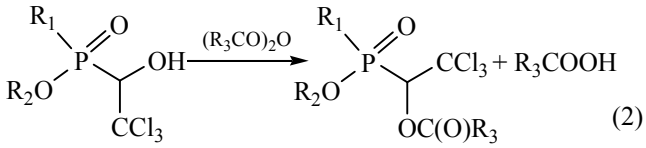
In our previous communication [1] we reported on a high level of antiesterase activity of (1-hydroxy-2,2,2-trichloroethyl)alkyl- and phenylphosphinic acid esters, which is largely due to non-enzymatic dehydrochlorination of these compounds to dichlorovinylphosphates and -phosphonates (1):



(1)

Such rearrangement is facilitated by the strong negative inductive effect of polyfluoroalkoxy residues on the phosphorus atom. In addition, the introduction of hydrophobic groups to eliminated or non eliminated parts of inhibitor molecule increases its irreversible inhibitory action towards esterases [2]. Therefore it is interesting to find out what factor contributes most of all to the influence of polyfluoroalkoxy groups on the level of antienzyme activity. This problem can be solved in two main ways. Firstly, the evaluation of biological activity of the compounds with protected hydroxy group. Secondly, the replacement of the trichloromethyl group for the less reactive one.

In this paper, we report on the synthesis of 1-acyloxy-2,2,2-trichloroethyl- phosphonates and phosphinates of general formula (**I**) (Table 1). The target compounds were obtained by the reaction of 1-hydroxy-2,2,2-trichloroethylphosphonates with anhydrides or chlorides of polyhalogen carboxylic acids:



(2)

Ia-Ig

The use of polyhalogen carboxylic acid derivatives as acylating agents is caused by the need to maximally prevent the process of releasing the hydroxy group of compounds **I** *in vitro* and their subsequent dehydrochlorination.

The structure of compounds **I** was confirmed by IR, ^{31}P NMR, and ^{19}F NMR spectroscopy (Table 2). IR spectra contain no hydroxy absorption band.

Compounds **I** were tested for biological activity *in vitro* against butyrylcholinesterase (BCHE, KF 3.1.1.8)

[1] For communication I, see [1].

Table 1. Yields, boiling and melting points, elemental analysis data for 1-acyloxy-2,2,2-trichloroetyl-phosphonates and -phosphinates **I**

Comp. no.	R_1	R_2	R_3	Yield, %	mp or bp, °C (*p*, mm Hg)	Found, %[a]		Formula	Calculated, %	
						C	H		C	H
Ia	C_6H_5	$OCH_2CF_2CF_2H$	CCl_3	85	86	28.3	1.55	$C_{13}H_9Cl_6F_4O_4P$	28.5	1.65
Ib	C_6H_5	$OCH_2(CF_2CF_2)_2H$	CCl_3	89	79	28.0	1.42	$C_{15}H_9Cl_6F_8O_4P$	27.8	1.40
Ic	C_6H_5	$OCH_2(CF_2CF_2)_3H$	CCl_3	92	73	27.4	1.11	$C_{17}H_9Cl_6F_{12}O_4P$	27.3	1.21
Id	C_6H_5	OCH_2CF_3	CCl_3	89	88	27.7	1.48	$C_{12}H_8Cl_6F_3O_4P$	27.9	1.56
Ie	OCH_3	OCH_3	CF_3	72	98 (0.06)	20.5	2.05	$C_6H_7Cl_3F_3O_5P$	20.4	2.00
If	OCH_3	OCH_3	CF_2CF_2H	70	106 (0.1)	21.6	2.12	$C_7H_8Cl_3F_4O_5P$	21.8	2.09
Ig	OCH_3	OCH_3	C_3F_7	68	120 (0.05)	21.0	1.64	$C_8H_7Cl_3F_7O_5P$	21.2	1.56

[a] Here and further, elemental analysis data are not given for phosphorus and fluorine, because of the distortion of the results due to the joint presence of the elements.

