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S-alkynyl esters of phosphorus thioacids as inhibitors of cholinesterases and as promising physiologically active compounds

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ABSTRACT. Data are given in the review on the anticholinesterase activity of 58 specially synthesised esters of phosphorus thioacids containing an actylenic bond in the thioester group. It was established that compounds containing an acetylenic group in the β and especially in the α position of the thioester residue display an inhibitory action many times greater than that of their saturated analogues. A phosphorylated enzyme is formed by the reaction of the actylenic organophosphorus inhibitors (OPIs) with the enzymes as in the case of reaction with the saturated analogues. It was shown that the acetylenic organophosphorus inhibitors possess high biological activity both for mammals and for arthropods. On replacing the phosphoryl oxygen (P=O) by sulphur (P=S) the toxicity of the acetylenic organophosphorus inhibitors for mammals was sharply reduced but was little changed for arthropods. This raises the possibility of obtaining highly selective insecto-acaricides. The mechanism of the antienzymic action of the acetylenic OPIs and the mechanism of detoxication of diethyl *S*-hexynyl dithiophosphate are considered.

The bibliography includes 44 references.

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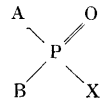
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I. Introduction

Among the substances influencing the transmission of nervous excitation in cholinergic synapses a significant place is occupied by derivatives (esters, amides, etc.) of phosphorus acids, many of which are used as insectoacaricides. Their bioligical action consists mainly of the inhibition of acetylcholinesterase (ACE), the enzyme causing the hydrolysis of acetylcholine (AC), the mediator of nervous excitation. It is well known[1] that under the stimulus of a nervous impulse, AC enters the synaptic gap and is reversily adsorbed onto a receptor which leads to excitation of the innervated cell. Due to the hydrolytic action of ACE the receptor is freed from AC and is transformed into its initial state acquiring the ability to take up a further portion of mediator. When ACE is inhibited the removal of AC from the receptor occurs very slowly, by diffusion alone, and the transmission of nervous impulses is disturbed which gives rise to serious disorders in the vital activity of organisms.

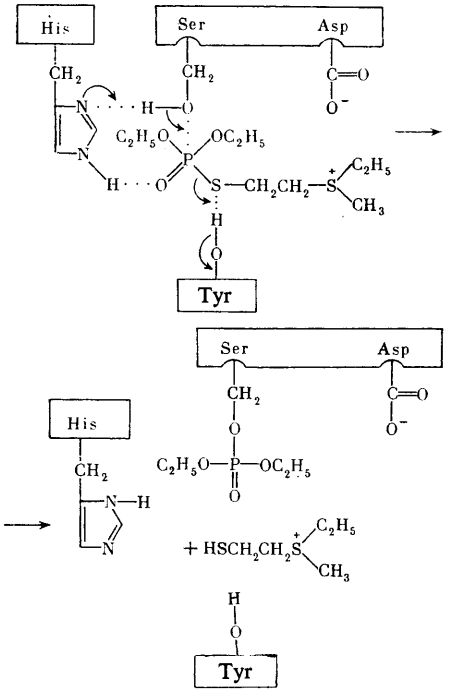
In addition to nervous tissue there are cholinesterases (CE) in erythrocytes and in the serum of many animals, in muscular tissue, etc. There are several varieties of CE which differ from one another not only in substrate specificity, i.e. the ability to hydrolyse choline esters of carboxylic acids, but also in sensitivity to inhibitors.[2,3]

Many derivatives of phosphoric, phosphonic and phosphinic acids of the following general formula are organophosphorus inhibitors (OPI) of cholinesterase.



where A and B are the same or different substituents at the phosphorus atom (alkyl, alkoxyl, amide, aryl, etc.) and X is a residue of an organic or inorganic acid (fluoride, cyano, phenol, mercaptan, etc.).

It has been demonstrated[4] that on reaction with cholinesterase these inhibitors phosphorylate the hydroxyl group of a serine residue which forms part of the catalytic centre of the enzyme. According to current ideas[5] the activation of the serine hydroxyl required to carry out this process occurs with the direct participation of the OPI by the following scheme.



The above scheme explains why thione OPI do not phosphorylate these enzymes. Unlike oxygen the sulphur atom is unable to form a stable hydrogen bond.[1,5]

The phosphorylated enzyme formed is a fairly stable compound. Spontaneous dephosphorylation takes place extremely slowly. Significant dephosphorylation occasionally leading to almost complete restoration of the cholinesterase catalytic activity (reactivation) is effected only by aromatic or aliphatic oximes.[6]

There is an anionic site in the active centre of the cholinesterases at a distance of 4–5 Å from the esterase site which interacts with the onium groups of substrates and of organophosphorus inhibitors increasing their adsorption to the active surface of the enzyme. Presumably[1] the anionic site is a carboxylate ion of a dibasic amino acid, such as aspartic or glutamic acid, forming part of the polypeptide chain of the enzyme. Both the esterase and the anionic sites are surrounded by hydrophobic regions on which hydrophobic adsorption of hydrocarbon radicals of substrates or inhibitors is effected thereby enabling the appropriate processes to occur at a higher rate.[7]

The anticholinesterase (anti-CE) activity of an OPI is characterised quantitatively by the bimolecular rate constant for the inhibition of the enzyme (k_{II}). In addition to the reaction conditions (pH, ionic strength, temperature and composition of the reaction medium) this activity depends on the nature of the enzyme and also on the structure of the substituents A and B and the fissile grouping.[7,8]

The presence of an onium atom in the β position of a thioalkyl radical, as in the compound Gd-42, viz. $(C_2H_5O)CH_3P(O)SCH_2CH_2\overset{+}{S}(C_2H_5)CH_3$, causes extremely high anti-CE activity sometimes exceeding the activity of its non-ionic analogue $(C_2H_5O)CH_3P(O).SCH_2CH_2SC_2H_5$ by a thousandfold.[1,2] Hydrophobic adsorption exerts a large effect on anti-CE activity. The value of k_{II} for the inhibition of human erythrocyte ACE by the compounds $(C_2H_5O).CH_3P(O)SC_nH_{2n+1}$ and $(C_nH_{2n+1}O)CH_3P(O)SC_4H_9$ is raised a hundredfold on increasing n from 1 to 7.[7]

Table 1. ω-Chlorobutynyl esters of phosphorus thioacids $RR'P(O)SCH_2C{\equiv}CCH_2Cl$.[18,19]

R	R′	Yield, %	Bp, °C (1.10^{-1} mm Hg)	d_4^{20}	n_D^{20}	Empirical formula
CH_3	C_2H_5O	72.5	122–123	1.2605	1.5283	$C_7H_{12}ClO_2PS$
CH_3	C_3H_7O	71.3	131–133	1.1918	1.5233	$C_8H_{14}ClO_2PS$
CH_3O	CH_3O	45.5	128–130	1.3015	1.5163	$C_6H_{10}ClO_3PS$
C_2H_5O	C_2H_5O	56.5	127–130	1.2441	1.5080	$C_8H_{12}ClO_2PS$
iso-C_3H_7O	iso-C_3H_7O	52	118–120	1.1635	1.4940	$C_{10}H_{18}ClO_3PS$
C_6H_5	C_2H_5O	48	162–163	1.2593	1.5721	$C_{12}H_{14}ClO_2PS$

It was shown recently[9,10] that a significant increase in the anti-CE activity of an OPI may be achieved by introducing an acetylenic grouping into the thioester residue. In some cases such phosphorus thioacid *S*-alkynyl esters supersede their saturated analogues in inhibitory activity by thousands and millions of times. Some of them inhibit the CE of arthropods more strongly than the CE of mammals, which is one source of selective toxicity. There is considerable practical interest in the *S*-alkynyl esters of dialkyl dithiophosphoric acids which acquire anti-CE activity by conversion into esters of monothiophosphoric acids by microsomal monooxidases. Such dithiophosphates display very high selective toxicity for arthropods and therefore are promising insecto-acaricides.[11]

To date a relatively large quantity of experimental data has been accumulated on the acetylenic OPIs which may be used for further study of the anti-CE activity and toxicity of this new and extremely intersting class of biologically active compounds.[9–17] The data presented in this review indicate the route for a purpose-directed search for highly selective insecto-acaricides. They are also of interest for the clarification of the mechanism of the interaction of the acetylenic OPIs with CEs.

II. Methods of synthesis of acetylenic organophosphorus inhibitors

The *S*-alkynyl esters of thiophosphoric and dithiophosphoric acids were synthesised by methods developed specially for this investigation.[13–15,18–28]

***S*-Butynyl esters of phosphorus thioacids** containing a triple bond in the β-position and a functional group in the ω-position of the thioester residue were obtained in the following way. The ω-chlorobutynyl esters of these acids were obtained initially by the reaction of the sodium or potassium salts of the appropriate phosphorus monothioacids with a 5-fold excess of 1,4-dichlorobutyne in alcohol (Table 1).[18]

$$RR'PSOM+ClCH_2C{\equiv}CCH_2Cl \rightarrow RR'P(O)SCH_2C{\equiv}CCH_2Cl,$$

R = CH_3, C_6H_5, CH_3O, C_2H_5, iso-C_3H_7O; R′ = CH_3O, C_2H_5O. iso-C_3H_7O, M = K or Na.

The products were subsequently converted by reaction with various nucleophilic reagents into ω-substituted *S*-butynyl esters of phsophorus monothioacids (Table 2). Sodium alkylmercaptides, dimethyl sulphide, dimethylamine, trimethylamine, triphenylphosphine and potassium dialkyl thiophosphate or thioacetate were used as nucleophilic reagents.[19]

$RR'P(O)SCH_2C{\equiv}CCH_2Cl \rightarrow$

- $\xrightarrow{R''SNa}$ $RR'P(O)SCH_2C{\equiv}CCH_2SR''$
- $\xrightarrow{S(CH_3)_2}$ $RR'P(O)SCH_2C{\equiv}CCH_2\overset{+}{S}(CH_3)_2Cl^-$
- $\xrightarrow{HN(CH_3)_2}$ $RR'P(O)SCH_2C{\equiv}CCH_2N(CH_3)_2$
- $\xrightarrow{N(CH_3)_3}$ $RR'P(O)SCH_2C{\equiv}CCH_2\overset{+}{N}(CH_3)_3Cl^-$
- $\xrightarrow{(C_6H_5)_3P}$ $RR'(O)SCH_2C{\equiv}CCH_2\overset{+}{P}(C_6H_5)_3Cl^-$
- $\xrightarrow{(iso\text{-}C_3H_7O)_2PSOK}$ $(iso\text{-}C_3H_7O)_2P(O)SCH_2C=CCH_2S$ $(iso\text{-}C_3H_7O)_2P(O)$
- $\xrightarrow{CH_3C(O)SK}$ $RR'P(O)SCH_2C{\equiv}CCH_2SC(O)CH_3$

O,O-Dimethyl and *O,O*-diethyl *S*-(4-acetoxybut-2-ynyl) thiophosphates (Table 2) were obtained by the reaction of 4-chlorobut-2-ynyl acetate with potassium dimethyl or diethyl thiophosphate.[20]

$$(RO)_2PSOK+ClCH_2C{\equiv}CCH_2OC(O)CH_3 \rightarrow$$
$$\rightarrow (RO)_2P(O)SCH_2C{\equiv}CCH_2OC(O)CH_3.$$

S-Propargyl esters of diethyl thiophosphoric acid were obtained by the reaction of the *O,O*-diethyl thiophosphates of potassium or monovalent copper with the appropriate propargyl halides.[12,21]

$$(C_2H_5O)_2PSOM + XCH_2C{\equiv}CR \rightarrow (C_2H_5O)_2P(O)SCH_2C{\equiv}CR,$$

R=H, Pr, Br; M=K, Cu; X=Cl, Br.

Table 2. ω-Substituted *S*-butynyl esters of phosphorus thioacids $RR'P(O)SCH_2C{\equiv}CCH_2R''$.[19,20]

R	R′	R″	Yield, %	Bp, °C (1.10^{-3} mm Hg)	d_4^{20}	n_D^{20}	Empirical formula
CH_3	C_2H_5O	C_2H_5S	24	125–130	1.1538	1.5335	$C_9H_{17}O_2PS_2$
CH_3	C_3H_7O	C_2H_5S	21	136–137	1.1319	1.5260	$C_{10}H_{19}O_2PS_2$
CH_3	C_4H_9O	C_2H_5S	20	140–145	1.1018	1.5198	$C_{11}H_{21}O_2PS_2$
CH_3	C_2H_5O	$CH_3C(O)S$	65	resin*	1.1993	1.5248	$C_9H_{19}O_3PS_2$
CH_3	C_2H_5O	$[(CH_3)_2\overset{+}{S}]Cl^-$	81	viscous oil	–	–	$C_9H_{18}O_2ClPS_2$
CH_3	C_2H_5O	$[(CH_3)_3\overset{+}{N}]Cl^-$	90	155–157	–	–	$C_{10}H_{21}O_2NClSP$
CH_3O	CH_3O	C_2H_5S	25	122–124	1.2005	1.5245	$C_8H_{15}O_3PS_2$
CH_3O	CH_3O	$CH_3C(O)S$	65	resin*	1.2552	1.5260	$C_8H_{13}O_4PS_2$
C_2H_5O	C_2H_5O	C_2H_5S	31	135–137	1.1548	1.5162	$C_{10}H_{19}O_3PS_2$
C_2H_5O	C_2H_5O	C_4H_9S	90	resin*	1.1110	1.5012	$C_{12}H_{23}O_3PS_2$
C_2H_5O	C_2H_5O	$[(CH_3)_2\overset{+}{S}]Cl^-$	75	glass	–	–	$C_{10}H_{20}O_3ClPS_2$
C_2H_5O	C_2H_5O	$[(CH_3)_2\overset{+}{N}]$	25	120–121	1.1045	1.4927	$C_{10}H_{20}O_3NPS$
C_2H_5O	C_2H_5O	$[(CH_3)_3\overset{+}{N}]Cl^-$	88	189–190**	–	–	$C_{11}H_{23}ClO_3NPS$
C_2H_5O	C_2H_5O	$CH_3C(O)S$	71	resin*	1.1994	1.5195	$C_{10}H_{27}O_4PS_2$
CH_3O	CH_3O	$CH_3C(O)O$	60	resin*	1.2206	1.4970	$C_8H_{13}O_5PS$
C_2H_5O	C_2H_5O	$CH_3C(O)O$	75	resin*	1.1744	1.4860	$C_{10}H_{17}O_5PS$

*Purified by column chromatography on silica gel.
**MP.

