# SYNTHESIS OF ORGANOPHOSPHORUS DERIVATIVES OF LUPININE AND EPILUPININE AND THEIR INTERACTION WITH CHOLINESTERASES

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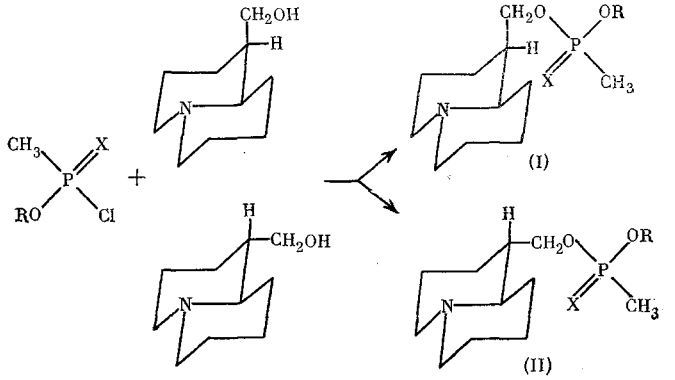
The search for novel, highly selective insecticides and acaricides is associated with studies of the relationship between the structures of the compounds and their anticholinesterase activity [1, 2].

In the search for highly specific cholinesterase inhibotors, we have synthesized some organophosphorus derivatives of lupinine [I] and epilupinine (II) and examined their anticholinesterase activity towards human erythrocyte acetylcholinesterase (ACE) and horse serum butyrylcholinesterase (BuCE). These compounds were synthesized as follows:




where R = $C_2H_5$, $C_3H_7$, $C_4H_9$, and $C_5H_{11}$; X = O or S. The properties of these compounds are given in Tables 1 and 2. The IR spectrum of O-ethyl-O-lupinanyl methylphosphonate contains absorption characteristic of the functional groups ($\nu$, $cm^{-1}$): 1277 (P=O), 1440 ($POC_2H_5$), 1070 (>N—), 1472 ($CH_3$), 1385 ($CH_2$) and trans-quinolizidine at 2685-2810. The IR spectrum of O-ethyl-O-lupinanyl methylthiophosphonate differs from this in showing absorption for the P=S group at 805 and 660 $cm^{-1}$.

The PMR spectrum of O-propyl-O-epilupinanyl methylthiophosphonate is in accordance with its structure. The complex signal at 3.7-4.3 ppm results from the superposition of the signals for the two $OCH_2$ groups. The asymmetrical doublet at 2.68 ppm is assigned to the two equatorial $\alpha$-protons of the quinolizidine ring, the doublet at 1.64 ppm to the P—$CH_3$ protons ($J_{H-P}$ = 15.2 Hz), and the triplet at 0.9 ppm to the protons of the terminal methyl group of the O-propyl radical. The signals for the remaining protons resonate at 1.0-2.0 ppm.

The anticholinesterase properties of the compounds were examined using ACE (EC 1.1.7) of specific activity 2 U/mg and BuCE (EC 1:1:8) of specific activity 28 U/mg (obtained from the Perm Research Institute for Vaccines and Sera). All the test compounds were reversible competitive inhibitors of both types of cholinesterase. The anticholinesterase activity was measured by the reversible inhibition constants $K_i$, which were found by the Lineweaver—Burk method [3]. Data for the reversible inhibitory activity towards ACE and BuCE with respect to structure are given in Table 3.

As will be seen from Table 3, in the interaction of O-alkyl-O-lupinanyl methylthiophosphonates with ACE, lengthening the alkyl radical from ethyl to pentyl results in an increase in reversible inhibitory activity by a factor of 4.4. Similar changes are seen in the case of O-alkyl-O-lupinanyl methylthiophosphonates. In the in-