from horse serum, acetylcholinesterase (ACHE, KF 3.1.1.7) from human blood erythrocytes, and an esterase isolated from a strain of Gram-positive soil bacterium *Bacillus subtilis* (EBs). It is worth to indicate the significantly higher level of antienzyme activity of polyfluoroacyloxy derivatives **Ia–Id** (Table 3) compared to non-fluorinated analogs. It should be emphasized that the enzyme inhibition constants k_{II} practically coincide with those for 1-hydroxy derivatives [1]. It is also interesting that "polyfluoroacylated trichlorfons" **(Ie, Ig, Ii)** are by 1–2 orders of magnitude more active than trichlorfon [1]. These facts allow us to suggest that the non-enzymatic dehydrochlorination (1) is not the only and the main reason of the high antiesterase activity of 1-hydroxyphosphonates and -phosphinates. The increase in the magnitude of k_{II} is likely due mainly to increased hydrophobic adsorption of the inhibitor on the enzyme's surface.

The correlation analysis by Gancia method using lipophilicity parameter log *P* and molecular volume value *V* allowed us to obtain the dependence for the suppression of *BCHE* by the drugs **I**:

$$\log k_{II} = -2.85 + 0.940\log P - 0.137(\log P)^2 + 0.00759V, \quad (3)$$
$$r\ 0.975,\ s\ 0.36.$$

The optimal value of log *P* for this group of inhibitors was 3.43, which is significantly less than similar parameters for the 1-hydroxyphosphinates [1]. The reason is probably an additional and significant influence of the value of the inhibitor molecule volume on the level of biological activity. This is confirmed by a sufficiently strict dependence of log k_{II}–*V* (Fig. 1), while improving the efficiency of irreversible esterase suppression by 2-3 orders of magnitude is due to an increase in the inhibitor molecule volume. The aforementioned fact confirms the importance of sorption of the compounds **I** on the hydrophobic region between the histidine imidazole ring and the hydroxy group of serine of the enzyme esterase center.

Sufficiently large values of k_{II}, at least an order of magnitude higher than the data for *afos,* have been found for the suppression of EBs by the drugs **I** (Table 3). However, the level of irreversible antiesterase action does not depend on structural features of the inhibitors. This is due, firstly, to the enzyme-resistant phosphorus–carbon bond for the bulky phenyl radicals of compounds **I**. Secondly, to a noticeable difference between the geometric dimensions of microbial

Table 2. NMR data for compounds **I**

Comp. no.	^{19}F NMR spectrum, δ, ppm			^{31}P NMR spectrum, δ, ppm
	OCH_2CF_2	CF_2	HCF_2	
Ia	–47.6	–60	–61.2	33.9
Ib	–41.5	–46.1; –51.1; –59.0	–60.5	34
Ic	–41.5	–44.8; –50.7; –58.8	–60.1	35
Id	–2.3[a]	–	–	34.2
Ie	–2.9[b]	–	–	9.6
If	–	–44.9	–59.1	9.8
Ig	–3.4[b]	–48.8; –41.0	–	9.3

[a] Chemical shift of trifluoroethoxy group. [b] Chemical shift of the terminal trifluoromethyl group.

Table 3. Antiesterase activity of 1-acyloxy-2,2,2-trichloroethylphosphonates and -phosphinates **I**

Comp. no.	R_1	R_2	R_3	log*P*	*V*, Å^3	Enzyme inhibition constant, k_{II}, l mol^{-1} min		
						AChE	BCHE	EBs
Ia	C_6H_5	$OCH_2CF_2CF_2H$	CCl_3	3.11	1022	–	4.57×10^6	2.00×10^5
Ib	C_6H_5	$OCH_2(CF_2CF_2)_2H$	CCl_3	4.78	1162	–	2.57×10^7	4.47×10^5
Ic	C_6H_5	$OCH_2(CF_2CF_2)_3H$	CCl_3	6.45	1297	–	2.82×10^7	3.98×10^5
Id	C_6H_5	OCH_2CF_3	CCl_3	2.99	1028	–	1.70×10^6	1.78×10^5
Ie	OCH_3	OCH_3	CF_3	2.65	758	2.51×10^4	3.98×10^4	–
If	OCH_3	OCH_3	CF_2CF_2H	2.76	808	3.24×10^5	2.51×10^5	–
Ig	OCH_3	OCH_3	C_3F_7	4.31	830	3.02×10^4	3.98×10^4	–
Ih[a]	OC_6H_5	OC_6H_5	CH_3	3.00	913	–	3.55×10^5	4.47×10^4
Ii	OCH_3	OCH_3	CH_3	1.53	753	6.03×10^3	6.03×10^3	–

[a] Compounds **Ih** и **Ii** are *afos* and *acetyldipterex* respectively [3].

esterase "pocket" containing active sites, from those of BCHE.

