Dialkoxy-phosphorylthiocholines, Alkoxy-methyl-phosphorylthiocholines and Analogous Choline Esters.

Syntheses, pK_a of Tertiary Homologues and Cholinesterase Inhibition

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The syntheses of S,ω-dimethylaminoethyl-dialkylthiophosphate, ω-dimethylaminoeththio-alkoxy-methyl-phosphine oxide and their corresponding oxygen phosphates are described. These tertiary amino esters have been quaternized with methyl iodide, yielding esters of thiocholine and choline. The alkoxy groups were ethoxy and *iso*propoxy groups. The protolytic dissociation constants in water $(pK)_a$ at 25°C of the dimethylaminoethyl esters have been determined and results obtained have been used in a discussion of the properties of the substituted phosphoryl groups. Compounds with sulfur-phosphoryl groups have significantly lower pK_a values than other analogous compounds investigated. The cholinesterase inhibiting properties of the compounds have been determined as pI_{50}. The esterase preparations were human plasma and hemolysate of human red cells. Thio derivatives are potent inhibitors and about 10^4 times as potent as their oxygen analogues. The chemical structures of the inhibitors in relation to their cholinesterase-inhibiting properties are discussed.

Choline esters of the aliphatic carboxylic acids, as *e.g.* acetylcholine, are considered to form addition compounds with cholinesterases as a first step in the sequence of reactions which lead to enzymic hydrolysis. The anchor points of the substrate are considered to be the carbonyl-ester and the ammonium parts of the molecule [1]. It has been shown that methyl-fluoro-phosphorylcholines are potent inhibitors of cholinesterases and it has been pointed out that phosphorylcholines are likely to react with the enzyme, as the first step in inhibition, in qualitatively the same way as substrates [2,3] do. The phosphoryl group in the inhibitors resembles the carbonyl group in substrates.

A new group of compounds related to choline esters has been introduced by Gosh and Newman [4]. It has been shown that S, ω-dialkyl-aminoethyl-dialkyl-thiophosphates are insecticides and methods for their syntheses are
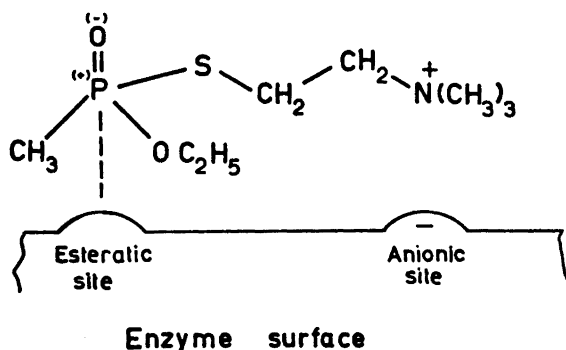
Fig. 1. Hypothetical model of the enzyme inhibitor complex formed during the inhibition of acetylcholinesterase by organophosphorylcholines.

given [5]. Diethoxy-phosphorylthiocholine and S, ω-dimethylaminoethyl-diethylthiophosphate have been shown to be potent cholinesterase inhibitors [6].

The present work deals with the syntheses and description of thio compounds of the types mentioned above, their oxygen analogues and alkoxy-methyl-phosphorylthiocholines (See Fig. 1.), their tertiary homologues and corresponding oxygen compounds.

The earlier described dialkoxy-phosphorylthiocholines, and the alkoxy-methyl-phosphorylthiocholines described in this paper, do presumably react with the cholinesterases as described above. (See Fig. 1.)

The bond energy between the esteratic site of the enzyme and the phosphoryl group is part of the total affinity to the enzyme. The degree of polarity in insecticides has been correlated to the toxicity by Peikow [7] and his general view can be applicated to the organo-phosphorylcholines. In the following an attempt will be made to use K_a values of the acid base equilibrium

$$RCH_2-CH_2-NH(CH_3)_2^+ = RCH_2-CH_2-N(CH_3)_2 + H^+$$
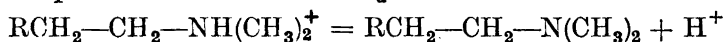
where R is the organophosphorus residue, for a discussion of the electronic distribution in the phosphoryl part of the inhibitor molecules. Through determinations of K_a values and inhibiting properties it is thus possible to discuss the affinity to cholinesterases in relation to the polarity.

