Electronic Structure and Biochemical Activities in Diethyl Phenyl Phosphates

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In the recent fifteen years thousands of organo phosphorus compounds have been found to be endowed with effective insecticidal properties and some of them—TEPP, parathion, paraoxon and so forth—are now in practical use[1]. It become apparent that the toxicity of these compounds to insect or to human being is mainly related to the reaction with the cholinesterase enzyme system in the body[2-5].

Diethyl substituted phenyl phosphates, which are treated in the present paper, have been tested in detail of their toxicities to insects, their effciencies of cholinesterase inhibition[3-5] and their hydrolysis constants[5-7], and each of the three has been found to be in line with the other[1,5,7]: phosphates are more toxic when their inhibiting activities are larger and when their hydrolysis constants are larger.

Diethyl phenyl phosphates are bound by a covalent bond to the esteratic site of cholinesterase in an irreversible phosphorylation, this inhibiting the hydrolysis of acetyl choline in a normal biochemical cycle. The enzyme-inhibiting mechanisms are proposed by Aldridge

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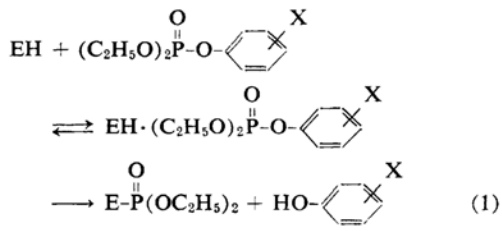
et al.[7-10] to be such as represented by the following scheme:



(1)

where EH is the enzyme, and the second step is assumed to be rate-determining. It was often said that the initial stage of the step is the attack of the electron-releasing, that is, the nucleophilic site or atom of cholinesterase on the P–O bond of phosphates, and this mechanism seems to be analogous to that of the alkali hydrolysis of the phosphates[1] and that the enzyme-inhibitory activity of the phosphate is, therefore, related to the lability of P–O bond[5,7]. And furthermore Aldridge estimated the strength of the bond by the ionization constant of the hydrolysis product[7]; the esters derived from strongly acidic phenols are highly toxic and vice versa. According to his opinion, paraoxon is toxic because *p*-nitrophenol is a strong acid.

In contrast with phosphates, it is interesting that highly purified parathion and its homologues—diethyl substituted phenyl thionophosphates— are little active as inhibitors of enzyme systems. They are oxidized in the tissue to the corresponding phosphates, which attack the cholinesterase, showing the poisoning action in vivo experiments[2,11,12].

The present authors have established the frontier electron theory, one of the quantum mechanical theories of chemical reactivity, of conjugated molecules, and found that the superdelocalizability, the index of reactivity in the theory, represents the experimental results better than those in other theories[13-15]. The theory has been successfully applied to biochemical problems as well, for instance, the carcinogenic activity of condensed aromatic hydrocarbons[16] and azo dyes[17], the plant growth activity of substituted benzoic acids[18], the nicotine-like activity of choline phenyl ethers[19] and the fungicidal activity of quinoline-*N*-oxides[20]. It is noteworthy, as theoretically indicated by the present authors, that these activities are directly related to the interaction of the conjugated molecule with a nucleophilic (electron releasing) atom or group of the tissue; and in this connection a discussion will be given in a later section.

Superdelocalizability on the *r*th atom of phenyl phosphates that is to be used in this paper is that for a nucleophilic reaction (the reagent attacking phosphates has been considered nucleophilic in nature[1,8]) and has the following form:

$$S_r^{(N)} = 2\sum_j^{\text{unocc}} \frac{(C_r^j)^2}{-\lambda_j} \qquad (2)$$

where C_r^j is the coefficient of the *r*th atomic orbital in the *j*th molecular orbital, λ_j is the coefficient of its energy $\varepsilon_j = \alpha + \lambda_j\beta$, α and β are the standard coulomb and resonance integrals, and the summation covers unoccupied orbitals. The larger its magnitude is, the more reactive the molecule is. The phosphorus atom is considered to offer one orbital and one electron conjugable with other atoms, as has been discussed in our previous paper on ATP[21]. The coulomb integral of the atom X and the resonance integral between the atoms X and Y are written as $\alpha + a_X\beta$ and $l\beta$, respectively; and in Table I are listed the energy parameters used in the paper, which are not so different from those in previous papers[17,18,21],*.