To answer the question posed at the beginning of this communication, we have synthesized a number of 1-hydroxyphosphonates containing not trichloromethyl but octafluorobutyl group **II**. It becomes impossible to split off a proton from the hydroxy group and therefore it excluded metabolic conversion of phosphonates to vinylphosphates [reaction (1)].

Compounds **IIa–IIc** were prepared according to a known reaction [4, 5]:

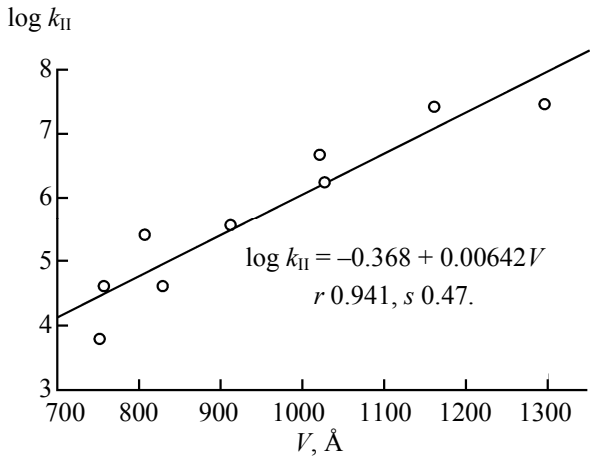


Fig. 1. Effect of the molecular volume *V* of 1-acyloxy-2,2,2-trichloroethylphosphonates and -phosphinates **I** on the level of antibutyrylcholinesterase activity.

$$(R_FO)_3P + H(CF_2CF_2)_2CHO\cdot H_2O \rightarrow (R_FO)_2P(O)CH(OH)(CF_2CF_2)_2H + R_FOH \quad (4)$$

II

The analytical data for hydroxyphosphonates **II** and their biological activity are presented in the Tables 4 and 5, respectively.

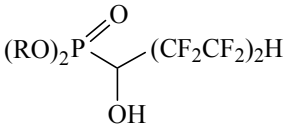
1-Hydroxyphosphonates and -phosphinates **II** are weak inhibitors of cholinesterase. It should be noted that an unexpected reversible inhibition of cholinesterase by 1-hydroxyphosphonate **IIc** is observed. The explanation of such a phenomenon can be as follows. It is known that in some cases combined inhibition of cholinesterase by organophosphorus compounds may occur [2]:

$$EIr \rightleftarrows E + In \rightleftarrows EIn \rightarrow EIn', \quad (5)$$

where E is enzyme, In is inhibitor, EIn is Michaelis–Menten complex, EIn' is phosphorylated enzyme, EIr is inhibitor complex, incapable to transform into EIn'.

Such scheme is common for reactions with equilibrium formation of an intermediate product. In this case the usual methods of studying the kinetics often cannot determine whether the intermediate product is on the reaction route, or formed in parallel [6]. In our case, the change in the concentration of inhibitor did not lead to a decrease of its activity, which confirms the formation of the complex EIr.

Table 4. Yields, boiling and melting points, elemental analysis data for 1-hydroxyoctafluoropentylphosphonates **II**



Comp. no.	R	Yield, %	bp, °C (*p*, mm Hg)	mp, °C	Found, %		Formula	Calculated, %	
					C	H		C	H
IIa	CF_3CH_2	89	140 (0.1)	44	23.1	1.39	$C_9H_7F_{14}O_4P$	22.7	1.48
IIb	$H(CF_2)_2CH_2$	92	148 (0.3)	39	23.9	1.45	$C_{11}H_9F_{16}O_4P$	24.5	1.68
IIc	$H(CF_2)_6CH_2$	72	162 (0.08)	–	24.2	0.90	$C_{19}H_9F_{32}O_4P$	24.3	0.96

Combined inhibition of cholinesterase happens not only if the phosphorus atom has bulky hydrophobic radicals. This effect also occurs in those cases where there are two alkoxy groups at the phosphorus atom, with the reversible component more pronounced in BCHE, not ACHE [2]. However, in our case, the reversible nature of inhibition was observed for both enzymes. This is due to the introduction of hydrophobic groups into substrate molecule leading to a weakening of anticholinesterase activity and the appearance of a reversible component. It should be particularly noted that the reversible inhibitor of esterases **IIc** has a very high hydrophobicity and a large volume of the molecule (Table 5). Therefore it is interesting to evaluate the biological activity of the compounds with easily cleaved hydrophobic groups. For such a role polyfluoroalkylphosphates are the best choice.

