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Phosphorylated Derivatives of Alkaloids and Nitrogen-containing Heterocycles—Cholinesterase Inhibitors

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The review deals with the synthesis and anticholinesterase activities of phosphorylated derivatives of certain alkaloids and nitrogen-containing heterocycles. It is shown that the conformational properties of the alkaloid and nitrogen-containing heterocycle residues in the composition of the organophosphorus inhibitor (OPI) molecule play an important role in the inhibition of the catalytic activity of cholinesterases. The type of inhibition of cholinesterases also varies as a function of chemical structure.
The bibliography includes 45 references.

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I. INTRODUCTION

A characteristic feature of many esters of quinquevalent phosphorus acids is the ability to inhibit irreversibly cholinesterases. This process is known to proceed via two main stages: the sorption of the inhibitor on the active surface of the enzyme and phosphorylation of this centre. The capacity of an organophosphorus inhibitor (OPI) for sorption on the active surfaces of cholinesterases depends on the presence in their molecules of onium, dipolar, and hydrophobic groups. The second stage of the inhibition process is determined by the electrophilic phosphorylating capacity of the OPI, which depends on the character of the substituents linked to the phosphorus atom[1-14].

The majority of investigators of the relation between the structure of OPI and their anticholinesterase activity have concentrated their attention on the electrophilic phosphorylating capacity of these substances. Many attempts have been made to find a relation between the electrophilic phosphorylating capacity of OPI and their anticholinesterase properties. For example, relations have been sought between the hydrolysability of OPI, the inhibitor concentrations which reduce the activity of cholinesterases by 50% (I_{50}), and the inhibition rate constants k_2, on the one hand, and the Hammett (σ), Taft (σ^*), and Kabachnik (σ^{ph}) constants, on the other. However, only in a few instances has a direct relation been observed between these parameters[1-13].

It has been shown in studies by Kabachnik, Brestkin, Michelson, Rozengart, Godovikov, and co-workers[15-17] that the hydrophobic interactions of the OPI with the active surface of the enzyme play a decisive role in the inhibition of cholinesterases by OPI. However, hitherto no account has been taken of the role of the OPI conformation in their interaction with cholinesterases. Sadykov and co-workers have therefore undertaken studies on the synthesis and anticholinesterase activities of organophosphorus compounds containing alkaloid residues. Since alkaloids can exist in various stable conformations, this made it possible to estimate the influence of the conformations of OPI on their ability to inhibit cholinesterases.

II. SYNTHESIS

In order to be able to investigate the questions raised above, phosphorylated derivatives of alkaloids and nitrogen-containing heterocycles with a logically varied structure were synthesised and their anticholinesterase activities were investigated. Phosphorylated lupinine, epilupinine, anabasine, cytisine, decahydroquinoline, tetrahydroquinoline, 8-hydroxyquinoline, α-, β-, and γ-pipecolines, 2,6-dimethylpiperidine, piperidine, and morpholine were chosen as objects of the investigation. These substances were synthesised by standard methods (see Schemes 1-3).[18-25]

The corresponding methiodides of the majority of these compounds were obtained. The physicochemical constants of the substances synthesised are listed in Tables 1-4.

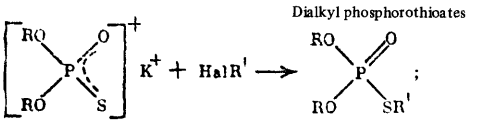
The anticholinesterase activities of the compounds synthesised were determined on enzyme preparations: acetylcholinesterase from human blood erythrocytes with a specific activity 2*E*/mg (EC 3.1.1.7) (ACE) and horse blood serum butyrylcholinesterase with a specific activity of 29*E*/mg (EC 3.1.1.8) (BuCE). The reversible and irreversible inhibiting activities were estimated in terms of the K_i (the reversible inhibition equilibrium constants) and k_2 respectively. These constants were found by standard methods described in the literature[26-28].

III. IRREVERSIBLE INHIBITION OF CHOLINESTERASES

The most characteristic feature of the interaction of OPI with cholinesterases is irreversible inhibition of these enzymes. As a rule phosphorothiolic esters give rise to the irreversible inhibition. A number of dialkyl phosphorothioates containing alkaloid residues in the thioester group were therefore synthesised and their antichlolinesterase activities were investigated.

Thus the study of the anticholinesteras activity of dialkyl phosphorothioates, obtained on the basis of the lupinine and epilupinine alkaloids [(I)-(XIV)] and their methiodides [(I')-(XIV')], showed that all the compounds

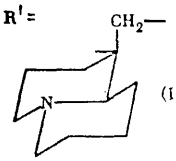
Scheme 1



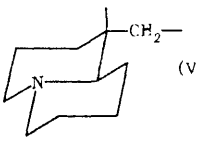
(I)-(XXXIII)

R = C_2H_5; n-C_3H_7; n-C_4H_9; n-C_5H_{11}; iso-C_3H_7; iso-C_4H_9; iso-C_5H_{11};

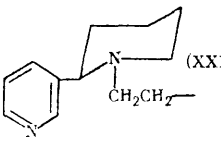
R' =



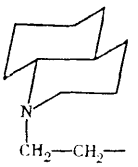
(I)-(VII);



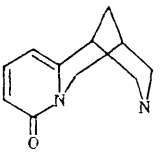
(VIII)-(XIV);



(XXII)-(XXVIII);



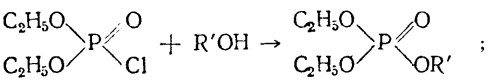
(XV)-(XXI);



(XXIX)-(XXXIII).

Scheme 2

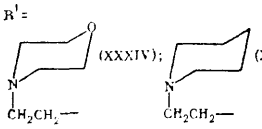
Diethyl phosphates



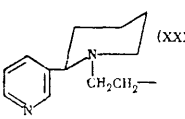
;

(XXXIV) — (XLVI)

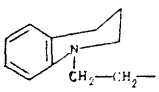
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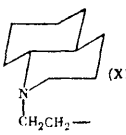
(XXXIV); (XXXV); (XXXVI); (XXXVII);



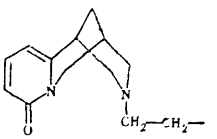
(XXXVIII);



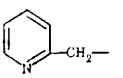
(XXXIX);



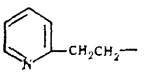
(XL);



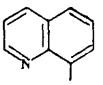
(XLI);



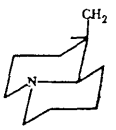
(XLII);



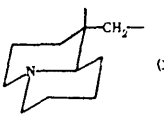
(XLIII);



(XLIV);



(XLV);

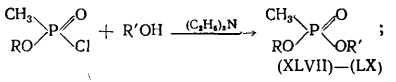


(XLVI).

are powerful irreversible inhibitors of cholinesterases[29,30]. Tables 5 and 6 show that an increase in the length of the straight-chain alkyl groups from ethyl to n-pentyl in a series of both types of OPI [(I)-(XIV)] leads to an enhancement of the anticholinesterase activity in relation to acetylcholinesterase (by factor of 27 and 90 for lupinine and epilupinine derivatives respectively). This shows that the dependence of the inhibiting activity on the length of the alkyl groups in O,O-dialky *S*-lupinanyl phosphorothioates is weaker than for the analogous epilupinine derivatives.

Scheme 3

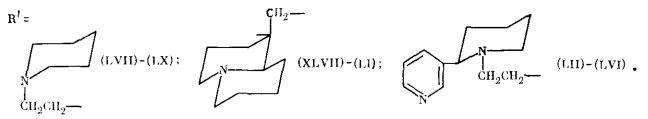
O, Dialkyl methylphosphonates



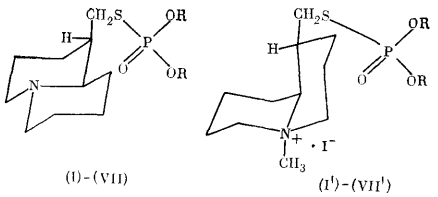
(XLVII)—(LX)

R=C_3H_7—C_6H_{13}; $CH_3O(CH_2)_n$; $C_2H_5O(CH_2)_n$;
$n = 2$—5

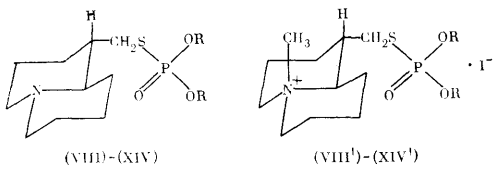
R' =



(LVII)-(LX); (XLVII)-(LI); (LII)-(LVI).