EXPERIMENTAL

The phosphorylcholine and phosphoryl-thio-choline esters were synthesized according to the scheme [5]
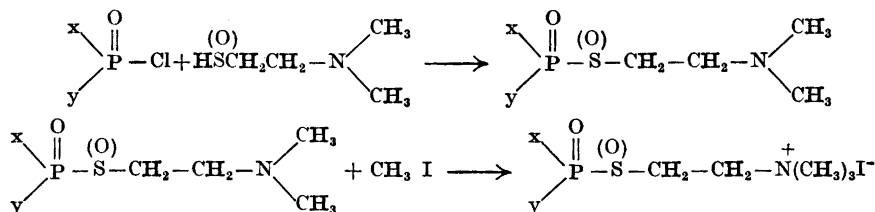
Two series were synthesized 1) x = y = C_2H_5O or iso-C_3H_7O and 2) x = CH_3 and y = C_2H_5O or iso-C_3H_7O.

The thio compounds (tertiary or quaternary) are extremely toxic and care must be taken while handling them to avoid direct contact with, or inhaling of, the compounds.

The free amino bases. One mole of dialkoxyphosphoryl chloride or alkoxy-methyl-phosphoryl chloride and one mole of dimethylaminoethanol or dimethylaminoethanthiol were mixed in 400 ml of ether with 1.05 moles of triethylamine. The reaction mixture was refluxed for one hour. Triethylammonium chloride was filtered off and the filtrate fractionated by distillation at reduced pressure. Yield ~60 %.

Hydrogen oxalates. One mole of the amino base (25 % solution in methanol) and 1.05 moles of oxalic acid (10 % solution in methanol) were mixed. The hydrogen oxalate which was precipitated by evaporation of the methanol is recrystallized from acetone to constant melting point (as a rule only once). Yield ~70 %.

Choline and thiocholine esters. One mole of the tertiary amino base and 1.5 moles of methyl iodide were refluxed in 2.5 l of ether. The methiodides precipitate in 5 h and were filtered off. Yield ~85 %.

Verification of the composition. The boiling points were rather approximately determined by the distillation by means of an ordinary thermometer and a McLeod manometer and shall not be considered as characteristic physical constants though reproducible in our distillation set up. Densities were determined by a pycnometer and the refractive index in an Abbe refractometer. Molar refractions were calculated from group and atomic refractions given by Vogel [8] and Holmstedt [9]. Melting points were determined on a Kofler Heizbank. Carbon and hydrogen contents were determined in an ordinary combustion oven which is possible if the sample is mixed with the double amount of vanadium pentoxide. Nitrogen content was determined according to Kjeldahl and sulfur content was determined by combustion with sodium peroxide in a Parr bomb and precipitation as barium sulfate. The content of oxalic acid was determined by permanganate titration at 60°C. The end point is rather vague but after some exercise reproducible results were obtained. (For data see Table 2). Finally the number of acid protons per molecule were determined by means of sodium hydroxide titration. (See determination of pK_a and Fig. 2).

The choline and thiocholine esters were prepared as iodides. The iodine content was determined by directly applied potentiometric argentometric titration. For data see Table 3.

Table 1. Physical data of the amino ester distillation fractions.

x y	M	B.p. °C/mm	d_4^{25}	n_D^{25}	R_D calc. found		M	B.p. °C/mm	d_4^{25}	n_D^{25}	R_D calc. found	
CH_3 C_2H_5O	195.2	56/0.05	1.0294	1.4320	49.3	49.2	211.3	80/0.06	1.0725	1.4780	55.8	55.8
CH_3 i-C_3H_7O	209.2	70/0.3	0.9992	1.4285	53.9	53.9	225.3	77/0.1	1.0501	1.4675	60.4	59.6
C_2H_5O C_2H_5O	225.2	60/0.05	1.0486	1.4220	54.9	54.6	241.3	85/0.1	1.0772	1.4655	61.4	61.8
i-C_3H_7O i-C_3H_7O	253.3	75/0.05	1.0042	1.4215	64.2	64.0	269.3	85/0.1	1.0296	1.4570	70.7	71.4