When 5-hydrooctafluoropentanal hydrate reacts with trimethylphosphite the products are the corresponding mixed phosphates, formed through phosphono-phosphate rearrangement of 1-hydroxyphosphonates [7]:

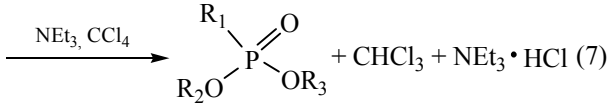
$$(R_FO)_3P + H(CF_2CF_2)_2CHO{\cdot}H_2O \rightarrow (RO)_2P(O)OCH_2(CF_2CF_2)_2H + ROH. \quad (6)$$

IIIa, IIIb

Other mixed phosphates and phosphonates were obtained by the Todd–Atherton reaction from the corresponding incomplete phosphites or phosphonites:

$$R_1(R_2O)P(O)H + R_3OH$$

IVa, IVb



$$\xrightarrow{NEt_3,\ CCl_4} \quad + CHCl_3 + NEt_3 \cdot HCl \quad (7)$$

IIIc–IIIg

Parameters of the mixed phosphates and phosphonates and their biological activity are given in Tables 6 and 7, respectively. Analysis of the biological activity of these compounds showed the low irreversible inhibition of esterases, especially ACHE. The following correlations have been found:

$$\log k_{II} = -3.30 - 0.671\log P - 0.056(\log P)^2 + 0.0122V, \quad (8)$$
$$r\ 0.916,\ s\ 0.46.$$

$$\log k_{II} = 1.46 + 4.88\log P - 0.350(\log P)^2 - 0.0124V, \quad (9)$$
$$r\ 0.998,\ s\ 0.12.$$

Correlation parameters [Eqs. (8), (9), Fig. 2] clearly indicate the importance of hydrophobic and steric parameters in evaluating the inhibitory activity of polyfluoroalkylphosphates. In addition, there is a coincidence of the main parameters of correlation equations with those for 1-hydroxy-2,2,2-trichloroethylphosphonates. In particular, the value of log P_{opt} was 5.09 [for a maximum of function, Eq. (9)], which is in good agreement with the value obtained earlier [1].

We also have found a reversible inhibition of ACHE and BCHE by di(1,1,5-trihydrooctafluoropentyl)ethylphosphonate **IIIg,** with the concentration

Table 5. Biological activity of 1-hydroxyoctafluoropentylphosphonates **II**

Comp. no.	Enzyme inhibition constant, k_{II}, l mol^{-1} min		log *P*	*V*, Å^3
	AChE	BCHE		
IIa	4.00×10^2	2.00×10^3	6.35	878
IIb	2.30×10^2	1.80×10^6	6.58	1004
IIc[a]	5.00×10^{-4}	2.50×10^{-4}	13.3	1510

[a] For compound **IIc** values of IC_{50} causing 50% reversible inhibition are given.

Table 6. Yields, boiling and melting points of the mixed polyfluoroalkylphosphates and -phosphonates **III**

Comp. no.	R_1	R_2	R_3	Yield, %	bp, °C (mm Hg)
IIIa	CH_3O[a]	CH_3	$CH_2(CF_2CF_2)_2H$	78	60 (0.5)
IIIb	CH_3O	CH_3	$CH_2(CF_2CF_2)_3H$	69	89 (0.5)
IIIc	C_2H_5O[b]	C_2H_5	$CH_2(CF_2CF_2)_2H$	70	69 (0.4)
IIId	*iso*-C_3H_7O	*iso*-C_3H_7	$CH_2CF_2CF_2H$	65	72 (1)
IIIe	*iso*-C_3H_7O	*iso*-C_3H_7	$CH_2(CF_2CF_2)_3H$	74	80 (0.7)
IIIf	C_2H_5	CH_2CF_3	CH_2CF_3	62	109 (7)
IIIg	C_2H_5	$CH_2CF_2CF_2H$	$CH_2CF_2CF_2H$	71	141 (4)

[a] Compounds **IIIa, IIIb** were obtained by the reaction (5) and described earlier [7]. [b] Compounds **IIIc–IIIg** were obtained by the reaction (6).

