NEW TYPE OF SELECTIVELY ACTING ORGANOPHOSPHORUS INSECTICIDES AND ACARICIDES

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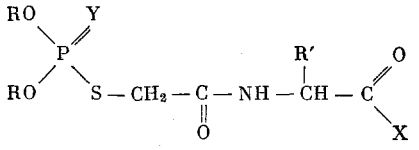
In recent years much attention is being devoted to the synthesis of insecticides and acaricides which exhibit low toxicity toward warm-blooded animals and a selective action toward insects and mites. Since organophosphorus insecticides belong to the type of cholinergic poisons, a search for selectively toxic compounds can be based either on the differences in the cholinesterases (CE) or parasympathetic receptors of arthropods and vertebrates, or on the differences in the metabolic transformations that the compounds undergo in the various organisms. In the first case the presence or absence of a complementarity of the inhibitor of the active CE or parasympathetic receptor surface should evidently have great importance; the important role of this factor was established recently [1]. It is possible to assume that the conditions of complementarity are different for warm-blooded animals and insects, and the selectivity of the action of compounds can depend on this. On the basis of these considerations and taking into account the fact that cholinesterases are purely protein enzymes, we postulated that a selectivity of action should be sought for organophosphorus inhibitors of CE that contain various amino acid moieties in the side chain. Here the selectivity of action should depend not only on the nature of the selected amino acids, but also on the order of their arrangement in the inhibitor molecule.

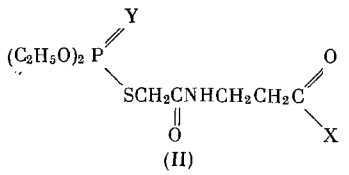
In the present paper we report the data on the synthesis and study of the toxic action on warm-blooded animals, insects and mites of organothiophosphorus compounds of general formula



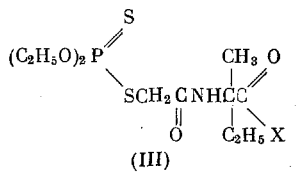
(I)

R = CH_3, C_2H_5, i-C_3H_7; R′ = H, CH_3, n-C_3H_7, i-C_3H_7, i-C_4H_9, $CH_2C_6H_5$, $CH_2C_6H_4OH$-p, $C_2H_4SCH_3$; X = OH, OC_2H_5, $NHCH_3$; Y = S or O,

and also of the corresponding β-alanine and isovaline derivatives



(II)



(III)

For compounds of this type it could be assumed that the amide group will play the role of substance fixative on the anionic portion of the enzyme, and the following amino acid moiety should then either facilitate or inhibit the formation of the Michaelis complex, depending on the complementarity of the adjacent links of the enzyme chain. Besides this, differences in the rate of enzymatic carboxyesterase of carboxyamidase hydrolysis in the organisms of warm-blooded animals and arthropods could be expected for compounds of this type, since it is known that the carboxyesterases and carboxyamidases of warm-blooded animals are more active than the corresponding enzymes of insects and mites.

It should be mentioned that two of the above-indicated type of compounds (I; R = CH_3, R' = H, X = OCH_3 and X = NH_2, Y = S) were known [2], but their synthesis was not associated by anybody with the above-

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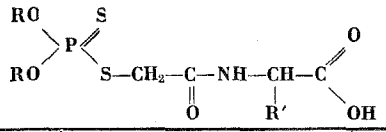
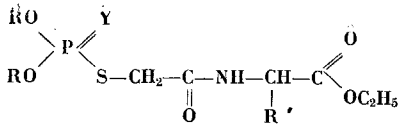
TABLE 1.