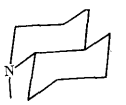
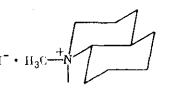
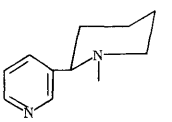
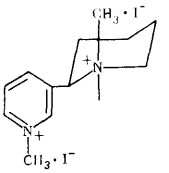
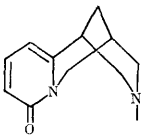
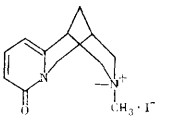
(I)-(VII) (I')-(VII')



(VIII)-(XIV) (VIII')-(XIV')

Similar changes in k_2 as a function of the length of the alkyl moieties of the alkoxy-groups are observed also for the corresponding methiodides (I')-(XIV'). Comparison of the inhibiting activities of the bases (I)-(XIV) with those of their methiodides (I')-(XIV') shows that the values of k_2 for all the methiodides are higher by a factor of 2-8 than for their uncharged analogues. Such a comparatively small increase of the anticholinesterase activity of the methiodides is explained by the fact that at pH 7.5 used in the experiments these compounds are largely protonated[29].

Table 2. *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]bases (XV)-(XXXIII) and their methiodides (XV')-(XXXIII').[21,23]

Compound	R	R'	n_D^{20}	M.p., °C	Yield, %
(XV)	C_2H_5		1.5020		71
(XVI)	n-C_3H_7		1.4931	—	53
(XVII)	n-C_4H_9		1.4950	—	76
(XVIII)	n-C_5H_{11}		1.4919	—	69
(XIX)	iso-C_3H_7		1.4900	—	75
(XX)	iso-C_4H_9		1.4938	—	81
(XXI)	iso-C_5H_{11}		1.4887	—	67
(XV')	C_2H_5		—	101	—
(XVI')	n-C_3H_7		—	94	75
(XVII')	n-C_4H_9		—	117	81
(XVIII')	n-C_5H_{11}		—	110	53
(XIX')	iso-C_3H_7		—	113	77
(XX')	iso-C_4H_9		—	87	83
(XXI')	iso-C_5H_{11}		—	127	61
(XXII)	C_2H_5		1.5201	—	59
(XXIII)	n-C_3H_7		1.5156	—	65
(XXIV)	n-C_4H_9		1.5001	—	68
(XXV)	n-C_5H_{11}		1.4982	—	65
(XXVI)	iso-C_3H_7		1.5112	—	74
(XXVII)	iso-C_4H_9		1.5061	—	66
(XXVIII)	iso-C_5H_{11}		1.5027	—	58
(XXII')	C_2H_5		—	104	54
(XXIII')	n-C_3H_7		—	117	65
(XXIV')	n-C_4H_9		—	121	77
(XXV')	n-C_5H_{11}		—	*	69
(XXVI')	iso-C_3H_7		—	*	49
(XXVII')	iso-C_4H_9		—	*	61
(XXVIII')	iso-C_5H_{11}		—	*	55
(XXIX)	C_2H_5		1.5245	—	51
(XXX)	n-C_3H_7		1.5203	—	67
(XXXI)	n-C_4H_9		1.5186	—	62
(XXXII)	n-C_5H_{11}		1.5162	—	76
(XXXIII)	iso-C_3H_7		1.5234	—	71
(XXIX')	C_2H_5		—	96	70
(XXX')	n-C_3H_7		—	109	62
(XXXI')	n-C_4H_9		—	115	75
(XXXII')	n-C_5H_{11}		—	141	59
(XXXIII')	iso-C_3H_7		—	123	51

* Oily product.

Table 1. *O,O*-Dialkyl *S*-lupinanyl phosphorothioates (I)-(VII), *O,O*-dialkyl *S*-epilupinanyl phosphorothioates (VIII)-(XIV), and their methiodides (I')-(VII') and (VIII')-(XIV').[20,22,24]

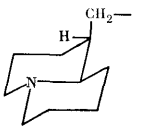
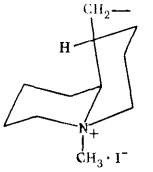
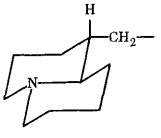
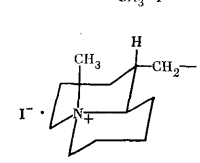
Compound	R	R'	n_D^{20}	$[\alpha]_D^{20}$	Compound	Methiodides		
						R'	m.p., °C	yield, %
(I)	C_2H_5		1.4911	—19.15	(I')		127	90
(II)	n-C_3H_7		1.4961	—24.10	(II')		107	87
(III)	n-C_4H_9		1.4921	—16.42	(III')		102	20
(IV)	n-C_5H_{11}		1.4902	—18.44	(IV')		114	69
(V)	iso-C_3H_7		1.4944	—9.25	(V')		120	84
(VI)	iso-C_4H_9		1.4911	—26.03	(VI')		111	75
(VII)	iso-C_5H_{11}		1.4890	—14.49	(VII')		107	63
(VIII)	C_2H_5		1.4950	+94.53	(VIII')		131	83
(IX)	n-C_3H_7		1.4981	+66.08	(IX')		103	67
(X)	n-C_4H_9		1.5005	+29.64	(X')		133	78
(XI)	n-C_5H_{11}		1.4853	+54.65	(XI')		96	59
(XII)	iso-C_3H_7		1.4943	+75.34	(XII')		137	64
(XIII)	iso-C_4H_9		1.4907	+78.06	(XIII')		119	58
(XIV)	iso-C_5H_{11}		1.4920	+70.40	(XIV')		105	70

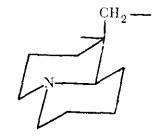
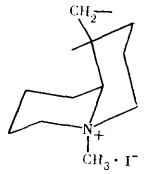
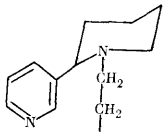
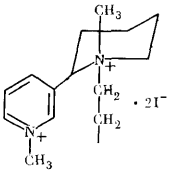
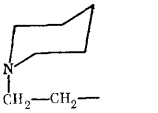
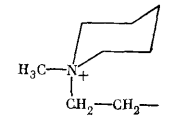
Table 3. Alkyl diethyl phosphates (XXIV)-(XLVI).[25]

Compound	n_D^{20}	d_4^{20}	MR_D found	MR_D calc.	Yield, %	M.p., °C
(XXXIV)	1.4500	1.1354	63.22	63.76	74	155—156
(XXXV)	1.4512	1.0565	67.03	66.73	71	151—153
(XXXVI)	1.4507	1.0519	71.34	71.35	64	*
(XXXVII)	1.4765	1.0856	76.16	76.48	58	*
(XXXVIII)	1.4868	1.0935	89.92	90.67	62	103—105
(XXXIX)	1.5210	1.1680	81.59	82.12	87	95—96
(XL)	1.4557	1.0510	82.46	83.01	63	*
(XLI)	1.5547	—	—	—	72	53—55
(XLII)	1.4640	1.0970	61.62	61.26	69	*
(XLIII)	1.4895	1.1273	66.38	65.85	58	*
(XLIV)	1.4075	1.0826	63.95	63.28	83	*
(XLV)	1.4674	1.0848	78.04	78.39	76	*
(XLVI)	1.4560	1.0661	77.80	77.39	78	82—84

Note. MR_D is the molecular refraction, m.p. are the melting points of the methiodides; the yields of the methiodides are nearly quantitative.

* Oily product.

Table 4. Dialkyl methylphosphonates (XLVII)-(LX) and their methiodides (XLVII')-(IX').[17-19]

Compound	R	R'	n_D^{20}	M.p., °C	$[\alpha]_D^{20}$	Yield, %
(XLVII)	$CH_3O(CH_2)_2$		1.5193		13.2	72
(XLVIII)	$C_2H_5O(CH_2)_2$		1.5103	—	12.4	69
(XLIX)	$CH_3O(CH_2)_3$		1.4920	—	20.5	55
(L)	$CH_3O(CH_2)_4$		1.4860	—	20.0	54
(LI)	$CH_3O(CH_2)_5$		1.4842	—	16.0	61
(XLVII')	$CH_3O(CH_2)_2$		—	45—46	—	*
(XLVIII')	$C_2H_5O(CH_2)_2$		—	48—48.5	—	*
(XLIX')	$CH_3O(CH_2)_3$		—	128—129	—	*
(L')	$CH_3O(CH_2)_4$		—	137—138	—	*
(LI')	$CH_3O(CH_2)_5$		—	142—143	—	*
(LII)	$CH_3O(CH_2)_2$		1.5113	—	47.1	68
(LIII)	$C_2H_5O(CH_2)_2$		1.5041	—	44.0	66
(LIV)	$CH_3O(CH_2)_2$		1.4985	—	45.4	56
(LV)	$CH_3O(CH_2)_4$		1.4905	—	40.0	58
(LVI)	$CH_3O(CH_2)_5$		1.4870	—	43.3	54
(LII')	$CH_3O(CH_2)_2$		—	94—95	—	*
(LIII')	$C_2H_5O(CH_2)_2$		—	113—114	—	*
(LIV')	$CH_3O(CH_2)_3$		—	98	—	*
(LV')	$CH_3O(CH_2)_4$		—	104—105	—	*
(LVI')	$CH_3O(CH_2)_5$		—	69—70	—	*
(LVII)	C_3H_7		1.4598	—	—	82
(LVIII)	C_4H_9		1.4632	—	—	76
(LIX)	C_5H_{11}		1.4697	—	—	79
(LX)	C_6H_{13}		1.4711	—	—	68
(LVII')	C_3H_7		—	81—81.5	—	92
(LVIII')	C_4H_9		—	70—71	—	86
(LIX')	C_5H_{11}		—	68—68.5	—	88
(LX')	C_6H_{13}		—	74.5—75.5	—	94

* Nearly quantitative yield.

