11. Reactions of Organic Compounds and Study Methods [Russian translation], Vol. 13, Khimiya (1964), p. 267.
12. V. A. Davankov, Dissertation, Moscow (1966).
13. Preparative Organic Chemistry [Russian translation], Khimiya (1964), p. 773.
14. Reactions of Organic Compounds and Study Methods [Russian translation], Vol. 13, Khimiya (1964), p. 310.
15. T. Ya. Medved and M. I. Kabachnik, Izv. Akad. Nauk SSSR, Otd. Khim. Nauk, 1954, 314.
16. Yu. P. Belov, Dissertation, Moscow (1975).
17. K. J. Laidler, Kinetics of Organic Reactions [Russian translation], Moscow (1966).
18. M. P. Tsyurupa, Dissertation, Moscow (1974).
19. W. C. Hamilton, Acta Cryst., 18, 502 (1965).

SYNTHESIS, REACTION WITH ESTERASES, AND TOXICITY OF O-ALKYL S-(CARBALKOXYMETHYLMERCAPTO)METHYL METHYLTHIOPHOSPHONATES

T. A. Mastryukova, A. É. Shipov,
Z. K. Emkuzheva, A. P. Brestkin,
I. L. Brik, Yu. E. Mandel'shtam,
A. N. Fedin, T. I. Nozhko,
Z. Tilyabaev, S. A. Roslavtseva,
Yu. S. Kagan, E. A. Ershova,
and M. I. Kabachnik

UDC 542.91:541.69:547.1'118

Previously [1] we had described the synthesis and physiological activity of compounds of general formula $CH_3(RO)P(S)S(CH_2)_nSCH_2COOR'$ (I) (R = CH_3, C_2H_5; R' = CH_3, C_2H_5, i-C_3H_7, i-C_4H_9; n = 1, 2), among which are included active insecticides and acaricides with a moderate toxicity toward warm-blooded animals. It was shown that the physiological activity of the (I) compounds depends to a large degree on the structure of the R and R' substituents. In this connection it was interesting to follow the effect of the R and R' groups on the ability of the monothio analogs of the (I) compounds to inhibit the esterases of warm-blooded animals and arthropoda and compare the obtained data with the toxicity of these compounds. In addition, it was previously established [2] that an increase in the size of the alkyl in the alkoxyl group on the phosphorus atom enhances (due to the hydrophobic interactions with the environment of the active center) the ability of methylthiophosphonates to inhibit the cholinesterases of warm-blooded animals. Consequently, it also seemed interesting to ascertain to what degree this relationship extends to the esterases of arthropoda.

We synthesized a number of O-alkyl S-(carbalkoxymethylmercapto)methyl methylthiophosphonates:

$$CH_3(RO)P(O)SCH_2SCH_2COOR' \text{ (II)} - \text{(XIV)}$$

R = CH_3, C_2H_5, C_3H_7, C_4H_9, i-C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}; R' = CH_3, C_2H_5, i-C_4H_9.

These compounds were obtained by the reaction of sodium O-alkyl methylthiophosphonates with carbalkoxymethyl chloromethyl sulfides. The constants of the obtained compounds and their elemental analysis data are given in Table 1.

To estimate the antienzymatic activity of compounds (II)-(XIV) we determined the bimolecular rate constants (k_2) of the reactions of these compounds with the acetylcholinesterase of human erythrocytes (ACE), the butyrylcholinesterase of horse serum (BuCE), the cholinesterase (CE_t) and carboxylesterase (CBE) of the nerve tissue of the American cockroach (Periplaneta americana L.), and also the I_{50} values for the cholinesterase of housefly heads (Musca domestica L.) (CE_m). The toxicity of the compounds was characterized by the LD_{50} values.

Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, Moscow. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 5, pp. 1131-1136, May, 1980. Original article submitted April 16, 1979.