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TABLE 1. Constants of O-Alkyl-O-lupinaryl Methylphosphorates and O-Alkyl-O-lupinanyl Methylthio phosphonates

| R | X | $n_D^{20}$ | $d_4^{20}$ | MR found | MR calc. | ΔMR | $[\alpha]_D$, benzene | Found, % C | Found, % H | Found, % P | Calculated, % C | Calculated, % H | Calculated, % P | Yield, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_2H_5$ | O | 1,4836 | 1,0836 | 72,50 | 72,64 | 0,14 | −43,80 | 56,25 | 8,93 | 10,82 | 56,73 | 8,72 | 11,27 | 30 |
| $C_3H_7$ | O | 1,4762 | 1,0700 | 76,75 | 77,25 | 0,70 | | 58,46 | 9,05 | 10,27 | 58,13 | 8,99 | 10,72 | 46 |
| $C_4H_9$ | O | 1,4800 | 1,0596 | 81,34 | 81,87 | 0,53 | | 60,08 | 9,44 | 10,12 | 59,40 | 9,24 | 10,23 | 35 |
| $C_5H_{11}$ | O | 1,4783 | 1,0500 | 85,65 | 86,36 | 0,71 | −42,72 | 61,20 | 9,68 | 9,47 | 60,56 | 9,46 | 9,78 | 63 |
| $C_2H_5$ | S | 1,5060 | 1,0847 | 79,68 | 80,13 | 0,45 | −33,0 | 53,47 | 8,51 | 10,53 | 53,61 | 8,24 | 10,65 | 49 |
| $C_3H_7$ * | S | 1,5113 | 1,0841 | 84,63 | 84,74 | 0,11 | | 54,91 | 8,87 | 10,23 | 55,08 | 8,52 | 10,16 | 50 |
| $C_4H_9$ | S | 1,5050 | 1,0733 | 89,12 | 89,37 | 0,25 | | 56,13 | 8,65 | 9,47 | 56,42 | 8,78 | 9,72 | 61 |
| $C_5H_{11}$ * | S | 1,5053 | 1,0565 | 93,12 | 93,98 | 0,86 | −28,92 | 57,97 | 8,90 | 9,22 | 57,66 | 9,01 | 9,31 | 62 |

*From research of A. A. Sadykova.

TABLE 2. Physicochemical Constants of O-Alkyl-O-epilupinaryl Methylphosphonates and O-Alkyl-O-epilupinaryl Methylthio phosphonates

| R | X | $n_D^{20}$ | $d_4^{20}$ | MR found | MR calc. | ΔMR | $[\alpha]_D$, benzene | Found, % C | Found, % H | Found, % P | Calculated, % C | Calculated, % H | Calculated, % P | Yield, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C_2H_5$ | O | 1,4860 | 1,0661 | 72,06 | 72,06 | 0,52 | 14,7 | 56,45 | 8,38 | 11,56 | 56,73 | 8,72 | 11,27 | 32,2 |
| $C_3H_7$ | O | 1,4802 | 1,0553 | 77,80 | 77,25 | 0,55 | – | 57,93 | 9,01 | 10,83 | 58,13 | 8,99 | 10,72 | 30 |
| $C_4H_9$ | O | 1,4776 | 1,0502 | 81,51 | 81,87 | 0,36 | – | 59,64 | 9,35 | 10,52 | 59,40 | 9,24 | 10,23 | 28 |
| $C_5H_{11}$ | O | 1,4658 | 1,0179 | 86,20 | 86,36 | 0,16 | 20,9 | 60,41 | 9,25 | 9,93 | 60,56 | 9,46 | 9,78 | 25 |
| $C_2H_5$ | S | 1,5110 | 1,0770 | 79,68 | 80,13 | 0,45 | 2,6 | 53,87 | 8,53 | 10,15 | 53,61 | 8,24 | 10,65 | 37,8 |
| $C_3H_7$ | S | 1,5016 | 1,0654 | 84,42 | 84,74 | 0,32 | – | 55,49 | 8,27 | 10,56 | 55,08 | 8,52 | 10,16 | 40 |
| $C_4H_9$ | S | 1,4959 | 1,0477 | 88,94 | 89,37 | 0,43 | – | 56,87 | 8,53 | 9,93 | 56,42 | 8,78 | 9,72 | 40 |
| $C_5H_{11}$ | S | 1,4939 | 1,0370 | 93,31 | 93,98 | 0,67 | 9,2 | 57,81 | 9,26 | 9,47 | 57,66 | 9,01 | 9,31 | 35,5 |

