SYNTHESIS AND ANTICHOLINE ESTERASE PROPERTIES OF S-ETHYNYL ESTERS OF THIOPHOSPHORIC ACIDS

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It has been already shown that S-butynyl esters of thiophosphoric acids containing the acetylenic bond in the β-position of the thioester radical, exhibit a pronounced insecto-acaracidal activity [1]. It was found that these organophosphoric inhibitors (OPI) have a higher anticholine esterase activity than their saturated analogs, and it was suggested that the "acetylenic effect", a value showing the ratio of the bimolecular constant (k_{II}) of the rate of inhibition of choline esterases (CE) to that of their saturated analogs, is related to the ability of the acetylenic group of OPI to react with that active center of the CE which has an affinity to the acetylenic group [2]. In this case, the value of the "acetylenic effect" should depend on the position of the acetylenic bond in the alkynylthiol residue of the OPI.

To verify this supposition, we synthesized S-ethynyl esters of thiophosphoric acids, containing an acetylenic bond in the α-position of the thioester group (Table 1) $(RO)_2P(O)\cdot SC{\equiv}CX$ (I) (R = alkyl; X = hydrogen, alkyl, alkylthiomethyl, diakoxyphosphorylthio group), and studied their anticholine esterase activity.

To synthesize these compounds, we developed a preparatory method, which consists in reacting dialkoxyphosphorylsulfenyl chlorides with the corresponding magnesium bromoacetylenides [3]. The structure of the compounds obtained was confirmed by elemental analysis and IR spectra. Thus in the IR spectra absorption bands in the 1280 cm^{-1} region (P=O) are always observed. For compounds with X = H, there are absorption bands in the 2065 cm^{-1} region,

TABLE 1. S-Ethynyl Esters of Thiophosphoric Acids $(RO)_2P(O)\cdot SC{\equiv}CX$

R	X	Yield, %	Bp, deg C (10^{-3} mm)	d_4^{20}	n_D^{20}	Empirical formula	Found/calculated, %		
							C	H	P
C_2H_5	H	21	72–76	1,2108	1,4683	$C_6H_{11}O_3PS$	37,87 / 37,11	5,78 / 5,67	15,21 / 15,97
C_3H_7	H	25	85–87	1,1273	1,4650	$C_8H_{15}O_3PS$	43,24 / 43,24	6,47 / 6,75	13,66 / 13,36
C_4H_9	H	20	98–100	1,0337	1,4580	$C_{10}H_{19}O_3PS$	48,27 / 48,00	7,26 / 7,61	12,36 / 12,42
C_2H_5	C_4H_9	30	100–104	1,0750	1,4688	$C_{10}H_{19}O_3PS$	47,60 / 48,00	7,67 / 7,61	12,15 / 12,42
C_2H_5	$CH_2SC_2H_5$	22	105–108	1,1639	1,4912	$C_9H_{17}O_3PS_2$	40,02 / 40,30	6,67 / 6,34	11,43 / 11,58
C_2H_5	$CH_2SC_4H_9$	19	–	1,1925	1,5150	$C_{11}H_{21}O_3PS_2$	44,68 / 44,59	6,38 / 7,09	10,73 / 10,47
C_2H_5	$S(O)P(OC_2H_5)_2$	18	–	1,1344	1,4892	$C_{14}H_{28}O_6P_2S_2$	40,17 / 40,19	6,69 / 6,69	14,94 / 14,83

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TABLE 2. Anticholine Esterase Activity of OPI $(RO)_2P(O)SYX$ (k_{II}, liter/mole·min)