 0568-5230/80/2904-0838$07.50

TABLE 1. Constants and Analyses of Compounds $CH_3(RO)P(O)SCH_2SCH_2COOR'$ (II)-(XIV)

Compound	R	R'	Yield, %	n_D^{20}	d_4^{20}	MR found	MR calc.	Found P (S), %	Empirical formula	Calc. P (S), %
(II)	CH_3	CH_3	41,0	1,5273	1,3114	57,29	57,63	12,42	$C_6H_{13}O_4PS_2$	12,68
(III)	CH_3	i-C_4H_9	46,0	1,5075	1,1945	71,41	71,48	10,65	$C_9H_{19}O_4PS_2$	10,82
(IV)	C_2H_5	CH_3	39,6	1,5156	1,2522	62,30	62,25	12,52 *	$C_7H_{15}O_4PS_2$	11,99
(V)	C_2H_5	C_2H_5	43,2	1,5100	1,2172	66,94	66,86	11,68	$C_8H_{17}O_4PS_2$	11,37
(VI)	C_2H_5	i-C_4H_9	76,8	1,5022	1,1659	76,06	76,10	9,91†	$C_{10}H_{21}O_4PS_2$	10,31
(VII)	C_3H_7	CH_3	62,9	1,5143	1,2310	66,64	66,86	11,27	$C_8H_{17}O_4PS_2$	11,37
(VIII)	C_4H_9	CH_3	75,0	1,5062	1,1906	71,48	71,48	11,56	$C_9H_{19}O_4PS_2$	10,82
(IX)	i-C_4H_9	CH_3	66,6	1,5072	1,1933	71,44	71,48	‡	$C_9H_{19}O_4PS_2$	–
(X)	i-C_4H_9	i-C_4H_9	64,1	1,4954	1,1193	85,62	85,33	9,44	$C_{12}H_{25}O_4PS_2$	9,43
(XI)	C_5H_{11}	CH_3	54,4	1,5028	1,1702	75,85	76,10	10,19	$C_{10}H_{21}O_4PS_2$	10,31
(XII)	C_6H_{13}	CH_3	71,0	1,5013	1,1522	80,43	80,71	(19,97)	$C_{11}H_{23}O_4PS_2$	(20,40)
(XIII)	C_7H_{15}	CH_3	60,3	1,4981	1,1349	84,84	85,33	(19,23)	$C_{12}H_{25}O_4PS_2$	(19,53)
(XIV)	C_8H_{17}	CH_3	60,1	1,4958	1,1069	90,19	89,95	(18,91)	$C_{13}H_{27}O_4PS_2$	(18,73)

*Found: C 32.25; H 6.04%. Calculated: C 32.55; H 5.85%.
†Found: C 40.10; H 7.08%. Calculated: C 39.98; H 7.05%.
‡Found: C 38.06; H 6.86%. Calculated: C 37.75; H 6.69%.

TABLE 2. Antienzymatic Activity and Toxicity of Compounds $CH_3(RO)P(O)SCH_2SCH_2COOR'$ (II)-(XIV)

Compound	R	R′	10^{-5} k_2, liter/mole · min				$10^5 \cdot I_{50}$, % CE_m	LC_{50}, %				LD_{50}, μg/kg American cockroach
			ACE	BuCE	CE_t	CBE		house-fly	rice weevil	black beet aphis	spider mite	
(II)	CH_3	CH_3	–	–	–	–	100,0 *	0,005	0,014	0,00003	0,00065	–
(III)	CH_3	i-C_4H_9	–	–	–	–	28,0	0,012	0,022	0,00005	0,0009	–
(IV)	C_2H_5	CH_3	6,7	0,28	0,92	5,0	350,0	0,0075	0,012	0,0001	0,00035	8
(V)	C_2H_5	C_2H_5	–	–	–	–	–	–	–	0,0003†	0,00045	–
(VI)	C_2H_5	i-C_4H_9	–	–	–	–	7,5	0,061	0,086	0,00022	0,00045	–
(VII)	C_3H_7	CH_3	48,0	2,5	11,0	34,0	100,0‡	0,029	0,021	0,00049	0,00074	12
(VIII)	C_4H_9	CH_3	3,8	0,31	0,46	46,0	100,0	0,015	0,034	0,0009	0,0008	16
(IX)	i-C_4H_9	CH_3	115,0	3,4	21,0	91,0	36,0	0,018	0,016	0,000014	0,0007	26
(X)	i-C_4H_9	i-C_4H_9	–	–	–	–	0,49	0,240	0,242	0,00005	0,009	–
(XI)	C_5H_{11}	CH_3	44,0	4,4	18,0	820,0	23,0	0,016	0,120	0,00013	0,00075	45
(XII)	C_6H_{13}	CH_3	30,0	11,0	25,0	3400,0	14,0	0,019	0,145	0,00052	0,0011	350
(XIII)	C_7H_{15}	CH_3	27,0	50,0	7,15	16,0	3,0	0,137	0,210	0,00060	0,0015	–
(XIV)	C_8H_{17}	CH_3	40,0	125,0	6,85	2150,0	800,0	0,157	0,175	0,0033	0,0014	470

*I_{16}.
†LC_{50} for greenbug.
‡I_{80}.

