ACTA CHEMICA SCANDINAVICA 11 (1957) 859—865

Methyl-fluoro-phosphorylcholines

Two Synthetic Cholinergic Drugs and Their Tertiary Homologues

L.-E. TAMMELIN

Research Institute of National Defence, Dept. 1, Sundbyberg 4, Sweden

The syntheses of ω-dimethylaminoethoxy-methyl-phosphoryl and ω-dimethylamino*iso*propoxy-methyl-phosphoryl fluorides and their methiodides, which are choline esters, are described. It is shown that the ω-dimethylaminoalkoxy-phosphoryl fluorides are liquid compounds which solidify by storage at room temperature. This is suggested to be caused by an intramolecular rearrangement that quaternizes the amino group and breaks the fluorine-phosphorus linkage giving the molecule a cyclic structure with a phosphorus-nitrogen bond. Hydrolysis of the fluorine-phosphorus bond has been shown in aqueous solutions of the synthesized methyl-fluoro-phosphoryl esters. At pH 8 and 25°C the tertiary amino esters are hydrolysed almost instantly and the choline esters considerably faster than the corresponding alkoxy esters. The choline esters are more toxic when injected intraperitoneally than alkoxy esters as *e. g.* methyl-*iso*propoxy-phosphoryl fluoride (Sarin) and are potent cholinesterase inhibitors.

Cholinesterase inhibitors can be classified as follows. *Compounds which in their chemical structure resemble the substrates* but are not or only slowly hydrolysed by the enzyme. They are bound to the enzyme by qualitatively the same kind of forces which primarily bind the substrates during the course of enzymic hydrolysis. An ionic bond between an ammonium group and the enzyme is essential in this case [1].

Compounds of the phosphorylating type which during the first step of the inhibition process are bound to the enzyme qualitatively in the same way as the carbonyl-ester group of the substrates. Neostigmine is one inhibitor of the first type and Sarin, Tabun, Parathion and DFP belong to the second type.

Sarin is *iso*-propoxy-methyl-phosphoryl fluoride [2]. Exchange of the *iso*-propanol residue in Sarin against a choline or β-methylcholine (ω-trimethylammonium*iso*propanol) halogenide residue leads to compounds which structurally have properties in common both with substrates of cholinesterase and phosphorylating inhibitors. The structural formulas of some compounds having affinity for cholinesterases are shown in the chart (Fig. 1) where the two last are the choline esters described in this paper.

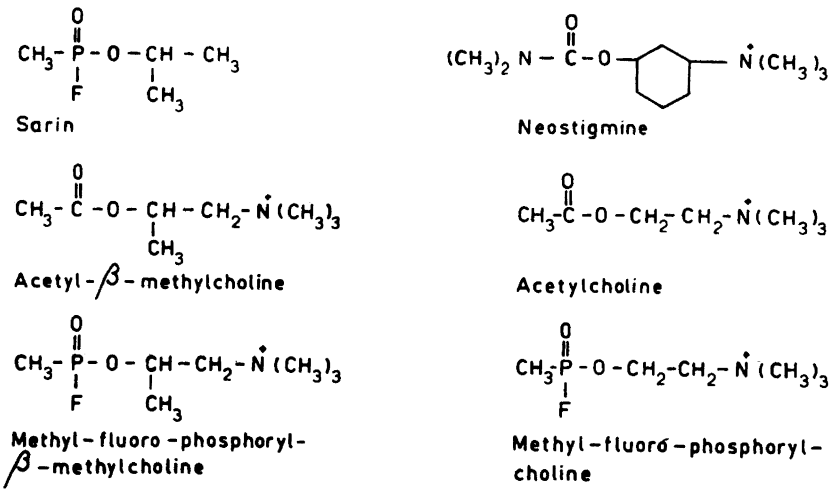
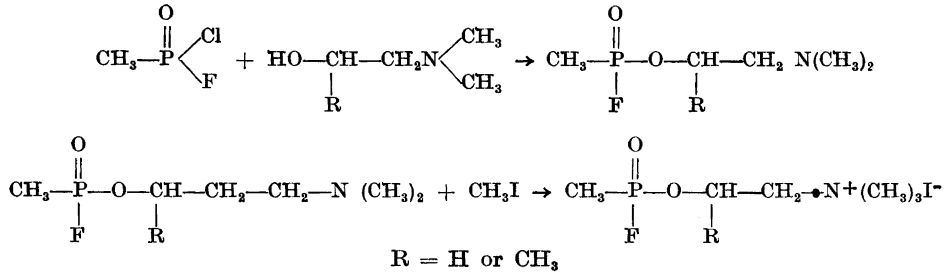


Fig. 1

SYNTHESES

The syntheses of the methyl-fluoro-phosphorylcholines were made according to the following scheme.



