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# PRELIMINARY COMMUNICATION

## STEREOSPECIFICITY IN THE METABOLISM OF THE CHIRAL ISOMERS OF FONOFOS BY MOUSE LIVER MICROSOMAL MIXED FUNCTION OXIDASE

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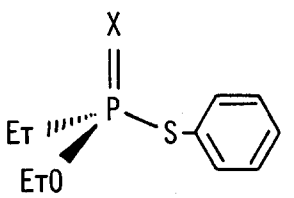
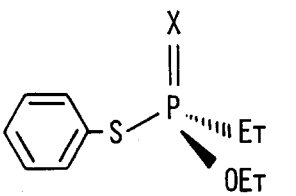
The metabolic conversion of phosphorothionate (P=S) triesters to the corresponding phosphate (P=O) ester is a well known activation reaction occurring in animals and plants. Since P=S esters are generally poor anticholinesterases, activation to the P=O ester is required for intoxication by organophosphorus insecticides containing the P=S moiety. This communication is concerned with the stereochemical course of P=S to P=O activation of the resolved isomers of fonofos (O-ethyl S-phenyl ethylphosphonodithioate) in the presence of mouse liver microsomal mixed function oxidase. This enzymatic desulfuration reaction proceeded with greater than 70 percent retention of configuration at the phosphorus center.

Mechanistic studies on the conversion of P=S to P=O esters by enzymatic and model oxidation systems have recently received considerable attention (1-7). While the stereochemistry of P=S to P=O conversion by peroxyacids has been examined (7), related studies with the microsomal mixed function oxidase enzymes have not been reported. We have prepared the enantiomers of fonofos (I and II) and fonofos oxon (III and IV) and established their absolute configuration by X-ray diffraction analysis and chemical correlation (8). Details of this work will be reported elsewhere.

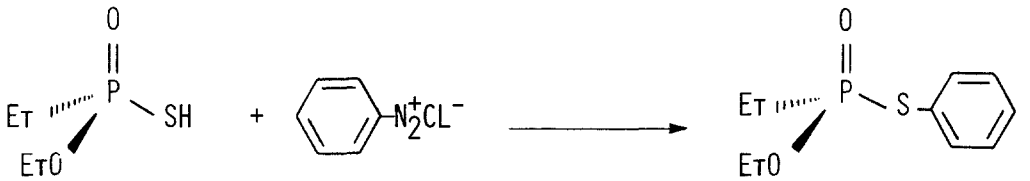



I, X = S, $(\underline{S})_P$ II, X = S, $(\underline{R})_P$

III, X = O, $(\underline{R})_P$ IV, X = O, $(\underline{S})_P$

Briefly stated, however, the absolute configuration of the fonofos isomers I and II was established by X-ray analysis of the corresponding p-bromo analog of fonofos (a solid, m.p. 29-31°) and relating it to fonofos by chemical correlation. The configurations of the fonofos oxon isomers were established through their synthesis from $(\underline{R})_P$-(+) and $(\underline{S})_P$-(-) O-ethyl ethylphosphonothioic acid (configuration assigned by X-ray analysis of the corresponding α-phenylethylamine salt) by reaction with benzene diazonium chloride as depicted below for the $(\underline{R})_P$ isomer. $(\underline{S})_P$ and $(\underline{R})_P$ refer to the two different stereochemical configurations of the phosphorus atom in each isomer according to the Cahn-Ingold-Prelog System (9).




$(\underline{R})_P$ ACID III, $(\underline{R})_P$

Enantiomers of $^{35}$S-phenyl fonofos were prepared by reaction between $^{35}$S-sodium benzenethiolate and the corresponding enantiomers of O-ethyl ethylphosphonochloridothioate in a 1,2-dimethoxyethane-ether solvent mixture. Enzymatic oxidations were carried out in 25-ml open Erlenmeyer flasks at 37.5°. The reaction mixture, in a final volume of 5 ml, consisted of Tris-HCl buffer (0.1 M), pH 7.8, glucose 6-phosphate (G-6-P, $1 \times 10^{-3}$ M), NADPH ($5 \times 10^{-4}$ M), G-6-P dehydrogenase (1 I.U.), $^{35}$S-phenyl-labeled fonofos isomer ($2 \times 10^{-4}$ M), added in 50 µl acetone, and female mouse liver microsomal suspension (17.5 mg protein). Each mixture was incubated for 2 hr and a total of 300 separate incubations were carried out for each enantiomer. The reaction was terminated by the addition of sodium chloride and acetone, the incubation mixtures were combined, and radiolabeled materials were extracted into ether by continuous liquid-liquid extraction for 24 hr. Approximately 95 percent of the charged radioactivity was recovered in ether. $^{35}$S-labeled fonofos oxon (III or IV) was isolated and purified as follows: solvent partitioning between acetonitrile and hexane, Florisil column chromatography using ether-hexane to separate biological materials, and finally by preparative thin-layer chromatography (t.l.c.) using Silica gel $GF_{254}$ (1 mm) and the same ether-hexane solvent. The oxons obtained from I and II were determined to be > 99 percent pure (disregarding isomeric content) as estimated by autoradiography of a t.l.c. plate and by gas-liquid chromatography (g.l.c.) analysis using an alkaline flame ionization detector. Other $^{35}$S-labeled materials isolated were diphenyl disulfide, diphenyl disulfide oxide, and unmetabolized fonofos.