Table 7. Anticholinesterase activity of the mixed polyfluoroalkylphosphates and -phosphonates **III**

Comp. no.	R_1	R_2	R_3	Enzyme inhibition constant, k_{II}, l mol^{-1} min		log*P*	*V*, Å^3
				AChE	BCHE		
IIIa	CH_3O	CH_3	$CH_2(CF_2CF_2)_2H$	4.30×10^2	4.79×10^4	3.31	724
IIIb	CH_3O	CH_3	$CH_2(CF_2CF_2)_3H$	2.00×10^2	1.51×10^6	4.98	879
IIIc	C_2H_5O	C_2H_5	$CH_2(CF_2CF_2)_2H$	2.20×10^3	3.98×10^5	4.00	799
IIId	*iso*-C_3H_7O	*iso*-C_3H_7	$CH_2CF_2CF_2H$	4.30×10^3	2.53×10^3	3.16	805
IIIe	*iso*-C_3H_7O	*iso*-C_3H_7	$CH_2(CF_2CF_2)_3H$	2.00×10^4	7.94×10^3	6.49	1170
IIIf	C_2H_5	CH_2CF_3	CH_2CF_3	8.00×10^2	4.27×10^2	2.27	658
IIIg[a]	C_2H_5	$CH_2CF_2CF_2H$	$CH_2CF_2CF_2H$	2.00×10^{-6}	2.00×10^{-6}	5.84	1137

[a] For the compound **IIIg** values of IC_{50} causing 50% reversible inhibition are given.

of the substrate, providing 50% inhibition of the enzyme by two orders of magnitude lower than for the phosphonate **IIc**.

Thus, the studies of antiesterase activity of polyfluoroalkoxy derivatives of phosphonic and phosphoric acids revealed that the most significant contribution to the level of enzyme inhibition make polyfluoro ester fragments. Moreover, esterase of microbial origin is more sensitive to hydrophobic substrates. Finding among the studied range of organophosphorus substances reversible inhibitors offers the prospect of using them as low-toxicity antidotes.

EXPERIMENTAL

The antiesterase activity was evaluated by a technique based on a comparison of the enzymatic hydrolysis of chromogenic substrate indophenylacetate before (v_0) and after (v_τ) contact with the inhibitor with incubation time (t) from 1 to 10 minutes [1].

The molecular volumes V and lipophilicity parameters log *P* for the target compounds were calculated using the software package HyperChem™ Release 7.52 for Windows Molecular Modeling System, after the geometric optimization of the structure by Fletcher-Reeves method.

The homogeneity of the compounds was confirmed either by GLC (on a LKhM-8MD instrument, stationary phase XE-60 10% on Chezasorb Inerton, detector katharometer, carrier gas helium, 40 ml/min) for liquid products or by TLC (on Silufol UV 254 plates, eluent acetone-hexane, 1:4) for solid products.

^{31}P and ^{19}F NMR spectra were recorded on the device, designed and built in St. Petersburg STI (operating frequency 16.2 and 37.6 MHz, external standard 85% H_3PO_4 and trifluoroacetic acid, respectively). IR spectra were recorded from mulls in mineral oil **Ia–Id**, or from films **Ie–Ig** on an IKS-29 instrument. Elemental analyses were performed on a CHN-3 analyzer.

(1,1,3-Trihydrotetrafluoropropyl)(1-trichloroacetoxy-2,2,2-trichloroethyl) phenylphosphinate (Ia). To a solution of 2.68 g of 1,1,3-trihydrotetrafluoropropyl)(1-hydroxy-2,2,2trichloroethyl)phenylphosphinate in 45 ml of dichloroethane, cooled to 0°C, 0.91 g of trichloroacetyl chloride was added dropwise with stirring, followed by 3.2 ml of 25% aqueous sodium hydroxide solution. The mixture was stirred at room temperature for further 2 h. The organic layer was separated, washed with water (3×25 ml), and dried with calcium chloride. Removing solvent in vacuo gave 3.1 g (85%) of the target product as a colorless crystalline substance, mp 86°C.