TABLE 3. Reversible Inhibition Constants ($K_i$) for O-Alkyl-O-lupinanyl Methylphosphonates (Io), O-Alkyl-O-lupinanyl Methylthiophosphonates (Is), O-Alkyl-O-epilupanyl Methylphosphonates (IIo), and O-Alkyl-O-epilupinanyl Methylthiophosphonates (IIs) with ACE and BuCE

| R | X | $K_i$ (M) | | ACE(I) / BuCE(II) | $K_i$ (M) | | ACE(II) / BuCE(II) | ACE(IIo) / ACE(IIs) |
|---|---|---|---|---|---|---|---|---|
| | | ACE | BuCE | | ACE | BuCE | | |
| $C_2H_5$ | O | $7{,}5\cdot10^{-4}$ | $5{,}2\cdot10^{-4}$ | 1,5 | $7{,}2\cdot10^{-4}$ | $2{,}8\cdot10^{-4}$ | 2,5 | 3,3 |
| $C_3H_7$ | O | $1{,}8\cdot10^{-4}$ | $1{,}3\cdot10^{-4}$ | 1,3 | $9{,}8\cdot10^{-4}$ | $4{,}7\cdot10^{-4}$ | 2,0 | 5 |
| $C_4H_9$ | O | $1{,}7\cdot10^{-4}$ | $8{,}5\cdot10^{-5}$ | 2,0 | $1{,}6\cdot10^{-5}$ | $1{,}8\cdot10^{-4}$ | 0,1 | 1 |
| $C_5H_{11}$ | O | $1{,}7\cdot10^{-4}$ | $1{,}6\cdot10^{-5}$ | 10 | $4{,}9\cdot10^{-5}$ | $1{,}0\cdot10^{-4}$ | 0,49 | 24,5 |
| $C_2H_5$ | S | $2{,}0\cdot10^{-4}$ | $6{,}6\cdot10^{-5}$ | 7,0 | $2{,}2\cdot10^{-4}$ | $4{,}0\cdot10^{-5}$ | 5,5 | 3,75 |
| $C_3H_7$ | S | $1{,}5\cdot10^{-4}$ | $1{,}6\cdot10^{-5}$ | 1,0 | $9{,}0\cdot10^{-5}$ | $4{,}6\cdot10^{-6}$ | 19,5 | 1,2 |
| $C_4H_9$ | S | $1{,}8\cdot10^{-5}$ | $3{,}7\cdot10^{-6}$ | 5,0 | $7{,}9\cdot10^{-6}$ | $6{,}7\cdot10^{-6}$ | 1,2 | 9,4 |
| $C_5H_{11}$ | S | $3{,}8\cdot10^{-5}$ | $2{,}5\cdot10^{-6}$ | 17 | $2{,}0\cdot10^{-6}$ | $5{,}8\cdot10^{-6}$ | 0,35 | 4,5 |

teraction of these compounds with BuCE, lengthening the alkyl radical increases the reversible inhibitory activity of O-alkyl-O-lupinanyl methylthiophosphonates and O-alkyl-O-lupinanyl methylthiophosphonates by factors of 32.5 and 26.4, respectively. All these lupinine derivatives showed specificity, even if only slight, towards BuCE. This effect was seen most clearly in the pentyl compounds.

Examination of the relationship between the $K_i$ values and the lengthening of the alkyl chain from $C_2H_5$ to $C_5H_{11}$ in the interaction of epilupinine methylphosphonates and methylthiophosphonates shows that the reversible inhibitory activity of the methylphosphonates is increased by a factor of 15, and of the methylthiophosphonates, by 110. In interaction with BuCE, the change from $C_2H_5$ to $C_5H_{11}$ results in only a small increase in reversible inhibitory activity. Lenghening the alkyl radical reduces the specificity towards ACE.

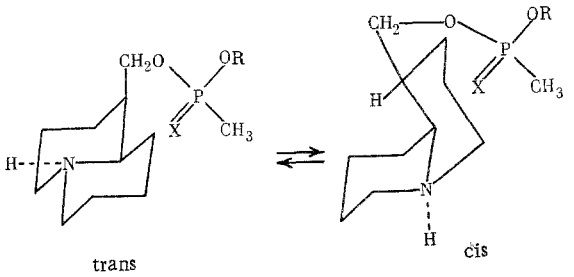
Comparing the $K_i$ values for O-alkyl-O-lupinanyl methylphosphonates with those for the corresponding methylthiophosphonates, and those for O-alkyl-O-epilupinanyl methylphosphonates with those for their thiono-analogs, it will be seen that changing from the methylphosphonate to the methylthiophosphonate results in an increase in reversible inhibitory activity towards both types of enzyme. It is noteworthy that replacement of the P=O group by P=S in the inhibitor molecule results in a decrease in the angle of rotation both of the lupinine and epilupinine compounds by an average of 12°, which undoubtedly affects the reversible inhibitory activity of these compounds.

