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-

creases with pH and concentration of O_2^* and is inhibited by hydrogen peroxide. The yield appears to be independent of dosage rate in the range from 2.19×10^{20} to 0.464×10^{20} ev./l. min. The initial yields of normal oxygen from the enriched oxygen appear in Table I.

TABLE I

γ-RAY INITIATED $O^{16}O^{18}$–H_2O^{16} EXCHANGE IN ALKALINE SOLUTION

Expt.	pH	$(O_2^*)_0$, mM	Dosage rate (ev./l. min.)	$(H_2O_2)_0$, mM	G_{O_2}[a]	G_{O_2}/mM O_2^*
1	2.15	1.17	2.19×10^{20}	0.0	0.75	0.64
2	6.0	1.21	2.19×10^{20}	.0	0.90	0.74
3	8.98	1.17	2.19×10^{20}	.0	2.4	2.1
4	9.65	0.68	2.19×10^{20}	.0	5.7	8.4
5	11.32	1.20	2.19×10^{20}	.0	36	30
6	11.91	0.987	2.19×10^{20}	.0	58	59
7	11.89	0.796	0.464×10^{20}	.0	48	60
8	11.65	0.114	2.19×10^{20}	.0	9.4	82
9	12.65	1.08	2.19×10^{20}	.0	120	111
10	11.62	1.18	2.19×10^{20}	.156	10.3	8.7

[a] G_{O_2} = molecules O_2 formed/100 ev. of absorbed γ-ray energy. (Energy absorption is based on $G_{Fe^{+++}} = 15.5$ for ferrous sulfate dosimeter.)

The yield of normal oxygen rises sharply in alkaline solution in the region above pH 9. Since the yield of free radicals produced by Co^{60} γ-rays is 2.61 H and OH/100 ev.,[1] it is evident that under the conditions studied, as many as 40 O_2^* molecules are converted to normal O_2 molecules/radical pair formed in the solution. This chain reaction is terminated by reaction with hydrogen peroxide as can be seen by comparing experiments 5 and 6 with 10.

During the course of this investigation it was also established that there is no thermal exchange of dissolved O_2^* with OH^- in aqueous solutions of pH 11.8. However, it was found that a thermal exchange of 0.5×10^{-6} M O_2^*/min. occurred in a solution containing 1.15 mM O_2^* and 0.18 mM normal hydrogen peroxide at a pH of 11.75. This rate is very small compared to the gamma ray induced rate in the experiments carried out at a dosage rate of 2.19×10^{20} ev./l. min. (see experiment 10). Owing to the fact that only of the order of 10^{-5} M hydrogen peroxide is formed during the course of the irradiation, a correction for the contribution of the thermal rate would be difficult to estimate without a more complete knowledge of the effect of hydrogen peroxide concentration on the exchange reaction.

As a result of recent work[2] on the free radical induced deuterium-water reaction, we have suggested the equilibrium

$$OH = O^- + H^+$$

to explain the drop in yield of hydrogen deuteride at a pH of 9.0. Since the $O_2^* + H_2O = O_2 + H_2O^*$ chain reaction begins at this pH and continues to develop at a pH of 12.65 we postulate participation of O^-, O_2^* and OH^- as indicated in the following mechanism

$$\left.\begin{aligned} H_2O + \gamma\text{-rays} &= H + OH \\ OH &= O^- + H^+ \end{aligned}\right\} \text{Initiation}$$

$$\left.\begin{aligned} O^- + O_2^* &= O^{*-} + O_2 \\ O^{*-} + OH^- &= O^- + O^*H^- \end{aligned}\right\} \text{Propagation}$$

$$\left.\begin{aligned} H + O_2^* &= HO_2^* \\ HO_2^* &= H^+ + O_2^{*-} \\ O^{*-} \text{ or } O^- + HO_2^- &= O^*H^- \text{ or } OH^- + O_2^- \\ O^{*-} \text{ or } O^- + O_2^{*-} &= O^{*-} \text{ or } O^- + O_2^* \end{aligned}\right\} \text{Termination}$$

The relatively stable O_2^- molecule ion and hydrogen peroxide or the ion HO_2^- are suggested as chain terminators.

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VITAMIN B_{12}. XXI. CRYSTALLINE α-RIBAZOLE PHOSPHATE AND ITS SYNTHESIS

Sir:

A crystalline phosphate of α-ribazole (1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole) has been obtained both as a degradation product of vitamin B_{12} and by synthesis. An amorphous barium salt of this phosphate(s) obtained by degradation of vitamin B_{12} was reported previously.[1]

α-Ribazole (2′ or 3′)-phosphate was separated from the acid hydrolyzate of vitamin B_{12} as the lead salt. The lead salt was converted to the phosphate with hydrogen sulfide; after countercurrent distribution (*n*-butanol–water) of the crude product, it crystallized from water–acetone mixtures. The crystalline phosphate melted at 240–241° dec. (micro-block). *Anal.* Calcd. for $C_{14}H_{19}N_2O_7P$: C, 46.93; H, 5.34; N, 7.82; P, 8.65. Found: C, 46.88; H, 5.57; N, 7.54; P, 8.39. The absorption spectra of aqueous solutions were: at *ca.* pH 2, maxima at 277 mμ ($E^{1\%}_{1\text{ cm.}}$ 217) and 285 mμ ($E^{1\%}_{1\text{ cm.}}$ 202); and at *ca.* pH 11, maxima at 249 mμ ($E^{1\%}_{1\text{ cm.}}$ 191), 280 mμ ($E^{1\%}_{1\text{ cm.}}$ 144), and 288 mμ ($E^{1\%}_{1\text{ cm.}}$ 136).

A crystalline brucine salt of α-ribazole (2′ or 3′)-phosphate also was obtained from the acid hydrolyzate of vitamin B_{12}. A methanol solution of the phosphate obtained from the above-mentioned lead salt was treated with a methanol solution of brucine. Concentration of the solution and cooling gave the crystalline dibrucine salt. It also crystallized from water; m.p. 169–175° (micro-block).

Anal. Calcd. for $C_{60}H_{71}N_6O_{15}P$: C, 62.81; H, 6.24; N, 7.33; P, 2.71. Found: C, 62.82; H, 6.28; N, 7.39; P, 2.85.

α-Ribazole (2′ or 3′)-phosphate was best prepared synthetically by phosphorylation of 5′-trityl-α-ribazole with diphenylchlorophosphonate.[2] After removal of the trityl and phenyl groups by acid hydrolysis, α-ribazole phosphate was isolated as the lead salt, which was decomposed with hydro-

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