Determination of the acid equilibrium constants, pK_a, of the ω-dimethylaminoesters

When hydrogen oxalates of the amino esters, i.e. BHOxH (see also heads of Tables 2 and 3) are dissolved in water, BH^+ and HOx^- are formed. Both ions show protolysis. The values of pK_a for the equilibrium, $BH^+ = B + H^+$, were determined at 25°C with an automatic recording titrator as described by Larsson and Hansen [10]. Hydrogen oxalates (BHOxH) of the compounds (B) were dissolved in 0.100 M potassium chloride (yielding $10^{-2.4}$ M solutions) and titrated with 0.1000 M sodium hydroxide. All solutions were kept carbon dioxide free. The titration curves were recorded and showed pH as a function of the amount of sodium hydroxide added. The pH scale of the equipment was standardized against two buffer solutions, 0.05 M potassium hydrogen phtalate, pH = 4.01, and 0.01 M borax, pH = 9.18.

For evaluation of pK_a the curves were converted to curves where the number of protons split off per BHOxH, Z, was shown as a function of pH [11] (Fig. 2). At every point of the recorded curve Z can be calculated from the equation:

$$Z = \frac{[H^+] + [Na^+] - [OH^-]}{[B] + [BH^+]}$$

where $[H^+]$ and $[OH^-]$ were computed from pH and $[Na^+]$ from the added amount of sodium hydroxide, and $[B]+[BH^+]$ is the total concentration of B or oxalate, that is $[B]+[BH^+] = [H_2Ox]+[HOx^-]+[Ox^{2-}]$. Using the law of electro-neutrality and the following relationships:

$$K_1' = \frac{a_{H^+}[HOx^-]}{[H_2Ox]} \; ; \; K_2' = \frac{a_{H^+}[Ox^{2-}]}{[HOx^-]} \; ; \; K_a' = \frac{a_{H^+}[B]}{[BH^+]}$$

Z may be expressed as

$$Z = \frac{\dfrac{a_{H^+}}{K_2'} + 2}{\dfrac{a_{H^+}^2}{K_1'K_2'} + \dfrac{a_{H^+}}{K_2'} + 1} - \frac{\dfrac{a_{H^+}}{K_a'}}{\dfrac{a_{H^+}}{K_a'} + 1}$$

Evaluation of the mixed diss. const. K_2' *and* K_a'. The curves in Fig 2 show that pK_2' and pK_a' are rather well apart and it was decided to use the preceding expression of Z to test if A—B and B—C in Fig 2 could be treated as separate titration curves. In this case pH at $Z = 0.5$ gives pK_2' and pH at $Z = 1.5$ gives pK_a'. Using the approximate values $K_2' = 10^{-4}$; $K_a' = 10^{-8}$ and $K_1' = 6.5 \times 10^{-2}$, Z was calculated at $a_{H^+} = K_2'$ giving $Z = 0.4995$ and at $a_{H^+} = K_a'$ giving $Z = 1.5001$. This shows that $pK_2' = $ pH at $Z = 0.5$ and $pK_a' = $ pH at $Z = 1.5$ with satisfactory accuracy. Moreover the experimental pH/Z curves have been shown to follow the theoretical curve, yielding equivalence points at $Z = 1$ and $Z = 2$. Errors in analysis, impurities in the substanses or simultaneous hydrolysis of the esters are easily recognized as deviations from the theoretical curves [11].

Evaluation of the second thermodynamic diss. const. K_2 *of oxalic acid.* The eight pH/Z curves at $Z = 0.5$ (part A—B, Fig. 2) showed pH = 3.93 ± 0.01. Thus $pK_2 = 3.93 ± 0.01$

$$pK_2 = pK_2' + \log \frac{f_{HOx^-}}{f_{Ox^{2-}}}$$ where f is the activity factor of the ion indicated. The ionic strength was 0.10. Values of f are from Bates [12]. $pK_2 = pK_2' + 0.324 = 4.25$.

