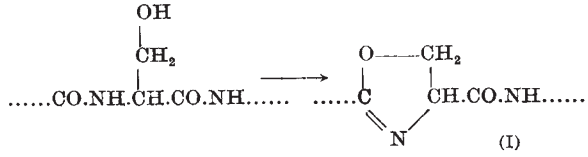
MECHANISM OF ESTERASE ACTION

Nature of the Reactive Serine Residue in Enzymes inhibited by Organo-Phosphorus Compounds

THERE is now overwhelming evidence[1] that the inhibition of cholinesterase, chymotrypsin and other esterases by di-*iso*propyl phosphorofluoridate and other organo-phosphorus compounds involves the phosphorylation of only one of the many serine residues present in the enzyme molecule. Serine itself does not react with di-*iso*propyl phosphofluoridate in aqueous solution[2], and there must be some special explanation for the exceptional reactivity of one of the serine residues in the sensitive enzymes. There are two obvious ways in which the reactivity of a serine side-chain may be enhanced: it may be influenced by some other side-chain (for example, histidine[1]) in its proximity, or it may be chemically modified.

One chemical modification of a serine side-chain to give a possibly more reactive grouping is cyclization to a Δ^2-oxazoline, (I):



(I)

The presence of Δ^2-oxazoline residues in intact proteins has been suggested before[3] and they have been postulated as intermediates in the N → O acyl migrations which occur when serine-containing peptides and proteins are treated with acid[4].

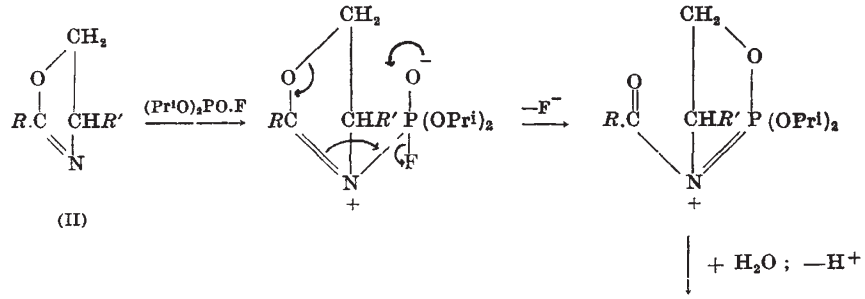


Fig. 1

We have, accordingly, studied the action of di-*iso*propyl phosphofluoridate in aqueous sodium bicarbonate at 37° on three representative Δ^2-oxazolines, namely, 2-methyl-Δ^2-oxazoline (II; R = Me; R' = H), 2-phenyl-Δ^2-oxazoline (II; R = Ph; R' = H), and 4-carbamoyl-2-phenyl-Δ^2-oxazoline (II; R = Ph; R' = —CO.NH$_2$). Although the last-named compound did not react, the two others did, the methyl compound more readily than the phenyl, giving products which yielded O-phosphoryl-ethanolamine (III; R' = H) on acid hydrolysis; control experiments showed that the reactions did not involve prior ring-opening to N-acyl-ethanolamines, followed by O-phosphorylation.

A reasonable course for the reaction is shown in Fig. 1. Our main argument, however, is not dependent on the validity of this mechanism.

As might be expected from the nucleophilic reaction of the oxazoline nitrogen postulated in the first stage, measurement of the basic strengths of the three Δ^2-oxazolines studied showed them to run parallel with the reactivity towards di-*iso*propyl phosphofluoridate, thus:

Δ^2-Oxazoline	Reactivity towards di-*iso*propyl phosphofluoridate	pK_a
2-Methyl-, (II; R = Me; R' = H)	++	5·5
2-Phenyl-, (II; R = Ph; R' = H)	+	4·4
4-Carbamoyl-2-phenyl-, (II: R = Ph; R' = CONH$_2$)	0	2·9

The decreases in basic strength observed on replacing a 2-methyl- by a 2-phenyl-group and on introducing an amide grouping at position 4 are expected consequences of the electron-attracting properties of the phenyl and amide groups.

It appears, from these results, that a Δ^2-oxazoline derivative will react with di-*iso*propyl phosphofluoridate in aqueous sodium bicarbonate, under conditions under which serine itself will not react, to give an O-phosphoryl-ethanolamine derivative, providing that it is sufficiently basic (pK_a not less than about 4). Whether or not a Δ^2-oxazoline peptide, as (I), will react under these conditions will depend on its basic strength; it seems likely that such a grouping will have a pK_a of just about 4, owing to the base-weakening effect of the adjacent peptide grouping, and may, therefore, be expected to react with di-*iso*propyl phosphofluoridate (and, presumably, also with analogous organo-phosphorus compounds) under such 'physiological' conditions.

We suggest, therefore, as a working hypothesis, that the singular serine residue present in the active centres of cholinesterase, chymotrypsin and other enzymes sensitive to di-*iso*propyl phosphofluoridate is, in fact, a Δ^2-oxazoline residue, as (I), and that it owes its unique reactivity to this chemical modification. Possible implications of this hypothesis for the mode of action of esterases are elaborated in the following communication.

Details of this work will be published elsewhere in due course; in the meantime, the experimental consequences are being actively pursued by one of us (H. N. R.) in collaboration with others.

G. R. Porter
H. N. Rydon
J. A. Schofield

Manchester College of Science and Technology,
Manchester, 1.

[1] For refs. see Aldridge, *Ann. Rep. Chem. Soc.*, **53**, 294 (1956).
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[4] Elliott, *Biochem. J.*, **50**, 542 (1952). Edmann and Josefsson, *Acta Chem. Scand.*, **10**, 148 (1956); *Nature*, **179**, 1189 (1957).