Toxicity, Acetylcholinesterase Inhibition and Metabolism of Enolic Phosphate Esters Resembling the Insecticide, Phosdrin

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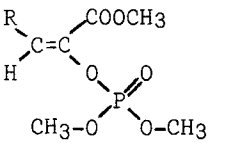
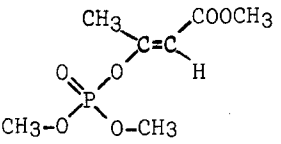
During the course of study of a series of homologs of P-enolpyruvate (WOODS, et al. 1970, 1972), three compounds were synthesized (I, II, III) that show striking similiarities to the insecticide, Phosdrin (IV).





cis-Phosdrin (IV)

R=CH_3-=I
R=$CH_3CH_2CH_2$-=II
R=$CH_3(CH_2)_5CH_2$-=III

Phosdrin and its derivatives can exist as either the *cis* or *trans* isomers which differ in their toxicities. The LD_{50} of the *cis* form in mice is near 2.0 mg/Kg, while the *trans* isomer has an LD_{50} of approximately 45.0 mg/Kg (SPENCER, 1961).

The extreme toxicity of this class of organophosphorous compounds toward both mammals and insects prompted us to investigate the toxicity of our compounds (I, II, III). Since the toxicity of Phosdrin and similar compounds is principally due to the inhibition of acetylcholine esterases and the consequences of this inhibition, we also investigated the anticholinesterase activity of our compounds. CASIDA, et al. (1958) reported detoxication of Phosdrin in mammalian tissue to dimethylphosphate as indicated by P^{32} tracer studies. Liver tissue appeared to be the major area of detoxication. More recently, MORELLO et al. (1967a, 1967b, 1968) have reported metabolism of *cis*-Phosdrin to *cis*-dismethyl Phosdrin while *trans*-Phosdrin was degraded to dimethylphosphate. The purpose of our investigation was to determine the toxicity and the metabolism of compounds I and II in rat liver homogenates.

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MATERIALS AND METHODS

Synthesis

Compounds I, II and III were prepared from the respective β-bromo-α-ketoacid methyl ester by reaction with an equimolar quantity of $(CH_3O)_3P$ in ether. The β-bromo-α-ketoacid methyl esters were prepared from the α-ketoacid methyl ester by reaction with bromine, (WOODS, et al. 1970).

The α-ketoacid methyl esters were synthesized from free α-ketoacids by esterification in absolute methanol-benzene with a catalytic amount of *p*-toluenesulfonic acid. Water from the esterification was removed by forming an azeotrope with benzene. The esters were finally isolated by vacuum distillation. NMR, IR and elemental analyses gave the expected data substantiating the identity of all intermediates and compounds, I, II and III.

Toxicity

The LD_{50} for compounds I and II were determined using ten 200-250g black, white or buff hooded rats (male and female) for each compound. Compounds I and II were dissolved in 0.9% sodium chloride solution and injected intraperitoneally. Commercial *cis, trans*-Phosdrin (65:35) was used as a control. Mortalities were recorded after 24 hours, although generally no changes were observed after 1-2 hours.

Metabolism Study

The procedures of MORELLO, et al. (1967a) were used with the following modifications. Rat liver homogenates were prepared in 0.9% NaCl. Nonenzymatic controls were used utilizing heat inactivated liver homogenates which were treated in a manner identical to the unheated samples. The homologs and Phosdrin were incubated for one and two hours at 37° at pH 7.5 (Tris-HCl buffer, 0.05M). After incubation the reaction was terminated with 6N HCl and extracted with chloroform. Both the chloroform and aqueous fractions were concentrated under vacuum. The sample was further concentrated and purified by descending chromatography using 95% acetone (aqueous) as the eluting solvent.

The final concentrates containing the metabolites, were chromatographed on Whatman Number 1 and 4 paper using 5:4:1 mixture of isopropyl alcohol, water, and ammonium hydroxide as the solvent. Phosphorous-containing spots were visualized using molybdic acid spray.

Acetylcholinesterase Determinations

The procedure used was essentially as reported by METCALF (1951). This method measures the amount of acetylcholine remaining after incubation at 37° for 30 minutes. Bovine erythrocyte acetylcholinesterase and acetylcholine chloride were used as the enzyme source and the substrate.

The pI_{50} values were determined for compounds I and II. Compound III was found to be insoluble at a concentration of 10^{-4}M, in phosphate buffer. Since I and II were shown to be ineffective inhibitors at 10^{-4}, no attempt was made to determine the pI_{50} for III. pI_{50} values were computed by plotting log % activity remaining *versus* the inhibitor concentration and represent the average of three separate determinations.

RESULTS AND DISCUSSION

Toxicity

Compounds I and II gave no deaths in 10 animals at doses up to 250 mg/Kg. Toxicity studies were discontinued at 250 mg/Kg of compound I and II. Due to the insolubility of III no toxicity studies were performed on this compound.

Metabolism

Compounds I and II were found to be metabolized to dimethyphosphate. Inorganic phosphate also appeared in the aqueous extract of the metabolism mixtures and probably originated in the liver extract. Rf values were used to verify the identity of the chromatographed metabolites. The metabolites were also co-chromatographed with known dimethylphosphate to verify the identity.

Acetylcholinesterase Inhibition

Table I summarizes the effect of compounds I and II on bovine erythrocyte acetylcholinesterase. The pI_{50} values for previously reported *cis* and *trans* Phosdrin are also listed. There are 2-3 orders of magnitude difference in the pI_{50} of compounds I and II and the Phosdrin isomers.

O'BRIEN (1963) has suggested that the effectiveness of a particular organophosphate ester depends upon: (1) The electrophillic character of the phosphorous group which interacts with the esteric site of the enzyme and (2) on the presence of a group that interacts electrostatically or through noncoulombic forces (VAN der VAALS or hydrophobic) with the anionic site. He concluded that the bonding sites should be at a distance equal to the distance between the esteric and anionic sites in cholinesterase, the distance being 4.5-5.9 Å.

TABLE I

Inhibition of Bovine Erythrocyte
Acetylcholinesterase by Enol Phosphate Esters (I, II)

Compound	I_{50}(M)	pI_{50}
I	2.5×10^{-3}	2.60
II	5.2×10^{-3}	2.28
Phosdrin		
cis	1.78×10^{-7}	6.75[1]
trans	1.78×10^{-5}	4.75[1]

MORELLO, et al. (1967a) have reported the distance between the phosphate and carbomethoxy ester group of Phosdrin (See Table II). They concluded that the distance between the phosphate and the carbomethoxy ester group of *trans*-phosdrin is too short for maximum bonding to occur at both sites. This is the case of our homologs (I and II) which have the phosphate and the carbomethoxy ester group separated by a distance of approximately 2.5Å. As indicated by the toxicity studies and acetylcholinesterase inhibition, the homologs (I and II) both have essentially the same toxicity and pI_{50}. This would be expected since both compounds are identical except for the alkyl groups on the vinyl carbon (C-3).

TABLE II

Distance between the Carbomethoxy Ester
and the Phosphate Groups of Phosdrin
and The Enol Phosphates (I, II)

Compound	Average Distance (Å)
I	2.5
II	2.5
Phosdrin	
cis	4.75[1]
trans	3.3[1]

[1]Values reported by MORELLO, et al. (1967a)

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