Tables 5 and 6 and Figs.1 and 2 show that there is a continuous increase of the anticholinesterase activity in relation to ACE of both series of OPI [(I)-(XIV)] with branched hydrocarbon moieties of the alkoxy-groups. A similar behaviour has been noted for the methiodides (I')-(XIV') but with higher constants k_2.

Table 5. The rate constants for the inhibition of ACE and BuCE (k_2, litre mol^{-1} min^{-1}) by *O,O*-dialkyl *S*-lupinanyl phosphorothioates (I)-(VII) and their methiodides (I') to (VII').[29,30]

Compound	R	$10^5 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(BuCE)}{k_2(ACE)}$	Compound	$10^5 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(BuCE)}{k_2(ACE)}$
(I)	C_2H_5	0.22	2.40	1000	(I')	0.63	4.20	660
(II)	n-C_3H_7	1.50	3.00	200	(II')	5.20	8.40	161
(III)	n-C_4H_9	3.50	59.0	1500	(III')	82.00	90.0	109
(IV)	n-C_5H_{11}	5.80	1.30	224	(IV')	140.0	49.0	35
(V)	iso-C_3H_7	0.10	0.45	450	(V')	0.40	0.92	230
(VI)	iso-C_4H_9	1.60	1.20	75	(VI')	4.90	2.80	56
(VII)	iso-C_5H_{11}	46.0	33	70	(VII')	150	60	40

Table 6. The rate constants for the inhibition of ACE and BuCE (k_2, litre mol^{-1} min^{-1}) by *O,O*-dialkyl *S*-epilupinanyl phosphorothioates (VIII)-(XIV) and their methiodides (VIII')-(XIV').[29,30]

Compound	R	$10^6 k_2$ (ACE)	$10^6 k_2$ (BuCE)	$\frac{k_2(BuCE)}{k_2(ACE)}$	Compound	$10^6 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(BuCE)}{k_2(ACE)}$
(VIII)	C_2H_5	0.02	4.80	240	(VIII')	0.16	1.30	81
(IX)	n-C_3H_7	0.38	6.00	16	(IX')	2.80	1.80	6
(X)	n-C_4H_9	1.90	60.0	31	(X')	10.0	7.10	7
(XI)	n-C_5H_{11}	1.80	100.0	55	(XI')	23.0	34.0	15
(XII)	iso-C_3H_7	0.014	3.20	235	(XII')	0.04	0.46	109
(XIII)	iso-C_4H_9	5.40	7.10	1.3	(XIII')	13.0	1.1	9
(XIV)	iso-C_5H_{11}	7.10	47.0	6	(XIV')	16.0	10.0	6

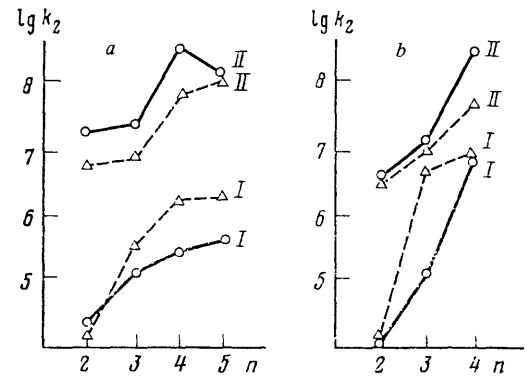


Figure 1. Dependence of $\lg k_2$ on the number n and degree of branching of carbon atoms in the alkyl moiety of the alkoxy-groups in the inhibition of ACE (continuous line I) and BuCE (continuous line II) by *O,O*-dialkyl *S*-lupinanyl phosphorothioates and in the inhibition of ACE (dashed line I) and BuCE (dashed line II) by *O,O*-dialkyl *S*-epilupinanyl phosphorothioates[29]; *a*) OPI with straight-chain alkyl groups; *b*) OPI with isoalkoxy-groups.

The greater inhibiting activity of these compounds in relation to BuCE than ACE is probably a consequence of the improved conditions for the hydrophobic sorption of the alkoxy-groups on the corresponding hydrophobic sections of BuCE. This effect is not associated with the inductive influence of the alkoxy-groups, since the pK_a of the corresponding tautomeric acids are very similar. The constants k_2 for the methiodides (I')-(XIV') in relation to BuCE are only slightly higher (by a factor of 1.5–3) compared with the k_2 for the bases (I)-(XIV).[29,30]

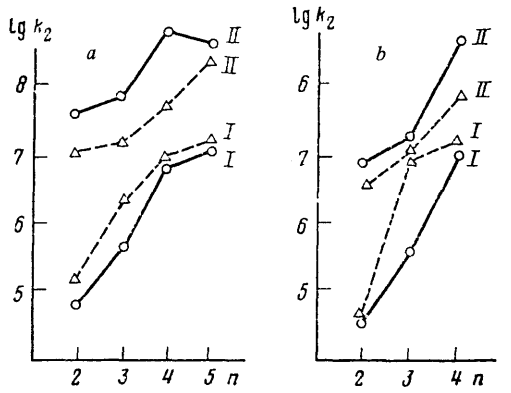
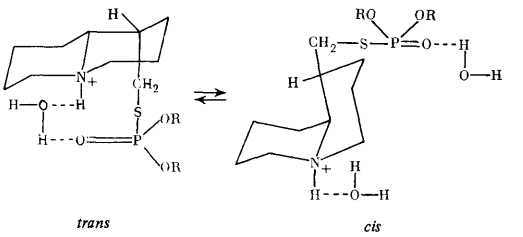


Figure 2. Dependence of lg k_2 on the number n and degree of branching of the carbon atoms in the alkyl moiety of the alkoxy-groups in the inhibition of ACE (continuous line I) and BuCE (continuous line II) by the methiodides of *O,O*-dialkyl *S*-lupinanyl phosphorothioates and in the inhibition of ACE (dashed line I) and BuCE (dashed line II) by the methiodides of *O,O*-dialkyl *S*-epilupinanyl phosphorothioates[29]: *a*) OPI with normal alkoxy-groups; *b*) OPI with isoalkoxy-groups.

Comparison of the anticholinesterase activities of the OPI investigated in relation to ACE and BuCE is of definite interest. In comparing the data obtained for ACE and BuCE, one may note that the ability of both series of OPI [(I)-(XIV)] and their methiodides [(I')-(XIV')] to inhibit BuCE depends more on the length of the alkyl moieties of the alkoxy-groups than in the case of ACE. On the other hand, the appearance of an onium group in the OPI molecule results in a sharp increase of the inhibiting activity in relation to ACE. These data emphasise yet again the tendencies of BuCE and ACE to undergo hydrophobic and ion-ion interactions respectively[15]. All the compounds investigated exhibited selectivity in their activity in relation to BuCE. For the lupinine derivatives (I)-(VII), this effect is very marked and the greatest selectivity is shown by compounds with ethyl (by a factor of 1000), n-butyl (by a factor of 1500), and isopropyl (by a factor of 450) moieties in the alkoxy-groups. On the other hand, in the analogous epilupinine compounds (VIII)-(XIV) this effect is pronounced only for the ethyl (by a factor of 240) and isopropyl (by a factor of 235) derivatives.

Comparison of the inhibiting activities in relation to ACE of the epilupinine derivaties (VIII)-(XIV) with those of the lupinine derivates (I)-(VII) showed that the OPI of the (VIII)-(XIV) series exhibit a more powerful anticholinesterase activity than the analogous compounds of type (I)-(VII). The opposite dependence on the structure of the part of the OPI molecule which is split off is observed on comparing the anticholinesterase activities of the same series of compounds with one another in the inhibition of BuCE. Consequently *O,O*-dialkyl *S*-lupinanyl phosphorothioates and their methiodides exhibit a greater selectivity in their activity in relation to BuCE than their epilupinine analogues. Such specificity in the activity of lupinine derivatives is apparently associated with the fact that in solution the protonated form of the luipinine residue exists mainly in two conformational states: one having a *trans*-junction with the quinolizidine ring and an axial hydroxymethyl group and the other having a *cis*-junction with the quinolizidine ring and an equatorial hydroxymethyl group[31,32]. It may be that the *cis*-conformer is better adapted to sorption on the anionic centre of BuCE; as a result of such sorption, the equilibrium between the *trans*- and *cis*-forms shifts towards the formation of the latter, as a result of which one observes the specificity of the activity of the lupinine derivatives in relation to BuCE.