Sterically hindered *S*-propargyl esters of diethyl thiophosphoric acid were obtained by the reaction of the copper(I) salt of diethyl phosphoric acid with the sterically hindered propargyl chlorides.

$$(C_2H_5O)_2PSOCu + HC{\equiv}CCR'R''Cl \rightarrow (C_2H_5O)_2P(O)SCR'R''C{\equiv}CH,$$

$$R'=R''=CH_3, \quad R'+R''=(CH_2)_5.$$

S-Ethynyl esters of dialkyl thiophosphates were obtained by three methods:

a) by the reaction of the potassium salts of *O,O*-dialkyl thiophosphates with substituted ethynyl bromides in the presence of equimolar quantities of copper(I) chloride (Table 3).[22]

$$(RO)_2PSOK + BrC{\equiv}CR' \xrightarrow{CuCl} (RO)_2P(O)SC{\equiv}CR',$$

R=CH_3, C_2H_5, C_3H_7 and R′=C_4H_9, $CH_2SC_2H_5$, $CH_2OC_2H_5$, $CH_2OC(O)CH_3$, C_6H_5, cyclo-C_6H_{11}, cyclohex-1-enyl.

b) by the reaction of the copper(I) salts of *O,O*-dialkyl thiophosphates with substituted ethynyl bromides (Table 4).[23]

$$(RO)_2PSOCu + BrC{\equiv}CR' \rightarrow (RO)_2P(O)SC{\equiv}CR',$$

R = C_2H_5 and R′ = C_4H_9, t-C_4Hh_9, $CH_2CH_2SC_2H_5$, cyclo-C_6H_{11}, 1-hydroxycyclohexyl, 1-acetoxycyclohexyl.

c) by the reaction of dialky phosphorylsulphenyl chlorides with substituted ethynyl magnesium bromides (Table 5).[24]

$$(RO)_2P(O)SCl + BrMgC{\equiv}CR' \rightarrow (RO)_2P(O)SC{\equiv}CR',$$

R = C_2H_5, C_3H_7 and R′ = H, C_4H_9, $CH_2SC_2H_5$, $CH_2SC_4H_9$.

Table 3. *S*-Ethynyl esters of dialkyl thiophosphoric acids $(RO)_2P(O)SC{\equiv}C-R'$.[22,23]

R	R′	Yield, %	d_4^{20}	n_D^{20}	Empirical formula
CH_3	$CH_2SC_2H_5$	75	1.3365	1.5130	$C_7H_{13}O_3PS_2$
CH_3	C_4H_9	78	1.1175	1.4845	$C_8H_{15}O_3PS$
CH_3	$CH_2OC_2H_5$	80	1.0575	1.4940	$C_7H_{13}O_4PS$
CH_3	$CH_2OC(O)CH_3$	75	1.3091	1.4989	$C_7H_{11}O_5PS$
CH_3	C_6H_5	70	1.2667	1.5780	$C_{10}H_{11}O_3PS$
CH_3	cyclo-C_6H_{11}	71	1.1279	1.5002	$C_{10}H_{17}O_3PS$
CH_3		75	1.2078	1.5425	$C_{10}H_{15}O_3PS$
C_2H_5	$CH_2SC_2H_5$	70	1.1648	1.4980	$C_9H_{17}O_3PS_2$
C_2H_5	C_4H_9	76	1.0758	1.4705	$C_{10}H_{19}O_3PS$
C_2H_5	$CH_2SC_4H_9$	81	1.1925	1.5155	$C_{11}H_{21}O_3PS_2$
C_2H_5	$CH_2OC_2H_5$	85	1.1482	1.4820	$C_9H_{17}O_4PS$
C_2H_5	$CH_2OC(O)CH_3$	81	1.2190	1.4859	$C_9H_{15}O_5PS$
C_2H_5	C_6H_5	72	1.2123	1.5640	$C_{12}H_{15}O_3PS$
C_2H_5		80	1.1235	1.5225	$C_{12}H_{19}O_3PS$
C_3H_7	$CH_2OC(O)CH_3$	82	1.1860	1.4904	$C_{11}H_{19}O_5PS$
C_3H_7	C_6H_5	71	1.1515	1.5370	$C_{14}H_{19}O_3PS$

Table 4. *S*-Ethynyl esters of diethyl thiophosphoric acids $(C_2H_5O)_2P(O)SC{\equiv}C-X$.[23]

X	Yield, %	d_4^{20}	n_D^{20}	Empirical formula
C_4H_9	60	1.0750	1.4700	$C_{10}H_{19}O_3PS$
t-C_4H_9	70	1.0432	1.4860	$C_{10}H_{19}O_3PS$
$CH_2CH_2SC_2H_5$	60	1.1470	1.5120	$C_{10}H_{19}O_3PS_2$
cyclo-C_6H_{11}	80	1.1063	1.4965	$C_{12}H_{21}O_3PS$
	81	1.1595	1.5055	$C_{12}H_{21}O_4PS$
	76	1.1394	1.4900	$C_{14}H_{23}O_5PS$

***S*-Alkynyl esters of diethyl phosphoric acid** containing an acetylenic bond in the γ-position of the thioester residue were synthesised by the alkylation of potassium diethyl thiophosphate with the appropriate bromoalkynes.

$$(C_2H_5O)_2PSOK + RC{\equiv}C(CH_2)_2Br \rightarrow (C_2H_5O)_2P(O)S(CH_2)_2C{\equiv}CR,$$

R=H, C_2H_5;

Alkylthio-substituted *S*-butynyl esters of *O,O*-dialkyl thiophosphoric acids containing a triple bond in the β-position of the thioester residue were obtained analogously to the corresponding esters of the phosphorus monothioacids through the intermediate ω-chlorobutynyl esters of these acids.[25,26]

$$(RO)_2P(S)SK+ClCH_2C{\equiv}CCH_2Cl \rightarrow (RO)_2P(S)SCH_2C{\equiv}CCH_2Cl,$$

$$(RO)_2P(S)SCH_2C{\equiv}CCH_2Cl+R'SNa \rightarrow (RO)_2P(S)SCH_2C{\equiv}CCH_2SR',$$

$R=CH_3$, C_2H_5 and $R'=C_2H_5$, C_4H_9.

Table 5. ***S*-Ethynyl esters of thiophosphoric acids $(RO)_2P(O)SC{\equiv}CX$.**[12,24]

R	X	Yield, %	Bp, °C (1.10^{-3} mm Hg)	d_4^{20}	n_D^{20}	Empirical formula
C_2H_5	H	21	72–76	1.2108	1.4683	$C_6H_{11}O_3PS$
C_3H_7	H	25	85–87	1.1273	1.4650	$C_8H_{15}O_3PS$
C_4H_9	H	20	98–100	1.0337	1.4580	$C_{10}H_{19}O_3PS$
C_2H_5	C_4H_9	30	100–104	1.0750	1.4688	$C_{10}H_{19}O_3PS$
C_2H_5	$CH_2SC_2H_5$	22	105–108	1.1639	1.4912	$C_9H_{17}O_3PS_2$
C_2H_5	$CH_2SC_4H_9$	19	–	1.1925	1.5150	$C_{11}H_{21}O_3PS_2$
C_2H_5	$S(O)P(OC_2H_5)_2$	18	–	1.1344	1.4892	$C_{14}H_{28}O_6P_2S_2$

Table 6. ***S*-Ethynyl esters of dialkyl dithiophosphoric acids $(RO)_2P(S)SC{\equiv}C-X$.**[27,28]

R	X	Yield, %	d_4^{20}	n_D^{20}	Empirical formula
CH_3	C_4H_9	78	1.1540	1.5215	$C_8H_{15}O_2PS_2$
CH_3	$CH_2SC_2H_5$	75	1.2384	1.5670	$C_7H_{13}O_2PS_3$
C_2H_5	C_4H_9	81	1.0797	1.5140	$C_{10}H_{19}O_2PS_2$
C_2H_5	$CH_2SC_2H_5$	85	1.0271	1.5486	$C_9H_{17}O_2PS_3$
C_2H_5	$CH_2SC_4H_9$	70	1.1545	1.5426	$C_{11}H_{22}O_2PS_3$

O,O-Diethyl *S*-(4-acetoxybut-2-ynyl) dithiophosphate was obtained by the reaction of 4-chlorobut-2-ynyl acetate with potassium *O,O*-diethyl dithiophosphate.[20]

$$(C_2H_5O)_2P(S)SK+CH_3C(O)OCH_2C{\equiv}CCH_2Cl \rightarrow (C_2H_5O)P(S)SCH_2C{\equiv}CCH_2OCOCH_3.$$

***S*-Ethynyl esters of dialkyl dithiophosphoric acids** were obtained by two methods:

a) by the reaction of dialkyl thiophosphoryl disulphides with lithium acetylides (Table 6),[27]

$$(RO)_2P(S)S{-}SP(S)(OR)_2+LiC{\equiv}CR' \rightarrow (RO)_2P(S)SC{\equiv}CR';$$

b) by the reaction of copper(I) salts of *O,O*-dialkyl dithiophosphoric acids with substituted ethynyl bromides (Table 7),[28]

$$(RO)_2P(S)SCu+BrC{\equiv}CR' \rightarrow (RO)_2P(S)SC{\equiv}CR',$$

$R'=C_4H_9$, C_6H_5, cyclo-C_6H_{11}, cyclohex-1-enyl,

$R=CH_3$, C_2H_5, C_3H_7, C_4H_9.

***S*-Alkynyl esters of diethyl dithiophosphoric acid** containing a triple bond at various distances from the phosphorus atom were obtained by the reaction of potassium *O,O*-diethyl dithiophosphate with the appropriate bromoalkynes.[28]

$$(RO)_2P(S)SK+Br(CH_2)_nC{\equiv}CR' \rightarrow (RO)_2P(S)S(CH_2)_nC{\equiv}CR',$$

$R'=H$, C_2H_5, C_3H_7, C_4H_9; $n=0, 1, 2$.

Table 7. Ethynyl esters of dialkyl dithiophosphoric acids $(RO)_2P(S)SC{\equiv}C-X$.[27,28]

R	X	Yield, %	d_4^{20}	n_D^{20}	Empirical formula
CH_3	C_4H_9	80	1.1540	1.5225	$C_{19}H_{15}O_2PS_2$
CH_3	C_6H_5	80	1.2920	1.6215	$C_{10}H_{11}O_2PS_2$
CH_3	cyclo-C_6H_{11}	68	1.1638	1.5460	$C_{10}H_{17}O_2PS_2$
CH_3		85	1.2306	1.5840	$C_{10}H_{15}O_2PS_2$
C_2H_5	C_4H_9	85	1.0790	1.5140	$C_{10}H_{19}O_2PS_2$
C_2H_5	C_6H_5	78	–	1.5925	$C_{12}H_{15}O_2PS_2$
C_2H_5	cyclo-C_6H_{11}	71	1.1191	1.5335	$C_{12}H_{21}O_2PS_2$
C_2H_5		90	1.1764	1.5635	$C_{12}H_{19}O_2PS_2$
C_3H_7	C_6H_5	80	1.1695	1.5735	$C_{14}H_{19}O_2PS_2$
C_4H_9	C_6H_5	90	1.1498	1.5700	$C_{16}H_{23}O_2PS_2$

***S*-Alkyl esters of *O,O*-dialkyl thio- and dithiophosphoric acids** were obtained by the reaction of the appropriate alkyl halides with the potassium salts of these acids (Table 8).

$$(RO)_2P(S)YK+BrCH_2CH_2R' \rightarrow (RO)_2P(Y)SCH_2CH_2R',$$

$R'=CH_2SC_2H_5$, CH_2OCOCH_3, t-C_4H_9, CH_2Br, C_6H_5,

cyclo-C_6H_{11}, cyclohex-1-enyl and Y = O or S.

The synthesis of *O,O*-diethyl *S*-(3-ethylthiopropyl) thio- and dithiophosphates was effected in the following way. First *O,O*-diethyl *S*-(3-bromopropyl) thio- or dithiophosphates were obtained by the reaction of the potassium salts of diethyl thio- and dithiophosphoric acid with 1,3-dibromopropane. The corresponding *S*-ethylthiopropyl thioesters were then obtained from them by the action of sodium ethylmercaptide.

$$(C_2H_5O)_2PSYK+BrCH_2CH_2CH_2Br \rightarrow (C_2H_5O)_2P(Y)SCH_2CH_2CH_2Br,$$

$$(C_2H_5O)_2P(Y)SCH_2CH_2CH_2Br+C_2H_5SNa \rightarrow (C_2H_5O)_2P(Y)S(CH_2)_3SC_2H_5,$$

Y = O or S.

***S*-Propargyl thioacetate** was obtained by the reaction of propargyl bromide or chloride with potassium thioacetate.[29]

$$CH_3C(O)SK+XCH_2C{\equiv}CH\rightarrow CH_3C(O)SCH_2C{\equiv}CH+KX,$$

X = Cl or Br.