Evaluation of the thermodynamic diss. const. K_a *of the ammonium ions.* The titration curves of the eight different substances showed at $Z = 1.5$ different pH values between the

Table 2. Analytical data of the ammonium ester hydrogen oxalates.

x y	Formula	M	% C		% H		% N		% oxalic acid		M.p. °C
			cacl.	found	calc.	found	calc.	found	calc.	found	
CH$_3$ C$_2$H$_5$O	C$_9$H$_{20}$NO$_7$P	285.2	37.9	37.3	7.1	6.8	4.9	4.8	31.6	32.4	85
CH$_3$ i-C$_3$H$_7$O	C$_{10}$H$_{22}$NO$_7$P	299.3	40.2	40.2	7.4	7.5	4.7	4.6	30.1	29.8	100
C$_2$H$_5$O C$_2$H$_5$O	C$_{10}$H$_{22}$NO$_8$P	315.3	38.1	37.6	7.0	7.0	4.5	4.4	28.6	28.6	112
i-C$_3$H$_7$O i-C$_3$H$_7$O	C$_{12}$H$_{26}$NO$_8$P	343.3	42.1	42.1	7.7	7.8	4.1	4.1	26.3	26.3	122

limits indicated in Fig. 2 part B—C. The pK_a' values found were converted to pK_a as follows:

$$pK_a = pK_a' + \log f_{BH^+} = pK_a' - 0.08$$

f_{BH^+} is the activity factor of the ammonium ions. The ionic strength was 0.10. The value of f_{BH^+} was estimated from values given by Kielland[13]. The pK_a values of the organo-phosphorus ammonium esters are shown in Table 5.

Determination of the cholinesterase inhibiting effect, p I$_{50}$.

The ability to inhibit cholinesterase was determined by means of an electrometric method[14]. The enzyme preparations were erythrocyte hemolysate and plasma from human blood. Hemolysate diluted 1/30 and plasma diluted 1/20 in a Michel buffer solution (pH = 8) were incubated at 25°C with the inhibitor and thereafter acetylcholine was added to a final concentration of $10^{-2.3}$ M. Two compounds, methyl-etoxy-phosphoryl thiocholine and the corresponding dimethylaminoethanthiol ester were tested on enzyme preparations free from phosphorylphosphatases, the plasma fraction IV. 6 and a homogenate of the electric organ from *Torpedo*. In both cases I_{50}-values were obtained which were in good agreement with those obtained from human blood.

The results from preliminary tests with varying incubation time indicated that two hours are sufficient to obtain maximum inhibition at 25° with the actual concentrations of enzymes and inhibitors. The enzyme activity (v) was measured at various inhibitor concentrations (I) and pI_{50} determined by plotting v/v' against pI (v' = uninhibited enzymic activity) and taking the pI value at $v/v' = 0.5$ as pI_{50}[15]. The results are given in Table 6. Inhibitor solutions stored overnight gave the same results as those freshly prepared. Reproducibility of the pI_{50} values is shown by the maximum deviations in series of three determinations being ± 0.2.

RESULTS

S,ω-Dimethylaminoethyl-O,O'-dialkylthiophosphates, ω-dimethylaminoeth-thio-alkoxy-methyl-phosphine oxides and the corresponding compounds with oxygen instead of sulfur have been prepared as free amines, hydrogen oxalates

Table 3. Analytical data of the ammonium thioester hydrogen oxalates.

$$\begin{array}{c} x \\ \diagdown \\ \diagup \\ y \end{array} P - S - CH_2 - CH_2 - N \overset{+H}{\diagup} \overset{CH_3}{\diagdown} \quad \overset{-OOC}{\underset{HOOC}{\big|}}$$

