ELSEVIER

Journal of Fluorine Chemistry 107 (2001) 155–158

JOURNAL OF FLUORINE CHEMISTRY

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Fluorinated phosphorus compounds Part 4. A lack of anticholinesterase activity for four tris(fluoroalkyl) phosphates

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Received 1 September 2000; accepted 13 October 2000

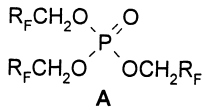
Abstract

Whilst examining the chemistry of a series of tris(fluoroalkyl) phosphates as potential fire retardants, concern was raised over their possible anticholinesterase activity. Bimolecular rate constants (k_i values) for the inhibition of bovine erythrocyte acetylcholinesterase (AChE) at 37°C and pH 7.4 were therefore sought for four fluoroalkyl phosphates: $(CF_3CH_2O)_3P{=}O$, $(C_2F_5CH_2O)_3P{=}O$, $(C_3F_7CH_2O)_3P{=}O$ and $(CF_3CH_2O)_2P(O)OCH_2C_2F_5$. Under the experimental conditions used, none of them inhibited AChE; it was only possible to define their k_i values as being less than 7–16 M^{-1} min^{-1}. They are therefore at least a factor of 10^5 less potent inhibitors than the nerve agents sarin or soman, and should be non-toxic via the mechanism of direct inhibition of AChE.

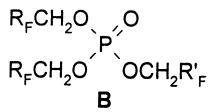
Keywords: Acetylcholinesterase; Bis(fluoroalkyl) fluoroalkyl phosphate; Cholinesterase; Fluorinated phosphate; Tris(fluoroalkyl) phosphate

1. Introduction

Recent studies from this laboratory addressed the synthesis of fluoroalkyl phosphoryl compounds [1–3]. In Part 3, we described the preparation of tris(fluoroalkyl) phosphates of general formulae **A** and **B** [4].[1] Whilst examining their chemistry, concern was raised over their possible anticholinesterase activity.

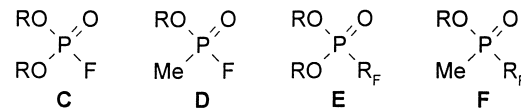


A



B

Inhibition of acetylcholinesterase (AChE) by organophosphorus molecules is generally responsible for their acute toxicity. The most notorious fluorine-containing inhibitors are the nerve gases that contain a phosphorus–fluorine bond [6,7]. They are represented by dialkyl phosphorofluoridates of structure **C** and alkyl methylphosphonofluoridates of structure **D**. Comparatively little is known about the anticholinesterase activity of phosphorus compounds containing fluoroalkyl groups. Surprisingly some dialkyl perfluoroalkyl phosphonates and phosphinates of structures **E** and **F** are also good inhibitors [8].



C **D** **E** **F**

Inhibitors react with the enzyme by a bimolecular displacement reaction in which a serine hydroxyl group in the active site makes a nucleophilic attack on the electrophilic phosphorus atom, displacing one of the groups on phosphorus. For fluoridates **C** and **D**, phosphylation of the enzyme occurs with loss of fluoride. For perfluoroalkyl compounds **E** and **F**, phosphylation occurs with presumed loss of fluoroalkane (alkaline hydrolysis of perfluoroalkyl derivatives of pentavalent phosphorus occurs under mild conditions and results in cleavage of the perfluoroalkyl group [9,10]).

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[1] Organic phosphorus compounds are named after the corresponding parent acids. Most of the substances described in this paper are derivatives of phosphoric acid $(HO)_3P{=}O$. Compounds of structure **A** are symmetrical phosphates or tris(fluoroalkyl) phosphates, while those of structure **B** are unsymmetrical phosphates or bis(fluoroalkyl) fluoroalkyl phosphates. The term phosphylated, as suggested by Hudson and Keay [5], is used to denote any group covalently bound through phosphorus.

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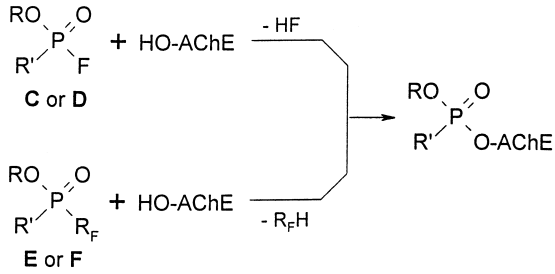
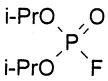
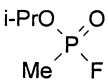
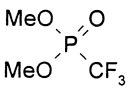
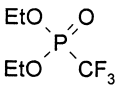
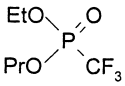
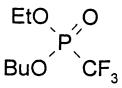
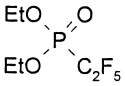
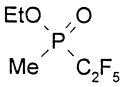