4-Chlorobut-2-ynyl acetate was obtained in one step in 65% yield by the reaction of 1,4-butynediol with acetyl chloride and then with thionyl chloride.[20]

$$CH_3C(O)Cl+HOCH_2C{\equiv}CCH_2OH+SOCl_2\rightarrow$$
$$\rightarrow CH_3C(O)OCH_2O{\equiv}CCH_2Cl.$$

Table 8. *S*-Alkyl esters of diethyl thio- and dithiophosphoric acids $(C_2H_5O)_2P(Y)SCH_2CH_2-X$.[28]

X	Y	Yield, %	d_4^{20}	n_D^{20}	Empirical formula
$CH_2SC_2H_5$	O	80	1.1378	1.4920	$C_9H_{21}O_3PS_2$
CH_2OCOCH_3	O	70	1.1440	1.4620	$C_9H_{19}O_5PS$
t-C_4H_9	O	90	1.0058	1.4610	$C_{10}H_{23}O_3PS$
CH_2Br	O	71	1.3420	1.4930	$C_7H_{16}O_3PSBr$
C_6H_5	O	78	1.1097	1.5170	$C_{12}H_{19}O_3PS$
cyclo-C_6H_{11}	O	70	1.0462	1.4802	$C_{12}H_{25}O_3PS$
	O	70	1.0677	1.4925	$C_{12}H_{23}O_3PS$
$CH_2SC_2H_5$	S	80	1.1161	1.5212	$C_9H_{21}O_2PS_3$
CH_2OCOCH_3	S	70	1.1570	1.4995	$C_9H_{19}O_4PS_2$

***S*-Hex-1-ynyl thioacetate** was obtained in one stage in the following way.[30] Hex-1-ynyllithium was obtained by the reaction of a freshly prepared soluton of butyllithium in ether with 1-hexyne and was then converted into the corresponding lithium mercaptide by reaction with sulphur.

$$C_4H_9C{\equiv}CH \xrightarrow{C_4H_9Li} C_4H_9C{\equiv}CLi,$$
$$C_4H_9C{\equiv}CLi \xrightarrow{S} C_4H_9C{\equiv}CSLi.$$

Reaction with acetyl bromide then gives *S*-hex-1-ynyl thioacetate.

$$C_4H_9C{\equiv}CSLi+CH_3C(O)Br\rightarrow CH_3C(O)SC{\equiv}CC_4H_9.$$

III. Anticholinesterase activity of acetylenic organophosphorus inhibitors

Partially purified water-soluble preparations of acetylcholinesterase of human erythrocytes (ACE) and butyrylcholinesterase of horse serum (BuCE) served as enzyme sources. In addition a brain homogenate from white mice (mouse CE), a homogenate of the heads of the fly *Musca domestica L.* (fly CE), homogenates of whole beet aphids *Aphis fabae Scop.* and grass aphids *Schizaphis gramina Rond.* (aphid CE) and spider mites *Tetranychus urticae Koch* (mite CE) were also used. The rates of cholinesterase hydrolysis of acetylthiocholine (ATC) before (v_0) and after (v_i) reaction with the various inhibitors were determined at 25 °C and pH 7.5 by Ellman's method.[31] Ellman's reagent was added after stopping the enzymic reaction in experiments with aphid CE.[32] The bimolecular rate constant (k_{II}) for the reaction of a cholinesterase with an OPI was determined at 25 °C and pH 7.5 by known methods[7,8] and was calculated from the formula:

$$k_{II}=\frac{2.303}{t[I]}\lg\frac{v_0}{v_i}(M^{-1}\,min^{-1}),$$

where v_i is the rate of enzymic hydrolysis of ATC after t min incubation of the CE with the OPI at a concentration [I] many times greater than the concentration of enzyme active centres; v_0 is the rate of enzymic hydrolysis of ATC in the absence of the OPI. The rate constant for the alkaline hyrolysis of the OPI (k_{OH}) was determined at 25 °C and pH 9.2 by a known method.[14]

Table 9. Anticholinesterase activity (k_{II}, M^{-1} min^{-1}) of acetylenic OPI $(RO)_2P(O)SCH_2C{\equiv}CCH_2R'$ and their saturated analogues $(RO)_2P(O)SCH_2CH_2CH_2CH_2R'$ (compounds numbered 'a').[8,9]

Compound No.	OPI		Anticholinesterase activity of OPI					
	R	R'	ACE	BuCE	mouse CE	fly CE	beet aphid CE	mite CE
(I)	CH_3	Cl	$2.8\cdot10^5$ (28)	$7.2\cdot10^5$ (36)	$6.2\cdot10^4$ (8)	$1.3\cdot10^6$ (19)	$4.8\cdot10^5$ (23)	$3.5\cdot10^5$ (125)
(II)	C_2H_5	Cl	$2.6\cdot10^5$ (10)	$5.8\cdot10^5$ (3)	$8.9\cdot10^4$ (12)	$2.5\cdot10^6$ (37)	$1.0\cdot10^5$ (3)	$6.5\cdot10^4$ (1.6)
(III)	CH_3	SC_2H_5	$3.2\cdot10^5$ (270)	$1.0\cdot10^6$ (1000)	$3.3\cdot10^5$ (140)	$7.0\cdot10^6$ (580)	$2.9\cdot10^6$ (2000)	$3.8\cdot10^6$ (7600)
(IV)	C_2H_5	SC_2H_5	$2.1\cdot10^5$ (260)	$2.9\cdot10^6$ (60)	$1.1\cdot10^5$ (48)	$3.2\cdot10^6$ (640)	$1.5\cdot10^4$ (7)	$6.5\cdot10^5$ (810)
(V)	C_2H_5	SC_4H_9	$3.6\cdot10^6$	$2.1\cdot10^7$	$2.8\cdot10^5$	–	–	$2.5\cdot10^5$
(VI)	CH_3	$OC(O)CH_3$	$1.4\cdot10^5$	$1.4\cdot10^5$	$1.0\cdot10^5$	$3.0\cdot10^5$	$1.8\cdot10^5$ $1.4\cdot10^2$	$2.8\cdot10^5$
(VII)	C_2H_5	$OC(O)CH_3$	$1.4\cdot10^5$	$3.3\cdot10^5$	$1.5\cdot10^5$	$4.5\cdot10^5$	$6.5\cdot10^5$	$2.7\cdot10^5$
(VIII)	CH_3	$SC(O)CH_3$	$4.4\cdot10^5$	$5.0\cdot10^5$	$2.9\cdot10^5$	$5.5\cdot10^6$	$1.8\cdot10^5$	$1.7\cdot10^6$
(IX)	C_2H_5	$SC(O)CH_3$	$6.8\cdot10^5$	$5.2\cdot10^6$	$3.8\cdot10^5$	$1.5\cdot10^7$	$1.3\cdot10^5$	$1.2\cdot10^6$
(X)	C_3H_7	$SC(O)CH_3$	$3.0\cdot10^6$	$3.8\cdot10^7$	$3.2\cdot10^6$	$4.0\cdot10^6$	$6.0\cdot10^5$	$1.3\cdot10^3$
(XI)	C_2H_5	OH	$2.9\cdot10^4$	$2.9\cdot10^5$	$2.0\cdot10^5$	$2.2\cdot10^6$	$4.8\cdot10^6$	$4.0\cdot10^5$
(XII)	C_2H_5	$\overset{+}{N}(CH_3)_3Cl^-$	$1.5\cdot10^6$	$1.0\cdot10^7$	$1.8\cdot10^6$	–	–	$1.7\cdot10^7$
(Ia)	CH_3	Cl	$1.0\cdot10^4$	$2.0\cdot10^4$	$7.8\cdot10^3$	$7.7\cdot10^4$	$2.1\cdot10^4$	$2.8\cdot10^3$
(IIa)	C_2H_5	Cl	$2.7\cdot10^4$	$1.7\cdot10^5$	$7.7\cdot10^3$	$6.7\cdot10^4$	$3.4\cdot10^4$	$4.0\cdot10^4$
(IIIa)	CH_3	SC_2H_5	$1.2\cdot10^3$	$1.0\cdot10^3$	$2.3\cdot10^3$	$1.2\cdot10^4$	$1.3\cdot10^3$	$5.0\cdot10^2$
(IVa)	C_2H_5	SC_2H_5	$8.0\cdot10^2$	$4.8\cdot10^4$	$2.3\cdot10^3$	$5.0\cdot10^3$	$2.2\cdot10^3$	$8.0\cdot10^2$

Footnote. Values of the acetylene effect q are given in parentheses (q = k_{II} acetylenic OPI/k_{II} saturated analogue).

1. *S*-Butynyl esters of phosphorus thioacids containing an acetylenic bond in the β-position of the thioester residue

The esters investigated were of *O,O*-dialkyl thiophosphoric acid, viz $(RO)_2P(O)SCH_2C{\equiv}CCH_2R'$, where R = CH_3, C_2H_5, C_3H_7, and R' = Cl, OH, SC_2H_5, SC_4H_9, $OC(O)CH_3$, $SC(O)CH_3$, and $\overset{+}{N}(CH_3)_3Cl^-$ (Table 9) and of *O*-ethyl thiophosphoric acids, viz. $(C_2H_5O)RP(O)SCH_2C{\equiv}CCH_2R'$, where R = CH_3, C_6H_5, and R' = Cl, SC_2H_5, $SC(O)CH_3$, $\overset{+}{N}(CH_3)_3Cl^-$ (Table 10). In these compounds the acetylenic group is at approximately the same distance from the

phosphorus atom as the sulphonium sulphur atom in the preparation Gd-42 (see above for formula). It was assumed that this highly reactive group may react with the anionic centre in cholinesterases and thereby affect the inhibitory activity of these compounds.

Table 10. Anticholinesterase activity (k_{II}, M^{-1} min^{-1}) of acetylenic OPI $R(C_2H_5O)P(O)SCH_2C{\equiv}CCH_2R'$ and their saturated analogues $R(C_2H_5O)P(O)SCH_2CH_2CH_2CH_2R'$ (compounds numbered 'a').[9]

Compound No.	OPI		Anticholinesterase activity of OPIs					
	R	R'	ACE	BuCE	mouse CE	fly CE	beet aphid CE	mite CE
(XIII)	CH_3	Cl	$3.1\cdot10^6$ (62)	$2.1\cdot10^6$ (1500)	$9.3\cdot10^6$ (140)	$1.0\cdot10^9$ (21 000)	$6.6\cdot10^6$ (51)	$9.0\cdot10^5$ (4.5)
(XIV)	C_6H_5	Cl	$4.0\cdot10^5$ (50)	$2.2\cdot10^5$ (8)	$4.7\cdot10^4$ (9)	$1.0\cdot10^5$ (31)	$9.0\cdot10^6$ (310)	$2.7\cdot10^4$ (11)
(XV)	CH_3	SC_2H_5	$6.6\cdot10^6$ (41)	$1.3\cdot10^6$ (20)	$2.9\cdot10^7$ (260)	$1.1\cdot10^9$ (38 000)	$5.3\cdot10^7$ (590)	$2.5\cdot10^7$ (1200)
(XVI)	C_6H_5	SC_2H_5	$6.2\cdot10^5$ (31)	$1.8\cdot10^6$ (8)	$4.6\cdot10^4$ (38)	$4.4\cdot10^5$ (44)	$5.6\cdot10^6$ (670)	$1.1\cdot10^5$ (85)
(XIIIa)	CH_3	Cl	$5.0\cdot10^4$	$1.4\cdot10^3$	$6.7\cdot10^4$	$4.8\cdot10^4$	$1.3\cdot10^5$	$2.2\cdot10^5$
(XIVa)	C_6H_5	Cl	$8.0\cdot10^3$	$2.7\cdot10^4$	$5.4\cdot10^3$	$3.2\cdot10^3$	$2.9\cdot10^4$	$2.4\cdot10^3$
(XVa)	CH_3	SC_2H_5	$1.6\cdot10^5$	$6.4\cdot10^4$	$1.1\cdot10^5$	$2.9\cdot10^4$	$9.0\cdot10^4$	$2.1\cdot10^4$
(XVIa)	C_6H_5	SC_2H_5	$2.0\cdot10^4$	$2.2\cdot10^5$	$1.2\cdot10^3$	$1.0\cdot10^4$	$8.4\cdot10^3$	$1.3\cdot10^3$

Footnote. Values of the acetylene effect q are given in parentheses.

Parallel experiments were carried out with saturated analogues of some of the acetylenic inhibitors in order to assess objectively the effect of the acetylenic group on the anticholinesterase (anti-CE) activity of the compounds. This effect was assessed quantitatively by the size of the 'acetylene effect' (q) which shows how many times the bimolecular rate constant for cholinesterase inhibition by the acetylenic compound was greater than the same inhibition constant of its saturated analogue.

It follows from the data of Tables 9 and 10 that the acetylenic compounds (I)–(IV) and (XIII)–(XVI) possess higher anti-CE activity than their saturated analogues. This is observed for all compounds without exception but varies within very wide limits depending on the nature of the enzyme and the structure of the inhibitor. The ethylmercapto-substituted thiophosphates (III) and (IV) exceed the chloro-substituted compounds (I) and (II) in the size of the acetylenic effect. This is linked with the fact that the inhibitory activity of an acetylenic OPI is practically independent of the nature of the substituent but for the saturated analogues the chloro-substituted compounds possess a higher anti-CE activity than the ethylmercapto-substituted compounds. It is characteristic that the structure of the phosphoryl portion of the inhibitor molecule shows a smaller influence on the acetylenic effect. Relatively close values of q were observed for mouse CE, fly CE and ACE both for the chloro-substituted esters of dimethyl and diethyl thiophosphoric acids (I) and (II) and for the ethylmercapto-subtituted derivatives of these acids (III) and (IV). The dimethyl thiophosphates surpassed by a little the corresponding diethyl thiophosphates in the size of q for the other CE.

There were no saturated analogues for compounds (V)–(XII). Experiments with them gave additional information on the link between the structure of the acetylenic OPI and their anti-CE activity. Dimethyl and diethyl esters of thiophosphoric acids with an $OC(O)CH_3$ substituent (VI) and (VII) displayed close inhibitory activity towards each of the CE investigated as was also seen in experiments with chloro- and ethylmercapto-substituted derivatives of these acids. Appreciable differences were also not observed in their anti-CE activity for various CE. For example the size of the inhibition constant for compound (VI) for fly CE, the most sensitive, was 3.0×10^5 M^{-1} min^{-1}, and for mouse CE, the least sensitive, was 1.0×10^5 M^{-1} min^{-1}, i.e. a threefold difference.

On the other hand a clearly marked dependence of inhibitory activity was shown by the acetylenic OPIs with a $SC(O)CH_3$ substituent (VIII)–(X) on the structure of the phosphoryl portion of the molecule for each CE and on the nature of the CE for each compound. Thus mammalian and aphid CE proved to be the most sensitive to the ester of dipropyl thiophosphoric acid and fly CE to the ester of diethyl thiophosphoric acid. In difference to the other enzymes mite CE displayed an extremely low sensitivity towards the ester of dipropyl thiophosphoric acid (X) $k_{II} = 1.3\times10^3$ M^{-1} min^{-1} which is 30 000 times less than k_{II} for BuCE, 3.8×10^7 M^{-1} min^{-1}. A large difference was detected in the sensitivity of the CEs investigated towards a diethyl thiophosphate with an OH susbtituent (XI). In the inhibition of ACE $k_{II} = 2.9\times10^4$ M^{-1} min^{-1} but in the inhibition of aphid CE $k_{II} = 4.8\times10^6$ M^{-1} min^{-1}.

The butylmercapto derivative (V) possesses a higher anti-CE activity than the methylmercapto derivative (IV) in relation to mammalian CE. Consequently in addition to the acetylenic group, the length of the hydrocarbon chain, which provides hydrophobic adsorption to the corresponding portion of the catalytic centre of the enzyme, enhances the anti-CE activity. Compound (XII), having a trimethylammonium group, surpasses all the other compounds, except for compound (X), in its anti-CE activity on reaction with mouse CE. The sensitivity of the other CEs to compound (XII) differed little from their sensitivity to certain uncharged actylenic OPIs.

Two general regularities must be mentioned in relation to the selectivity of the inhibitory action of the actylenic OPIs investigated. a) Among the mammalian enzymes BuCE proved to be the most sensitive to each of the OPIs investigated which is in agreement with numerous literature data on the higher inhibitory activity of phosphates in relation to BuCE than to ACE.[2] b) Among the arthropod enzymes, fly CE possesses the highest sensitivity towards the OPIs investigated with few exceptions.