Laboratory code	R	R′	Yield, %	mp, °C	Found, %				Calculated, %				R_f hexane–acetone (3:2) SiO_2·10% H_2O	LC_{50}	
					C	H	N	P	C	H	N	P		greenbug*	spider mite†
III-124	CH_3	H	27,5	105—106				11,1 11,3				11,3	0,20		
III-125	CH_3	CH_3	7,0	72—74			4,9 4,9				4,9				
III-116	C_2H_5	H	83,0	74,5—76	32,2 32,4	5,5 5,5	4,2 4,3		31,9	5,4	4,6		0,59	0,024	Nontoxic
III-117	C_2H_5	CH_3	82,8	98—99,5	34,8 34,8	5,8 6,0		9,5 9,4	34,3	5,8		9,8	0,60	0,03	
III-148	C_2H_5	‡	90,0	Oil			4,4 4,3				4,4		0,28		
III-127	C_2H_5	i-C_3H_7	95,1	69—70			4,2 4,4				4,1		0,31 **	0,10	
III-159	C_2H_5	n-C_3H_7	90,7	89—90	38,9 38,7	6,6 6,5			38,5	6,5				3,5	0,28
III-164	C_2H_5	††	90,0	123—124	38,9 38,9	6,4 6,5	4,4 4,6		38,5	6,5	4,1			0,87	0,32
III-135	C_2H_5	i-C_4H_9	94,4	66,5—68,5			4,1 4,4				3,9		0,51 **	0,12	Nontoxic
III-119	C_2H_5	$CH_2C_6H_5$	91,7	91,5—92	46,6 46,6	5,7 5,6	3,8 3,8	7,4 7,3	46,1	5,7	3,6	7,9	0,69	0,30	"
III-150	C_2H_5	$C_2H_4SCH_3$	85,2	92—92,5	35,2 35,0	5,7 5,6	3,8 4,0		35,2	5,9	3,7		0,32	0,057	"
EG-6	i-C_3H_7	H	80,0	77,5—78	36,6 36,6	6,2 6,1	4,1 4,3	9,0 9,1	36,5	6,1	4,2	9,4	0,61		
EG-9	i-C_3H_7	CH_3	80,0	67—68	38,6 38,4	6,5 6,6	4,2 4,1	9,2 9,2	38,5	6,5	4,1	9,0	0,40	0,20	"
EG-7	i-C_3H_7	i-C_4H_9	90,0	Oil	43,5 43,2	7,4 7,6	3,6 3,6	7,8 8,0	43,6	7,3	3,6	8,0	0,48		
	Thiophos													0,001—0,008	0,005—0,015

*Counted on the 2nd day. †Counted on the 5th day. ‡β-Alanine derivative (formula II). **SiO_2 · 6% H_2O.
††Isovaline derivative (formula III).

TABLE 2.