R = alkyl, R' = alkyl or alkoxy, R_F = fluoroalkyl

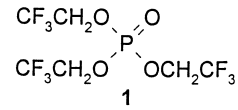
The bimolecular rate constant k_i is generally considered the most reliable criterion to measure the inhibitory power. Some rate constants for the inhibition of acetylcholinesterase by compounds of general formulae **C–F** are given in Table 1. Variations in anticholinesterase activity can be explained by at least two factors. One is the reactivity of the phosphorus atom which determines the rate of phosphylation. The second is the ease of binding between the inhibitor and the enzyme to form a complex prior to phosphylation; hydrophobic, electronic and steric effects of the groups on phosphorus determine the efficiency of this interaction.

Table 1
Rate coefficients for the inhibition of human erythrocyte AChE by various fluorinated organophosphorus compounds at 25°C and pH 7.5 [7][a]

Compound	k_i (M^{-1} min^{-1})
 **DFP**	10×10^4
 **sarin**	2×10^7
	5×10^2
	3×10^3
	3×10^4
	2×10^6
	5×10^5
	4×10^3

[a] DFP = diisopropyl fluorophosphate (or diisopropyl phosphorofluoridate).

The effect of fluorination of the ester groups in organophosphates on anticholinesterase activity has not been examined before. Inhibition rates of acetylcholinesterase for some unfluorinated trialkyl phosphates have been reported by Bracha and O'Brien [11,12] but their accuracy has been questioned due to the presence of trace impurities that are more potent inhibitors [13]. The purpose of the present study was to determine the anticholinesterase activity of fluoroalkyl phosphates **1–4**.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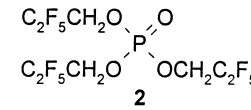
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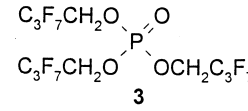
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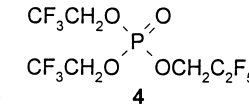

4

These phosphates are considerably larger in size than their unfluorinated counterparts and more susceptible to alkaline hydrolysis. They were prepared in a state of high purity and incubated with bovine erythrocyte acetylcholinesterase at physiological temperature and pH.

2. Experimental details

2.1. Materials

Bovine erythrocyte AChE (EC 3.1.1.7) and acetylcholine iodide (AChI) were purchased from Sigma Chemicals (Dorset, UK). Standard solutions of NaOH were purchased from BDH Laboratory Supplies (Leicester, UK). The fluoroalkyl phosphates studied were synthesised at Porton Down using a method described previously and doubly distilled [4]. They were >99% pure by GC-MS and multinuclear NMR analysis (^1H, ^{13}C, ^{19}F and ^{31}P).

2.2. Apparatus

The AChE inhibition experiments were performed at 37°C and pH 7.4 using a Metrohm automatic titration apparatus to pH-stat the reaction. This apparatus comprised a 713 pH Meter, a 614 Impulsomat and two 765 Dosimats (one containing 0.01 M NaOH solution, the other containing 0.01 M AChI solution). The reaction was monitored using a Kipp and Zonen y-t pen recorder. A Haake DC10 was used to control the water bath temperature and circulate water at 37°C to the jacket surrounding the reaction vessel.

2.3. Preparation and storage of solutions

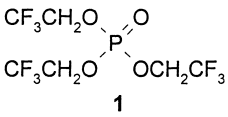
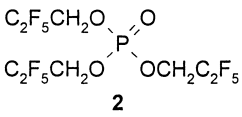
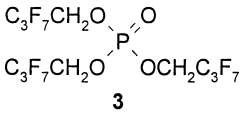
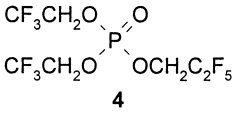
The fluoroalkyl phosphates were dissolved in propan-2-ol (analytical reagent grade) at concentrations ranging from

0.39 to 0.18 M. The solutions were stored at room temperature and used throughout the day. The acetylcholinesterase was dissolved in 0.1 M NaCl containing pH 7.4 phosphate buffer (5×10^{-3} M) to a specific activity of 5 μM units ml^{-1}, where 1 μM unit will hydrolyse 1 μM of acetylcholine to choline and acetate per minute at pH 8 and 37°C. The solid AChE had an activity of 0.35 μM units mg^{-1}. The AChE solution was stored in a refrigerator when not in use. Acetylcholine iodide and standard sodium hydroxide were dissolved in deionised water and used throughout the week. The AChI solutions (0.1 and 0.01 M) were stored in a refrigerator when not in use.