It follows from the data of Table 10 that a sharp increase in anti-CE activity is also observed for the thiophosphonates on introducing an acetylenic group into the OPI molecule. Thus for the methyl esters (XIII) and (XV) the size of k_{II} in relation to fly CE proved to be exceptionally high (10^9 M^{-1} min^{-1}) and these compounds may be considered to be highly selective inhibitors of this enzyme. Such an enormous increase in anti-CE activity over the saturated analogues (XIIIa) and (XVa) cannot be explained by the higher phosphorylating ability of the acetylenic compounds since the introduction of an acetylenic group into the β-position of the thioalkyl residue increases the rate constant for alkaline hydrolysis by only 5–6 times. It must be noted that the saturated methylthiophosphonates strongly inhibit not fly CE but the other CEs, compound (XIIIa) inhibits the aphid CE and mite CE and compound (XVa) inhibits mouse CE and ACE.

In contrast to the thiophosphates, the nature of the substituent for the methylthiophosphonates (XIII) and (XV) showed a less clear influence on the acetylene effect. On reacting these compounds with ACE, fly CE and mouse CE the size of q varied little with the nature of the substituent. For aphid CE and mite CE the size of q was greater than when reacting these enzymes with ethylmercapto-substituted derivatives, and for BuCE than with chloro-substituted compounds.

With few exceptions the *S*-butyl esters of phenylthiophosphonic acid (XIV) and (XVI), in comparison with similar esters of methylphosphonic acid (XIII) and (XV), proved to be less strong inhibitors of the CEs investigated. For example, a large difference was observed towards esters of these acids for mite CE for all three substituents. There was a particularly large difference in the sensitivity of fly CE to compounds with substituents Cl and SC_2H_5. For the chloro-substituted derivatives (XIII) and (XIV) this difference was 10000 times and for the ethylmercapto-substituted derivatives (XV) and (XVI) 2500 times.

The thioesters of phenylthiophosphonic acid were surpassed by the corresponding methylthiophosphonates in the size of the acetylene effect. For the majority of the CEs investigated the size of q did not exceed 50 (Table 10), and only for aphid CE were they relatively large, viz. 310 for chloro-substituted and 670 for ethylmercapto-substituted compounds. As for the methylthiophosphonates, the influence of the substituent on the acetylene effect for phenylthiophosphonates is displayed in different ways for the investigated CEs. For arthropod and mouse CE the size of q is greater for ethylmercapto-substituted compounds [(XIV) and (XV)], on the other hand for ACE the size of q is greater for chloro-substituted compounds and for BuCE there is no difference in the values of q for these compounds.

From the experimental data given in this section the problem arises as to which position of the acetylenic bond in the *S*-butynyl group provides the greatest anti-CE activity. The acetylenic group, with its high π-electron density, may react by forming a type of π-complex not only with the carboxyl group of the anionic site of the CE, but also with other groups such as alcohols, amino groups, etc. Consequently the optimum effect of the acetylenic grouping may not necessarily be displayed at the β-position.

2. *S*-Alkynyl esters of diethyl phosphoric acid with the acetylenic group at various positions in the thioester residue

The anti-CE activity of two sets of compounds of general formula $(C_2H_5O)_2P(O)SX$ has been investigated (Table 11). In compounds (XVII)–(XIX), the first set, the group X is a hexynyl group C_6H_9 in which the acetylenic bond is found in the α-, β- or γ-positions relative to the sulphur atom, i.e. X = $-C\equiv CC_4H_9$, $-CH_2.C\equiv CC_3H_7$, $-CH_2CH_2C\equiv CC_2H_5$. In compounds (XX)–(XXII), the second set, the position of the acetylenic bond and the length of the *S*-alkynyl chain are chained. i.e. X = $-C\equiv CH$, $-CH_2C\equiv CH$, $-CH_2CH_2C\equiv CH$. The anti-CE activity of the saturated analogues of these compounds was studied. Compound $(C_2H_5O)_2P(O)SC_6H_{13}$ is the saturated analogue for the three compounds of the first set, each compound of the second set has its own analogue.

It follows from the data of Table 11 that inhibitors with an actylenic bond in the α-position possess the greatest anti-CE activity. In their ability to inhibit CEs they surpassed such strong OPIs as the onium compounds, such as the methylsulphomethylate of *O,O*-diethyl S-[β-(phenylmethylamino)ethyl] thiophosphate.[35] The k_{II} for this compound when inhibiting ACE was 2.5×10^7 M^{-1} min^{-1}, and when inhibiting BuCE was 6.8×10^7 M^{-1} min^{-1}. Compound (XX) gave the same value for the constant when inhibiting ACE, viz. 2.3×10^7 M^{-1} min^{-1}, but compound (XVII) gave the even larger value of 1.0×10^8 M^{-1} min^{-1} when inhibiting BuCE (Table 11).

Table 11. Anticholinesterase activity (k_{II}, M^{-1} min^{-1}) of OPIs of structure $(C_2H_5O)_2P(O)SX$ with various positions of the acetylenic group in the thioether residue.[10,12]

Compound No.	OPI X	Anticholinesterase activity of OPIs					
		ACE	BuCE	mouse CE	fly GE	beet aphid CE	mite CE
(XVII)	$C\equiv CC_4H_9$	$9.5\cdot10^6$ (2500)	$1.0\cdot10^8$ (3100)	$7.1\cdot10^6$ (7100)	$1.5\cdot10^8$ (169 000)	$4.6\cdot10^7$ (6600)	$6.0\cdot10^7$ (8600)
(XVIII)	$CH_2C\equiv CC_3H_7$	$1.4\cdot10^4$ (3.7)	$1.0\cdot10^6$ (30)	$7.0\cdot10^3$ (7.0)	$3.0\cdot10^5$ (34)	$1.7\cdot10^4$ (2.4)	$4.0\cdot10^4$ (5.7)
(XIX)	$CH_2CH_2C\equiv CC_2H_5$	$4.0\cdot10^3$ (1)	$1.2\cdot10^5$ (3.6)	$6.6\cdot10^3$ (6.6)	$1.1\cdot10^5$ (12.3)	$1.7\cdot10^4$ (2.4)	$3.0\cdot10^4$ (4.3)
(XX)	$C\equiv CH$	$2.3\cdot10^7$ (82 000)	$5.6\cdot10^7$ (210 000)	$2.2\cdot10^7$ (220 000)	$3.8\cdot10^8$ (2 700 000)	$1.4\cdot10^7$ (140 000)	$4.8\cdot10^7$ (480 000)
(XXI)	$CH_2C\equiv CH$	$2.2\cdot10^5$ (3000)	$4.0\cdot10^5$ (600)	$7.0\cdot10^4$ (1400)	$1.2\cdot10^5$ (3000)	$1.5\cdot10^4$ (500)	$6.3\cdot10^4$ (300)
(XXII)	$CH_2CH_2C\equiv CH$	$4.0\cdot10^3$ (6.3)	$2.4\cdot10^4$ (4.9)	$1.7\cdot10^3$ (5.0)	$1.1\cdot10^4$ (0.6)	$2.0\cdot10^3$ (0.7)	$2.6\cdot10^3$ (0.09)
(XXIII)	$C(CH_3)_2C\equiv CH$	$6.6\cdot10^4$	$2.4\cdot10^5$	$5.9\cdot10^4$	$8.5\cdot10^5$	$9.0\cdot10^4$ *	–
(XVIIa)	C_6H_{13}	$3.8\cdot10^3$	$3.3\cdot10^4$	$1.0\cdot10^3$	$8.9\cdot10^3$	$7.0\cdot10^3$	$7.0\cdot10^3$
(XXa)	C_2H_5	$2.7\cdot10^2$	$2.7\cdot10^2$	$1.0\cdot10^2$	$1.1\cdot10^2$	$1.0\cdot10^2$	$1.0\cdot10^2$
(XXIa)	C_3H_7	$0.7\cdot10^2$	$0.7\cdot10^3$	$0.5\cdot10^2$	$0.4\cdot10^2$	$0.3\cdot10^2$	$2.0\cdot10^2$
(XXIIa)	C_4H_9	$6.4\cdot10^2$	$4.9\cdot10^3$	$2.9\cdot10^2$	$1.8\cdot10^4$	$3.0\cdot10^3$	$3.0\cdot10^4$

*Grass aphid CE was used in this experiment.
Footnote. Values of the acetylene effect q are given in parentheses.

Moving the acetylenic bond in the thioalkynyl group from the α- to the β-position leads to a sharp fall in the inhibition rate constant for the CE investigated extending to two to three orders of magnitude (Table 11). In line with this the sizes of the acetylene effect were also sharply reduced. The values of q for compound (XVII) of the first series with the acetylenic bond in the α-position were in the thousands but with the acetylenic bond in the β-position [compound (XVIII)] q was in the range 2.4–34.

A large difference in values of q was also observed for the corresponding compounds of the second series. Thus for compound (XX) the size of q when inhibiting fly CE ws 2.7×10^6 but for compound (XXI) with the acetylenic bond in the β-position q was 3000. Further movement of the triple bond to the γ-position of the thioester residue showed a less marked effect in the compounds of both the first and second series.

It seemed of interest to compare the anti-CE activity of compounds (XVII) and (XX) and also compounds (XVIII) and (XXI). Replacement of a hydrogen atom in compound (XX) by a C_4H_9 group [(compound (XVII)] leads to an increase in the inhibitory activity in relation to arthropod CE and BuCE and caused a small reduction in this activity in relation to mouse CE and ACE. Replacement of the acetylenic hydrogen in compound (XXI) by a C_3H_7 group [compound (XVIII)] and replacement of two other hydrogen atoms in the alkylthio residue of this compound by methyl groups [compound (XXIII)] proved to have a weak effect. Here also

Table 12. Anticholinesterase activity (k_{II} M^{-1} min^{-1}) of acetylenic OPIs $(RO)_2P(O)SC{\equiv}CR'$ and their saturated analogues $(RO)_2P(O)SCH_2CH_2R'$ (compounds numbered 'a').[10,11,15]

Compound	OPI		Anticholinesterase activity of OPIs					
	R	R'	ACE	BuCE	mouse CE	fly CE	beet aphid CE	mite CE
(XX)	C_2H_5	H	$2.3\cdot10^7$ (820 000)	$5.6\cdot10^7$ (210 000)	$2.2\cdot10^7$ (220 000)	$3.0\cdot10^8$ (2 700 000)	$1.4\cdot10^7$ (140 000)	$4.8\cdot10^7$ (480 000)
(XXIV)	C_3H_7	H	$1.5\cdot10^7$	$4.0\cdot10^8$	$1.4\cdot10^6$	–	–	–
(XXV)	C_4H_9	H	$6.2\cdot10^5$	$5.7\cdot10^7$	$1.4\cdot10^6$	–	–	–
(XXVI)	CH_3	$CH_2OC(O)CH_3$	$9.3\cdot10^6$	$1.1\cdot10^8$	$3.4\cdot10^6$	$1.3\cdot10^7$	$1.4\cdot10^7$ *	$4.8\cdot10^7$
(XXVII)	C_2H_5	$CH_2OC(O)CH_3$	$1.7\cdot10^7$ (68 000)	$8.5\cdot10^8$ (610 000)	$5.0\cdot10^7$ (500 000)	$2.1\cdot10^8$ (210 000)	$3.0\cdot10^9$ * (30 000 000)	$1.6\cdot10^9$ (10 000 000)
(XXVIII)	C_3H_7	$CH_2OC(O)CH_3$	$1.9\cdot10^7$	$3.6\cdot10^8$	$2.3\cdot10^7$	$5.7\cdot10^7$	$1.5\cdot10^7$ *	$8.0\cdot10^4$
(XXIX)	CH_3	$CH_2SC_2H_5$	$2.0\cdot10^7$	$5.1\cdot10^7$	–	–	–	–
(XXX)	CH_3	$CH_2OC_2H_5$	$7.3\cdot10^6$	$3.7\cdot10^7$	–	–	–	–
(XXXI)	C_2H_5	$CH_2OC_2H_5$	$1.0\cdot10^9$	$3.4\cdot10^9$	–	–	–	–
(XXXII)	C_2H_5	$CH_2OC_3H_7$	$2.1\cdot10^7$	$2.2\cdot10^8$	$1.2\cdot10^7$	$1.7\cdot10^8$	$2.5\cdot10^8$	$1.6\cdot10^7$
(XXXIII)	C_2H_5	$CH_2OC_4H_9$	$5.7\cdot10^7$	$2.4\cdot10^8$	$6.1\cdot10^7$	$2.6\cdot10^9$	$1.7\cdot10^8$	$1.3\cdot10^7$
(XXXIV)	C_3H_7	$CH_2OC_4H_9$	$6.5\cdot10^7$	$9.8\cdot10^8$	$6.7\cdot10^7$	$1.7\cdot19^9$	$4.8\cdot10^8$	$2.1\cdot10^6$
(XXXV)	C_2H_5	$CH_2CH(COOC_2H_5)_2$	$4.5\cdot10^6$	$3.0\cdot10^7$	$5.0\cdot10^6$	–	$3.5\cdot10^7$ *	–
(XVII)	C_2H_5	C_4H_9	$9.5\cdot10^6$ (2 500)	$1.0\cdot10^8$ (3 000)	$7.1\cdot10^6$ (7 100)	$1.5\cdot10^9$ (169 000)	$4.6\cdot10^7$ (6 600)	$6.0\cdot10^7$ (86 000)
(XXXVI)	C_2H_5	$CH_2SC_2H_5$	$7.3\cdot10^6$ (4 000)	$2.1\cdot10^8$ (5 200)	$5.0\cdot10^6$ (7 100)	$5.2\cdot10^8$ (55 000)	$5.0\cdot10^7$ (500 000)	$5.0\cdot10^7$ (50 000)
(XXXVII)	C_2H_5	$CH_2SC_4H_9$	$2.3\cdot10^7$	$1.0\cdot10^7$	$1.0\cdot10^7$	$1.0\cdot10^9$	$7.5\cdot10^7$	$2.0\cdot10^7$
(XXXVIII)	C_2H_5	$C(CH_3)_3$	$1.7\cdot10^7$ (14 000)	$2.8\cdot10^8$ (80 000)	$2.0\cdot10^6$ (5 000)	$7.0\cdot10^6$ (11 000)	$1.8\cdot10^7$ * (11 000)	–
(XXXIX)	C_2H_5	$CH_2CH_2SC_2H_5$	$1.7\cdot10^7$ (21 000)	$6.3\cdot10^7$ –	$2.0\cdot10^7$ (8 700)	$1.5\cdot10^8$ (30 000)	$1.3\cdot10^7$ * (6 000)	–
(XL)	C_2H_5	$CH_2CH_2\overset{+}{S}(CH_3)C_2H_5$	$4.0\cdot10^6$ (220)	$4.5\cdot10^7$ –	$1.2\cdot10^7$ (520)	$3.0\cdot10^8$ (3 300)	$4.8\cdot10^7$ * (900)	–
(XXa)	C_2H_5	H	$2.7\cdot10^2$	$2.7\cdot10^2$	$1.0\cdot10^2$	$1.1\cdot10^2$	$1.0\cdot10^2$	$1.0\cdot10^2$
(XXVIIa)	C_2H_5	$CH_2OC(O)CH_3$	$2.5\cdot10^1$	$1.4\cdot10^3$	$1.0\cdot10^2$	$1.0\cdot10^3$	$1.0\cdot10^2$ *	$1.0\cdot10^2$
(XVIIa)	C_2H_5	C_4H_9	$3.8\cdot10^3$	$3.3\cdot10^4$	$1.0\cdot10^3$	$8.9\cdot10^3$	$7.0\cdot10^2$	$8.0\cdot10^2$
(XXXVIa)	C_2H_5	$CH_2SC_2H_5$	$1.8\cdot10^3$	$4.0\cdot10^3$	$7.0\cdot10^2$	$9.6\cdot10^3$	$1.0\cdot10^2$	$1.0\cdot10^3$
(XXXVIIIa)	C_2H_5	$C(CH_3)_3$	$8.0\cdot10^2$	$3.5\cdot10^3$	$4.0\cdot10^2$	$6.2\cdot10^2$	$1.6\cdot10^2$ *	–
(XXXIXa)	C_2H_5	$CH_2CH_2SC_2H_5$	$8.0\cdot10^2$	–	$2.3\cdot10^3$	$5.0\cdot10^3$	$2.2\cdot10^3$ *	–
(XLa)	C_2H_5	$CH_2CH_2\overset{+}{S}(CH_3)C_2H_5$	$1.8\cdot10^4$	–	$2.3\cdot10^4$	$9.0\cdot10^4$	$5.3\cdot10^4$ *	–