Laboratory code	R	R′	Y	Yield, %	mp, °C	Found, %				Calculated, %				Rf hexane–acetone (3:2) SiO_2 · 10% H_2O	LC_{50}	
						C	H	N	P	C	H	N	P		green-bug*	spider mite†
EG-4	CH_3	H	S	31,0	68—69	31,9 31,7	5,3 5,4	4,8 4,8	10,2 10,3	31,9	5,4	4,6	10,2	0,38	0,006	0,034
EG-5	CH_3	CH_3	S	71,0	82—83	34,6 34,9	6,0 5,9	4,6 4,4	9,9 9,7	34,3	5,8	4,4	9,8	0,45	0,012	0,012
III-120	C_2H_5	H	S	88,2	58,5—60	36,4 36,5	6,0 6,0	4,6 4,4	9,1 9,0	36,5	6,1	4,2	9,4	0,54	0,015	0,015
III-122	C_2H_5	CH_3	S	88,2	55—56			4,1 4,3	9,1 9,1			4,1	8,8	0,52	0,018	0,006
III-140	C_2H_5	‡	S	95,0	Oil			4,0 4,0	9,0 9,1			4,1	8,8	0,44 **	0,0075	0,20
III-141	C_2H_5	*i*-C_3H_7	S	83,2	53,5—54,5	41,9 41,9	7,0 7,5	4,0 4,0	8,0 7,8	42,0	7,1	3,8	8,3	0,70 **	0,17	0,0027
III-162	C_2H_5	*n*-C_3H_7	S	90,0	Oil				8,1 8,2				8,3		0,28	0,0072
III-166	C_2H_5	††	S	81,3	50,5—51,5	42,2 41,9	6,8 6,6			42,0	7,1				0,057	0,004
III-138	C_2H_5	*i*-C_4H_9	S	85,2	31—32	44,0 44,0	7,6 7,3		8,4 8,3	43,6	7,3		8,0	0,71 **	0,30	0,015
III-126	C_2H_5	$CH_2C_6H_5$	S	90,9	46—47	48,3 48,3	5,8 6,0	3,2 3,3	7,2 7,1	48,8	6,2	3,3	7,4	0,57	0,30	0,04
III-154	C_2H_5	$C_2H_4SCH_3$	S	78,0	45,5—46	38,8 38,6	6,5 6,5		7,3 7,6	38,7	6,4		7,7	0,55	0,0058	0,045
III-157	C_2H_5	$CH_2C_6H_4OH$	S	81,0	47—48			3,6 3,5	7,4 7,2			3,2	7,1	0,32	7,0	0,12
EG-8	*i*-C_3H_7	H	S	52,0	29—30	40,4 40,3	6,5 6,8	4,1 4,2	8,6 8,4	40,3	6,8	4,0	8,7	0,58	0,13	Nontoxic
EG-10	*i*-C_3H_7	CH_3	S	85,0	36—37	42,1 42,4	7,0 7,1	3,6 3,8	8,3 8,4	42,0	7,0	3,8	8,3	0,65 **	0,02	0,60
EG-11	*i*-C_3H_7	$CH_2C_6H_5$	S	74,0	59—61			3,2 3,0				3,1		0,64 **		
III-155	C_2H_5	H	O	57,7	34—35	38,7 38,8	6,4 6,5	4,2 4,3	9,8 10,0	38,3	6,4	4,5	9,9	0,43		
EG-33	C_2H_5	CH_3	O	40,0	46—47	40,4	6,8	4,5 4,4	9,2 9,0	40,4	6,8	4,3	9,5	0,25	0,025	0,0055
EG-35	C_2H_5	‡	O	36,0	Oil	40,6 40,7	7,1 7,0	4,6 4,6		40,4	6,8	4,3		0,20		
III-156	C_2H_5	*i*-C_3H_7	O	50,0	35,5—36,5	44,3 44,2	7,3 7,5	3,9		43,9	7,4	3,9				
EG-29	C_2H_5	*i*-C_4H_9	O	75,0	41—42			4,0 4,0				3,8		0,47		
EG-31	C_2H_5	$C_2H_4SCH_3$	O	76,0	38—40	40,5 40,5	6,7 6,8			40,3	6,8			0,34	0,088	0,005
	Thiophos														0,001—0,008	0,005—0,01

*Counted on the 2nd day.

†Counted on the 5th day.

‡ β-Alanine derivative (formula II).

** SiO_2 · 6% H_2O.

††Isovaline derivative (formula (III).

developed concepts regarding a search for selectively acting compounds. The derivatives of the amino acids and esters of amino acids (I, II, and III; X = OH or OC_2H_5) were obtained by us by the reaction of the corresponding chloroacetyl derivatives of the amino acids with the sodium and potassium salts of dialkyl dithiophosphates and dialkyl monothiophosphates, for example

$$(RO)_2PSSK + ClCH_2C(O)NHCH(R')COX \longrightarrow (RO)_2PSSCH_2C(O)NHCH(R')COX + KCl$$

Their constants, yields and analyses are given in Tables 1 and 2. The purity of the obtained compounds was followed by thin-layer chromatography (TLC). The attention is attracted to the fact that the melting points of the derivatives of the free amino acids (I, II, and III, X = OH, Y = S, see Table 1) are higher than for the corresponding derivatives of the amino acid esters (I, II, and III, X = OC_2H_5, Y = S, see Table 2). These data are in full agreement with the changes in the melting points observed for the chloroacyl derivatives of the amino acids and their esters. Only compounds III-125 and EG-5 are an exception. It could also be mentioned that the derivatives of the dithioacid esters have higher melting points than the esters of the corresponding monothioacids, which is also characteristic for the derivatives of the monothio and dithio esters of phosphorus acids. The obtained compounds are readily soluble in the common organic solvents and are difficultly soluble in water. However, some of them, namely III-124, III-125, and EG-4 (see Tables 1 and 2), the derivatives of dimethyl dithiophosphate, and also of diethyl monothiophosphate (see Table 2, III-155, etc.), proved to be quite readily soluble in water, which apparently could be the reason for the relatively low yields of these compounds.