Both the dimethylaminoethoxy-methyl-phosphoryl fluoride and the dimethylamino*iso*propoxy-methyl-phosphoryl fluoride are colourless, water soluble oils with amine-like odours. They are unstable and are transformed into solid compounds by storage at room temperature.

Methyl-fluoro-phosphoryl choline iodide and methyl-fluorophosphoryl-β-methylcholine iodide are both colourless, water soluble crystalline compounds. *They are extremely toxic and care must be taken while handling them* to avoid direct contact with or the inhaling of the crystalline powder

Methyl-fluoro-phosphoryl chloride was synthesized from methyl-phosphoryl dichloride according to the schemes given by Collomp [4] as cited by Bocquet [5].

*ω-Dimethylaminoethoxy-methyl-phosphoryl fluoride and ω-dimethylamino*iso*propoxy-methyl-phosphoryl fluoride.* 2-dimethylaminoethanol (1 mole) is slowly added to 1 mole of methyl-fluoro-phosphoryl chloride which is dissolved in anhydrous ether (150 ml)

Table 1.

		M	% C		% H		% N		% F		B. p. °C/mm	M. p. °C	n_D^{25}	d_4^{25}	Yield %	R_D	
			calc.	found	calc.	found	calc.	found	calc.	found						calc.	found
2-Dimethylamino-ethoxy-methyl-phosphoryl fluoride	$C_5H_{13}FNO_2P$	169.1	35.5	34.9	7.8	7.7	—	—	11.3	10.7	40/0.2		1.4130	1.14	60	38.8	37.0
2-Dimethylamino-1-methylethoxy-methylphosphoryl fluoride	$C_6H_{15}FNO_2P$	183.2	39.3	39.2	8.3	8.2	—	—			40/0.1		1.4150	1.06	65	43.4	43.2
									% I								
Methyl-fluoro-phosphoryl-choline iodide	$C_6H_{16}FINO_2P$	311.1	23.2	23.2	5.2	5.1	4.5	4.6	40.8	40.5		152			75		
Methyl-fluoro-phosphoryl-β-methylcholine iodide	$C_7H_{18}FINO_2P$	325.1	25.9	25.9	5.7	5.6	4.3	4.1	39.1	38.5		84			85		

and 1.1 moles of triethylamine. The reaction mixture is refluxed for one hour. After cooling the precipitate formed is filtered off and the final product is isolated from the filtrate by means of distillation; for data see Table 1. The liquid compounds obtained are not very stable and form by storage at room temperature solid compounds which do not react with methyl iodide. It is thus recommended to do the next step immediately after the distillation.

Methyl-fluoro-phosphorylcholine iodide and methyl-fluorophosphoryl-β-methylcholine iodide The tertiary esters described earlier are quaternized by means of methyl iodide. One mole of the ester is treated with 1.1 moles of methyl iodide in ether solution at 25°C. A colourless precipitate is formed which is filtered off after six days and washed by ether and a small amount of alcohol; for data see Table 1.

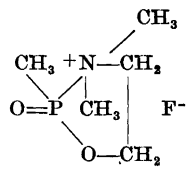
REARRANGEMENT OF THE DIMETHYLAMINOALKYL ESTERS

The dimethylaminoethoxy-methyl-phosphoryl and dimethyl-amino*iso*-propoxy-methyl-phosphoryl fluorides are liquid compounds which by storage at room temperature in sealed glass bottles are gradually transformed quantitatively into solid compounds. This occurs without noticeable pressure changes in the bottles and takes a few days for the ethyl derivative and a few months for the *iso*propyl derivative.

The solid ethyl derivative was used for an investigation in order to elucidate the kind of intramolecular rearrangement that causes the formation of the solid compounds.