*Grass aphid CE was used in experiments.
Footnote. Values of the acetylene effect q are given in parentheses.

a small reduction was observed in the anti-CE activity in relation to ACE. It is characteristic that compound (XXII) with the acetylenic bond in the γ-position is a weaker inhibitor than its saturated analogue in relation to arthropod CEs, giving a value of $q < 1$.

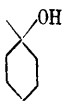
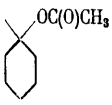
3. *S*-Alkynyl esters of dialkyl thiophosphoric acids containing hydrophobic or onium groups in the alkylthio residue

OPIs of general formula $(RO)_2P(O)SC{\equiv}CR'$ have been investigated where the acyclic hydrophobic group R' = C_4H_9, $CH_2SC_2H_5$, $CH_2SC_4H_9$, $CH_2CH_2SC_2H_5$, $CH_2SC(O)CH_3$, $CH_2OC(O)CH_3$, $CH_2OC_2H_5$, $CH_2OC_3H_7$, $CH_2OC_4H_9$, $CH_2CH(COOC_2H_5)_2$, and R = CH_3, C_2H_5, C_3H_7, C_4H_9 (Table 12). In the experiments with these compounds a study was made of the dependence of their anti-CE activity on the structure of the group R' which is capable of hydrophobic adsorption onto appropriate portions of the enzyme active surface. In addition, comparison of the anti-CE activity of compounds with various R but with the same R' made it possible to assess the effect of the structure of the phosphoryl group of the OPIs. These same problems were also investigated in experiments with compounds containing cyclic hydrophobic groups R', such as phenyl, cyclohexenyl and cyclohexyl and groups R = CH_3 and C_2H_5 (Table 13). The influence of onium groups was investigated in experiments with the inhibitor $(C_2H_5O)_2P(O)SC{\equiv}CCH_2CH_2\overset{+}{S}(CH_3)C_2H_5$ (Table 12).

It follows from the data of Table 12 that replacement of the hydrogen atom of the acetylenic group in the alkylthio residue of compound (XX) with various acyclic hydrophobic groups leads to a significant increase in the anti-CE activity in relation to BuCE. Only the substituents $-CH_2CH(COOC_2H_5)_2$ and $-CH_2SC_4H_9$ did not increase it but reduced the inhibitory action on this CE a little [compounds (XXXV) and (XXXVII)]. In the majority of cases the substituent reduced or did not change the anti-CE activity in relation to the other CE.

For example, compound (XXXVIII) inhibits these CE more weakly than compound (XX). In this case the hydrophobic t-butyl group does not aid but seemingly hinders absorption of the acetylenic inhibitor molecule to the active surface of the enzyme. Only a few substituents increased the anti-CE activity of an inhibitor for certain CE. Thus compound (XX) is surpassed in anti-CE activity by compounds (XVII) and (XXXVII) in experiments with fly, beet aphid and spider mite CE, by compound (XXVII) in experiments with aphid and mite CE and by compound (XXXVIII) in experiments with fly and aphid CE.

Table 13. Anticholinesterase activity (k_{II}, M^{-1} min^{-1}) of acetylenic OPI $(RO)_2P(O)SC{\equiv}CR'$ and their saturated analogues $(RO)_2P(O)SCH_2CH_2R'$ (compounds numbered 'a').[9,12]

Compound No.	OPI		Anticholinesterase activity of OPIs				
	R	R'	ACE	BuCE	mouse CE	fly CE	grass aphid CE
(XLI)	CH_3	C_6H_5	$1.8\cdot10^5$ ($1\,800$)	$1.3\cdot10^7$ ($16\,000$)	$5.5\cdot10^5$ ($5\,500$)	$7.3\cdot10^7$ ($20\,000$)	$5.0\cdot10^5$ ($5\,000$)
(XLII)	CH_3		$6.4\cdot10^5$ ($1\,500$)	$1.9\cdot10^7$ (240)	$6.4\cdot10^5$ ($4\,300$)	$1.6\cdot10^8$ ($3\,900$)	$2.3\cdot10^5$ (300)
(XLIII)	CH_3	*цикло*-C_6H_{11}	$2.0\cdot10^8$ ($2\,000\,000$)	$2.2\cdot10^9$ ($1\,500\,000$)	$1.0\cdot10^8$ ($1\,000\,000$)	$2.3\cdot10^9$ ($300\,000$)	$1.5\cdot10^8$ ($1\,500\,000$)
(XX)	C_2H_5	H	$2.3\cdot10^7$ ($82\,000$)	$5.6\cdot10^7$ ($210\,000$)	$2.2\cdot10^7$ ($220\,000$)	$3.0\cdot10^8$ ($2\,700\,000$)	$1.4\cdot10^7$ * ($140\,000$)
(XLIV)	C_2H_5	C_6H_5	$9.0\cdot10^6$ ($2\,100$)	$1.3\cdot10^8$ ($10\,000$)	$7.9\cdot10^6$ ($10\,000$)	$1.4\cdot10^8$ ($14\,000$)	$8.0\cdot10^6$ ($27\,000$)
(XLV)	C_2H_5		$8.5\cdot10^6$ (450)	$4.3\cdot10^8$ ($1\,400$)	$3.3\cdot10^6$ (400)	$7.0\cdot10^8$ ($1\,300$)	$9.5\cdot10^5$ (53)
(XLVI)	C_2H_5	cyclo-C_6H_{11}	$3.1\cdot10^8$ ($18\,000$)	$1.2\cdot10^9$ ($19\,000$)	$8.0\cdot10^8$ ($670\,000$)	$8.0\cdot10^8$ ($24\,000$)	$1.4\cdot10^9$ ($400\,000$)
(XLVII)	C_2H_5		$8.0\cdot10^6$	$2.3\cdot10^8$	$9.0\cdot10^5$	$1.2\cdot10^8$	$9.0\cdot10^7$
(XLVIII)	C_2H_5		$3.0\cdot10^6$	$4.8\cdot10^7$	$4.1\cdot10^6$	$7.5\cdot10^8$	$5.2\cdot10^6$
(XLIX)	C_3H_7	C_6H_5	$1.8\cdot10^7$	$1.8\cdot10^8$	$1.3\cdot10^7$	$3.6\cdot10^8$	$4.4\cdot10^7$ *
(XLIa)	CH_3	C_6H_5	$1.0\cdot10^2$	$8.0\cdot10^2$	$1.0\cdot10^2$	$3.7\cdot10^3$	$1.0\cdot10^2$
(XLIIa)	CH_3		$4.3\cdot10^2$	$8.0\cdot10^4$	$1.3\cdot10^2$	$4.3\cdot10^4$	$7.7\cdot10^2$
(XLIIIa)	CH_3	*цикло*-C_6H_{11}	$1.0\cdot10^2$	$1.5\cdot10^3$	$1.0\cdot10^2$	$8.0\cdot10^3$	$1.0\cdot10^2$
(XXa)	C_2H_5	H	$2.7\cdot10^2$	$2.7\cdot10^2$	$1.0\cdot10^2$	$1.1\cdot10^2$	$1.0\cdot10^2$ *
(XLIVa)	C_2H_5	C_6H_5	$4.3\cdot10^3$	$1.3\cdot10^4$	$8.0\cdot10^2$	$1.0\cdot10^4$	$3.0\cdot10^2$
(XLVa)	C_2H_5		$1.9\cdot10^4$	$3.1\cdot10^5$	$1.5\cdot10^4$	$5.5\cdot10^5$	$1.8\cdot10^4$
(XLVIa)	C_2H_5	cyclo-C_6H_{11}	$1.7\cdot10^4$	$6.3\cdot10^4$	$1.2\cdot10^3$	$3.3\cdot10^4$	$3.3\cdot10^3$

*Beet aphid CE was used in experiments.

Footnote. Values of the acetylene effect q are given in parentheses.

Replacement of a hydrogen atom in compound (XX) by phenyl [compound (XLIV)] and cyclohexenyl [compound (XLV)] residues led to an increase in the anti-CE activity for BuCE and to a decrease for mouse CE, aphid CE and ACE (Table 13). Compound (XLIV) proved to be less strong in relation to fly CE, but compound (XLV) was a stronger inhibitor than compound (XX). Again, replacement of hydrogen on the acetylenic bond in compound (XX) by a cyclohexenyl group [compound (XLVI)] is accompanied by a significant strengthening of the inhibitory activity in relation to all the CE investigated. For aphid CE the size of k_{II} for this compound was 1.4×10^9 M^{-1} min^{-1}, which is two orders of magnitude greater than for compound (XX). It is characteristic that compounds (XLVII) and (XLVIII), into the cyclohexyl residue of which OH and $OC(O)CH_3$ groups respectively have been introduced, possess many times lower anti-CE activity than compound (XLVI).

A reduction in k_{II} is observed in many cases on replacing the acetylenic hydrogen atom by hydrophobic susbtituents but an appreciable increase always occurred in the value of k_{II} on analogous replacement of hydrogen in the saturated analogue (XXa) [i.e. compounds (XVIIa), (XXXVa), (XXXVIIIa), (XXXIXa), (XLa), (XLIVa), (XLVa), (XLVIa)], seemingly as a result of the increase in the adsorption rate of inhibitors onto the active surface of the CE caused by the hydrophobic adsorption of substituents onto the correpsonding hydrophobic portions of this surface. The cyclohexenyl derivative (XLVa), and not the cyclohexyl compound (XLVIa), was the strongest inhibitor, in relation to all the CE, of the saturated analogues with cyclic substituents [Table 13, compounds

(XXIVa), (XLVa), (XLVIa)] in contrast to the acetylenic compounds. This occurred in spite of the high hydrophobicity of cyclohexyl groups.

It is possible that the difference noted in the effectiveness of substituents is explained by the fact that when many acetylenic compounds are adsorbed onto the enzymes these substituents do not make contact with the hydrophobic portions of the active surface. The linear acetylenic group orients the acetylenic compound on the enzyme differently from the orientation of the saturated analogue. Overall the impression is created that the interaction with the anionic centre of the CE active site does not play a major role in the inhibition process in the case of the acetylenic OPIs.

It is seen from the data shown in Tables 12 and 13 that the values of the acetylenic effect vary between very wide limits and depend on both the structure of the compound and on the source of the enzyme. As already mentioned above the value of q for compound (XX) was very high. Replacement of the hydrogen atom on the acetylenic bond of this compound by groups of various structure led, as a rule, to a reduction in the size of q. Only on replacing the hydrogen atom by a cyclohexyl group [compound (XLVI)] was there an increase in q for the inhibition of mouse and aphid CE. The absence of a correlation between the change in the size of k_{II} and q for an acetylenic analogue is worthy of attention. For example on replacing the hydrogen atom in compound (XX) by a cyclohexyl group [compound (XLVI)] the size of k_{II} for fly CE was increased almost threefold (3×10^8 M^{-1} min^{-1} and 8×10^8 M^{-1} min^{-1} respectively) but the size of q was reduced by more than 100 times (2 700 000 and 24 000 respectively). The same picture was observed on replacing the hydrogen atom in compound (XX) by a butyl group [compound (XVII)] when inhibiting BuCE and arthropod CE. These divergences between the changes of k_{II} and q are explained by the fact that replacement of the hydrogen atom by certain groups in the saturated analogue (XXa) causes an increase in k_{II} several times greater than the analogous replacement in the acetylenic compound (XX). For example, replacement by a cyclohexyl group [compound (XLVI)] in the acetylenic compound (XX) leads to an increase in k_{II} for fly CE of less than threefold but replacement of hydrogen by this group [compound (XLVIa)] in the saturated compound (XXa) leads to an increase of k_{II} for the same enzyme of 300-fold.

The dependence of inhibiting ability on the length of the alkyl chain in the phosphoryl portion of the OPI molecule has been investigated for certain compounds. It must be noted first that the esters of dimethyl thiophosphoric acid, both acetylenic [(XXVI), (XXX), (XLI)–(XLIII)] and saturated [(XLIa), (XLIIa), (XLIIIa)], display in the majority of cases a lower anti-CE activity than their diethyl homologues (XXVII), (XXXI), (XLIV), (XLV), (XLVI), (XLIVa), (XLVa) and (XLVIa). Only in the case of the cyclohexyl derivatives (XLIII) and (XLVI) and only for fly CE and BuCE was the ratio reversed. These two compounds surpass significantly all the known acetylenic OPIs in anti-CE activity. Further lengthening of the alkyl chain in the phosphoryl portion of the OPI molecule leads to a different result depending on the enzyme and on the structure of the group R′ on the acetylene. On investigating a series of acetylenic OPIs with R′ = H [compounds (XX), (XXIV), (XXV)] it was discovered that the diethyl thiophosphate possesses the greatest activity in relation to mouse CE and ACE but the dipropyl thiophosphate possessed the greatest in relation to BuCE. This agrees with the results obtained previously for ACE and BuCE in experiments with $(RO)_2P(O)SC_2H_4\overset{+}{N}(CH_3)_2C_6H_{11}$, where R = C_2H_5, C_3H_7, C_4H_9 and $(RO)_2P(O)SC_2H_4\overset{+}{S}(CH_3)C_2H_5$, where R = C_2H_5, C_3H_7.[34] It was characteristic that grass aphid CE proved to be the more sensitive to the diethyl thiophosphate.[35]

However, in the series of OPIs with R′ = $CH_2OC(O)CH_3$ the diethyl thiophosphate (XXVII) was a more active inhibitor of all the CE, except for ACE, than the dimethyl thiophosphate (XXVI) and the dipropyl thiophosphate (XXIII). Among the compounds with R′ = $CH_2OC_4H_9$ the ester of diethyl thiophsophoric acid [compound (XXXIII)] inhibits mite CE more strongly, the ester of dipropyl thiophosphoric acid [compound (XXXIV)] BuCE and aphid CE, but mouse and fly CE and ACE were equally sensitive to both OPIs. The dipropyl derivative [compound (XLIX)] of the OPI series with R′ = C_6H_5 was a stronger CE inhibitor than the diethyl derivative (XLIV) in all cases.