The derivatives of the methylamides (I and II, X = $NHCH_3$, Y = S) were obtained by us by reacting the acids (I and II, X = OH) with an excess of p-nitrophenol in the presence of dicyclohexylcarbodiimide [3] and subsequent decomposition of the formed p-nitrophenyl esters with methylamine according to the scheme

$$(RO)_2P(S)SCH_2C(O)NHCH(R')COOH \xrightarrow[(C_6H_{11}N)_2C]{p\text{-}NO_2C_6H_4OH} (RO)_2P(S)SCH_2C(O)NHCH(R')COOC_6H_4NO_2\text{-}p \xrightarrow{CH_3NH_2}$$

$$\rightarrow (RO)_2P(S)SCH_2C(O)NHCH(R')C(O)NHCH_3$$

The constants, yields, analyses and TLC data of the compounds synthesized in this manner are given in Table 3.

It should be mentioned that we were unable to obtain the methylamides of the above-indicated types of amino acids by the direct reaction of the free acids (I and II, X = OH) with methylamine in the presence of dicyclohexylcarbodiimide, apparently because of the high basicity of methylamine. The synthesis of these compounds via the carbonic acid anhydrides [4-6] by the scheme

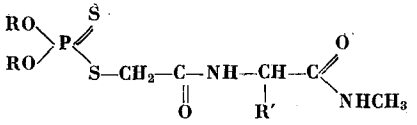
$$(RO)_2P(S)SCH_2C(O)NHCH_2COOH \xrightarrow[(C_2H_5)_3N]{C_2H_5OC(O)Cl} (RO)_2P(S)SCH_2C(O)NHCH_2C(O)OC(O)OC_2H_5 \xrightarrow{CH_3NH_2}$$

$$\rightarrow (RO)_2P(S)SCH_2C(O)NHCH_2C(O)NHCH_3$$

gave only very low yields of the methylamides.

In harmony with the above-discussed assumptions, the obtained compounds proved to be highly selective insecticides and acaricides, with a comparatively low toxicity toward warm-blooded animals. As was to be expected, the least toxic of them proved to be the free acids. For them the LD_{50} (per os on white mice) ranged from 1200 to 2000 mg/kg and higher. The toxicity of the esters was somewhat greater, but also for them in the series of dithio derivatives the LD_{50} ranged from 500 mg/kg to 1000 mg/kg. The corresponding monothiophosphate esters are more toxic than the dithio esters, since the LD_{50} for III-122 is 1000, and for EG-33 it is 290 mg/kg. The most toxic are the methylamide derivatives (I and II; X = $NHCH_3$). Here the LD_{50} ranges from 50 to 250 mg/kg. The results of testing the obtained compounds for their insecticidal and acaricidal activity are given in the last two columns of Tables 1-3. Common for most of the compounds is their greater acaricidal activity when compared with the insecticidal activity.

If the free acids are excluded, which naturally possess only slight insecticidal and acaricidal properties, then in the series of esters and methylamides a clearly expressed selectivity is observed, depending on the nature of the amino acid moiety. Thus, if III-140 (β-alanine derivative) is a comparatively powerful insecticide, close to Thiophos, and a quite weak acaricide, then the corresponding α-alanine derivative (III-122) is a powerful acaricide (like Thiophos) and only an average insecticide (see Table 2). For the corresponding valine derivative (III-141) the acaricidal properties are expressed even more strongly and exceed those of Thiophos by a factor of two; in this connection the compound is practically devoid of insecti-

TABLE 3. 