Compound	R	Y	X	Source of enzyme					
				human erythrocytes	horse blood serum	brain of mice	black sugar beet aphid	cobweb mite	head of house fly
(I)	C_2H_5	C≡C	H	$2{,}3\cdot10^7$	$5{,}6\cdot10^7$	$2{,}2\cdot10^7$	$1{,}4\cdot10^7$	$4{,}8\cdot10^7$	$3{,}0\cdot10^8$
	C_3H_7	The same	H	$1{,}5\cdot10^7$	$4{,}0\cdot10^8$	$1{,}4\cdot10^6$	–	–	–
	C_4H_9	»	H	$6{,}2\cdot10^5$	$5{,}7\cdot10^7$	$1{,}4\cdot10^6$	–	–	–
	C_2H_5	»	C_4H_9	$9{,}5\cdot10^6$	$1{,}0\cdot10^8$	$7{,}1\cdot10^6$	$4{,}6\cdot10^7$	$7{,}0\cdot10^7$	$1{,}5\cdot10^9$
	C_2H_5	»	$CH_2SC_2H_5$	$7{,}3\cdot10^6$	$2{,}1\cdot10^8$	$5{,}0\cdot10^6$	$5{,}0\cdot10^7$	$5{,}0\cdot10^7$	$5{,}2\cdot10^8$
	C_2H_5	»	$CH_2SC_4H_9$	$2{,}3\cdot10^7$	$1{,}0\cdot10^7$	$1{,}0\cdot10^7$	$7{,}5\cdot10^7$	$2{,}0\cdot10^7$	$1{,}0\cdot10^9$
(II)	C_2H_5	CH_2CH_2	H	$2{,}7\cdot10^2$	$2{,}7\cdot10^2$	$<1{,}0\cdot10^2$	$1{,}0\cdot10^2$	$1{,}0\cdot10^2$	$1{,}1\cdot10^2$
	C_2H_5	CH_2CH_2	C_4H_9	$3{,}8\cdot10^3$	$3{,}3\cdot10^4$	$1{,}0\cdot10^3$	$7{,}0\cdot10^3$	$0{,}8\cdot10^3$	$8{,}9\cdot10^3$
	C_2H_5	CH_2CH_2	$CH_2SC_2H_5$	$1{,}8\cdot10^3$	$4{,}0\cdot10^3$	$7{,}0\cdot10^2$	$<1{,}0\cdot10^2$	$<1{,}0\cdot10^3$	$9{,}6\cdot10^3$
(III)	C_2H_5	$CH_2C\equiv C$	$CH_2SC_2H_5$	$2{,}1\cdot10^5$	$2{,}9\cdot10^6$	$1{,}1\cdot10^5$	$1{,}5\cdot10^4$	$6{,}5\cdot10^5$	$3{,}2\cdot10^6$
(IV)	C_2H_5	$CH_2CH_2CH_2$	$CH_2SC_2H_5$	$8{,}0\cdot10^2$	$4{,}8\cdot10^4$	$2{,}3\cdot10^3$	$2{,}2\cdot10^3$	$8{,}0\cdot10^2$	$5{,}0\cdot10^3$

and those with X = C_4H_9 and $CH_2SC_2H_5$, at 2210 cm^{-1} (C≡C). The stretching vibration bands of the H–C≡ groups are noticeably broadened and shifted into the low-frequency region. Their doublet character, a maximum with a frequency of 3220 and 3290 cm^{-1} differs distinctly. The low-frequency band should be apparently related to the intramolecular hydrogen bond P=O... H–C≡.

For comparison, we synthesized certain saturated analogs of the S-ethynyl thioesters, i.e., S-alkyl and S-ethylthiopropyl esters of O,O-diethylthiophosphoric acid (II).

$$(C_2H_5O)_2P(S)OK + BrCH_2CH_2X \rightarrow (C_2H_5O)_2P(O)SCH_2CH_2X$$
(II)

where X = H, C_4H_9, and $CH_2SC_2H_5$.

To estimate the anticholine esterase activity of the compounds obtained, we determined the k_{II} with respect to acetylcholine esterase (ACE) of human erythrocytes, the ACE from the brain of mice, butylcholine esterase (BuCE) from the horse blood serum, the ACE from heads of house flies (*Musca domestica*), the CE from black sugar beet aphids (*Apchis fabae* Koch.), and the CE from cobweb mites (*R. urticae* Koch.).

Table 2 shows that all the ethynyl esters of thiophosphoric acids are characterized by high anticholine esterase activity (k_{II} $10^6 - 10^9$ liter/mole·min). Among the enzymes from warm-blooded animals, BuCE is distinguished by the highest sensitivity toward these compounds, and among the arthropoda enzymes – the ACE from heads of house flies.

A replacement of hydrogen atom in the alkylthio residue of compound (I) by a butyl group leads to an inappreciable increase in the anticholine esterase activity with respect to BuCE and ACE from heads to house flies (two- and fivefold, respectively), while with respect to other enzymes the k_{II} values do not change appreciably. Introduction of the S atom into the γ-position of the alkylthiol radical also does not lead appreciably to the appearance of a selectivity of action of the S-ethynyl esters of thiophosphoric acid with respect to CE of different origins.

It should be noted that OPI studied containing an acetylenic bond in the α-position of the thioester group have a much higher anticholine esterase activity than the analogous S-butynyl esters of thiophosphoric acid with an acetylenic bond in the β position [2]. For comparison, in Table 2, values of k_{II} are given for O,O-diethyl S-(ω-ethylthiobut-2-ynyl) thiophosphate (III) and its saturated analog (IV).