Dimethyl-(1-trifluoroacetoxy-2,2,2-trichloroethyl)phosphonate (Id). To a solution of 2.3 g of dimethyl(1-hydroxy-2,2,2-trichloroethyl)phosphonate in 40 ml of dichloroethane 2.1 g of trifluoroacetic acid anhydride was added dropwise at room temperature. The reaction mixture was heated at 40°C with stirring for 2 h. Then the solvent and trifluoroacetic acid were distilled off in a vacuum. Fractional distillation gave 2.3 g (72%) of the target product. Polyfluoroacyloxy trichlorfon derivatives **If, Ig** were obtained in a similar manner.

(1,1,3-Trihydrotetrafluoropropyl)ethylphosphonite. 13.2 g of 1,1,3-trihydrotetrafluoropropanol were placed in a 4-neck flask equipped with a stirrer, a reflux condenser, and a dropping funnel and cooled to 0°C. 6.6 g of ethylphosphonic acid dichloride was added dropwise, maintaining the temperature in the flask below 3°C. The reaction mixture was kept at room temperature for 0.5 h, and then hydrogen chloride was removed by bubbling dry air through it for 5 h. Fractional distillation of the residue afforded 12.6 g (78%) of the product, bp 103°C (10 mm Hg). For (1,1,5-trihydrooctafluoropentyl)ethylphosphonite: bp 131°C (12 mm Hg).

Di(1,1,7-trihydrododecafluoroheptyl)(1-hydroxy-5-hydrooctafluoropentyl)-phosphonate (IIb). To a solution of 2.5 g of 5-hydrooctafluoropentanal hydrate in 40 ml of benzene 10.2 g of tris(1,1,7-trihydrododecafluoroheptyl)phosphite was added dropwise with stirring, keeping the temperature of the reaction mixture in the range of 20–25°C. After stirring for further 2 h solvent was distilled off and the residue subjected to fractional distillation yielding 6.8 g of the target product. Other phosphonates **II** were obtained in the same way.

1,1,5-Trihydrooctafluoropentyldiethylphosphate (IIIc). To a mixture of 5 g of triethylamine and 11.5 g of 1,1,5-trihydroperfluoropentanol-1 in 150 ml of carbon tetrachloride 6.9 g of diethylphosphite was added with stirring maintaining the temperature around 10 °C. Reaction mixture was stirred for 1 h at room temperature and for 1 h at 35–40°C. The precipitate formed was filtered off and the solvent was removed at a reduced pressure. Fractional distillation of the residue gave 12.9 g (70%) of the target product, bp 69°C (0.4 mm Hg).

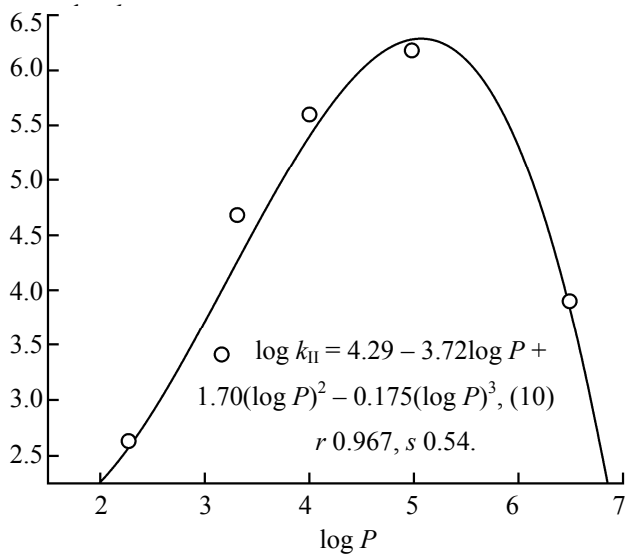


Fig. 2 Effect of the lipophilicity of the phosphates **III** on BCHE inhibition constant.

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