COMMUNICATIONS TO THE EDITOR

REACTION OF CERTAIN OCTAMETHYLPYROPHOSPHORAMIDE DERIVATIVES WITH CHYMOTRYPSIN[1]

Sir:

Octamethylpyrophosphoramide (Schradan) is a very effective systemic insecticide, although it is non-toxic to insects on contact or injection.[2] *In vivo* it is converted in plant[3] and animal tissues[4,5] to an anticholinesterase. *In vitro* oxidation of Schradan by permanganate produces an anticholinesterase which differs from the biological derivatives.[6] The chemical nature of these active products is not known.

Many organophosphates inhibit the enzymatic activity of chymotrypsin through a stoichiometric reaction involving introduction of one organophospho-residue per mole of enzyme.[7] In the present study, purified Schradan[8] at a concentration of 1.5 molar caused no inhibition of a chymotrypsin solution containing 10 micrograms of enzyme per ml. but conversion to a chymotrypsin inhibitor was achieved by oxidation with permanganate, incubation with liver slices or by growing pea plants. The permanganate and liver derivatives were effective inhibitors at an estimated concentration of 5×10^{-5} molar under the same conditions.

The nature of the active product from permanganate oxidation was investigated by reaction with chymotrypsin and subsequent analysis for phosphorus,[9] dimethylamine,[10] monomethylamine,[11,12] formaldehyde[13] and nitrogen.[14] The oxidation product, prepared by reaction of equimolar Schradan and permanganate in aqueous solution at *p*H 6.5, was added to a chymotrypsin solution until nearly complete inhibition of enzymatic activity (manometric esterase assay) was effected. A similar sample with un-reacted Schradan served as a control. These samples were purified by salt precipitations and dialysis and then analyzed. The formaldehyde was liberated from boiling 2.4 *N* HCl; the other constituents were determined from an acid hydrolysate. The chymotrypsin treated with Schradan contained no phosphorus, amines, or formaldehyde; the sample treated with oxidized Schradan contained these constituents in the molar ratios indicated in Table I.

TABLE I

ANALYSIS OF CHYMOTRYPSIN INHIBITED BY OXIDIZED OCTAMETHYPYROPHOSPHORAMIDE

	Experimental ratio	Theoretical ratio[a]
Chymotrypsin	1.00	1.00
Phosphorus	0.95	1.00
$(CH_3)_2NH$	1.48	1.50
CH_3NH_2	0.60	0.50
HCHO	0.51	0.50

[a] Assumes mole for mole reaction of oxidized Schradan with chymotrypsin.

The data show that one mole of organophosphate from the oxidized Schradan combined with each mole of chymotrypsin and that half of these attached groups liberated monomethylamine and formaldehyde on treatment with acid. It would therefore appear that half of the combining moeties contained an oxidized group, and that equal combination occurred with either moiety after cleavage of the pyrophosphate bond. The oxidation activated the acid anhydride linkage, since it converted Schradan to a substance that reacted with chymotrypsin and was easily hydrolyzed by water or alkali.

These results are consistent with the hypothesis that the oxidation forms an amine oxide-like structure which may attract electrons from the phosphorus and thereby activate the acid anhydride linkage to produce a reactive phosphorylating agent. The close agreement between the theoretical and the experimental ratios in Table I support this hypothesis. Additional supporting evidence was obtained by oxidizing the inactive compounds bis-(dimethylamino)-*p*-nitrophenyl phosphate and bis-(dimethylamino)-fluorophosphine oxide to produce derivatives which readily inactivated chymotrypsin.

Partially oxidized samples of Schradan displayed both contact and systemic insecticidal properties without increased toxicity to white rats.

Similar studies on the nature of the liver and plant metabolites are in progress.

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FREE RADICAL INITIATED $O^{16}O^{18}$–H_2O^{16} EXCHANGE REACTION IN AQUEOUS SOLUTIONS

Sir:

A γ-ray initiated chain conversion of isotopically enriched dissolved oxygen (designated O_2^*) to normal dissolved oxygen (O_2) has been found in alkaline solutions. The yield of this reaction in-