x y	Formula	M	% C calc.	% C found	% H calc.	% H found	% N calc.	% N found	% S calc.	% S found	% oxalic acid calc.	% oxalic acid found	M.p. °C
CH₃ C₂H₅O	C₉H₂₀NO₆PS	301.3	35.9	36.3	6.7	6.8	4.7	4.6	10.6	10.8	30.0	30.1	102
CH₃ i-C₃H₇O	C₁₀H₂₂NO₆PS	315.3	38.1	38.5	7.0	7.2	4.5	4.4	10.1	10.2	28.6	29.0	143
C₂H₅O C₂H₅O	C₁₀H₂₂NO₇PS	331.3	36.3	37.7	6.7	7.1	4.2	4.1	9.6	9.7	27.2	26.8	116
i-C₃H₇O i-C₃H₇O	C₁₂H₂₆NO₇PS	359.4	40.2	40.0	7.3	7.7	3.9	3.8	8.9	9.0	25.8	25.8	120

and methiodides (see Tables 1, 2, 3 and 4). The methiodides are choline or thiocholine ester iodides.

The dimethylaminoethyl-phosphoryl esters are water soluble colorless liquid compounds which are faintly discolored by storage in closed bottles at room temperature. The S,ω-dimethyl aminoethyl-diethylthiophosphate is not quite stable and preparations solidify after some months of storage. The hydrogen oxalates and methiodides are water soluble colorless crystalline compounds. It is apparent that the compounds hydrolyse very slowly because there is no effect on pK_a determinations nor on the cholinesterase inhibition studies if the experiments are performed within a few hours after preparation of solutions.

Table 4. Analytical data of the quaternary ammonium ester iodides.

$$\begin{array}{c} x \\ \diagdown \\ \diagup \\ y \end{array} P - O - CH_2 - CH_2 - \overset{+}{N}(CH_3)_3 \; I^- \qquad \begin{array}{c} x \\ \diagdown \\ \diagup \\ y \end{array} P - S - CH_2 - CH_2 - \overset{+}{N}(CH_3)_3 \; I^-$$

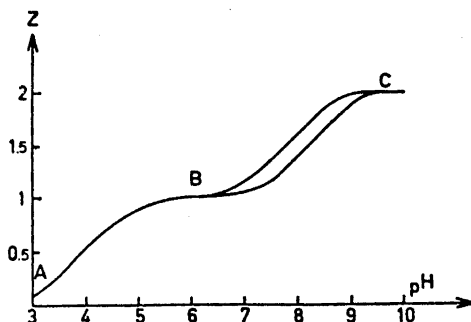| x y	Formula	M	% I calc.	% I found	M.p. °C	Formula	M	% I calc.	% I found	M.p. °C
CH₃ C₂H₅O	C₈H₂₁INO₃P	337.2	37.7	37.0	100	C₈H₂₁INO₂PS	353.2	35.9	35.9	110
CH₃ i-C₃H₇O	C₉H₂₃INO₃P	351.2	36.2	36.2	114	C₉H₂₃INO₂PS	367.2	34.6	34.9	164
C₂H₅O C₂H₅O	C₉H₂₃INO₄P	367.2	34.6	35.3	100	C₉H₂₃INO₃PS	383.2	33.1	33.2	138
i-C₃H₇O i-C₃H₇O	C₁₁H₂₇INO₄P	395.2	32.2	32.1	172	C₁₁H₂₇INO₃PS	411.3	30.9	30.7	154

Fig. 2. Titration curves obtained with hydrogen oxalates of ω-dimethylaminoethyl-organo-phosphoryl esters (BHOxH) as titrand and sodium hydroxide as titrator. Z is the number of protons split off per mole BHOxH. A—B shows titration of OxH⁻ and B—C of BH⁺. The two B—C curves limits the family of curves obtained from different B shown together with pK_a in Table 5.

Hydrogen oxalates of dimethylaminoethyl esters can be prepared in a pure state, as has been shown in the preparative part of this paper and confirmed by the titration curves (Fig. 2). The pH-scale was fixed by means of two buffer solutions. In determinations of the thermodynamic equilibrium constants pK_a, pH at $Z = 0.5$ offers a third possibility in controlling the pH-scale. The obtained value of the second dissociation constant (pK_2) of oxalic acid is well in accordance with earlier determinations. (Found 4.25, earlier values 4.22 and 4.29)[16]. The pH-scale is thus satisfactorily fixed and the repro-ducability of the ammonium pK_a determinations can be controlled by the A-B part of the (Z/pH) diagram (Fig 2). This part of the titration curve, which admittedly could be considered to be a complication, has thus been shown to be of value in checking the reliability of the experiments. Hydrogen oxalates of amines are therefore suitable for determinations of ammonium pK_a. The pK_a values of the compounds investigated in this paper are shown in Table 5.

