DIFFERENTIAL GLUTATHIONE-DEPENDENT DETOXICATION OF TWO GEOMETRICAL VINYL ORGANOPHOSPHORUS (MEVINPHOS) ISOMERS

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Recent investigations in this laboratory offered an explanation for the different toxicities of the cis and trans isomers of dimethyl-1-carbomethoxy-1-propen-2yl-phosphate, mevinphos (Phosdrin) and of dimethyl-1,3, dicarbomethoxy-1-propen-2yl-phosphate (Bomyl) (Spencer, 1961; Morello et al 1967).

The present communication presents evidence showing that the enzymatic detoxication of cis Phosdrin is reduced glutathione-dependent, the products of the reaction being S-methyl glutathione and cis monomethyl-1-carbomethoxy-1-propen-2yl-phosphate (cis desmethyl-Phosdrin). Trans Phosdrin, on the contrary, is metabolized to dimethyl phosphate by a glutathione independent enzyme(s).

MATERIAL AND METHODS

Cis and trans Phosdrin were prepared as described by Spencer (1958) and Morello et al (1967). Methyl glutathione (courtesy Dr. I.G. Walker) and glutathione were products of Calbiochem and Eastman Organic Chemicals respectively. Cis desmethyl-Phosdrin was prepared as described by Spencer (1958). All other chemicals used were of the best quality commercially available. The mice were female albino, supplied by Rolfsmeyer Farms, Madison, Wis.

Cis and trans Phosdrin detoxication

Mouse liver homogenates were prepared in 0.25 M sucrose or 0.9% sodium chloride (1:4, W:V) and centrifuged at 10,000 x g for 10 min. Part of the

supernatant (5ml) was dialized for 6 hours against 0.25 M sucrose, 0.001 M phosphate buffer (pH 7.5) or 0.9% sodium chloride, 0.001 M phosphate buffer (pH 7.5), with one change at 3 hrs. Incubations were carried out in 10 ml erlenmeyer flasks in a water bath shaker.

After incubation, appropriate dilutions were made, and an aliquot was tested against bovine red cell cholinesterase to evaluate the quantity of organophosphate remaining (Vardanis and Crawford, 1964; Morello et al 1967).

Identification of the glutathione metabolite

Mouse liver homogenates were prepared in sodium chloride and centrifuged at 45,000 x g for 1 hour. The supernatant (35 ml) was dialized, as described above, against 0.9% NcCl containing 0.1% mercaptoethanol. Incubation was at 35° for 40 min.

Each incubation flask contained dialized mouse liver supernatant equivalent to 7 g wet weight; cis Phosdrin, 1.4 mM; reduced glutathione, 3.2 mM; tris-buffer, 20 mM (pH 7.5). The total volume was 50 ml. At the end of incubation the mixture was boiled for 3 min, filtered, concentrated to 3 ml under vacuum at 30° and filtered again. The filtrate (referred to below as "product of the reaction") was used for chromatography. Treatment with iodoacetamide was done as reported by Johnson (1966).

RESULTS AND DISCUSSION

Table I shows that mouse liver supernatant lost 75% of its cis-Phosdrin degrading activity on dialysis, and that the addition of reduced glutathione fully restored the original activity. Trans Phosdrin detoxication was not affected to any considerable extent by dialysis or the addition of reduced glutathione. The small amount of cis-Phosdrin-degrading activity in the dialized supernatant is probably due to glutathione remaining after dialysis (e.g. protein-bound).

Glutathione and methyl glutathione have very similar chromatographic mobility, but can be resolved after treatment with iodoacetamide (Table II and also Johnson, 1966). The "product of the reaction" shows one spot at R_F 0.31 which after treat-

Table I

The Cis and Trans Phosdrin Detoxication

System	% Detoxication	
	Cis Phosdrin	Trans Phosdrin
Liver supernatant	94	91
Liver supernatant + reduced glutathione	99	93
Dialized liver supernatant	24	81
Dialized liver supernatant + reduced glutathione	96	90
Control	4	0

Incubation in 15 mM phosphate buffer (pH 7.5) for 15 min at 35°. Mouse liver supernatant equivalent to 100 mg of liver. Phosdrin 0.3 mM; reduced glutathione (when added) 4 mM. Incubation volume was 1 ml. Control represents incubations carried out without supernatants or supernatants that were treated in a boiling water bath for 3 min.

ment with iodoacetamide is resolved to two spots of similar intensity with R_F values of 0.31 and 0.16, corresponding to methyl glutathione and glutathione respectively. It should be noted that glutathione was added in approximately