The anti-CE activity of compounds therefore depends on the nature of the enzyme and on the structure of the OPI in both the phosphoryl and the cleavable portions of the molecule.

The change in anti-CE activity on converting the acetylenic compound (XXXIX) with a sulphide sulphur atom into the derivative (XL) with a sulphonium sulphur atom was unusual. In this case the cationic group provides a reduction in the size of k_{II} for warm-blooded CE and an insignificant increase (2–4 times) for fly CE and aphid CE while the rate constant for alkaline hydrolysis of the sulphonium derivative was many times greater than that for the sulphide (2.9×10^{-1} and 4.4×10^{-4} min^{-1} respectively).

It was characteristic that the acetylenic effect for compounds with a sulphide sulphur atom (XXXIX) was significantly greater than for the derivative with sulphonium sulphur atom (XL) for all CEs. This is linked with the fact that replacement of sulphide sulphur atom in the saturated prototype [compound (XXXIXa)] by sulphonium [compound (XLa)] leads to a strengthening of the anti-CE activity in relation to each CE. The k_{II} constants are increased 10–25 times, the alkaline hydrolysis constant is increased 75 times.

The low effect on introducing an onium grouping is explained to a significant extent by the fact that in compounds (XL) and (XLa) the onium atom is placed at a significantly large distance from the phosphorylmercapto group ($-CH_2CH_2CH_2CH_2\overset{+}{S}=$), compared with the optimum distance ($-CH_2CH_2\overset{+}{S}=$) for example in preparation Gd-42 mentioned above. The rigidity of the *S*-alkynyl residue also plays a particular role in the case of the acetylenic OPIs. It is possible that the hypothesis on the absence or small extent of reaction with the anionic site of a CE for all groups of acetylenic OPIs is correct.

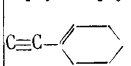
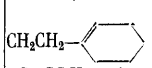
4. Analysis of the mechanism of the anticholinesterase action of acetylenic OPIs

a) Alkaline hydrolysis

The mechanism of the interaction of acetylenic OPIs with the cholinesterases is an extremely important problem. It might have been thought that the acetylenic group, which shows an electron-accepting effect, increases the electrophilicity of the phosphorus atom and thereby increases the phosphorylating ability of a compound. The rate constant for alkaline hydrolysis (k_{OH}) of the organophosphorus inhibitors is a fairly reliable measure of this ability. Experiments have shown that the values of k_{OH} for the acetylenic OPIs are in reality higher than the values for their saturated analogues (Table 14). They are particularly high for compounds with an acetylenic bond in the α-position. For compound (XX) $k_{OH} = 2.2 \times 10^{-3}$ min^{-1} but for its saturated analogue (XXa) this constant was 290 times smaller.

Such a strengthening of the phosphorylating ability does not agree quantitatively in any way with the enormous size of the acetylene effect which ranges from 82 000 for ACE to 2 700 000 for fly CE for the same compound (XX) (Table 11). In addition the colossal difference observed in the values of the acetylene effect for a single compound against several CEs cannot be explained by the change in k_{OH}.

Table 14. Rate constants for the alkaline hydrolysis (k_{OH}, min^{-1}) of the acetylenic OPIs $(C_2H_5O)_2P(O)SR$ and their saturated analogues (compounds number 'a').[12–14]

OPI		k_{OH}, min^{-1}	OPI		k_{OH}, min^{-1}
Compound No.	R		Compound No.	R	
(XVII)	$C\equiv CC_4H_9$	$1.2\cdot10^{-3}$ (150)	(XX)	$C\equiv CH$	$2.2\cdot10^{-3}$ (290)
(XVIIa)	C_6H_{13}	$8.0\cdot10^{6}$	(XXa)	CH_2CH_3	$7.7\cdot10^{-6}$
(XVIII)	$CH_2C\equiv CC_3H_7$	$2.2\cdot10^{-5}$ (2.7)	(XXXVI)	$C\equiv CCH_2SC_2H_5$	$2.9\cdot10^{-4}$ (17)
(XIX)	$C_2H_4C\equiv CC_2H_5$	$2.0\cdot10^{-5}$ (2.6)	(XXXVIa)	$CH_2CH_2CH_2SC_2H_5$	$1.7\cdot10^{-5}$
			(XXXIX)	$C\equiv CCH_2CH_2SC_2H_5$	$4.4\cdot10^{-4}$ (81)
(XLV)		$4.4\cdot10^{-3}$ (115)	(XXXIXa)	$CH_2CH_2CH_2CH_2SC_2H_5$	$5.4\cdot10^{-6}$
(XLVa)		$3.8\cdot10^{-5}$	(XL)	$C\equiv CCH_2CH_2\overset{+}{S}(CH_3)C_2H_5$	$2.9\cdot10^{-4}$ (740)
(XLVI)	$C\equiv CC_6H_{11}$-cyclo	$2.4\cdot10^{-3}$ (70)	(XLa)	$CH_2CH_2CH_2CH_2\overset{+}{S}(CH_3)C_2H_5$	$3.9\cdot10^{-4}$
(XLVIa)	$CH_2CH_2C_6H_{11}$-cyclo	$3.4\cdot10^{-5}$			

Footnote. Values of the acetylene effect q are given in parentheses.

At first glance the non-correspondence between the acetylene effects in the alkaline hydrolysis reaction and in the reaction with CE may be a reason to propose a different mechanism for the irreversible inhibition of enzymes by acetylenic OPIs, for example not phosphorylation but alkylation of the active site. It is well known[36] that derivatives of α-propynylamine inhibit monoamine-oxidases irreversibly by alkylation of the enzyme. However such a hypothesis encounters serious objections. It was established previously[7] that on increasing the thioalkyl residue from SCH_3 to SC_7H_{15} in compounds $(C_2H_5O)CH_3P(O)SR$ the anti-CE activity was significantly increased but the size of k_{OH} was practically unchanged. This was explained by the fact that the size of the bimolecular rate constant for the reaction of OPIs with CE is determined mainly by the rate of formation of the enzyme–inhibitor complex which is the first stage of the reaction and not by the rate of phosphorylation which is the second stage. According to this analogy it is also obvious that the observed non-correspondence between the increase of k_{II} and k_{OH} on replacing an ethylenic by an acetylenic group in the S-alkyl residue may be explained by the significant acceleration of the adsorption of an acetylenic OPI onto the CE active surface. This increase of the adsorption rate may not be the same for the various CEs which differ in the structure of their active centres.

b) Change of anticholinesterase activity of acetylenic OPIs under various conditions

Specially conducted investigations[36] have shown that acetylenic OPIs possess some properties characteristic of typical OPIs. One of such properties is the loss of the ability of an OPI to inhibit a CE irreversibly on replacement of the phosphoryl oxygen by a sulphur atom, which was mentioned in the introduction. It has been established[11] that in contrast to compound (XVII) its dithio analogue $(C_2H_5O)_2P(S)SC\equiv CC_4H_9$ does not inhibit CE. Another acetylenic dithio derivative $(CH_3O)_2P(S)SC\equiv CC_4H_9$ does not possess anti-CE activity.

Considerable similarity has been observed between acetylenic and the usual OPIs in the character of the change in k_{II} vlaue on changing the pH and in the action of tetraalkylammonium ions.

The effect of tetraalkylammonium ions on the bimolecular rate constant for the reaction of ACE with an OPI was investigated at 25 °C and pH 7.5 and was assessed by the value of the inhibition constant K_i[37] which was found from the equation

$$k_{II,\,i} = k_{II}\frac{1}{(1+[I]/K_i)},$$

where [I] is the concentration of the irreversible inhibitor. Experimental points plotted on a graph having coordinates $1/k_{II}$ and [I] lay on a straight line cutting the abscissa axis at a distance equal to $-K_i$.

The effect of pH on the rate of interaction of cholinesterase with OPI was investigated at various values from 3 to 11.

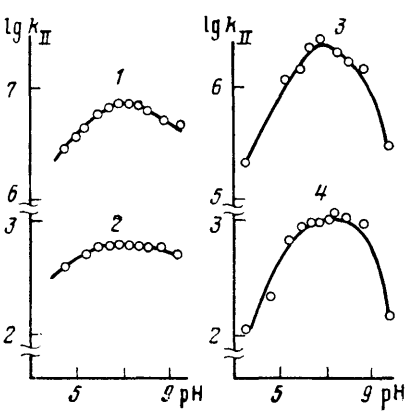


Figure 1. Dependence of the reaction rate of OPIs with ACE on pH; *1*, $(C_2H_5O)_2P(O)SC\equiv CC_2H_4SC_2H_5$; *2*, $(C_2H_5O)_2P(O)SCH_2.CH_2C_2H_4SC_2H_5$; *3*, $(C_2H_5O)_2P(O)SC\equiv CC_6H_{11}$-cyclo; *4*, $(C_2H_5O)_2.P(O)SCH_2CH_2C_6H_{11}$-cyclo.

The dependence of the size of k_{II} on pH when inhibiting ACE with an acetylenic compound and its saturated analogue is expressed by similar curves but the values of the pH optima of these curves were close and were in the pH range 7–8 (Fig. 1). It was established in experiments with ACE[38] that tetraalkylammonium ions reduced to a varying degree the interaction of this enzyme with the acetylenic OPIs (XXXIX) and (XLIV) and with their saturated

analogues (XXXIXa) and (XLIVa). The values of K_i proved to be practically identical for the tetraalkylammonium ions investigated (Table 15). It is evident that the changes in conformation and the degree of ionisation of the functional groups on the active surface of the enzyme occurring on changing pH or by the action of tetraalkylammonium ions proved to be the same when reacting cholinesterases with acetylenic OPI and with their saturated analogues. This indicates that the introduction of an acetylenic bond into the OPI molecule does not change the main features of the mechanism of inhibition of CE.

Table 15. Reversible inhibition constants (K_i, M) for ACE of human blood erythrocytes by tetraalkylammonium ions on reacting the enzyme with acetylenic OPI or their saturated analogues.[38]

OPI		K_i, M		
Compound No.	Formula	$(CH_3)_3N^+$	$(C_2H_5)_4N^+$	$(C_4H_9)_4N^+$
(XXXIX)	$(C_2H_5O)_2P(O)SC{\equiv}CC_2H_4SC_2H_5$	$1.2\cdot10^{-3}$	$1.6\cdot10^{-3}$	$5.1\cdot10^{-5}$
(XXXIXa)	$(C_2H_5O)_2P(O)S(CH_2)_4SC_2H_5$	$1.5\cdot10^{-3}$	$1.8\cdot10^{-3}$	$5.6\cdot10^{-5}$
(XLIV)	$(C_2H_5O)_2P(O)SC{\equiv}C-C_6H_5$	$4.0\cdot10^{-3}$	$3.3\cdot10^{-3}$	$7.2\cdot10^{-5}$
(XLIVa)	$(C_2H_5O)_2P(O)SC_2H_4-C_6H_5$	$6.1\cdot10^{-3}$	$5.0\cdot10^{-3}$	$6.0\cdot10^{-5}$

c) Reactivation

The bimolecular rate constant for reactivation ($k_{II,re}$ M^{-1} min^{-1}) was determined at 25 °C and pH 7.5 and calculated from the formula:

$$k_{II,re} = 2.303/\tau[R]\ \lg(v_0 - v_i)/(v_0 - v_\tau),$$

where v_0 is the initial rate of the enzymic hydrolysis of substrate, v_i the residual rate of hydrolysis after reaction of the enzyme with the OPI, v_τ the rate of hydrolysis of substrate after τ min reaction of the inhibited enzyme with reactivator of concentration [R]. In this formula $(v_0 - v_i)$ is directly proportional to the initial quantity of phosphorylated enzyme and $(v_0 - v_\tau)$ to the quantity of phosphorylated enzyme remaining after partial reactivation at time τ. 1,1-Trimethylene bis(4-pyridinaldoxime) dibromide (TMB-4)[38] was used as reactivator.

The most convincing proof of the phosphorylation of CEs on reaction with acetylenic OPI are the results of experiments on the reactivation of ACE inhibited by various OPIs. It follows from the data of Table 16 that ACE, inhibited by derivatives of diethyl phosphoric acid such as the acetylenic inhibitors (XXXIX) and (XLVI) and their saturated analogue (XXXIXa) and by the well known OPI paroxon, are reactivated by TMB-4 at identical rates. The observed similarity of the reactivation rate constants indicates that a phosphorylated enzyme of the same structure $E-O-P(O)(OC_2H_5)_2$ is formed by reaction of ACE with these compounds which differ markedly in structure. Similar results were also obtained in experiments with the acetylenic derivatives of dimethyl phosphoric acid (XLIII) and (XLIV) and with methyl-paroxon. In this case the reactivation rate constants were a little greater than in the experiments with the derivatives of diethyl phosphoric acid.