2.4. Kinetic methods

All reactions were performed in water at 37°C at an ionic strength of 0.1 M in NaCl. The reaction vessel initially contained 5 ml NaCl solution (0.1 M) and 0.5 ml AChE solution (5 μM units ml^{-1}). Acetylcholine iodide (0.10 ml of 10^{-1} M) was then added, giving a total AChI concentration of 1.8×10^{-3} M. The pH-stat was set to maintain the reaction at pH 7.4 by adding NaOH solution (0.01 M). The rate of addition was monitored on a y-t pen recorder. Acetylcholine iodide solution (10^{-2} M) was added at the same rate to maintain the substrate concentration. This reaction was allowed to run for approximately 1 min, after which the fluoroalkyl phosphate in propan-2-ol solution was added. The volume of solution added was 0.1 ml; initial experiments had shown that 0.1 ml of the fluoroalkyl phosphate solution, when added to 5 ml of 0.1 M NaCl solution at 37°C, was about the limit of solubility of these compounds.

Table 2
Rate coefficients for the inhibition of bovine erythrocyte AChE by some fluoroalkyl phosphates at 37°C and pH 7.4

Organophosphorus compound[a]	k_i (M^{-1} min^{-1})
 **1**	<7
 **2**	<10
 **3**	<16
 **4**	<10

[a] Fluoroalkyl phosphates **1–4** are poor inhibitors of bovine erythrocyte acetylcholinesterase at 37°C and pH 7.4.

3. Results of the AChE inhibitions

None of the four compounds studied inhibited the acetylcholinesterase. Any potential reaction between AChE and these compounds was observed for at least 15 min, i.e. assuming a first order inhibition reaction, $t_{(1/2)} > 15$ min, hence

$$k_{obs} < 0.69/15\ min^{-1}$$

or

$$k_{obs} < 5 \times 10^{-2}\ min^{-1}$$

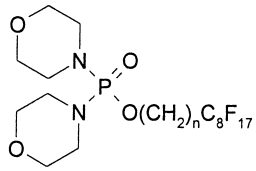
where $t_{(1/2)}$ is the half-life of the reaction and k_{obs} the observed first order rate.

Second order rate coefficients or k_i values, obtained from k_{obs} divided by the inhibitor concentration, are used to define inhibitory potency. Since slightly different concentrations of the fluoroalkyl phosphates **1–4** were used, the maximum k_i value for each also varied slightly. The values obtained are given in Table 2.

4. Discussion

Under the experimental conditions used, it was shown that fluoroalkyl phosphates **1–4** do not inhibit AChE. Since no inhibition was observed, it was possible only to define their inhibition rates as shown in Table 2. They are at least a factor of 10^5 less potent inhibitors of bovine erythrocyte acetylcholinesterase than the nerve agents sarin or soman [7]. Comparison with the data of Brestkin et al. [8], which was measured using a different enzyme, suggests that they are several orders of magnitude less potent inhibitors than the dialkyl perfluoroalkyl phosphonates or phosphinates (refer to Table 1). Fluoroalkyl phosphates **1–4** should be non-toxic via the mechanism of direct inhibition of acetylcholinesterase. However, this work does not exclude the possibility that metabolic activation of these compounds to more potent inhibitors of AChE might occur *in vivo*, or that they will be toxic by a mechanism unrelated to AChE inhibition. Toxicity studies in animals would be needed to rule out these possibilities.

The low affinity of fluoroalkyl phosphates **1–4** for acetylcholinesterase is due to their hydrophobic character and the poor leaving ability of the $-OCH_2R_F$ moiety. In this regard, they are reminiscent of the dimorpholinophosphates **5a–b** reported by Sadtler et al. [14]. These compounds, in which the perfluoroalkyl group on phosphorus is similarly attached through a $-OCH_2$ spacer, have been proposed as components of fluorocarbon emulsions for breathing applications in medicine.



n = 2 **5a** LD_{50} ip mice > 2 g/kg
n = 11 **5b** LD_{50} iv mice > 2 g/kg

Compounds **5a–b** had very low acute toxicity to mice when administered by intraperitoneal (ip) or intravenous (iv) injection [14]. It can be concluded that polyfluoroalkoxy groups on phosphorus give rise to molecules that are much less hazardous to handle than those containing P–F or P–R_F groups.

Acknowledgements

Thanks to Linda Knutsen and James Riches for their interest in this work and to the Ministry of Defence UK for financial support.

References

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