Laboratory code	R	R′	Yield, %	mp, °C	Found, %				Calculated, %				R_f hexane–acetone (3:2) SiO_2·6% H_2O	LC_{50}	
					C	H	N	P	C	H	N	P		greenbug*	spider mite†
III-130	C_2H_5	H	64,6	87—88,5	34,7 34,8	5,8 6,0	9,1 9,0		34,4	6,1	8,9		0,13	0,027	0,013
III-133	C_2H_5	CH_3	71,0	114—115			8,6 8,8				8,5		0,16	0,052	0,0016
III-149	C_2H_5	‡	72,5	101—102	36,6 36,6	6,3 6,4	8,4 8,5		36,6	6,4	8,5		0,12**	0,0027	0,0027 ††
III-134	C_2H_5	i-C_3H_7	74,1	137—138	41,0 40,8	7,3 7,3	8,1 8,1		40,4	7,1	7,9		0,33	0,01	0,025
III-160	C_2H_5	n-C_3H_7	74,1	112—113			8,1 8,0	9,2 9,1			7,9	8,7		3,0	0,012
III-136	C_2H_5	i-C_4H_9	76,0	88—89			7,6 7,8				7,6		0,40	0,45	0,085
III-132	C_2H_5	$CH_2C_6H_5$	76,2	131,5—132	47,3 47,5	6,3 6,3			47,5	6,2			0,27	0,20	Nontoxic
III-152	C_2H_5	$C_2H_4SCH_3$	57,5	106,5—107,5	37,0 37,3	6,6 6,4			37,1	6,5			0,29**	0,07	0,046 ††
EG-15	i-C_3H_7	CH_3	72,0	114—115			7,9 8,1				7,9		0,38	0,4	Nontoxic
EG-16	i-C_3H_7	i-C_3H_7	78,0	135—136			7,7 7,8				7,3		0,44	0,27	*
				Thiophos										0,001—0,008	0,005—0,015

*Counted on the 2nd day. †Counted on the 5th day. ‡β-Alanine derivative (formula II).
**SiO_2 · 10% H_2O. ††Counted on the 3rd day.

cidal activity. It is interesting that the methylamide obtained from α-alanine, III-133 (see Table 3), is more powerful than the corresponding ester as an acaricide, but weaker as an insecticide. This compound, despite its relatively high toxicity for warm-blooded animals (LD_{50} 120 mg/kg), retains an astonishing selective action for mites.

Selectivity of action is apparently not a specific property of structures (I) or (II) (X = OC_2H_5, $NHCH_3$, Y = S), and depends on the nature of the amino acid. Thus, in structure (I) the glycine derivatives is completely nonselective [see Table 2, III-120; the LC_{50} (lethal coefficient) for both insects and mites is 0.015], while in structure (II) the β-alanine derivative (see Table 3, III-149) also lacks a selectivity of action. It is highly effective for both insects and mites (LC_{50} 0.0027). Since in the above-discussed compounds all of the changes occur only in the side chain, at some distance from the natural phosphorylating center [the grouping $(C_2H_5O)_2P(S)SCH_2C(O)NH$ remains unchanged], then the selectivity of action must be attributed to changes in the sorption conditions, depending on the complementarity of the surface of the enzymes (cholinesterases or detoxifying enzymes).

As a result, great possibilities open up on the studied series of compounds in the search for selectively acting compounds.

EXPERIMENTAL

The N-chloroacetyl derivatives of the amino acids were obtained by previously described methods – by the chloroacylation of amino acids [7-9]. The esters of the amino acids were obtained by the Fischer method [10], with their isolation from the hydrochlorides by treatment with a chloroform solution of ammonia [11]. The N-chloroacetyl derivatives of the amino acid esters were obtained by the Fischer method [12, 13], including those obtained and not obtained previously. For N-chloroacetyl-O-ethylvaline the yield was 85.3%; bp 112-112.5° (2 mm); n_D^{20} 1.4670; d_4^{20} 1.1360. Found %: C 48.8; H 7.0. MR 54.16. $C_9H_{16}ClNO_3$. Calculated %: C 48.8; H 7.3. MR 54.14. For N-chloroacetyl-O-ethylnorvaline the yield was 70%, bp 116 to 117° (2 mm); mp 38°. Found %: C 49.2, 49.2; H 7.2, 7.2; N 6.6, 6.4. $C_9H_{16}ClNO_3$. Calculated %: C 48.8; H 7.3; N 6.4. For N-chloroacetyl-O-ethylisovaline the yield was 84%; mp 46.5-47.5° (from heptane). Found %: C 48.9, 48.7; H 7.3, 7.2. $C_9H_{16}ClNO_3$. Calculated %: C 48.8; H 7.3. For N-chloroacetyl-O-ethyl-β-alanine the yield was 87.9%; bp 127.5-128° (3 mm); n_D^{20} 1.4742; d_4^{20} 1.2147. Found %: C 42.9, 42.9; H 6.3, 6.2. MR 44.83. $C_7H_{12}ClNO_3$. Calculated %: C 43.4; H 6.2. MR 44.91.