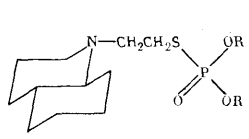
The fact that the methiodides of *O,O*-dialkyl *S*-lupinanyl phosphorothioates, which are known to have a *cis*-junction with the quinolizidine ring of the lupinine fragment and an equatorial thiophosphoryl group, exhibit an analogous specificity in their activity in relation to BuCE may serve as confirmation of the foregoing observations.

The *trans*-quinolizidine ring of the epilupinine derivatives remains unchanged on protonation or conversion into the methiodide and apparently approaches more closely the anionic centre of ACE. This results in a higher activity in relation to ACE.[33]

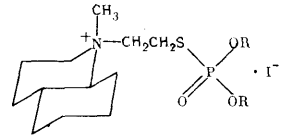
Thus one may note that the very high specificity of the activity of *O,O*-dialkyl *S*-lupinanyl phosphorothioates, and their methiodides is determined by the overall effect of the correspondence to the active centre of BuCE of the alkoxy-groups of the OPI molecule, on the one hand, and of the lupinine fragment on the other. The higher specificity of the activity of the epilupinine derivatives in relation to ACE compared with the lupinine derivatives is in all probability associated with the more effective sorption of the *trans*-quinolizidine ring of the epilupinine residue by the anionic centre of ACE rather than the *cis*-form of the quinolizidine ring in the lupinine derivatives.

As shown above, the inhibiting activities of compounds containing the lupinine and epilupinine derivatives depend significantly on the conformations of these fragments.

The anticholinesterase activitives of *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinolines (XV)-(XXI) and their methiodides (XV')-(XXI') have been investigated; here the decahydroquinoline residue has a structure similar to those of the lupinine and epilupinine residues, but the nitrogen atom is located in one of the rings and not between two rings[33,34].



(XV)-(XXI)



(XV')-(XXI')

All the compounds investigated proved to be inhibitors of cholinesterases with an irreversible type of activity (Table 7). Table 7 shows that decahydroquinoline derivatives exhibit a powerful inhibiting activity in relation to ACE and that the length and degree of branching of the alkoxy-group have little influence on the inhibiting activity. Only the di-isopropoxy-derivative, which is less active by approximately two orders of magnitude than the remaining compounds, is an exception. An analogous behaviour has been observed also for the corresponding methiodides (XV')-(XXI').

Table 7. The rate constants for the inhibition of ACE and BuCE (k_2, litre mol^{-1} min^{-1}) by *N*-[β-(dialkoxyphosphinyl)-mercaptoethyl]decahydroquinolines (XV)-(XXI) and their methiodides (XV')-(XXI').[33,34]

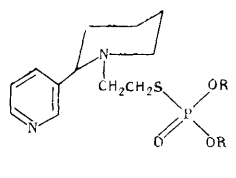
Compound	R	$10^7 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(\text{BuCE})}{k_2(\text{ACE})}$	Compound	$10^7 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(\text{BuCE})}{k_2(\text{ACE})}$
(XV)	C_2H_5	1.3	0.98	7	(XV')	0.55	2.1	4.1
(XVI)	n-C_3H_7	2.3	1.0	4	(XVI')	1.0	3.4	3.0
(XVII)	n-C_4H_9	4.5	3.6	8	(XVII')	3.3	9.0	3.0
(XVIII)	n-C_5H_{11}	2.7	3.5	13	(XVIII')	2.2	11.0	5.0
(XIX)	iso-C_3H_7	0.021	1.2	582	(XIX')	0.015	3.9	260
(XX)	iso-C_4H_9	2.4	26	100	(XX')	2.7	81	30
(XXI)	iso-C_5H_{11}	6.5	42	64	(XXI')	5.0	93	18.6

N-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinolines are more active in relation to BuCE than ACE. The dependence of the inhibiting activity on the length of straight-chain alkyl groups leads to a sharp increase of the anticholinesterase activity. An analogous behaviour has been observed also for the methiodides. It is noteworthy that the greatest selectivity in their activity is shown by the di-isoalkoxy-derivatives (by a factor of 582 for the di-isopropoxy-derivative, by a factor of 100 for the di-isobutoxy-derivative, and by a factor of 64 for the di-isopentoxy-derivative)[33]. Comparison of the anticholinesterase activities of the decahydroquinoline derivatives with those of the corresponding dialkyl phosphorothioates derived from lupinine and epilupinine shows that *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinolines are stronger inhibitors in relation to ACE than *O,O*-dialkyl *S*-lupinanyl phosphorothioates. This difference between the inhibiting activities can apparently be accounted for by the difference between the structures and conformational states of the decahydroquinoline (*trans*) and lupinine (*cis*) residues. In relation to BuCE, the inhibiting activity of the decahydroquinoline derivatives with straight-chain alkoxy-groups is inferior to that of the analogous lupinine compounds. The opposite is observed in the series of OPI with isoalkoxy-groups[34].

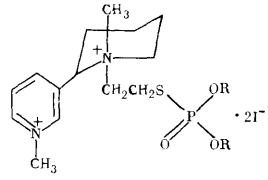
Comparison of the constants k_2 for *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinolines with those of the corresponding *O,O*-dialkyl *S*-epilupinanyl phosphorothioates shows that the anticholinesterase activities of both types of compounds (except the diethoxy- and di-isopropoxy-derivatives) in relation to ACE differ little. However, the methiodides of the decahydroquinoline derivatives are stronger ACE inhibitors than the OPI with the epilupinine fragment. This is apparently associated with the different basicities of the decahydroquinoline and epilupinine residues. *N*-[β-di-isoalkoxyphosphinyl)mercaptoethyl]decahydroquinolines are more active in relation to BuCE than the corresponding *O,O*-di-isoalkyl *S*-epilupinanyl phosphorothioates[33,34].

Thus the introduction of the bulky decahydroquinoline group in the β-position of the thioester group of the OPI instead of the lupinine and epilupinine groups led to a significant alteration of the inhibiting activity in relation to both types of cholinesterases. Comparison of the anticholinesterase properties of the decahydroquinoline derivatives with those of the dialkyl phosphorothioates derived from lupinine and epilupinine demonstrated yet again the sensitivity of the anionic centre of ACE to transoid saturated bicyclic systems. The change in the position of the nitrogen atom in the bicyclic system enhances the inhibiting activity in relation to ACE.

Whereas OPI containing residues of mononitrogen bases in the component which is split off were discussed above, we shall now deal with the anticholinesterase properties of the OPI containing the residue of the alkaloid anabasine [α-(β-piperidinyl)pyridine] in the β-position of the thioesters group and shall compare the data obtained with the inhibiting activities of the analogous decahydroquinoline derivatives[35].



(XXII)-(XXVIII)



(XXII')-(XXVIII')

All the compounds investigated proved to be irreversible cholinesterase inhibitors (Table 8).

Table 8. The rate constants for the inhibition of ACE and BuCE (k_2, litre mol^{-1} min^{-1}) by *N*-[β-dialkoxyphosphinyl)-mercaptoethyl]anabasines (XXII)-(XXVIII) and their methiodides (XXII')-(XXVIII').[35]

Compound	R	$10^5 k_2$ (ACE)	$10^6 k_2$ (BuCE)	$\frac{k_2(\text{BuCE})}{k_2(\text{ACE})}$	Compound	$10^6 k_2$ (ACE)	$10^7 k_2$ (BuCE)	$\frac{k_2(\text{BuCE})}{k_2(\text{ACE})}$
(XXII)	C_2H_5	1.3	0.8	6	(XXII')	0.39	0.15	4
(XXIII)	n-C_3H_7	7.4	2.4	3.5	(XXIII')	3.6	0.53	1.5
(XXIV)	n-C_4H_9	35	7.1	2	(XXIV')	14	2.5	2
(XXV)	n-C_5H_{11}	10	7.7	7	(XXV')	15	4.9	3
(XXVI)	iso-C_3H_7	0.53	4.9	93.7	(XXVI')	0.5	1.3	24
(XXVII)	iso-C_4H_9	18	10	5.5	(XXVII')	33	6.2	2
(XXVIII)	iso-C_5H_{11}	47	90	2	(XXVIII')	50	1.1	0.22

The irreversible inhibiting activity of *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]anabasines in relation to ACE increases with increase of the length of the alkyl groups from ethyl to n-butyl. On further increase in their length, the activity falls.

The irreversible inhibiting activity of *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]anabasines in relation to BuCE is higher than in relation to ACE. These differences depend to a

large extent on the length and degree of branching of the alkoxy-groups. The smallest difference is observed for the dibutyl derivatives (by a factor of two) and the highest for the di-isopropyl derivative (by a factor of 93).[17,35]

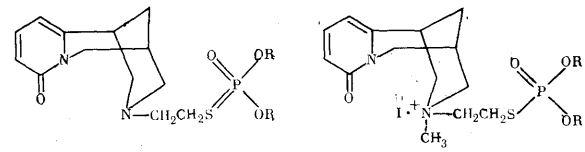
Thus the appearance of a second nitrogen-containing group in the thioester group of the OPI which is to be eliminated has a major influence on the anticholinesterase activity in the case of BuCE. This influence is determined to a large extent by the character of the hydrophobic alkyl moieties of the alkoxy-groups sorbed on the hydrophobic sections of the active surface of the enzyme in the region of the esterase centre.