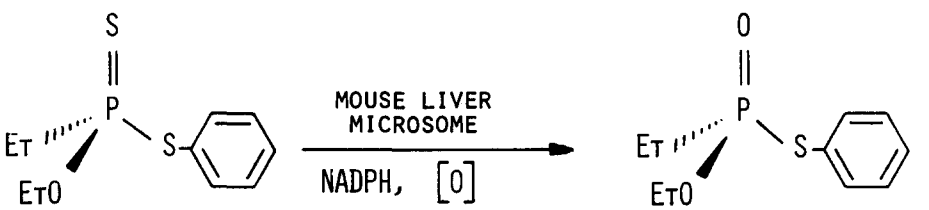
Data for the specific rotation, $[\alpha]_D^{25}$ (25°, sodium D line, 1 dm cell in cyclohexane), of starting $(\underline{R})_P$ and $(\underline{S})_P$ fonofos and the isolated oxons are presented in Table 1. Two separate experiments were conducted with the $(\underline{S})_P$ isomer. The degree of stereospecificity for each reaction was calculated by comparing the specific rotation of the isolated oxon with that of the corresponding fonofos oxon [$(\underline{R})_P$ +112.64° (c 1.02), $(\underline{S})_P$ -121.70° (c 0.636)] which was synthesized from the respective isomeric O-ethyl ethylphosphonothioic acid. Values for percent retention in Table 1 were obtained by summing the specific rotation of the isolated oxon and one-half of the difference in specific rotations between the synthesized oxon and isolated oxon, and dividing the sum by the specific rotation of the synthesized oxon. The values given must be regarded as estimates owing to differences in the specific rotations of the synthesized isomers of fonofos and fonofos oxon.

TABLE 1. Specific rotations of starting $(\underline{R})_P$ and $(\underline{S})_P$ fonofos and respective oxons isolated after incubation of each fonofos isomer with mouse liver microsomal mixed function oxidase

| Fonofos | $[\alpha]_D^{25}$ (Cyclohexane) | Fonofos oxon | $[\alpha]_D^{25}$ (Cyclohexane) | Stereospecificity |
|---|---|---|---|---|
| $(\underline{S})_P$ | +138.40°(c 0.301)* | $(\underline{R})_P$ | +49.78°(c 0.298) | 72% Retention |
| $(\underline{S})_P$ | +138.40° | $(\underline{R})_P$ | +61.83°(c 0.307) | 77% Retention |
| $(\underline{R})_P$ | -114.56°(c 0.309) | $(\underline{S})_P$ | -69.29°(c 0.533) | 78% Retention |

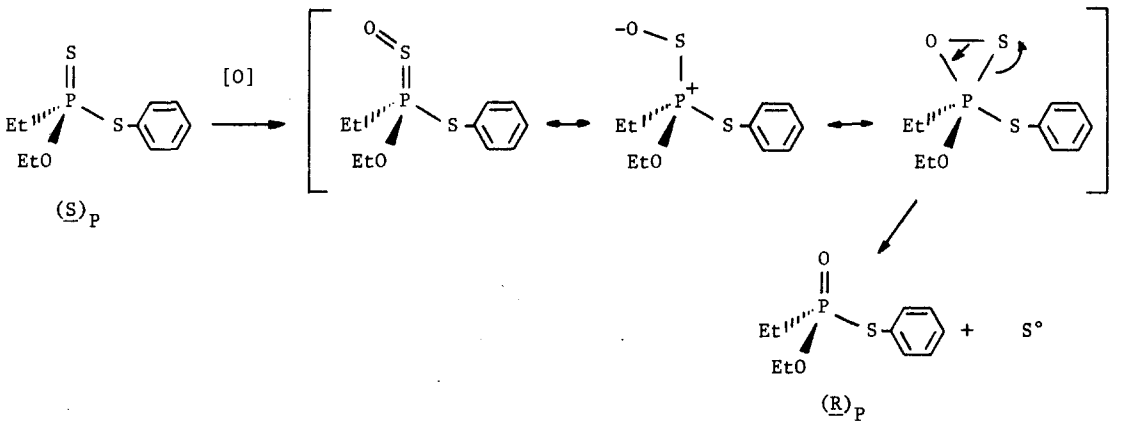
*Rotations were determined in a Rudolph high precision polarimeter; c is the concentration in g/100 ml.

The results indicate that the P=S to P=O activation reaction mediated by mouse liver microsomal oxidase proceeds predominantly with retention of configuration, as illustrated below with $(\underline{S})_P$ fonofos. Thus, the stereochemical course of the reaction is consistent




I, $(\underline{S})_P$ II, $(\underline{R})_P$

with the mechanism proposed by Ptashne et al. (2) for the oxidative desulfuration of parathion to paraoxon in which initial attack by oxygen on the thiono sulfur is postulated.



Although retention of configuration remains the principal course of the oxidative desulfuration reaction, evidently inversion also occurs to a significant extent (20-30%). Inversion probably proceeds with initial attack of oxygen on the phosphorus atom, followed by elimination of sulfur. In this regard, the stereochemical course of peroxy acid oxidation of a chiral phosphinothioate ester to the corresponding oxon is reported to be dependent on the nature of the peroxy acid and acidity of the reaction medium, with inversion occurring in the presence of strong acids (7). Further work is in progress on the behavior of $^{35}S$-labeled fonofos isomers in other model oxidation systems and in whole animals.

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