Preparation of Derivatives of Amino Acids and Their Esters. A mixture of 0.03 mole of the chloroacetyl derivative of the amino acid or amino acid ester and 0.032 mole of the potassium salt of the dialkyl dithiophosphate, or the sodium salt of diethyl thiophosphate, in 50 ml of absolute alcohol (methyl, ethyl, or isopropyl, depending on the taken salt) was heated at 60-70°, with stirring, for 2.5 h.* After cooling the mixture the KCl(NaCl) precipitate was filtered, washed with a little alcohol, and the filtrate was evaporated in vacuo. The residue was dissolved in ether and washed 2-3 times with small portions of cold water. In the case of the dimethyl dithiophosphate derivatives the residue was dissolved in ethyl acetate and washed once with ice water. The ether solution of the compounds was dried over anhydrous Na_2SO_4 and the ether was removed in vacuo. The residue usually crystallized on rubbing with hexane; in such case it was recrystallized from a mixture of either benzene or ether with hexane. The diethyl thiophosphate derivatives were purified by freezing out from a solution in ether at –78°. The constants of the compounds, their yields, and the analytical and TLC data are given in Tables 1 and 2.

Preparation of Derivatives of Methylamides of Amino Acids. To a solution of 0.01 mole of the acid (I, II; X = OH) and 0.012 mole of p-nitrophenol in 15 ml of absolute ethyl acetate, cooled to 0°, was added a solution of 0.01 mole of dicyclohexylcarbodiimide in 10 ml of ethyl acetate. The mixture was cooled for another 30 min and then kept at room temperature for 2 h. The precipitate of dicyclohexylurea was filtered and washed with a little ethyl acetate. The filtrate was evaporated in vacuo and the residue was dissolved in 20 ml of absolute $CHCl_3$. A stream of dry methylamine (excess) was passed through the chloroform solution until a precipitate ceased to deposit. The precipitate of methylammonium p-nitrophenolate was filtered, washed with $CHCl_3$, and the filtrate was washed with three portions of 1 N NH_3 solution (a total of 70 ml) and with two portions of water (a total of 50 ml). The chloroform solution was dried over anhydrous Na_2SO_4, the $CHCl_3$ was removed in vacuo, and the residue was recrystallized from a mixture of chloroform and hexane. The constants, yields and analyses of the obtained compounds are given in Table 3.

*In the case of the dimethyl dithiophosphate derivatives an alcohol solution of the potassium salt was added slowly to a solution of the chloroacetyl derivative.

The toxicity of the compounds was tested on white mice, with injection of an aqueous emulsion of the compound into the stomach through a sound. The LD_{50} was determined by the Miller–Tainter probit analysis method.

The contact insecticidal and acaricidal activity of the compounds was determined on the greenbug and spider mite. The compounds were applied by spraying the experimental arthropods. The LC_{50} was calculated on the basis of the obtained experimental data from the theoretical curve using probit analysis.

CONCLUSIONS

1. A number of trialkylthio- and dithiophosphates were obtained, containing the moieties of amino acids, their esters, and their methylamides.

2. Both the pesticidal activity and the selectivity of action of the obtained compounds are strongly dependent on the nature of the amino acid moiety.

LITERATURE CITED

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