One may conclude that the presence of two nitrogen-groups in the leaving part of the OPI molecule, which exists largely in the protonated form under the experimental conditions (pH 7.5), impairs the conditions for the hydrophobic sorption of the inhibitor; the alkoxy- and onium groups have a non-concerted influence, which decreases the inhibiting activity [35,36].

The compounds of the onium type which have been investigated show very slight differences in the inhibition of ACE and BuCE, the activities of the majority of them being similar in relation to both enzymes with the exception of the diazopropyl derivative, whose activity is selective in relation to BuCE, and the di-isopentyl derivative, which tends to be more active in relation to ACE (Table 8).[35]

Comparison of the anticholinesterase activities of the *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]anabasines with those of the *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]-decahydroquinolines investigated previously showed that the dialkyl phosphonothioates containing the anabasine group have an only slightly weaker inhibiting activity in relation to ACE than the analogous decahydroquinoline derivatives. *N*-[β-(Diethoxyphosphinyl)mercaptoethyl anabasine, whose anticholinesterase activity is inferior to that of *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinoline by a factor of 10 and the activity of the corresponding methiodide is lower by a factor of 14, is an exception. The different sorbabilities on the anionic centre of ACE of the leaving components of the OPI compared probably have an effect in this instance. The fact that a further increase of the length of the alkoxy-groups smoothes out the differences between the inhibiting activities of decahydroquinoline and anabasine derivatives is apparently associated with the possibility that in the case of the decahydroquinoline analogues there may be an effect of the "non-concerted" sorption on the active surface of ACE,[36] as a result of which small differences between the constants k_2 are observed for these compounds as a function of the length and degree of branching of the alkyl moieties of the alkoxy-groups (by a factor of 2–4). However, since the anabasine fragment of the OPI molecule is less effectively sorbed on the anionic centre of ACE than the decahydroquinoline fragment, they may exhibit a tendency towards an increase of the anticholinesterase activity as a function of the length and degree of branching of the alkyl moieties of the alkoxy-groups (the activity increases by a factor of 30 on passing from C_2H_5 to C_4H_9). The slight differences between the inhibiting activities of the OPI of the two types (with the exception of the diethoxy-analogues) are associated with the increase in the length and the degree of branching of the alkoxy-groups, on the one hand, and with the difference between the structures of the alkanethiol components, on the other. Changes in the phosphoryl component of the molecules of *N*-[β-(diethoxyphosphinyl)mercaptoethyl]anabasines enhance the possibility of "productive" sorption, while similar changes in *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]decahydroquinolines lead to an enhancement of the "unproductive" sorption on the active centre of acetylcholinesterase[17,33,35].

Comparison of the anticholinesterase properties of dialkyl phosphorothioates derived from anabasine with the analogous decahydroquinoline compounds in relation to BuCE shows that the activities of *N*-[β-(diethyoxyphosphinyl)mercaptoethyl]-anabasine and its methiodide are inferior (by a factor of 12 to 13) to those of the analogous decahydroquinoline derivatives. In all probability, the active centres of both ACE and BuCE respond to the differences between the structures of the leaving components of the molecules of the two types of OPI. An increase in the length of the alkoxy-groups results to some extent in a greater similarity of the inhibiting activities of the compounds compared. However, as for ACE, the anabasine derivatives are 4–5 times less active than the analogous dialkyl phosphorothioates derived from decahydroquinoline. Thus the appearance of a second nitrogen-containing group in the leading moiety of the OPI molecule influences the activity in relation to BuCE more than in relation to ACE. It was of interest to follow the variation of the anticholinesterase activities of *N*-[β-(dialkoxyphosphinyl)-mercaptoethyl] cytisines (XXIX)–(XXXIII) and their methiodides (XXIX′)–(XXXIII′), in which the conformational state of the cytisine residue differs sharply from those of lupinine, epilupinine, decahydroquinoline, and anabasine[37].



(XXIX)–(XXXIII) (XXIX′)–(XXXIII′)

Table 9 shows that all the compounds investigated proved to be inhibitors with a combined type of activity†. In relation to ACE, the irreversible inhibiting capacity increases with increase in the length of the alkyl groups up to the n-butyl derivative, after which there is a fall. The replacement of straight-chain alkyl groups by the isopropyl group results in a sharp decrease of the true degree of reversible inhibition of the enzyme by the inhibitor (k_a), which is apparently caused by the inadequate complementarity of the di-isopropoxy-derivative and the ACE active centre[33,37].

Table 9. The rate constants of the inhibition of ACE and BuCE by *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]cytisines (XXIX)–(XXXIII) and their methiodides (XXIX′)–(XXXIII′)* [37]

Compound	R	ACE			BuCE			
		$10^5 k_a$, litre mol^{-1}min^{-1}	$10^7 K_{ir}$, mol litre^{-1}	$10^7 K_{is}$, mol litre^{-1}	$10^4 k_a$, litre mol^{-1}min^{-1}	$10^7 K_{ir}$, mol litre^{-1}	$10^7 K_{is}$, mol litre^{-1}	k_a(BuCE)/k_a(ACE)
(XXIX)	C_2H_5	0.63	710	15	0.35	43	5.0	5.5
(XXX)	n-C_3H_7	1.2	9.6	6.5	3.3	3.7	0.18	2.7
(XXXI)	n-C_4H_9	35	1.3	2.3	9.0	4.8	2.1	2.6
(XXXII)	n-C_5H_{11}	74	1.3	4.8	9.6	3.6	5.4	13.2
(XXXIII)	iso-C_3H_7	0.35	12	7.4	5.1	4.6	0.2	145
(XXIX′)	C_2H_5	1.1	66	1.5	0.61	3.1	0.5	5.4
(XXX′)	n-C_3H_7	3.1	6.0	0.21	8.0	0.41	0.62	2.7
(XXXI′)	n-C_4H_9	11.0	2.2	0.21	14	0.15	0.31	1.2
(XXXII′)	n-C_5H_{11}	23	4.5	0.64	17	0.1	0.91	8.2
(XXXIII′)	iso-C_3H_7	0.64	490	0.17	7.0	0.2	0.75	105.9

* For the significance of k_a, K_{ir}, and K_{is}, see Brestkin and Godovikov[36].

† A number of studies, reviewed by Brestkin and Godovikov[36], have been devoted to this type of inhibition.

In relation to BuCE, the test compounds are more active than in relation to ACE. An increase in the length of the alkyl moieties of the alkoxy-groups increases the inhibiting activity. In contrast to ACE, BuCE is relatively insensitive to the replacement of the straight-chain alkyl groups by the isopropyl group. It is noteworthy that *N*-[β-(di-isopropoxyphosphinyl)mercaptoethyl]cytisine shows the greatest selectivity in relation to BuCE (by a factor of 145). This selectivity is apparently associated with the less effective sorption of the di-isopropoxy-derivative on the active surface of ACE.

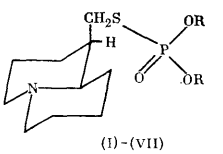
The reversible inhibiting activity in relation to ACE increases on passing from the ethyl to the propyl derivative, but a further increase in the length of the alkyl group has little influence on the reversible inhibiting activity of the organophosphorus compound investigated. It must be emphasised that the reversible inhibitors derived from cytisine exhibit selectivity in their activity with respect to BuCE.

The quaternisation of the nitrogen atom results in a slight increase of the anticholinesterase activity in relation to both enzymes (by a factor of 2–3), but the selectivity in relation to BuCE then falls. It follows from the foregoing that, compared with the previous compounds, *N*-[β-(dialkoxyphosphinyl)mercaptoethyl]cytisines and their methiodides proved to be significatnly weaker inhibitors with irreversible activity. The impairment of the inhibiting activity of cytisine derivatives may be associated with the steric hindrance of the cytisine residue in the sorption of the inhibitor on the active surface of the enzyme[37].