The crystalline solid obtained has the same percent composition as the initial liquid. (Found: C 35.0; H 7.7; F 11.6. Calc. for $C_5H_{13}FNO_2P$ (169.1): C 35.5; H 7.8; F 11.3). The solubility in ether is low which is a difference from the liquid isomer. No melting point is found since the compound decomposes at 200°C. A reineckate precipitated from aqueous solution gives negative results when tested for fluorine according to Widmark [6]. Potentiometric titrations on aqueous solutions show that the compound has no protolytic properties in the pH range 3—10. The initial pH was slightly acid but the sodium hydroxide consumption is practically none between these pH limits. Investigations of hydrolytic properties using a "pH-stat" [7] shows no hydrolysis in a pH range from 10 to 6. In 0.1 M HCl, however, hydrolysis occurs and the hydrolysate has been shown to give equivalent amounts of three acids when investigated by means of potentiometric titration. One of the equivalents emanates from the hydrochloric acid yielding a salt with an amino group. Identical titration curves are obtained from hydrolysates of the liquid isomer. The amino pK_a is in both cases the same and about 7.6. Water solutions of the solid isomer give no Schoeneman [8] reaction whereas the liquid does. The Schoeneman reaction gives positive results on compounds containing $=\overset{\overset{\|}{O}}{P}-Hal$.

The high melting point, the low solubility in ether and the lack of protolytic properties indicate that a quaternary nitrogen compound has been formed. The lack of fluorine in the reineckate and the negative Schoeneman reaction indicate that an ammonium fluoride has been formed. The hydrolysis which occurs only at low pH values indicates a P—N bond [9]. A cyclic compound with the structure:



would have these properties and is thus suggested for this compound. By hydrolysis of the P—N bond in such a structure the same organophosphorus acid can be expected to be obtained as from hydrolysis of P—F in the liquid form. Identical amino pK_a values of the hydrolysis products have been found a fact which supports the suggested structure.

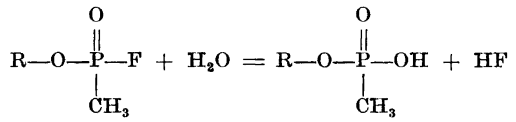
HYDROLYSIS

Sarin has been shown to be hydrolysed in aqueous solutions yielding the acid methyl-*iso*propoxy-hydroxy-phosphinoxide and hydrogen fluoride [10]. Choline and dimethylaminoethyl esters of the carboxylic acids have been shown to yield carboxylic acid and corresponding alcohol in aqueous solutions [1,11]. The P—O—C linkage is known to be hydrolyzable but the reaction rate at moderate pH values is known to be comparatively slow [12].

The qualitative course of the hydrolysis of the ω-dimethyl-aminoalkoxy-methyl-phosphoryl fluorides has been investigated as follows.

Aqueous solutions of the liquid compounds have been shown to yield fluorine ions which were precipitated as PbClF. The calculated amount of fluorine ions has been found after less than 2 h. Acidimetric potentiometric titrations have shown that equivalent amounts of two acids are obtained by the hydrolysis at room temperature.

The results show that the hydrolysis can be described with the following formula:



Using a "pH-stat" [7] the reaction rate of the hydrolysis has been determined at pH 8 with the following results. The half lives of the dimethylaminoethyl and dimethylamino*iso*propyl esters are very short and could not be determined. The choline and β-methyl-choline esters have half lives of 9 and 23 min, respectively. The corresponding value of Sarin [12] is 320 min. At pH = 6 the half life of the dimethylaminoethyl ester was 7 min. A more detailed investigation of the qualitative course and kinetics of the hydrolysis of the two choline esters has been done by Larsson [12]. The present results show, however, that the hydrolysis of these compounds is so fast, that special care must be taken in the determinations of toxicity and of enzyme inhibiting properties.

TOXICITY AND CHOLINESTERASE INHIBITION

Preliminary investigations of the toxicities have shown that aqueous solutions of dimethylaminoethoxy- and dimethylamino*iso*propoxy-methyl-phosphoryl fluorides show relatively low toxicity whereas the choline and β-methyl-choline esters are highly toxic. It was shown that when injected intraperitoneally into white mice both substances kill 50 % of the animals in doses close to 0.1 mg/kg body weight. The choline esters are thus more than three times

as toxic as Sarin, and they are among the most toxic synthetic compounds known. The low toxicity of the tertiary amino esters may be due to their rapid hydrolysis but also to other properties. A detailed description of the pharmacology of the compounds described in this paper is given by Fredriksson [13].

