The Reaction of Ethyl Hydrogen Methylphosphonate with *p*-Nitrobenzonitrile Oxide: Its Relevance to the Possible Reactivation of "Aged" Phosphonylated Acetylcholinesterase

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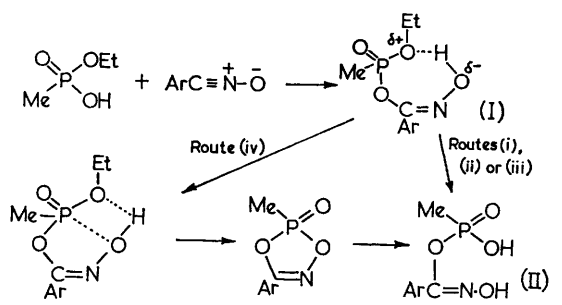
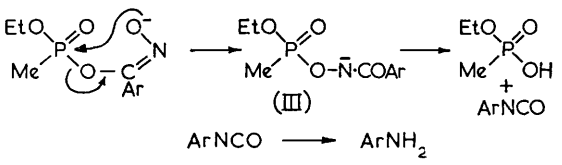
ETHYL HYDROGEN METHYLPHOSPHONATE has been shown to react, under anhydrous conditions, with *p*-nitrobenzonitrile oxide to give ethyl α-hydroxyimino-*p*-nitrobenzyl methylphosphonate (I), which, although relatively stable in the solid state in the absence of light, hydrolyses within a few minutes in the presence of water at 20° to give the stable hydrogen α-hydroxyimino-*p*-nitrobenzyl methylphosphonate (II). The rate of this remarkably fast hydrolysis has been measured in the pH range 2—4 in 2% aqueous ethanol, and the results (*e.g.*, $k_1 = 0{\cdot}65$ min.$^{-1}$ at 25° and pH 3·5; $E_a = 14{\cdot}9$ kcal. mole^{-1}) indicate that at pH 2 the hydrolysis proceeds *ca.* 2×10^7 faster than the hydrolysis of ethyl *p*-nitrophenyl methylphosphonate to give ethanol under these conditions.[1] The observed constancy of the rate in the range pH 2—3·5 suggests intramolecular catalysis involving the protonated form of the oxime (*i.e.*, =NOH rather than =N–O$^-$ or =N–OH$_2^+$), and that further protonation of (I) is not necessary. Several possibilities then exist, including (i) attack by water on phosphorus with elimination of ethanol, (ii) attack by water on the ethyl group, (iii) unimolecular loss of ethanol or (iv) concerted attack by the oxime oxygen on phosphorus with expulsion of ethanol and the formation of an easily cleaved five-membered ring.

Current experiments are designed to distinguish between these possibilities.

Consistent with the more rigid transition state demanded by this example of neighbouring group participation is the negative entropy of activation[2] of −19 e.u.

At pH 5 a different reaction of ethyl α-hydroxyimino-*p*-nitrobenzyl methylphosphonate occurs, to give *p*-nitroaniline. A likely explanation in this case involves nucleophilic attack, possibly intramolecular, by the oxime anion on phosphorus, a reaction known to be favourable in some circumstances[3] (*vide infra*), followed by expulsion of the best available leaving group, in this case the phosphonate-substituted *p*-nitrobenzhydroxamate anion, rather than ethoxide, to give the product of rearrangement (III), which under these conditions would undergo a Lössen rearrangement leading to *p*-nitroaniline.[4]





In accord with these observations, the related α-hydroxyimino-*p*-nitrobenzyl dihexylphosphinate, $Hex_2P(O)OC(Ar)$:NOH, formed from dihexylphosphinic acid and *p*-nitrobenzonitrile oxide, had a pK_a-value of 5·6, indicating a significant concentration of the oxime anion at pH 5, and was

stable in the pH range 2—4, but gave *p*-nitroaniline at pH 5.

These observations are relevant to the therapy of organophosphorus-inhibited acetyl cholinesterase (ChEOH) *in vivo*. This enzyme, which is inhibited by reaction with phosphonylating agents, can be reactivated by nucleophilic reaction with oximes,[3] thus providing a therapy. This therapy becomes ineffective when secondary alkyl esters of phosphorus are used,[5] as a result of loss of the alkyl group to give a phosphonate anion, resistant to further attack by the oxime anion.[5,6] This process is known as ageing.[3]

The experiments briefly reported above now provide a model for the reactivation of the aged enzyme in as much that a one-step process is now available whereby the process of realkylation of the phosphonate anion creates an oxime moiety close to the phosphonate centre. Variation, both of the alkyl group in the phosphonate and of the nitrile oxide is expected to provide some degree of the control over the mode of reaction which is required in order to make this system a viable model for the reactivation of "aged" phosphonylated cholinesterase.

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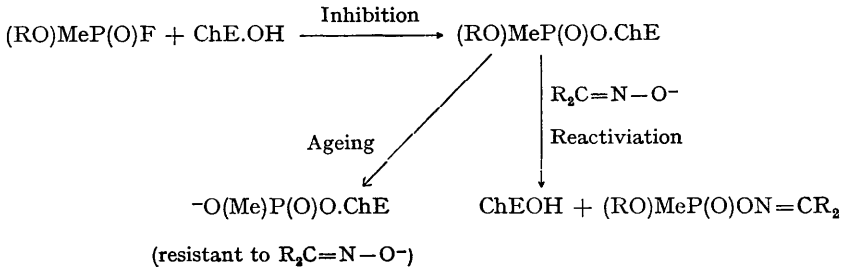

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