When the anticholine esterase activity of compounds (I) is compared with that of their saturated analogs (II) it follows that the introduction of the acetylenic bonds always lead to a sharp increase in the ability of the OPI to inhibit the choline esterase. Thus, Table 3 shows that the values of the acetylenic effect (α) reach an order of magnitude of 10^3–10^6, while in analogous compounds containing the acetylenic bond in the β-position (the (III)/(IV) pair), they vary within one to two orders of magnitude.

The very high acetylenic effect of the substituted ethynyl esters of thiophosphoric acid can be explained by both the high electrophilic phosphorylating ability of these compounds, and by the optimal position of the acetylenic bond with respect to that portion of the ac-

TABLE 3. Values of α-k_{II} (Y=C≡C)/k_{II} (Y=CH_2CH_2) of $(C_2H_5O)_2P(O)SYX$

Pairs of compounds	X	Source of enzyme					
		human erythrocyte	horse blood serum	brain of mice	black sugar beet aphid	cobweb mite	head of mice
(I)/(II)	H	$1\cdot10^5$	$2\cdot10^5$	$>2{,}2\cdot10^5$	$>1{,}4\cdot10^5$	$>4{,}8\cdot10^5$	$3\cdot10^6$
	C_4H_9	$2{,}5\cdot10^3$	$3\cdot10^3$	$>7{,}1\cdot10^3$	$7{,}0\cdot10^3$	$8{,}8\cdot10^4$	$1{,}7\cdot10^5$
	$CH_2SC_2H_5$	$4\cdot10^3$	$5{,}2\cdot10^3$	$7{,}1\cdot10^3$	$>5\cdot10^5$	$>5{,}0\cdot10^4$	$5{,}5\cdot10^4$
(III)/(IV) *	$CH_2SC_2H_5$	$2{,}4\cdot10^2$	60	48	6,8	$8\cdot10^2$	$6{,}4\cdot10^2$

* $\alpha = k_{II}(Y=CH_2C\equiv C)/k_{II}(Y=CH_2CH_2CH_2)$.

tive center of CE having an affinity to the acetylenic group. It is possible that these two factors act simultaneously.

EXPERIMENTAL

S-Ethynyl Esters of Thiophosphoric Acids (I). A 0.1-mole portion of XC≡CH in 30 ml of THF was added at 20°C in the course of 1 h to a solution of EtMgBr (prepared from 0.11 mole of EtBr, 0.11 mole of Mg in 50 ml of THF), and the mixture was left to stand for another 1 h at 20°C. The reaction mixture was added in the course of 1 h to 0.1 mole of diethoxyphosphorylsulfenyl chloride in 100 ml of ether at −20°C. The temperature was allowed to rise gradually to 20°C, and the mixture was left to stand for 1 h. It was then decomposed by 5% HCl at 0°C. The organic layer was separated and washed with a saturated $NaHCO_3$ solution, followed by ice water, and dried over Na_2SO_4. The solvent was evaporated and the residue distilled in vacuo. The physical constants, yields and data of elemental analysis are listed in Table 1.

S-Alkyl Esters of Diethylthiophosphoric Acid (II). $BrCH_2CH_2X$ was added to 0.1 mole of potassium salt of diethylthiophosphoric acid in 100 ml of alcohol. The mixture was boiled for 4 h, filtered, and solvent evaporated, and the residue distilled in vacuo. Given are: X, bp, °C (mm Hg), n_D^{20}; H, 61-62 (0.8), 1.4563 (cf. [4]: 78-79 (1)), 1.4570); C_4H_9, 102-103 (0.8), 1.4583 (cf [5]: 125 (1)); $CH_2SC_2H_5$, —, 1.4920.

Determination of Anticholine Esterase Activity. As the enzyme sources we used the ACE of human erythrocytes and BuCE from horse blood serum. (The partially purified preparations were produced at the Perm Scientific Research Institute of Vaccines and Blood Sera), the ACE from the brain of mice (brain homogenate), the CE from sugar-beet aphids and CE of cobweb mites (homogenate of whole animals). The CE activity was determined by the Ellman method [6] using acetylthiocholine or propionylthiocholine (for CE from cobweb mites) as the substrate in the concentration of $1\cdot10^{-3}$ M at 30°C (for the determination of the activity of CE from aphids at 25°C). The anticholine esterase activity of OPI was expressed in the form of a bimolecular rate constant (k_{II}, liter/mole·min) of the enzyme inhibition.

CONCLUSIONS

Introduction of acetylenic bond into the α-position of the thioester group of thiophosphoric acids leads to a sharp intensification of the ability of these compounds to inhibit choline esterase of different origins.

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