Comparison of the $K_i$ values for the O-alkyl-O-lupinanyl methylphosphonates with those for O-alkyl-O-epilupinanyl methylphosphonates, and of those for O-alkyl-O-epilupinanyl methylthiophosphonates with those for O-alkyl-O-lupinanyl methylthiophosphonates, shows (Table 3) that the epilupinine derivatives show specificity towards ACE, and that this specificity is greatest in the thiono-compounds.

In contrast to their behavior with ACE, these inhibitors behave differently towards BuCE. For instance, comparison of the $K_i$ values for the lupinine derivatives with those for the epilupinine derivatives shows that inhibitors in which the lupinine residue is present are specific towards BuCE. This difference in the effects of lupinine and epilupinine inhibitors towards ACE and BuCE may be rationalized as follows: lupinine is known to differ from epilupinine not only structurally, but also in its conformational state. Thus, in lupinine the hydroxymethyl group is axially oriented. Under some conditions, the trans-quinolizidine ring can undergo inversion. It has been found [4, 5] that lupinine exists in solution in a labile conformational state as a result of inversion at the nitrogen atom. Protonation of the nitrogen results in the fusion of the quinolizidine ring changing from trans- to cis-, and the axial hydroxymethyl group changes to equatorial. The trans-quinolizidine ring in epilupinine does not undergo a similar change on protonation of the nitrogen atom.

The display of specificity in epilupinine derivatives towards ACE appears to be due to the stability of the conformational state of the trans-quinolizidine system and the greater tendency of the epilupinine residue to undergo sorption at the active site of ACE.

The specificity of action of lupinine derivatives towards BuCE, on the other hand, is evidently due to the conformational lability of the quinolizidine ring. Thus, when the anticholinesterase activity changes (pH 7.5), protonation of the nitrogen atom in lupinine occurs with a corresponding change in conformation of the trans-quinolizidine ring to the cis-form. It may be that the cis-fused quinolizine ring, with an equatorial phosphoryl group, is more prone to sorption at the active site of BuCE. When this occurs, the dynamic equilibrium between the trans- and cis-conformers is shifted to the right:



trans cis

With respect to the increase in reversible anticholinesterase activity on replacement of the P=O by the P=S group, especially towards ACE, this could be due to the greater tendency of methylthiophosphonates to undergo hydrophobic sorption as a result of the lower electronegativity of sulfur as compared with oxygen in the methylphosphonates.

## EXPERIMENTAL

IR spectra were obtained on a Specord 75-IR instrument in vaseline lubrication, and PMR spectra on a XL-200 in $CCl_4$.

The O-alkyl methylphosphonyl chlorides and O-alkyl methylthiophosphonyl chlorides were obtained as described in [6-8], and the epimerization of lupinine was carried out as in [9]. Anticholinesterase activity was measured as described in [3].

O-Ethyl-O-lupinanyl methylphosphonate. To 14.25 g (0.1 mole) of O-ethyl chloromethylphosphonate in dry ether was added with stirring and cooling a mixture of 16.9 g (0.1 mole) of lupinine and 10.1 g (0.1 mole) of $Et_3N$, and the mixture was stirred for 3 h and kept overnight. The progress of the reaction was followed by TLC (grade II alumina) using the system benzene–ether–methanol, 10:5:2. The solid was filtered off, washed with dry ether, the extract dried over anhyd. sodium sulfate, and the ether distilled off. The residue was purified by chromatography on grade II alumina, with ether as eluant.

Obtained similarly were the O-alkyl-O-lupinanyl methylphosphonates, O-alkyl-O-lupinanyl methylthiophosphonates, O-alkyl-O-epilupinanyl methylthiophosphonates.

## CONCLUSIONS

Some esters of O-alkyl methylphosphonic acid and thioesters of O-alkyl methylthiophosphonic acid derived from the alkaloids lupinine and epilupinine have been prepared. These were found to be reversible competitive inhibitors of acetylcholinesterase and butyrylcholinesterase.

## LITERATURE CITED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