It has been confirmed that the *dialkoxy-phosphorylthiocholines* and tertiary homologues are potent inhibitors of acetylcholinesterase. Koelle [6] used rabbit

Table 5. The thermodynamic acid constants (pK_a) at 25°C of some ω-dimethylaminoethyl organo-phosphoryl esters. Carboxylic acid esters are from an earlier paper [19]. Reproducabi-lity ± 0.01.

Organo phosphorus residue / Amino ethyl residue	CH₃ O ∥ P— C₂H₅O	CH₃ O ∥ P— C₃H₇O	O ∥ (C₂H₅O)₂P—	O ∥ (*iso*C₃H₇O)₂P—	O ∥ CH₃C ∣	O ∥ C₂H₅C ∣	O ∥ C₃H₇C—
—OC₂H₄N(CH₃)₂	8.14	8.24	8.03	8.06	8.35	8.29	8.31
—SC₂H₄N(CH₃)₂	7.80	7.77	7.73	7.70	8.27	8.28	8.32

Table 6. The negative logarithm of the molar concentrations of some organophosphoryl-cholines and corresponding dimethylaminoethyl esters which reduce cholinesterase activity to 50 % in enzyme preparations of human plasma (P) and erythrocytes (E). Reproducability ±0.2

Amino ethyl residue \ Organo phosphorus residue	CH_3\\ C_2H_5O/ $P-$ (O)		CH_3\\ C_3H_7O/ $P-$ (O)		C_2H_5O\\ C_2H_5O/ $P-$ (O)		C_3H_7O\\ C_3H_7O/ $P-$ (O)	
	P	E	P	E	P	E	P	E
$-SC_2H_4N(CH_3)_2$	7.3	8.8	6.4	8.1	8.0	7.9	7.0	5.8
$-SC_2H_4N^+(CH_3)_3$	7.9	9.1	7.1	8.4	8.9	8.4	7.6	6.4
$-OC_2H_4N(CH_3)_2$	<4	<4	<4	4.6	<4	4.6	<4	<4
$-OC_2H_4N^+(CH_3)_3$	<4	<4	4.4	4	4.5	<4	<4	4.7

brain and rabbit erythrocytes as enzyme sources. In the present experiments human erythrocytes were used. Moreover, it has been shown that these organophosphorus compounds are equally or more potent inhibitors of the cholinesterase from human plasma than of the cholinesterase from human erythrocytes.

It has been shown that *alkoxy-methyl-phosphorylthiocholines* and tertiary homologues are more potent inhibitors than the dialkoxy derivatives of the acetylcholinesterase from human erythrocytes. The alkoxy-methyl-phosphoryl compounds are however less potent as inhibitors of human blood plasma cholinesterase.

It has been found that the dialkoxy-phosphorylcholines, alkoxy-methyl-phosphorylcholines and tertiary homologues are weak inhibitors of cholinesterases.

It was found that complete reaction between cholinesterases and the inhibitors is obtained in two hours. The pI_{50} values obtained after two hours incubation time are summarized in Table 6.
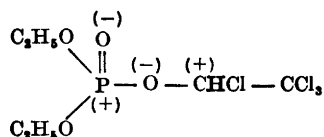
DISCUSSION

A question of importance in syntheses of the thio compounds is the position of the sulfur atom. The method of synthesis results in compounds where the sulfur is genetically situated between the dimethylaminoethyl and phosphoryl groups. A subsequent isomerisation is not probable since it has been made probable that

$$= \overset{S}{\underset{\|}{P}}-O-CH_2-CH_2-N(CH_3)_2 \text{ yields } = \overset{O}{\underset{\|}{P}}-S-CH_2-CH_2-N(CH_3)_2$$
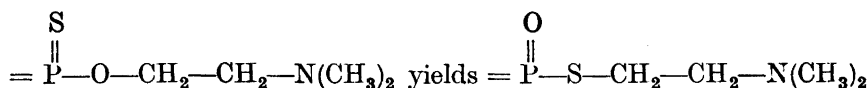
when heated [5,20]. Moreover the Z/pH diagrams show that the products obtained are homogeneous, which would be improbable if rearrangements occurred. The distillates described in Table 1 are rather crude due to difficulties in the dis-

tillation of the compounds which combine high boiling points with thermolability. However, the molar refractions show that the distillates on the whole have the predicted composition.