Results and Discussion

We calculated the superdelocalizability and the electron density on various positions of fifteen diethyl substituted phenyl phosphates, assuming that in the enzyme-inhibition the intermediate complex formation does not affect the electronic structure, and comparing with experimental results, found that the super-

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* The energy parameter of the sulfur atom seems unsettled yet. Here we adopted $a_{=S} = 0.8$ so that the difference $\Delta a = a_{=O} - a_{=S}$ comes to 1.2 in accordance with Pullmans'[22].
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TABLE I. ENERGY PARAMETERS EMPLOYED IN THE CALCULATION*

Phosphate part	$a_P=-1$		
	$a_{=O}=2$	$l_{P=O}=1$	
	$a_{=S}=0.8$	$l_{P=S}=0.85$	
	$a_{OC_2H_5}=2$	$l_{P-OC_2H_5}=0.6$	
	$a_{-O-}=2$	$l_{P-O-}=0.6$	
Substituent			
Nitro	$a_N=1$	$l_{C'-N}=1$	$a_{C'}=0.2$
	$a_O=1.5$	$l_{N-O}=1$	
Formyl	$a_{=O}=2$	$l_{C=O}=\sqrt{2}$	$a_C=0.2$
Cyano	$a_{\equiv N}=1$	$l_{C\equiv N}=1$	$a_C=0.1$
Chloro	$a_{Cl}=2$	$l_{C-Cl}=0.8$	$a_{C'}=0.5$
Methyl	$a_{CH_3}=3$	$l_{C-CH_3}=0.7$	
Methoxy	$a_{OCH_3}=2$	$l_{C-OCH_3}=0.9$	
Dimethylamino	$a_{CH_3}=3$	$l_{CH_3-N}=0.7$	
	$a_N=1$	$l_{N-C'}=1$	$a_{C'}=0.1$

* C' means the carbon atom adjacent to the substituent.

TABLE II. SUPERDELOCALIZABILITY AND TOTAL π ELECTRON DENSITY ON PHOSPHORUS ATOM AND ALKALI HYDROLYSIS CONSTANT, CHOLINESTERASE INHIBITORY ACTIVITY AND INSECTICIDAL ACTIVITY OF DIETHYL SUBSTITUTED PHENYL PHOSPHATES

Substituents	$S_P^{(N)}$ Superdelocalizability	q_P Total π electron density	Alkali hydrolysis constant (min^{-1})*1	Median inhibitory molar concentration (fly-brain ChE)*1	Insecticidal activity*3
2,4-Dinitro	1.132	0.278	5.7×10^{-3}	3.0×10^{-9}	+
o-Nitro	1.120	0.279	(3×10^{-4})*2	5.0×10^{-8}	++
p-Nitro	1.119	0.279	2.7×10^{-4}	2.6×10^{-8}	+++
p-Formyl	1.106	0.279		1.5×10^{-7}	−
p-Cyano	1.106	0.280		1.3×10^{-7}	+++
2,4,6-Trichloro	1.100	0.280	7.9×10^{-5}	6.0×10^{-9}	++
2,4-Dichloro	1.100	0.280	4.8×10^{-5}	5.0×10^{-7}	+
o-Chloro	1.099	0.280	5.1×10^{-5}	2.0×10^{-5}	±
p-Chloro	1.099	0.280	3.2×10^{-5}	3.0×10^{-5}	±
Non	1.097	0.281	9.2×10^{-5}	1.0×10^{-8}	−
m-Nitro	1.097	0.281	9.8×10^{-5}	5.0×10^{-8}	++
p-Methyl	1.097	0.281		1.0×10^{-3}	−
m-Methoxy	1.096	0.281	8.9×10^{-6}	1.3×10^{-4}	−
p-Methoxy	1.096	0.281		1.0×10^{-3}	−
p-Dimethylamino	1.094	0.282	1.9×10^{-6}	4.0×10^{-7}	+