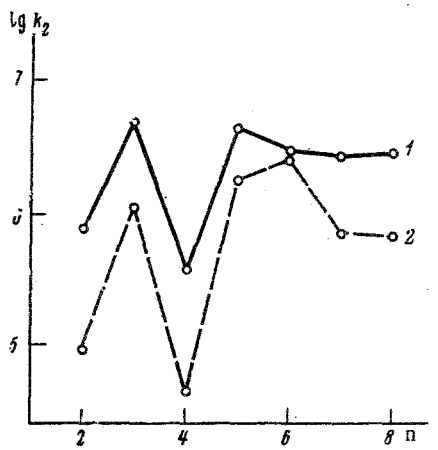

Fig. 1. Constants for inhibition of ACE (1) and CE_t (2) by compounds $CH_3(RO)P(O)SCH_2SCH_2COOCH_3$ as a function of number of carbon atoms (n) in the alkyl radical R.

In the experiments on white mice the compounds were administered perorally (for the entire series the LD_{50} values did not exceed 25 mg/kg), while in the experiments on cockroaches they were deposited on the external integuments. For the housefly, rice weevil (Calandra oryzae L.), black beet aphis (Aphis fabe L.), and spider mite (Tetranychus urticae Koch) we determined either the contact insecticidal or acaricidal activity (LC_{50}). The data on the antienzymatic activity and toxicity of the compounds are given in Table 2.

From Table 2 it can be seen that for compounds (IV), (VII), (VIII), and (XI)-(XIV) with straight-chain substituents (with a constant R' = CH_3) the k_2 value in the case of ACE and CE_t is determined by the size of the R radical (Fig. 1). Here the compounds with R = C_3H_7 (VII) and C_5H_{11} (XI) have the highest inhibiting effect toward ACE, while the compound with R = C_6H_{13} (XII) has the highest inhibiting effect toward CE_t. The I_{50} values for CE_m also change in a similar manner, with R = C_7H_{15} (XIII) having the maximum activity, while the biggest differences in I_{50} reach two orders of magnitude. In the case of BuCE, when R increases from C_2H_5 to C_8H_{17}, k_2 increases 460 times, while in the case of CBE it increases by nearly three orders of magnitude (with a maximum when R = C_6H_{13}). As a result, in the experiments in vitro, on the whole a tendency is observed for k_2 to increase with increase in the alkyl R for all of the investigated enzymes.

However, the opposite picture exists in the experiments in vivo, and here for all of the test objects the toxicity of the compounds decreases steadily with increase in the R substituent from C_2H_5 to C_8H_{17}. Analogous relationships are observed in the series of compounds (III), (VI), and (X) with R' = i-C_4H_9: the anticholinesterase activity toward CE_m increases with increase in the radical R from CH_3 to i-C_4H_9, while the toxicity for flies decreases. Apparently, together with an increase in the antienzymatic activity in vitro with increase in R, the nonspecific sorption of these compounds in vivo increases, which leads to a decrease in their toxicity.

EXPERIMENTAL

The carbalkoxymethyl chloromethyl sulfides were obtained as described in [1, 3, 4].

O-Alkylmethylthiophosphonic Acids. The acids were obtained by a modification of the method given in [5]. With vigorous stirring, to a solution of 12 g (0.21 mole) of KOH in 15 ml of water at 75-85°C was added in drops 0.1 mole of the O-alkyl methylchlorothiophosphonate [6]. When exothermic reaction started the external heating was removed and the addition of the acid chloride was continued at such a rate that the temperature of the mixture remained at 75-85°. The mixture was heated for another hour at 95-100°, extracted with ether, the aqueous layer was treated with 15-20 ml of conc. HCl, the mixture was extracted with either ether (for R = CH_3 and C_2H_5) or benzene, the extracts were dried over Na_2SO_4 and, having removed the solvent, the acid was either distilled or used as such to obtain the Na salt. Modification of the method given in [5] made it possible to increase the yield

of the acids by 5-30% and avoid marked heat evolution of the mixture. The constants of the acids were in good agreement with the literature data [5].