According to Perkow [7] the electronic distribution in organophosphorus insecticides can be described by the following formula:

$$
\begin{array}{c}
 \overset{(-)}{} \\
C_2H_5O \quad \overset{(-)}{O} \\
\diagdown \overset{\parallel}{} \overset{(-)}{} \overset{(+)}{} \\
P - O - \overset{(+)}{CHCl} - CCl_3 \\
\diagup \overset{(+)}{} \\
C_2H_5O
\end{array}
$$

which seems rather probable. The same author has shown that a fewer number of chlorine atoms in the chloroethyl group gives less potent insecticides. This is interpreted as being due to a decreased polarity in the organophosphorus residue. It is also pointed out that the reaction with proteins is favored by a high polarity in cases where the reaction is an addition of the insecticide or if it is a phosphorylation as in cholinesterase. In the last case the increased polarity is said to cause a weakening of the $\overset{(+)\ (-)\ (+)}{P - O - C}$ bridge with consequently enhanced phosphorylation.

The thio compounds show as a group considerably higher cholinesterase inhibiting properties than corresponding oxygen compounds. The present data are not sufficient to make it possible to judge definitely whether the present organophosphorus compounds phosphorylate cholinesterases, or are added as such or as metabolites although the first alternative seems probable for the thio inhibitors. However, a difference might be that the oxygen compounds are added as such and the thio compounds phosphorylate through breakage of the P—S bond. The relatively high hydrolysability of compounds containing the P-S-C group [17] supports the hypothesis of phosphorylation.

The thio compounds show also as a group lower pK_a values than the oxygen compounds. If the viewpoints of Eucken [18] on chloroacetic acid are applied qualitatively on these admittedly more complicated compounds the low pK_a values in the organophosphorus compounds can be understood as follows. The dipole resulting from the formal charges of atoms in the sphere around the phosphoryl group has, because of the found low pK_a values, a repellent effect on the proton added to the amino group and is, due to asymmetry, more efficient in the thio compounds. The difference in polarity indicated by the pK_a values in oxygen and thio compounds seems, however, too small to explain the relatively high cholinesterase inhibiting potency of the thio compounds as being due only to an increased dipole-dipole attraction between inhibitor and enzyme. A comparison between the pK_a values in phosphoryl esters and carbonyl esters [19] (e.g., ω-dimethylaminoethhyl acetate) indicates that the polarity of the $-\overset{\overset{\textstyle O}{\parallel}}{C}-S-C$ system is lower than of the $=\overset{\overset{\textstyle O}{\parallel}}{P}-S-C$ system.

Steric effects of the alkoxy groups in the thio compounds on the affinity for cholinesterases has been demonstrated in two ways. Di*iso*propoxy-phosphorylthiocholine is less active an inhibitor than the diethoxy compound and the same difference is shown by the tertiary homologues although the pK_a values and thus the reactivities should be similar. Moreover, dialkoxy and alkoxy-methyl compounds showed different patterns in affinity for acetylcholinesterase and serum esterase, effects which should be contributed to steric phenomena. The relatively high affinity for acetylcholinesterase compared to that for serum esterases of the alkoxy-methyl-phosphoryl compounds is unique among organophosphorus esters.

It seems probable that the compounds studied in this paper will become valuable tools in pharmacological studies of cholinergic effects due to their stability and interesting relations to cholinesterases. The comparison between thiocholine and dimethylaminoethanthiol diethoxy-phosphoryl esters made by Koelle and Steiner [6] is already one excellent example.

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