*1 Taken from Ref. 5.
*2 Estimated from the data of Ref. 6.
*3 Deduced from the data of median lethal dosage to various insects of Refs. 3, 4 and 5. +++ : very highly active, ++ : highly active, + : active, ± : less active, − : almost inactive.

delocalizability for a nucleophilic reaction on the phosphorus atom has close correlations with the case of alkali hydrolysis, and therefore, with the cholinesterase (ChE) inhibitory power and insecticidal activity, as is clearly seen in Table II. The total electron density on the phosphorus atom seems to show similar relations but even the largest difference is 0.004 at the utmost and would not be enough to elucidate large differences in the hydrolysis constants. Indexes of other positions or for other kinds of reaction (electrophilic and radical) had no such parallelism. These results suggest that in the alkali hydrolysis of phosphates the attack of hydroxyl anion to the phosphorus atom is rate-determining and the susceptibility of P–O bond to hydrolysis is nothing but the reactivity of the phosphorus atom, and would support the existing interpretation that both to the enzyme-inhibitory and to the insecticidal activities the hydrolytic attack of a nucleophilic site, probably of the esteratic site of cholinesterase, makes a controlling contribution.

Interactions of the substrates with the anionic site of cholinesterase, which become important in the hydrolysis of acetylcholine, are also probable for some phosphates; and they will

serve as a cause of discrepancy between activities of alkali hydrolysis and of esterase-inhibition, since in the former the reagent is a hydroxyl group only and has no subsidiary anionic site. In this series of compounds, however, these interactions do not seem to be significant, for we could find no such index on any atom in the substituted phenyl group, sterically suitable for the interactions, that would rationary explain the discrepancy.

In Table II, 2,4-dinitrophenyl phosphate is less toxic to insects than expected from the inhibitory power, this being explained as that it would be hydrolytically deactivated in its way to the effective site because of its too large reactivity[5]. This situation is well understood in terms of the superdelocalizability and its threshold for an effective insecticide seems to lie between 1.20 and 1.25. The lack of insecticidal toxicity of *p*-formylphenyl phosphate which could not be elucidated by experimental results[5] is difficult to explain even by this theoretical study, and some other factors would have to be considered.

Furthermore the striking difference of activities between phosphates and thionophosphates is to be discussed. We calculated as an example the superdelocalizability on the phosphorus atom of diethyl *p*-nirophenyl thionophosphate, which amounted to 1.056. Comparing this value with those of diethyl *p*-nitrophenyl phosphate (1.119) and other phosphates discussed above (1.132~1.094), one would clearly see the reason why the thionophosphate in itself is inactive in the inhibition of the enzyme system in vitro. As most thionophosphates substituted by other substituents are less reactive than the *p*-nitro compound, they are well understood to be inactive. The metabolic oxidation to phosphates thus has a meaning, we may say, in elevating the reactivity for a nucleophilic attack.

The nucleophilic nature of the cholinesterase inhibition and toxicity of phenyl phosphates, that is, the nature that the phosphate is attacked by an electron-releasing group, is very interesting in connection with our theoretical indication that the carcinogenic activity and at the same time the anti-tumor activity of conjugated compounds appear to be nucleophilic in their essential character as well as some other biochemical activities, for instance, the anti-fungal activity[20] or nicotine like activity[19]. The high nucleophilic reactivity of the phosphates in question suggests to us, that they would be endowed with the carcinogenic ability or with the carcinostatic ability, though the active site in the tissue will be different in each activity. In fact, paraoxon was found to have the carcinostatic activity[23]. On this point a more elaborate discussion will be given elsewhere[24].

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