Table 16. Bimolecular rate constants ($k_{II,r}$) for the reactivation of inhibited acetylcholinesterase [reactivator was TMB-4, 1,1-trimethylene-bis(4-pyridinaldoxime) dibromide].[38]

Inhibitor $(C_2H_5O)P(O)R$		$k_{II,re}$, M^{-1} min^{-1}	Inhibitor $(CH_3O)_2P(O)R$		$k_{II,re}$, M^{-1} min^{-1}
Compound No.	R		Compound No.	R	
(XLVI)	$SC{\equiv}C-C_6H_5$	$0.82\cdot10^4$	(XLIII)	$SC{\equiv}C-C_6H_5$	$1.1\cdot10^4$
(XXXIX)	$SC{\equiv}CC_2H_4SC_2H_5$	$1.0\cdot10^4$			
(XXXIXa)	$SCH_2-CH_2C_2H_4SC_2H_5$	$0.97\cdot10^4$	(XLIV)	$SC{\equiv}C-C_6H_5$	$1.4\cdot10^4$
Paraoxon	$O-C_6H_5-NO_2$	$0.92\cdot10^4$	Methyl-paraoxon	$O-C_6H_5-NO_2$	$1.2\cdot10^4$

d) Anticholinesterase activity of hexynyltrimethylammonium ions

The results obtained form a basis for considering that the inactivation of cholinesterase by acetylenic OPIs is brought about by esterification of a serine hydroxyl by the appropriate phosphoric acid. Experiments seemed to confirm the initial conclusion that the origin of the acetylene effect should be sought in the effect of the acetylenic bond on the first stage of the inhibition, viz. on the rate of formation of the enzyme–inhibitor complex. Consequently the possibility of an interaction of the C≡C triple bond with structures located either in the region of the anionic site of the enzyme or near the phosphorylated serine hydroxyl has been investigated.[12]

It is known that tetraalkylammonium ions are reversible inhibitors of CE. It is highly probable that their action is effected by adsorption with the formation of simple ion pairs with the carboxylate anion at the anionic point of the enzyme. It has to be clarified whether the triple bond shows a strengthening action on the adsorption of such compounds. Quaternary ammonium ions containing alkynyl groups have been investigated.[14] However the experiments carried out on the inhibition of ACE and BuCE by such quaternary ammonium ions (Table 17) show that the introduction of an acetylenic bond into any position of the hexyl group [compound (LIII)] had practically no effect on the ability of hexynyl-trimethylammonium ions [compounds (L)–(LII)] to inhibit reversibly the catalytic activity of the enzymes. The same values of I_{50} were obtained in experiments with trimethylpropynylammonium salts (LIV) and (LV). In reality there is no binding of the acetylenic group in the region of the anionic site of a CE.

The serine hydroxyl may be a binding region. Many authors have proposed its presence in the esterase site of cholinesterase.[1] This hypothesis will be considered below.

e) Cholinesterase hydrolysis of acetylenic substrates

It was mentioned above that acetylenic OPIs, like typical OPIs, phosphorylate the active site of CEs. Further, experiments with ammonium salts show that the acetylenic group does not show any effect on processes occurring in the region of the anionic site of the

enzyme. Nevertheless the acetylene effect (q) is present. Two alternative hypotheses may be proposed. Either the acetylenic group accelerates the formation of the enzyme–inhibitor complex by being adsorbed onto the active surface of the enzyme elsewhere than in the region of the anionic site and this adsorption improves the steric conditions for the attack by the phosphorus atom on the serine hydroxyl of the enzyme very effectively, or the triple bond participates directly in the phosphorylation of the enzyme, i.e. by reducing appreciably the energy of activation in this part of the reaction but not changing the final result. In both cases it remains unclear what occurs to the *S*-alkynyl residue after fission of the thioester bond. Does it remain bound to the enzyme or is it transferred into the reaction medium? The fact that the reactivation rate constants for ACE inhibited by acetylenic OPIs are practically equal to the reactivation rate constants for ACE inhibited by their saturated analogues does not rule out the possibility of forming a product of interaction of the acetylenic bond with any portion of the active surface of the enzyme. If the rate of freeing ACE from the remainder of the *S*-alkynyl group by the action of the reactivator is significantly greater than the rate of dephosphorylation then the presence of a product from the reaction of this group with the enzyme is by no means reflected in the size of the reactivation rate constant.

Table 17. Anticholinesterase activity (I_{50}, M) of trimethylhexynylammonium ions, with the acetylenic bond at different positions in the hexynyl chain, and of their saturated analogue.[14]

Inhibitor		I_{50}, M	
Compound No.	formula	ACE	BuCE
(L)	$(CH_3)_3\overset{+}{N}C{\equiv}CC_4H_9Br^-$	$1.0\cdot10^{-3}$	$2.7\cdot10^{-3}$
(LI)	$(CH_3)_3\overset{+}{N}CH_2C{\equiv}CC_3H_7\cdot Br^-$	$3.0\cdot10^{-4}$	$2.0\cdot10^{-4}$
(LII)	$(CH_3)_3\overset{+}{N}C_2H_4C{\equiv}CC_2H_5\cdot Br^-$	$1.1\cdot10^{-3}$	$7.4\cdot10^{-2}$
(LIII)	$(CH_3)_3\overset{+}{N}C_6H_{13}Br^-$	$2.1\cdot10^{-3}$	$6.5\cdot10^{-3}$
(LIV)	$(CH_3)_3\overset{+}{N}CH_2C{\equiv}CH\cdot Br^-$	$3.5\cdot10^{-3}$	$7.5\cdot10^{-3}$
(LV)	$(CH_3)_3\overset{+}{N}CH_2C{\equiv}CH\cdot I^-$	$1.3\cdot10^{-3}$	$1.5\cdot10^{-2}$

The experiments carried out on the reactivation of the enzyme showed convincingly that the acetylenic OPIs, like the usual OPIs, phosphorylate cholinesterases but they do not give an answer to the problem of the fate of the *S*-alkynyl group.

There are considerable methodological difficulties in the detection in the reaction medium of an acetylenic mercaptan or a product of its conversion during experiments on the inhibition of CE by acetylenic OPIs. In these experiments the concentration of CE active centres in the reaction medium usually does not exceed 1×10^{-9} M and consequently the possible concentration of the *S*-alkynyl residue or its conversion product may not be more than the value indicated. This is a thousand times less than the minimum concentration of SH groups which can be determined by contemporary chemical methods.

The interaction of cholinesterase with *S*-alkynyl esters of thioacetic acid, viz. $CH_3C(O)SCH_2C{\equiv}CH$ [compound (LVII)] and $CH_3C(O)SC{\equiv}CC_4H_9$ [compound (LVIII)] has been investigated in order to clarify this problem.[38]

If the acetylenic group forms a stable complex with the enzyme then these compounds should be irreversible inhibitors. Experiments showed that both compounds were hydrolysed, although slowly, by both ACE and BuCE. Ellman's reagent, present during the enzymic hydrolysis, pointed to the formation of a compound possessing an SH group in the reaction medium. It is possible that this compound is either an acetylenic mercaptan, which reacts with the Ellman reagent at the moment of fission from the substrate molecule, or a product of its conversion. Control experiments, in which the Ellman reagent is added after the end of enzymic hydrolyis, also showed the presence of a product with an SH group. When such a reaction mixture was protected from access to oxygen of the air the concentration of SH groups remained practically unchanged for an extended period. Consequently a stable product with an SH group is formed from the unstable *S*-alkynyl group split off from the substrate molecule or from the acetylenic OPI molecule. It is possible that the conversion of the *S*-alkynyl group occurs by the following scheme:

$$HS{-}C{\equiv}C{-}R \rightarrow S{=}C{=}CHR \xrightarrow{H_2O}$$
$$\rightarrow S{=}C(OH){-}CH_2R \rightarrow HS{-}C(O){-}CH_2R,$$

$$HSCH_2C{\equiv}CH \rightarrow HSC{\equiv}C{-}CH_3 \rightarrow S{=}C{=}CH{-}CH_3 \xrightarrow{H_2O}$$
$$\rightarrow HSC(O){-}CH_2CH_3.$$

Evidently such conversion products of the *S*-alkynyl group are also obtained on alkaline hydrolysis of both acetylenic OPIs and of the acetylenic substrates.

As was established in experiments on the alkaline hydrolysis of acetylenic OPI and substrates,[38] the number of micromoles of alkali spent in the neutralisation of the products formed was almost twice the number of micromoles taken in the experiments on the compounds, i.e. the alkali is bound not only by the appropriate phosphoric or acetic acid but also by the conversion product of the acetylenic mercaptan. According to the data obtained the acid properties of this product exceed those of the usual mercaptans, which is confirmation of the scheme shown.

It was established further[38] that the catalytic activity of the cholinesterases was unchanged during enzymic hyrolysis of the acetylenic substrate. As follows from Fig. 2, the quantity of compound (LVIII) taken is hydrolysed completely by BuCE. A new portion of this compound added to the reaction mixture at the same concentration is hydrolysed at the same rate. The slopes of the curves in coordinates of 'concentration of thiol product formed' against 'time' in the initial period of enzymic hydrolysis for the two portions of substrate proved to be equal. Analogous results were obtained in experiments with compound (LVII). Consequently the products of enzymic hydrolysis do not cause irreversible inhibition of the catalytic activity of the enzyme.

The *S*-alkynyl esters of thioacetic acid therefore act only as substrates in relation to the cholinesterases. The low rate for enzymic hydrolysis is most likely caused by slow desorption of the resulting mercaptan. Evidently among the numerous examples of cholinesterase hydrolysis of various carboxylic acid esters the

hydrolysis of acetylenic substrates is a rare case in which the rate of enzymic hydrolysis is limited by the desorption of the reaction product.[39]

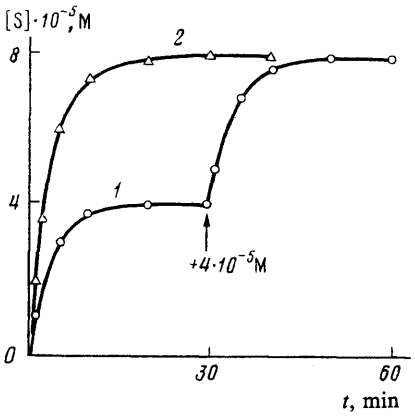


Figure 2. Cholinesterase hydrolysis of substrate $CH_3C(O).SC{\equiv}CC_4H_9$ (S) at different initial concentrations: *1*, 8 × 10^{-5} M, *2*, 4 × 10^{-5} M with subsequent addition of a new portion of substrate (indicated by the arrow).[38]

IV. Toxicity of acetylenic organophosphorus inhibitors

The method of determining the toxicity of OPIs for white mice on subcutaneous administration and the method of determining the contact toxicity for the beet aphid *Aphis fabae Scop.*, the rice weevil *Calandra oryzae L.*, the imago and larvae of the fourth instar of the Colorado beetle *Leptinotarsa decemlineata Say.*, larvae of the second instar of the Podisus bug *Podisus maculiventris Say.* and the spider mite *Tetranychus urticae Koch* have been described previously.[15] The mouse toxicity was characterised by the LD_{50}, the dose of the substance in mg kg^{-1} causing death of 50% specimens, and the toxicity for arthropods by the size of the LC_{50}, the concentration of compound in mg $litre^{-1}$ causing death of 50% specimens also 24 h after applying the OPI.

Only a small proportion of the compounds shown in Tables 12 and 13 were used in the toxicological investigation. Mostly these were compounds with high anti-CE activity (Table 18). The test objects for the toxic action of these compounds were white mice, beet aphids, rice weevils and spider mites. It follows from the data of Table 18 that the *S*-ethynyl esters of dialkyl thiophosphoric acids possess high insecticidal activity, exceeding that of the standard (chlorofos for beetles, carbofos for aphids) 10–100 times in certain cases. At the same time their saturated analogues proved to have low toxicity for arthropods. The introduction into the OPI molecule of an acetylenic bond in the α-position relative to the phosphoryl-mercapto group leads to a significant enhancement of the anti-CE activity and of the insecticidal properties. The efficiency of such enhancement depends on the OPI structure and is manifested in a different way for each compound for the arthropods investigated.

It is generally acknowledged that CE inhibition is the main reason for the toxic action of the organophosphorus insecto-acaricides.[40] However a direct link between the toxicity of the organophosphorus compounds and their inhibitory activity towards the CE of the given representatives of the arthropods or mammals exists in those fairly rare cases when compounds close in structure are investigated. The absence of such a link is explained chiefly by differences in losses of compound during the time taken to be transferred from the site of administration in the organism to the synaptic CE and in non-uniform conversions during metabolism.[40,41]

Table 18. Toxicity of *S*-ethynyl esters of dialkyl thio- and dithiophosphoric acids $(RO)_2P(Y)SC{\equiv}CX$ and their saturated analogues $(RO)_2P(Y)SCH_2CH_2X$ (compounds numbered 'a').[15]

Compound No.	OPI		LD_{50}, mg kg^{-1} mice		LC_{50}, mg $litre^{-1}$					
					beet aphid		beetle		spider mite	
	R	X	Y=O	Y=S	Y=O	Y=S	Y=O	Y=S	Y=O	Y=S
(LVI)	CH_3	C_4H_9	2.7 (175)	470	4 (15)	60	30 (0.7)	20	100 (10)	1000
(XXIX)	CH_3	$CH_2SC_2H_5$	9.2 (42)	390	57 (1)	53	500 (0.8)	400	300 (1.3)	400
(XVII)	C_2H_5	C_4H_9	0.45 (470)	235	45 (0.7)	30	200 (0.5)	90	10 (30)	300
(XXXVI)	C_2H_5	$CH_2SC_2H_5$	3.5	210	45 (0.8)	39	100 (2)	200	10 (40)	400
(XXXVII)	C_2H_5	$CH_2SC_4H_9$	60 2.4 (192)	460	48 (3)	150	150 (2)	300	200 (1.5)	300
(XXVII)	C_2H_5	$CH_2OC(O)CH_3$	0.5 (540)	270	12 (12)	150	700 (10)	7000	700 (4)	3000
(XLVI)	C_2H_5	C_6H_{11}-cyclo	1.2 (416)	500	0.1 (1)	0.1	3 (0.3)	1	7 (7)	50
(XLIV)	C_2H_5	C_6H_5	8.2 (57)	465	50 (2.4)	120	300 (3.3)	1000	100 (1)	100
(XVIIa)	C_2H_5	C_4H_9	160 (2.5)	400	*	*	*	*	*	*
(XXXVIa)	C_2H_5	$CH_2SC_2H_5$	24 (12.5)	300	*	*	*	*	*	*
(XXVIIa)	C_2H_5	$CH_2OC(O)CH_3$	210 (1.9)	400						
Standards		chlorophos	–		–		300		–	
		carbophos			30		–		–	
		metaphos			–		–		20	

*Compounds of low toxicity for arthropods. Death of test objects at concentration of 500 mg $litre^{-1}$ for mites and 100 mg $litre^{-1}$ for aphids were only 5–10%.

Footnote. Values of the thione effect *T* are given in parentheses [for mammals $T = LD_{50}(Y = S)/LD_{50}(Y = O)$ and for arthropods $T = LC_{50}(Y = S)/LC_{50}(Y = O)$].