Preliminary investigations of the cholinesterase inhibiting properties have been done using an electrometric method [14]. The enzyme preparations were erythrocyte hemolysate and plasma from human blood. Before the addition of substrate the enzyme solution in Michel buffer solution was incubated for 30 min at 25°C with the inhibitors in varying concentrations. The substrate was acetylcholine, the concentration of which was 7.3×10^{-3} M. Starting pH was 8.

The negative logarithm of the inhibitor concentration in moles/l that causes 50 % inhibition of the enzyme activity in the system described above (pI_{50}) was determined. The rapid hydrolysis of the compounds made these determinations uncertain and the results obtained are to be considered as minimum values of pI_{50}. Methyl-fluoro-phosphorylcholine showed a pI_{50} of about 10 for erythrocytes and a pI_{50} of about 8.4 for plasma. Methyl-fluoro-phosphoryl-β-methyl-choline showed a $pI_{50} = 8.4$ for both erythrocytes and plasma. The corresponding pI_{50} values of Sarin are 8.8 for erythrocytes and 8.4 for plasma.

There are many factors influencing the pI_{50} values such as spontaneous or enzymic hydrolysis of the inhibitor. It is, however, evident from the experimental results that the inhibitors investigated are very potent, and it is highly probable that both of them are more potent than Sarin.

DISCUSSION

The present work originates from the idea that an ammonium group added to the structure of Sarin would yield a compound with more potent pharmacodynamic actions. On the whole this has been verified. It is, however, not quite evident that an added ionic bond between the inhibitor and vital proteins is the reason for the increased potency. Comparisons between Sarin and methyl-fluoro-phosphoryl-β-methylcholine on a simple structural basis are not very elucidating because of the great difference which has been found in the reactivity of the molecules by hydrolysis experiments. An increased reactivity of the P—F bond as an explanation for the increased inhibiting property of the ω-ammonium esters seems as valid as theories about the significanse of an ionic bond. Both effects are likely to contribute but it is impossible from the present experiments to judge which one is dominant.

The choline esters described in this paper are not very likely to become of direct practical value, as therapeutics or insecticides, and should not, contrary to Sarin, be considered as potential war gases, because of their physical properties and low chemical stability. However, Fredriksson [13] has shown that they are valuable tools in studies of cholinergic receptor effects.

My sincere thanks are due to the Director of the Research Institute of National Defence, Dept. 1, Professor G. Ljunggren, for his kind interest in this work.

REFERENCES

1. Wilson, I. B. *J. Biol. Chem.* **208** (1954) 123.
2. Bonnaud, X. *Protar* **14** (1948) 113.
3. Kinnear, A. M. and Perren, E. A. *J. Chem. Soc.* **1952** 3437.
4. Collomp, *Bull. Inf. Scient. Min. Guerre (Sect. techn. de l'Armée)*, 23/G., 28 (1949) Paris.
5. Bocquet, I. R. *Contribution á l'étude de la synthése des halogeno phosphates d'alkyle radioactifs et de l'inhibition de la cholinestérase par ces toxiques organophosphorés.* Diss. *Acta Med. Belgica* (1956).
6. Widmark, G. *Acta Chem. Scand.* **7** (1953) 1395.
7. Larsson, L. and Hansen, B. *Svensk Kem. Tidskr.* **68** (1956) 521.
8. Epstein, J. and Bauer, V. E. *Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy*, Feb 27 – March 2, (1956).
9. Larsson, L. *Acta Chem. Scand.* **7** (1953) 306.
10. Epstein, J., Bauer, V. E., Saxe, M. and Demek, M. M. *J. Am. Chem. Soc.* **78** (1956) 4068.
11. Larsson, L. *Acta Chem. Scand.* **8** (1954) 1017.
12. Larsson, L. *Acta Chem. Scand.* **11** (1957) *In press.*
13. Fredriksson, T. *Arch. int. pharmacodyn.* **XX** (1957) 394.
14. Tammelin, L.-E. *Scand. J. Clin. Lab. Invest.* **5** (1953) 267.

Received February 27, 1957.