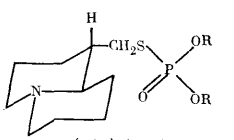
IV. COMPARISON OF THE ACTIVITIES OF THE OPI DERIVED FROM LUPININE AND EPILUPININE

Tables 10 present data for the effect on the cholinesterases of various species of mammals and arthropods by *O,O*-dialkyl *S*-lupinanyl phosphorothioates and *O,O*-dialkyl S-epilupinanyl phosphorothioates[30,38], which make it possible to discover a number of characteristic features. In the vast majority of cases, the OPI investigated proved to be stronger inhibitors of the ACE from the heads of files than of the mammalian ACE. This applies particularly to the first members of the series of compounds containing both straight-chain and branched groups. For example, the ACE from the heads of flies proved to be 15000 more sensitive to *O,O*-diethyl *S*-lupinanyl phosphorothioate than the ACE from human erythrocytes; the sensitivity in relation to the di-isopropyl derivative of lupinine was higher only by a factor of 250. In the case of epilupinine derivatives, the opposite relation between the sensitivities of the ACE from the heads of flies and human erythrocytes was observed, the increase in sensitivity being greater for the di-isopropyl compound (by a factor of 1000) than for the ethyl compound (by a factor of 550). It is noteworthy that some of the compounds investigated have the unique ability to suppress the ACE from the heads of flies: the absolute bimolecular inhibition rate constants reached approximately 10^8 litre mole^{-1} min^{-1}. An increase in the length of the straight-chain alkyl group in lupinine derivatives appreciably enhanced the anticholinesterase activity in relation to the mammalian enzyme. In contrast to this, the sensitivity of the ACE from the heads of flies to the OPI fell sharply with increase in the length of the alkyl group. Thus the compound with the C_5H_{11} group proved to be 120 times less active than the ethyl derivative. An increase in the length of the iso-groups increased the ability to suppress the mammalian ACE but had virtually no influence on the ACE from the heads of flies. In epilupinine derivatives an increase in the length of the straight-chain alkyl group had a very insignificant and indefinite influence on their ability to inhibit the ACE from the heads of flies. Indeed, the effect of epilupinine derivatives on the mammalian ACE did not differ from that of the analogous lupinine compounds. With increase in the length of both straight-chain and branched alkyl groups, the anticholinesterase activity increased significantly. This was manifested most clearly in relation to the ACE from human blood erythrocytes: on passing from C_2H_5 to C_5H_{11}, the activity increased by a factor of 90 and on passing from iso-C_3H_7 to iso-C_5H_{11} the activity rose by a factor greater than 500. The facts enumerated above indicate significant differences between the structures of the active surfaces of the cholinesterases from mammals and flies. It has been noted[39] that in the insect cholinesterase the distance between the anionic and esterase centres is greater than in the mammalian cholinesterase. However, it is difficult to account for the data obtained in terms of these differences alone. The results showing that the hydrophobic region around the anionic centre in the ACE from the heads of flies is more bulky and wider than in the ACE from human erythrocytes are of great interest. One canot rule out the possibility that this can justify the hypothesis of a greater complementarity between the bulky heterocyclic residue in the OPI investigated and the anionic centre of the fly cholinesterases compared with the mammalian enzyme. The fact that very small conformational differences between lupinine and epilupinine are sufficient to have a pronounced influence on the anticholinesterase effect of the OPI is quite probably also associated with precisely this factor. As regards the sharp decrease of the anticholinesterase activity of the OPI in relation to the ACE from the heads of flies with increase in the length of the hydrocarbon group, there is no doubt whatever that the cause lies in the difference between the hydrophobic environments of the esterase centres in the mammalian and insect enzymes. It may be that on this part of the active surface of the insect enzyme, the hydrophobic regions are not very large and that immediately beyond them there are hydrophilic regions which not only do not promote but actually actively hinder the sorption of large hydrocarbon groups, which leads to a pronounced decrease of the anticholinesterase effect.

Table 10. The rate constants for the inhibition of ACE of different origins ($10^5\ k_2$, litre mol^{-1} min^{-1}) by *O,O*-dialkyl *S*-lupinanyl phosphorothioates (I)-(VII) and *O,O*-dialkyl *S*-epilupinanyl phosphorothioates (VIII)-(XIV).[42]



(I)-(VII)

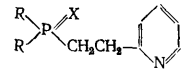


(VIII)-(XIV)

R	(I)–(VII)			(VIII)–(XIV)		
	from mouse brain	from rabbit erythrocytes	from heads of flies	from mouse brain	from rabbit erythrocytes	from heads of flies
C_2H_5	0.21	0.11	3400	0.24	0.77	110
n-C_3H_7	0.36	75	1200	1.4	4.5	190
n-C_4H_9	3.6	25	290	4.1	3.6	17
n-C_5H_{11}	0.12	0.52	28	4.1	3.6	17
iso-C_3H_7	0.002	0.16	25	0.12	0.04	140
iso-C_4H_9	0.28	2.1	120	7.0	1.2	19
iso-C_5H_{11}	3.0	3.8	34	12	6.6	62

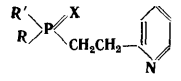
V. REVERSIBLE INHIBITION OF CHOLINESTERASES

The reversible inhibition of the catalytic activity of cholinesterases is less characteristic of organophosphorus inhibitors. The purely reversible inhibition of cholinesterases by organophosphorus compounds was first demonstrated[40] in relation to a series of β-(2-pyridyl)ethylphosphine oxides and sulphides (Table 11).



Subsequently, it was shown that the alkyl esters of quinquevalent phosphorus oxyacids are capable of inhibiting cholinesterases only reversibly[41–44].

Table 11. The rate constants for the reversible inhibition of cholinesterases (K_i, mol litre^{-1}) [40] by the compounds



R	R′	X	K_i, mol litre^{-1}	
			BuCE	ACE
C_2H_5	C_2H_5	O	$2.6\cdot10^{-4}$	$5.0\cdot10^{-4}$
C_2H_5	C_6H_5	O	$2.6\cdot10^{-4}$	$32\cdot10^{-3}$
C_2H_5	C_6H_5	S	$4.2\cdot10^{-5}$	$6.2\cdot10^{-4}$
C_6H_5	C_6H_5	O	$3.7\cdot10^{-5}$	$4.6\cdot10^{-4}$
C_6H_5	C_6H_5	S	$3.6\cdot10^{-6}$	$3.8\cdot10^{-4}$
Pyridine	—	—	$1.2\cdot10^{-2}$	$2.5\cdot10^{-2}$

An analogous effect has been observed for substituted alkyl esters of phosphoric and methylphosphonic acids containing alkaloid and certain heterocyclic residues.

Thus the study of the anticholinesterase activities of diethyl phosphate derivatives of morpholine, piperidine, α-pipecoline, anabasine, tetrahydroquinoline, decahydroquinoline, cytisine, α-hydroxymethylpyridine, α-hydroxyethylpyridine, 8-hydroxyquinoline, lupinine, and epilupinine [compounds (XXIV)–(XLVI) except compound (XXXVII)] showed that all the compounds investigated are competitive reversible inhibitors of ACE (Table 12). The dependence of the inhibiting activity on the structure of the cationic group of the organophosphorus inhibitor appears to be extremely complex. Thus the morpholine derivative does not inhibit the catalytic activity of acetylcholinesterase at all, the inhibiting activity increasing on going over to the piperidine, α-pipecoline, and then anabasine derivatives. However, the condensation of cyclohexane and benzene rings with the piperidine ring (the decahydroquinoline and tetrahydroquinoline derivatives) does not alter significantly the anticholinesterase activity. In all these compounds the nitrogen atom is present in one ring, while its introduction into the position between two rings leads to a decrease of the inhibiting activity of the given substances (the lupinine and epiluinine derivatives). The 8-hydroxyquinoline derivative is the most active inhibitor in this series of substances. Its activity is apparently due to the correspondence of the distances between the phosphorus and nitrogen atoms in the inhibitor and between the carbonyl carbon atom and nitrogen in acetylcholine. Weak inhibiting properties in relation to acetylcholinesterase can apparently be explained by the fact that the phosphate group of the inhibitor is not complementary in relation to the active centre of this enzyme[25].

Table 12. The anticholinesterase properties of the esters of *O,O*-diethylphosphoric acid containing heterocyclic groups[25].

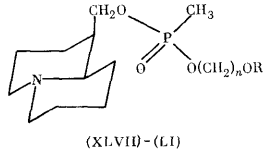
Compound	ACE	BuCE	Compound	ACE	BuCE
	K_i, mol litre^{-1}	K_i, mol litre^{-1}		K_i, mol litre^{-1}	K_i, mol litre^{-1}
(XXXIV)	0	0	(XLI) *	19%	$2.6\cdot10^{-4}$
(XXXV)	$1.15\cdot10^{-3}$	$2.60\cdot10^{-5}$	(XLII) **	$2.50\cdot10^{3}$	$1.4\cdot10^{4}$
(XXXVI) *	27%	$6.70\cdot10^{-5}$	(XLIII) **	$4.90\cdot10^{3}$	$2.9\cdot10^{5}$
(XXXVIII)	$6.60\cdot10^{-4}$	$2.70\cdot10^{-4}$	(XLIV)	$2.60\cdot10^{-4}$	$4.8\cdot10^{-6}$
(XXXIX)	$3.60\cdot10^{-3}$	$6.70\cdot10^{-3}$	(XLV)	$1.15\cdot10^{-3}$	$1.4\cdot10^{-4}$
(XL)	$3.10\cdot10^{-4}$	$7.1\cdot10^{-4}$	(XLVI)	$2.20\cdot10^{-4}$	$1.1\cdot10^{-5}$

*The percentage inhibition at a concentration of 7.2×10^{-3} M is quoted.