O-Alkyl S-(Carbalkoxymethylmercapto)methyl Methylthiophosphonates (II)-(XIV). With stirring, to 0.052 mole of the sodium O-alkyl methylthiophosphonate in 40 ml of abs. alcohol or acetone was added 0.05 mole of the carbalkoxymethyl chloromethyl sulfide in 10 ml of alcohol or acetone. The mixture was heated for 3 h at 60-70° (or at acetone reflux), filtered, the filtrate was evaporated in vacuo, and the residue was dissolved in 40-50 ml of ether or benzene, washed in succession with cold water, chilled satd. $NaHCO_3$ solution, and water, and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was chromatographed on a column packed with SiO_2 L100/160 μ using as eluant a mixture of hexane and acetone in a ratio that gradually changed from 98:2 to 3:2. The fractions were checked by TLC on the same support, using either a 4:1 or 3:2 hexane–acetone mixture as the eluant.

To determine the antienzymatic activity we used the commercial ACE (acetylhydrolase of EC 3.1.1.7 acetylcholine), with a specific activity of 1 E/mg, and BuCE (acylhydrolase of EC 3.1.1.8 acylcholines), with a specific activity of 9.6 E/mg, which are manufactured by the Perm Scientific Research Institute of Vaccines and Serums. The activity of the ACE and BuCE was determined by the potentiometric titration method (substrate = acetylcholine chloride) as described in [7]. The homogenizate of the thoracic ganglia of the American cockroach served as the source of the CE_t and CBE. The activity of the enzymes was determined colorimetrically: for CE_t as described in [8] (substrate = acetylthiocholine iodide), and for CBE as described in [9] (substrate = p-nitrophenyl acetate). The bimolecular rate constants (k_2) for the reaction of the esterases with the inhibitors were determined and calculated as described in [7], using one of the indicated methods to check the residual activity of the enzymes.

CONCLUSIONS

1. A number of O-alkyl S-(carbalkoxymethylmercapto)methyl methylthiophosphonates was obtained by reacting the sodium salts of methylthiophosphonic acids with halo derivatives.

2. It was found for the obtained compounds that increasing the size of the alkyl in the alkoxyl group on the phosphorus atom leads to an increase in the reaction rate with the esterases of warm-blooded animals and arthropoda, whereas the toxicity of the compounds decreases for the arthropoda.

LITERATURE CITED

1. T. A. Mastryukova, Z. K. Emkuzheva, A. É. Shipov, Yu. S. Kagan, E. A. Ershova, K. N. Savchenko, S. A. Roslavtseva, N. A. Guseva, T. N. Kaluzhina, V. P. Golubeva, and M. I. Kabachnik, Izv. Akad. Nauk SSSR, Ser. Khim., 1979, 637.
2. M. I. Kabachnik, A. P. Brestkin, N. N. Godovikov, M. J. Michelson, E. V. Rozengart, and V. I. Rozengart, Pharmacol. Rev., 22, 335 (1970).
3. W. J. Brehm and T. Levenson, J. Am. Chem. Soc., 76, 5389 (1954).
4. T. M. Voronkina, I. T. Strukov, and M. F. Shostakovskii, Zh. Obshch. Khim., 32, 2097 (1962).
5. A. A. Abduvakhabov, N. N. Godovikov, and M. I. Kabachnik, Chemistry of Organic Phosphorus Compounds [in Russian], Nauka, Leningrad (1967), p. 3.
6. M. I. Kabachnik, N. N. Godovikov, D. M. Paikin, M. P. Shabanova, N. M. Gamper, and L. F. Efimova, Zh. Obshch. Khim., 28, 1568 (1958).
7. V. A. Yakovlev, Kinetics of Enzymatic Catalysis [in Russian], Nauka, Leningrad (1965), p. 115.
8. I. L. Brik, Yu. E. Mandel'shtam, and V. Nemets, Khim. v Sel'skom Khoz., 15, No. 3, 40 (1977).
9. G. L. Ellman, Biochem. Pharmacol., 7, 88 (1961).