Table II

Identification of the glutathione metabolite

Compounds	R_F	R_F (After treatment with iodoacetamide)	
Standard reduced glutathione	0.32		0.19
Standard methyl-glutathione	0.37	0.34	
St. red. glutathione + St. methyl-glutathione	0.35	0.32	0.18
Product of the reaction	0.31	0.31	0.16

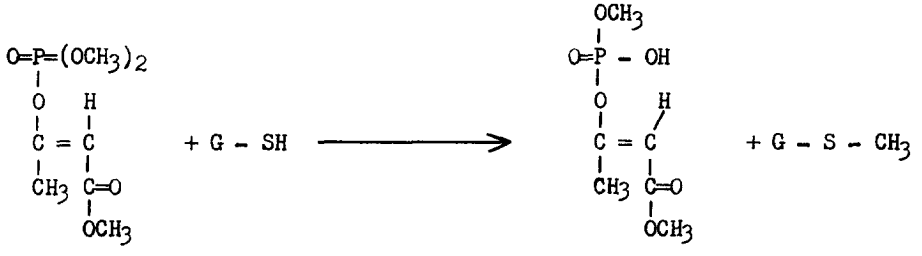
Ascending chromatography on Whatman No. 1 paper. Solvent mixture: butan-1-ol:acetic acid:water:mercaptoethanol (11:4:5:0.1). Chromatograms developed with ninhydrin reagent. Standards 50 µg per chromatogram; product of the reaction equivalent to 100 µg of reduced glutathione in the incubation mixture. See Materials and Methods for incubation procedure.

100% excess with respect to cis Phosdrin, assuming a reaction molar ratio of 1:1. A spot of R_F 0.1 was noted in chromatograms with standard glutathione, as well as with "product of the reaction"; this spot was greatly diminished by the use of mercaptoethanol in the solvent system and probably corresponds to oxidized glutathione.

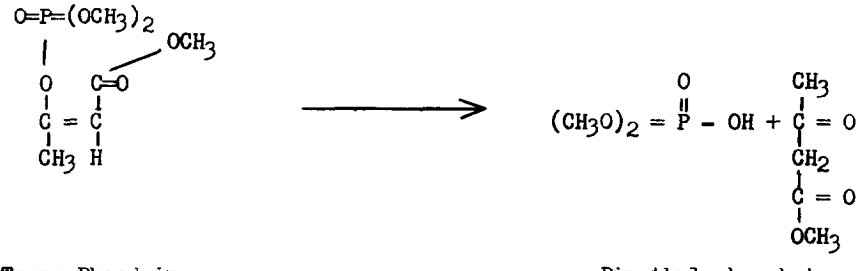
From an incubation mixture similar to the one used for the results of Table II we have identified cis desmethyl-Phosdrin and dimethyl phosphate as metabolites of cis and trans Phosdrin, respectively. These findings, as well as those on the metabolism of the Bomyl isomers, will be reported elsewhere. The present results, as well as those reported previously (Morello et al 1967), offer an interesting example of marked differences in the biological properties of a compound, as a consequence of geometrical isomerism.

The importance of glutathione-dependent enzymatic detoxication of organophosphorus compounds has recently become evident. Methyl parathion and Sumithion

PROPOSED METABOLIC PATHWAY FOR THE DEGRADATION OF CIS AND TRANS PHOSDRIN BY MOUSE LIVER



Cis-Phosdrin Glutathione Cis-desmethyl Phosdrin Methyl glutathione



Trans Phosdrin Dimethyl phosphate

(both dimethyl phosphorus compounds) have been reported to be metabolized to their respective desmethylated derivatives by a glutathione dependent preparation (Fukami and Shishido 1966), whereas the same preparation degrades ethyl parathion very slowly. Methyl iodide is degraded by rat liver in a similar way (Johnson 1966). Our results show that trans Phosdrin and both Bomyl isomers are not degraded to any great extent by a glutathione-dependent system.

Because the number of compounds studied till now is relatively small, it is perhaps premature to generalize with regard to the stereospecificity of the glutathione-dependent enzymatic degradation. Nevertheless, it is striking that compounds degraded to their respective desmethylated derivatives (e.g. methyl parathion, Sumithion, cis Phosdrin, methyl iodide) have their methyl groups relatively free. On the other hand some dimethyl phosphates (e.g. trans-Phosdrin, cis and trans Bomyl) whose methyl groups are partially covered (by carboxyester group(s) in the examples above) are not degraded via demethylation to any significant extent.

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