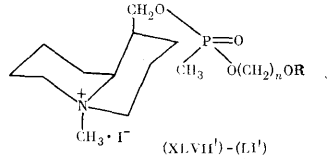
**These substances proved to be irreversible inhibitors and were assessed in terms of the bimolecular interaction constants k_2.

It can be seen from the foregoing that phosphates are very weak reversible inhibitors of both types of enzymes. However, they in fact exhibit a definite selectivity in relation to butyrylcholinesterase. Therefore, bearing in mind that methylphosphoric acid derivatives have a more specific activity in relation to acetylcholinesterase, for which hydrophobic interactions are less typical, the anticholinesterase activities of derivatives of methylphosphonic acids containing a hydrophilic group in the non-leaving part of the molecule and the residues of the alkaloids anabasine, lupinine and piperidine in the leaving group were therefore investigated. It was assumed that the specific structure of the anabasine and lupinine residues, in which there is a considerable hydrophobic environment around the nitrogen atom, promotes the formation of comparatively stable enzyme-inhibitor complexes [17,18].

The study of the inhibiting activities of alkoxyalkyl lupinanyl methylphosphonates (XLVII)–(LI) showed that there is a tendency, albeit small, towards an increase of the inhibiting activity in relation to ACE and BuCE with increase of the length of the alkoxy-group (Table 13, and Fig. 3). [45]



(XLVII)–(LI)



(XLVII′)–(LI′)

There is every reason to suppose that the change in inhibiting activity noted above is associated with an increase of the ability of the OPI to be sorbed on the active surfaces of the enzymes via a hydrophobic mechanism. However, the presence of the hydrophilic oxygen atom in the alkyl moiety in the alkoxy-group hinders this process and thereby reduces the efficiency of the inhibitors.

The methiodides of (*O*-alkoxyalkyl)methylphosphinyllupinines are less active reversible inhibitors of ACE and BuCE than their uncharged analogues. Evidently, the

charge, interacting at the anionic site, does not ensure the most effective hydrophobic sorption (Fig. 4). It is of interest to note that the inhibiting activities of both types of compounds depend not only on the distance between the oxygen and phosphorus atoms but also on the position of the former in relation to the alkyl moiety of the alkoxy-group. The presence of the oxygen atom in the β-position in the alkoxy-group to some extent interferes with hydrophobic sorption.

Table 13. The constants for the reversible inhibition of cholinesterases (K_i, mol litre^{-1}) by compounds (XLVII)–(LI) and (XLVII')-(LI').[45]

Compound	R	n	$10^4 K_i$ (ACE)	$10^4 K_i$ (BuCE)	Compound	$10^3 K_i$ (ACE)	$10^3 K_i$ (BuCE)
(XLVII)	CH_3	2	3.61	2.5	(XLVII')	4.2	1.25
(XLIX)	CH_3	3	2.87	1.2	(XLIX')	1.30	0.72
(L)	CH_3	4	2.02	0.93	(L')	0.74	0.65
(LI)	CH_3	5	1.32	0.53	(LI')	0.63	0.47
(XLVIII)	C_2H_5	2	2.94	1.8	(XLVIII')	2.42	1.04

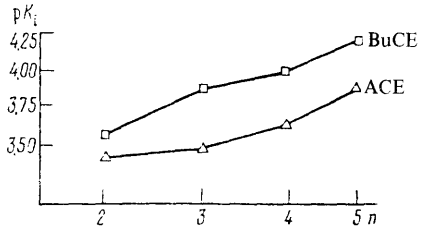


Figure 3. Dependence of the inhibition constant (pK_i) on the length of the alkoxy-group in compounds of type (V).[17,45]

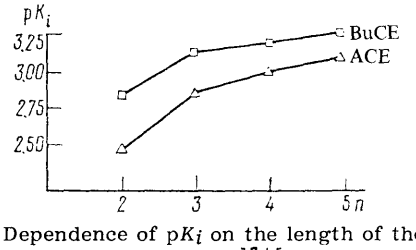
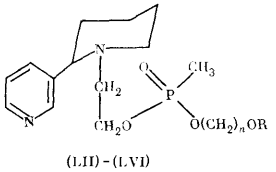
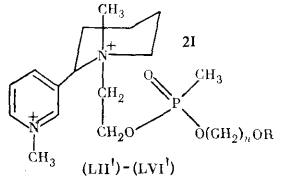


Figure 4. Dependence of pK_i on the length of the alkoxy-group in compounds of type (VI).[17,45]

N-β-(ω-Alkoxyalkoxy)methylphosphinyloxyethylanabasines (LII)–(LVI) and their dimethiodides (LII')–(LVI') proved to be reversible inhibitors with competitive activity[45].



(LII)-(LVI)



(LII')-(LVI')

21

An increase in the length of the alkyl moiety of the alkoxy-group entails a small (three fold) increase of the inhibiting activity in relation to both acetylcholinesterase and butyryl-cholinesterase.

An increase in the overall length of the alkoxy-group is accompanied by an increase of the inhibiting activity in relation to both enzymes, which is very low in the case of butyryl-cholinesterase and is appreciable in the case of acetylcholinesterase (Table 14, Fig. 5).

Table 14. The constants for the reversible inhibition of cholinesterases (K_i, mol litre^{-1}) by N-[β-(ω-alkoxyalkoxy)-methylphosphinyloxyethyl]anabasines (LII)–(LVI) and their methiodides (LII')-(LVI').[17,45]

Compound	R	n	$10^4 K_i$ (ACE)	$10^4 K_i$ (BuCE)	Compound	$10^4 K_i$ (ACE)	$10^5 K_i$ (BuCE)
(LII)	CH_3	2	9.6	1.88	(LII')	6.4	2.96
(LIV)	CH_3	3	5.4	1.01	(LIV')	3.6	3.08
(LV)	CH_3	4	3.7	0.735	(LV')	3.08	4.6
(LVI)	CH_3	5	2.85	0.545	(LVI')	5.50	2.94
(LIII)	C_2H_5	2	7.62	1.265	(LIII')	4.30	1.24

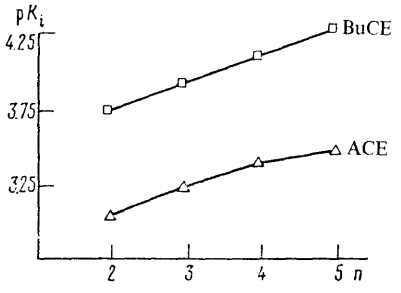


Figure 5. Dependence of pK_i on the length of the alkoxy-group in compounds of type (LII)–(LVI).[17,45]

Table 15. The constants for the reversible inhibition of cholinesterases (K_i, mol litre^{-1}) by N-[β-(alkoxyethyl-phosphinyloxyethyl)piperidines (LVII)–(LX) and their methiodides (LVII')-(LX').[17,45]

Compound	R	$10^4 K_i$ (ACE)	$10^4 K_i$ (BuCE)	Compound	$10^4 K_i$ (BuCE)
(LVII)	C_3H_7	4.26	1.47	(LVII')	1.32
(LVIII)	C_4H_9	3.21	1.61	(LVIII')	1.05
(LIX)	C_5H_{11}	2.06	1.015	(LIX')	0.677
(LX)	C_6H_{13}	1.55	0.307	(LX')	0.364

N-β-(Alkoxymethylphosphinyloxyethyl)piperidines (LVII)–(LX) are reversible competitive inhibitors of cholinesterases.

The activities of the test compounds in relation to butyryl-cholinesterases are inferior to those of analogous anabasine and lupinine derivatives. The average K_i is 1×10^{-4} M, decreasing almost by a factor of 5 in the series of substances investigated (Table 15).

Piperidine derivatives are also weaker inhibitors of acetylcholinesterase than the corresponding anabasine and lupinine derivatives.

The methiodides of *N*-β-(alkoxymethylphosphinyloxyethyl)-piperidines (LVII')-(LX') have the same inhibiting effect on butyrylcholinesterases as their uncharged analogues (Table 15, and Fig.6).