It follows from Tables 12 and 13 that of the *S*-ethynyl esters of diethyl phosphoric acid the strongest inhibitors of CE are compounds (XXVII) and (XLVI), while compound (XLVI) (Table 18) is surpassed by compound (XVII) in its toxic action on mice. Compounds (XVII), (XXXVI) and (XXXVII) surpass compound (XXVII) in their toxic action on mites, although these compounds are weaker than compound (XXVII) in anti-CE activity by a ratio of 30–80. Only for aphids was a satisfactory link

observed between the anti-CE activity and toxicity of these compounds.

Since the toxicities of the investigated thiophosphoric acid esters for mice are relatively large these compounds do not satisfy contemporary requirements for highly selective insecto-acaricides. It is known that replacement of a phosphoryl (P=O) group by a thiophosphoryl (P=S) group in an OPI leads to a reduction in toxicity since thione derivatives are devoid of anti-CE activity and their toxic action *in vivo* is brought about only after enzymic oxidative desulphurisation which occurs at different rates in different forms of animal.[40,41] This reaction is provided in the system by microsomal oxygenases with cytochrome P-450 as the central unit. This process is usually accomplished significantly more rapidly in arthropods than in mammals, which possess a relatively low activity of these enzymes.[41] As a result the replacement of a phosphoryl group in OPIs by a thiophosphoryl group markedly reduces their toxicity for mammals and in the same way increases the selectivity of their action on arthropods. The selective toxicity of compounds in this case is assessed most graphically (Table 18) by the size of the 'thione effect' (T) which is the ratio of the toxicity of the thione compound ($LD_{50,S}$ and $LC_{50,S}$) to the toxicity of its oxygen analogue ($LD_{50,O}$ and $LC_{50,O}$).

Acetylenic (8) and saturated (3) derivatives of dithiophosphoric acids, which are analogues of the monothio compounds, have been synthesised and investigated.[15] In the text they will be referred to by the number of the oxygen compound with the index S. Their toxicities and the size of the thione effect are given in the same Table 18. The thione derivatives investigated differ from one another in toxicity to a larger degree than do the oxygen analogues. For example, for beetles the toxicity of compound (XLVI,S) has a $LC_{50,S}$ value of 1 mg litre^{-1}, but the toxicity of compound (XXVII,S) has a value of 7000 mg litre^{-1}.

It was extremely important and unexpected that on replacing a phosphoryl by a thiophosphoryl group in the acetylenic compounds the toxicity was changed very little for arthropods but was strongly reduced for mammals. Some acetylenic derivatives of dithiophosphoric acid proved to be 100 times less toxic for mice than the corresponding derivatives of monothiophosphoric acid. The size of the thione effect for compounds (XVII), (XXVII) and (XLVI) was more than 400. This sharp fall in toxicity for mammals on replacing the phosphoryl oxygen by sulphur in OPIs is observed for the first time. In contrast to the acetylenic compounds the saturated analogues are characterised by significantly smaller values of the thione effect. Values of T were from 2 to 12 which is natural for the usual organophosphorus insecto-acaricides.

The size of the thione effect for arthropods was in the range 0.5 to 1 in all cases. A thione effect equal to 1 might be regarded as the result of complete conversion of the dithio into the monothio compound by oxidative desulphurisation by certain isoenzymes of cytochrome P-450. Obviously such a assumption is naive as values of the thione effect of less than 1 contradict this conclusion. Such low values for the thione effect may be explained by the presence in addition of further oxidative desulphurisation and deocmposition processes which are catalysed both by oxidases and by other enzymes.[41] Low values for the thione effect were detected for compounds (LVI) and (LVI,S) in experiments with other representatives of the arthropods, viz. Colorado beetle and Podisus bug. It follows from Table 19 that in all cases the size of the thione effect was less than 1, particularly in experiments with Podisus (T = 0.09).

In order to clarify the mechanism of the thione effect for the acetylenic organophosphorus compounds the interaction has been studied[38] of compounds of general formula $(C_2H_5O)_2P(Y)R$, where R = $C{\equiv}CC_4H_9$, $SC{\equiv}CC_4H_9$, and Y = O or S, and also of their saturated analogues with cytochrome P-450 from Wistar rat liver microsomes and from the abdomen of houseflies. The effect of the OPIs on cytochrome P-450 was studied by a known procedure.[17]

Table 19. Toxicity (LC_{50}, mg litre^{-1}) of compounds $(CH_3O)_2P(Y).SC{\equiv}CC_4H_9$ for Colorado beetles and for Podisus bugs.[41]

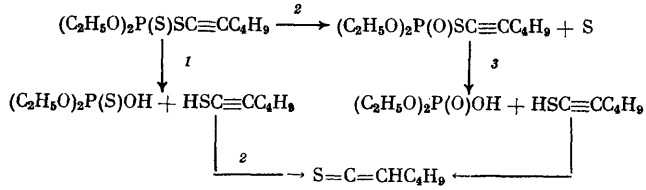
Y	LC_{50}, mg litre^{-1}		
	Colorado beetle		Podisus bug
	imago	larvae IV instar	larvae II instar
O	1900	1500	75
S	790 (0.42)	1050 (0.7)	6.5 (0.09)

Table 20. Reaction of compounds $(C_2H_5O)_2P(Y)R$ with cytochrome P-450.[17]

OPI			Decrease in cytochrome P-450 content, %	
compound No.	R	Y	rat liver microsomes	fly abdomen microsomes
(XXXVII, S)	$SC{\equiv}CC_4H_9$	S	22.2±2.5	60±6.5
(XXXVII)	$SC{\equiv}C_4H_9$	O	22.2±1.7	27.5±1.5
(XXXVIIa, S)	$SCH_2CH_2C_4H_9$	S	0	10.5±3.7
(XXXVIIa)	$SCH_2CH_2C_4H_9$	O	0	0
(LVII)	$C{\equiv}CC_4H_9$	O	0	0
(LVIIa)	$CH_2CH_2C_4H_9$	O	0	0

It is seen from Table 20 that after incubation of cytochrome P-450 from rat liver microsomal fractions in the presence of NADPH, destruction of enzyme occurs only by the action of the acetylenic thioesters of monothio- and dithiophosphoric acids (XXXVII) and (XXXVII,S) but in the case of cytochrome P-450 from fly abdomen destruction also occurs in the presence of the saturated compound, the *S*-hexyl dithiophosphate (XXXVIIa,S). The analogous *S*-hexyl monothiophosphate (XXXVIIa), having no thiol group like the esters of the phosphonic acids (LVII) and (LVIIa), does not react with the microsomal cytochrome P-450. It is obvious that the destructive action is not possessed by the compounds (XXXVII), (XXXVII,S) and (XXXVIIa,S) themselves but by products of their conversion. One of the most probable conversion routes for esters of phosphorus thioacids may be cleavage of the thioester bond by certain cytochrome P-450 isoenzymes. A normal mercaptan is formed from thioesters with a saturated R group. The mercaptans formed in the case of the acetylenic

thioesters are unstable and are readily converted into thioketenes as shown in the scheme.



The ability of thioketenes to react with any compound containing a labile hydrogen atom is well known.[17] It may therefore be taken that butylthioketene, formed by the metabolism of the acetylenic compounds (XXXVII) and (XXXVII,S), reacts with the appropriate groups in the haem region of cytochrome P-450 causing its destruction. It is known from the literature[42] that destruction of cytochrome P-450 is observed when organic compounds with unsaturated bonds add to the protoporphyrin ring of haem. The thioketene formed causes destruction of the monooxygenase and in this way reduces the rate of detoxification of acetylenic OPIs thereby aiding the retention of their toxicity. The saturated mercaptans formed on deactivation of the saturated analogues (XXXVIIa) and (XXXVIIa,S) are incapable of destroying the enzyme.

Another metabolic route for the thione esters of phosphorus acids is their activation by the process of oxidative desulphurisation. It is also effected in a microsomal monooxygenase system but seemingly by other cytochrome P-450 isoenzymes. A sulphur atom is formed which it is claimed is able to bind covalently with the cytochrome active centre causing its degradation.[43] In actual fact diethyl *S*-hexyldithiophosphate (XXXVIIa,S) displays a destructive action on fly abdomen cytochrome P-450. Only the sulphur atom formed in the oxidative desulphurisation of this compound may possess this action in this case since the other metabolite, hexylmercaptan, cannot exert a degradative action on a cytochrome as follows from the experiments with compound (XXXVIa). In addition it is evident from Table 20 that compound (XXXVIIa,S) does not possess a destructive action on the family of isoenzymes of rat liver cytochrome P-450, which evidently indicates the insignificant content of the isoenzyme effecting the oxidative desulphurisation in rat liver microsomes compared with fly abdomen microsomes.

Insect cytochrome P-450 is therefore able to activate the dithio derivatives used but rat cytochrome P-450 is less well suited for this.

The reasons stated explain logically why compound (XXXVII) reduces the content of cytochrome P-450 to the same extent in rat liver microsomes and in fly abdomen microsomes but compound (XXXVII,S) acts more strongly on the cytochrome P-450 from fly abdomen microsomes.

The introduction of a thioethynyl group into an OPI molecule, which contains a phosphoryl group, leads to high toxicity not only as a result of an increase in anticholinesterase activity but also by reducing its detoxification rate by destroying specific cytochrome P-450 isoenzymes. These processes proceed at approximately the same rate in mammals and arthropods.

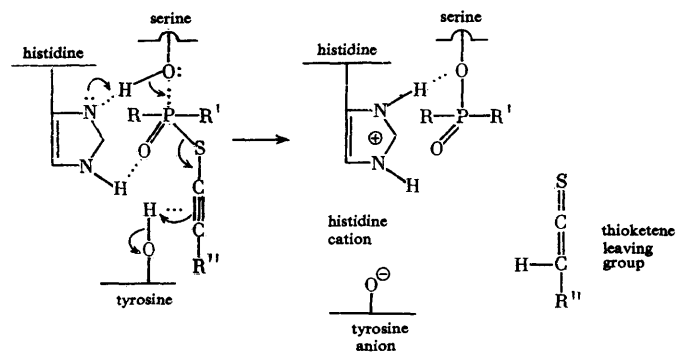
The activation of *S*-ethynyl esters of dialkyl dithiophosphoric acid by specific cytochrome P-450 isoenzymes in mammals and arthropods proceeds at different rates. These processes occur at a higher rate in arthropods than in mammals. Evidently these are the chief factors in the display of the thione effect observed by us.

V. Conclusion

The results of the investigations carried out show that thioesters of monothioacids of phosphorus containing an acetylenic bond in the thioester group are a new class of organophosphorus inhibitors possessing very high anticholinesterase activity.

Analysis of the mechanism of action of these OPIs on CE leads to the conclusion that the role of the acetylenic group consists of the formation of an enzyme–inhibitor complex which is then converted into a phosphorylated enzyme or of the significant acceleration of the phosphorylation step leading to the same phosphorylated enzyme. In connection with the first hypothesis it must be said that to date there are no clear data on the location of the binding portion. It may only be assumed that it is not in the region of the anionic site but is a small distance from the esterase centre. It is probably impossible to consider the binding zone as strictly fixed, its location may vary within known limits when reacting the enzyme with inhibitors of various structure. This follows from Koshland's theory[44] on the induced fit of an enzyme active centre to the structure of susbtrates and inhibitors when reacting with them. The correctness of this conclusion is indicated by the fact that OPIs with an acetylenic bond in the β-position are characterised by high values for the acetylenic effect. For example compound (XV), $CH_3(C_2H_5O)P(O)SCH_2C{\equiv}CCH_2SC_2H_5$, on reaction with arthropod CE gives inhibition rate constants k_{II} of 2.5×10^7 to 1.1×10^9 M^{-1} min^{-1} and values of the acetylene effect q of 590 to 38 000. Consequently the part of the enzyme binding the actylenic group to the active surface is accessible not only to compounds with the acetylenic group in the α-position but also to compounds with a β-acetylenic group. In the latter case it is evident that there are definite restrictions since the disposition of the functional portions of the binding onto the active surface of the enzyme corresponding to a compound with an α-positioned acetylenic bond will not correspond to a compound with a β-acetylenic bond.

It is possible to represent the second hypothesis by an extended scheme of phosphorylation of the esterase site of the enzyme, comprising a serine hydroxyl group, a histidine imidazole ring and a tyrosine phenolic hyroxyl with the inhibitor acetylenic group forming a π-complex bond with the tyrosine proton.



Lengthening of the conjugation chain in which transfer of a proton from tyrosine to the acetylenic bond and from serine to histidine occurs and there is remote transfer of an electron pair from the histidine nitrogen to the tyrosine oxygen must sharply reduce the activation energy of the phosphorylation. It is possible that at such an orientation of the OPI at the moment of attack adsorption onto the anionic point of the CE loses its value. It is understood that the

normally phosphorylated enzyme must be formed as a result of such a process but the rate of its formation must be appreciably higher.

It is not ruled out that these two hypotheses do not contradict one another. All depends on the time (moment) of forming the π-complex of the tyrosine phenolic hydroxyl with the acetylenic π-bond.

The effect of the acetylenic bond depends markedly on the structure of the compound. Compound (XX), $(C_2H_5O)_2$.P(O)SC≡CH, the simplest of the acetylenic OPIs investigated, possesses very high anti-CE activity. Replacement of the acetylenic hydrogen in this compound by various hydrocarbon residues does not increase but reduces anti-CE activity in relation to many CE in the majority of cases. A substituent with a sulphonium grouping proved to be weakly effective. Consequently the acetylenic OPIs with the substituents indicated above are not oriented on the catalytic centre of the enzyme in the best way. As is known[44] the complementarity of the inhibitor molecule with the enzyme active surface, which causes 'productive' adsorption in the final account, depends generally on the compatibility of the various forms of their interaction such as ion–ion, ion–dipole, dipole–dipole, hydrophobic and, in our case, on the orientation of the acetylenic group as well. When these interactions are compatible the adsorbed OPI molecule is oriented on the active centre so that the phosphorus atom and the serine hydroxyl of the enzyme are spatially drawn together and phosphorylation occurs. When the interactions are 'incompatible' the inhibitor molecule is disposed so that the phosphorus atom and the serine hydroxyl are spatially separate, the acetylenic bond is unable to participate and phosphorylation is hindered.

High physiological activity and selectivity of action are characteristics of acetylenic OPIs. Selectivity in relation to arthropods is sharply increased on replacing the phosphoryl group in them by a thiophosphoryl. The toxicity for mice is thereby reduced, occasionally by a hundredfold, while for OPIs without an acetylenic group it is reduced by no more than 20 times.[40] The toxicity of the same acetylenic OPI for arthropods is changed very little by this and in several cases the opposite effect is observed. The toxicity of esters of dithiophosphoric acid exceeds the toxicity of esters of monothiophosphoric acid.

The properties of the acetylenic organophosphorus compounds which are so unusual require further study of the metabolic features of these compounds in mammals and arthropods.

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