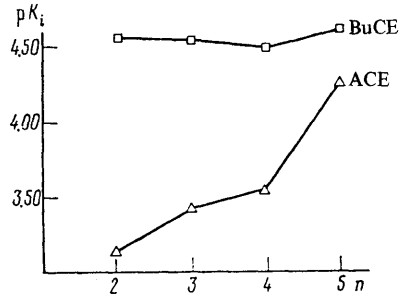


Figure 6. Dependence of pK_i on the length of the alkoxy-group in compounds of type (LVII)-(LX).[17,45]

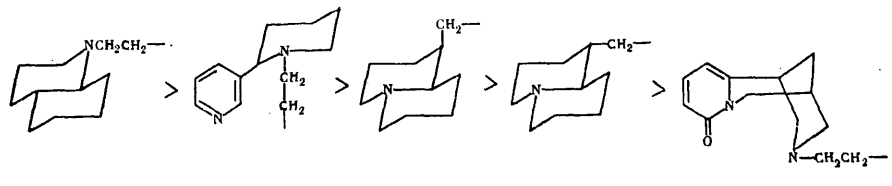
Thus the presence of an oxygen atom in the alkyl moiety of the reversible inhibitors, both with and without positive charges in the molecule, has an appreciable influence on the character of their interaction with cholinesterases. One should note that, for both the lupinine and anabasine derivatives, the characteristics of the changes in the anticholinesterase activity are similar. On this basis, one may conclude that the hydrophilic oxygen atom in the alkoxy-group of the reversible organophosphorus inhibitor in general reduces the inhibiting activity. Furthermore, one should also note that the reversible inhibiting activity depends significantly also on the structure of the heterocyclic residue present in the leaving component of the OPI molecule.

VI. CONCLUSION

Thus, comparison of the anticholinesterase activities of the *O,O*-dialkyl phosphorothioates which we investigated, containing the residues of the alkaloids lupinine, epilupinine, anabasine, cytisine, and decahydroquinoline in the alkanethiol component of the organo phosphorus inhibitor, showed that all the compounds exhibit a greater tendency towards interaction with the horse blood serum butyrylcholinesterase than with the acetylcholinesterase from human blood erythrocytes. Similar properties of dialkyl phosphorothioates were known previously but in our studies the selectivity of the activity of the above substances in relation to butyrylcholinesterase depends not only on the structure of the phosphoryl component but also on the bulk and conformational possibilities in the leaving part of the inhibitor molecule. Dialkyl phosphorothioates containing the lupinine residue proved to be more highly specific inhibitors of butyrylcholinesterase than the epilupinine derivatives. Under the same experimental conditions and for identical structures of the phosphoryl component of the lupinine and epilupinine derivatives, the differences between the conformational states of the protonated forms of lupinine and epilupine in solution can be appropriately regarded as the cause of the selectivity of their activity in relation to butyrylcholinesterase[17,37]. The protonated forms of *O,O*-dialkyl *S*-lupinanyl phosphorothioates apparently exist in two conformational states, as in the case of acyl-lupinanes: having a *trans*-junction with the quinolizidine ring and an axial hydroxymethyl group and having a *cis*-junction with the quinolizidine ring and an equatorial hydroxymethyl group. These two conformers are in dynamic equilibrium. It may be that the *cis*-conformer is better adapted to sorption on the anionic centre of butyrylcholinesterase, as a result of which the sorption equilibrium is displaced towards the formation of the *cis*-form and this entails the observed specificity of the activity of lupinine derivatives in relation to butyrylcholinesterase. The conformation of the epilupinine derivatives under the experimental conditions at pH 7.5 to 7.8 remains unchanged, i.e. it has a *trans*-junction with the quinolizidine ring and an equatorial hydromethyl group[29,30-33,38].

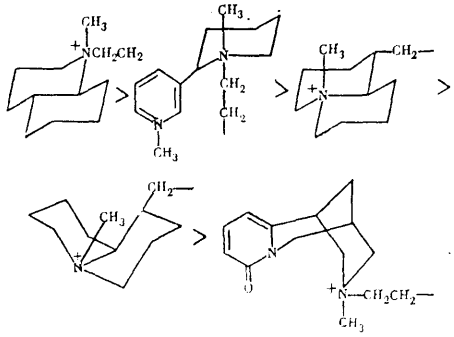
Comparison of the anticholinesterase activities of the diethyl phosphorothioates containing the residues of the above alkaloids as well as cytisine, anabasine, and decahydroquinoline made it possible to set up a series based on the relative inhibiting activities as a function of the structure of the leaving components of the organophosphorus compounds investigated: the constants k_2 in relation to the ACE from the heads of flies are 9.3×10^5 for *N*-[β-(diethoxyphosphinyl)-mercaptoethyl]anabasine, 1.8×10^4 for *N*-[β-(diethoxyphosphinyl)mercaptoethyl]cytisine, and 1.3×10^6 litre mol^{-1} min^{-1} for *N*-[β-(diethoxyphosphinyl)mercaptoethyl]decahydroquinoline. In terms of the strength of the irreversible inhibition of human blood erythrocyte acetylcholinesterase, the inhibitors $(C_2H_5O_2)P(O)SR$ can be arranged in the following sequence as a function of the substituent R:

Scheme 4



On the other hand, the sequence of their methiodides is as follows:

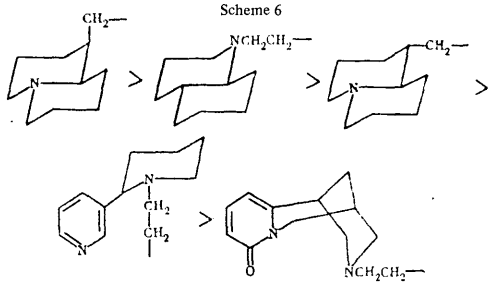
Scheme 5



It is seen from the above sequences that an increase in the complexity of the piperidine ring in the test substances results in a marked decrease of the irreversible inhibiting activity in relation to the erythrocyte acetylcholinesterase, which confirms the existence of a weakly hydrophobic framework around the anionic centre in the acetylcholinesterase from human erythrocytes. In the second sequence the methiodide of *O,O*-diethyl *S*-lupinanyl phosphorthioate is less active than the analogous epilupinine derivative, which is apparently due to the influence of the conformation change in the lupinine derivative on conversion into the methiodide[33].

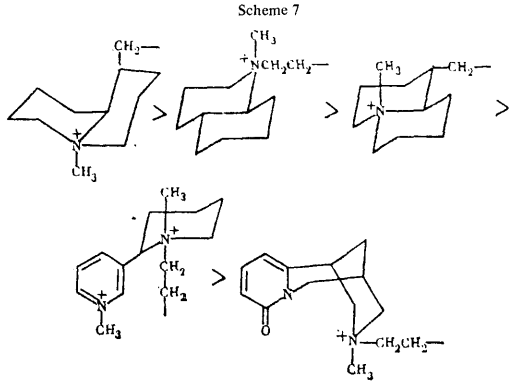
Another picture is observed when the above inhibitors act on butyrylcholinesterase. Butyrylcholinesterase is known to tend to undergo hydrophobic interactions and the hydrophobic framework around the anionic centre in this enzyme is more extensive than in acetylcholinesterase. The given sequence shows that the anionic centre of butyrylcholinesterase has a definite boundary and tends to sorb labile bicyclic systems and transition to systems containing groups such as the pyridine and quinolizidine groups results in a sharp decrease of the reversible inhibiting activity:

Scheme 6



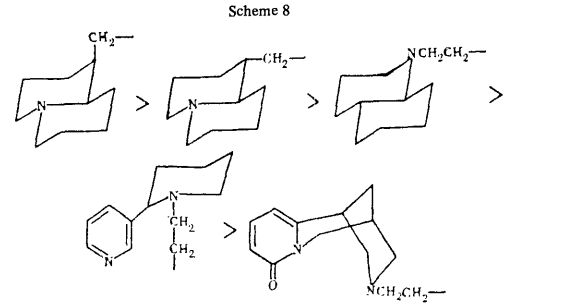
An analogous behaviour has been observed also for the methiodides of the test compounds:

Scheme 7



The compounds investigated proved to be very powerful inhibitors of the cholinesterase from the heads of flies with the exception of the cytisine derivative, which exhibited a very weak inhibiting activity in its interaction with all the enzymes investigated:

Scheme 8



Comparison of these series of relative inhibiting activities of the compounds, namely in their interaction with butyrylcholinesterase, shows that the first four compounds of the last sequence are very powerful inhibitors of both the acetylcholinesterase from the heads of flies and of butyrylcholinesterase. This suggests that in all probability the hydrophobic frameworks around the anionic centres of horse blood serum butyrylcholinesterase and the acetylcholinesterase from the heads of flies have a definite similarity in their steric structures[33].

The study of the acetylcholinesterase activities of *O,O*-dialkyl *S*-lupinanyl phosphorothioates and the corresponding epilupinanyl derivatives with straight-chain substituents in relation to the acetylcholinesterase from the heads of flies showed that the inhibiting activity of the lupinine derivatives decreases sharply with increase in the length of the alkoxy-groups in the inhibitor molecules; an analogous but less marked behaviour has been observed also for the epilupinine derivatives. The hydrophobic component in the region of the esterase centre of the acetylcholinesterase from the heads of flies is apparently small compared with the mammalian enzyme and immediately beyond it there are hydrophilic regions which hinder the sorption of large hydrocarbon groups, reducing the acetylcholinesterase activity. The investigations have yielded data which made it possible to discover differences between the structures of the active centres of mammalian cholinesterases and acetylcholinesterase from the heads of flies.

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