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METABOLISM OF *O*-ETHYL S,S-DIPROPYL PHOSPHORODITHIOATE IN RATS AND LIVER MICROSOMAL SYSTEMS*

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Abstract—After administration of *O*-ethyl-^{14}C and S,S-dipropyl-^{14}C-labeled *O*-ethyl S,S-dipropyl phosphorodithioate to rats, chloroform-extractable radioactivity was recovered from the urine only from rats treated with the propyl-^{14}C-labeled compound. It contained traces of methyl propyl sulfide, methyl propyl sulfoxide, and methyl propyl sulfone, products of S-methylation and subsequent oxidation of the propyl thiolate ion released from *O*-ethyl S,S-dipropyl phosphorodithioate. The major water-soluble metabolite isolated from rat urine, liver microsomes and supernatant was *O*-ethyl S-propyl phosphorothioic acid. Rat urine also contained *O*-ethyl phosphoric acid, S-propyl phosphorothiolic acid and S,S-dipropyl phosphorodithioic acid. Rat and rabbit liver supernatant enzymes were able to *O*-de-ethylate *O*-ethyl S,S-dipropyl phosphorodithioate in the presence of reduced glutathione. S-ethylglutathione-ethyl-^{14}C was produced when the supernatant preparations were incubated with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate, indicating that glutathione acted as an ethyl acceptor.

O-ETHYL S,S-dipropyl phosphorodithioate (Mocap) is a newly introduced material for the control of some nematodes and soil insects in crop situations.[1] This compound, although a phosphorodithioate, is structurally different from the typical phosphorodithioate insecticides in the sense that neither of the two alkyl sulfur groups involved in ester linkages with phosphorus are double-bonded, as is the usual case. Because of this important structural difference, *O*-ethyl S,S-dipropyl phosphorodithioate should be expected to behave differently in biological systems from the conventional phosphorothionate insecticides, which generally must be oxidized to phosphates to gain significant biological activity.[2] The resulting compounds are then more susceptible to hydrolysis at the P—S—ester or P—O—ester bonds.[3]

Menzer *et al.*[4] have shown that *O*-ethyl S,S-dipropyl phosphorodithioate is metabolized in bean and corn plants to ethyl propyl sulfide, ethyl propyl sulfoxide, ethyl propyl sulfone, propyl disulfide and *O*-ethyl S-propyl phosphorothioic acid. S,S-dipropyl phosphorodithioate was not recovered from plants. In the metabolic scheme proposed, the propyl thiolate ion occupied a central place. It attacked and dealkylated the parent compound to form ethyl propyl sulfide and it was also thiophilic in forming propyl disulfide.

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With respect to the metabolism of related sulfur-containing compounds, thiophenol has been shown to be readily S-methylated *in vivo*, followed by oxidation to the sulfoxide and sulfone.[5] Thiouracil was detoxified by methylation of the thiol group to form 2-methylthiouracil.[6] Both thiophenol and 5-mercaptouracil also formed S-glucosides in insects.[7] Williams[8] reported that ethyl mercaptan administered to mice and guinea pigs was excreted as methyl ethyl sulfone, a product of S-methylation and subsequent oxidation. [2,2-Dichloro-*N*-(β-hydroxy)-α-(hydroxymethyl)-*p*-(methylsulfinyl) phenethyl] acetamide and its sulfide also undergo S-methyl oxidation in animals.[9] Parke[10] has noted that mercaptans may result from the reduction of disulfides in animals and may undergo methylation to form methylalkyl sulfides, which are oxidized to the corresponding sulfones.

This investigation was undertaken to evaluate the metabolism of *O*-ethyl S,S-dipropyl phosphorodithioate in rats, both *in vivo* and *in vitro* and to compare this with the previously reported behavior of the compound in plants.

METHODS AND MATERIALS

Chemicals

Radiochemicals. *O*-ethyl S,S-dipropyl phosphorodithioate-ethyl-α-^{14}C (specific activity, 1·25 mc/m-mole) and -propyl-α-^{14}C (specific activity, 2·8 mc/m-mole) were obtained from Nuclear Research Chemicals, Orlando, Fla. The radiochemicals were chromatographed on silicic acid columns before use and were ascertained to be radiochemically pure.

Synthesis of methyl propyl sulfide. 1-Propanethiol (7·6 g, 0·1 mole) was refluxed with metallic sodium (2·3 g, 0·1 mole) at 60° for 1 hr. Excess unreacted sodium was removed and the mercaptide was then refluxed with methyl iodide (14·2 g, 0·1 mole) at 35° for 45 min. The mixture was allowed to cool and was then fractionally distilled.[11] The fraction boiling at 95° was collected (5·9 g, 65 per cent yield). It was very soluble in chloroform, acetone and ethanol. An infrared spectrum determined on a Beckman model IR8 spectrophotometer was consistent with the desired structure. Thin-layer chromatography on Silica gel gave an R_f of 0·82 in hexane–acetone (2:1) and 0·62 in hexane–chloroform (3:2). Spots appeared white on a pale yellow background when exposed to iodine vapors and bright reddish-brown when sprayed with palladium chloride solution after exposure to iodine vapors.

Synthesis of methyl propyl sulfoxide and methyl propyl sulfone. Methyl propyl sulfoxide and methyl propyl sulfone were synthesized according to the procedure of Menzer *et al.*[4] for the synthesis of the ethyl propyl sulfoxide and sulfone analogs, using methyl propyl sulfide as the starting material. The yield of methyl propyl sulfoxide was 69 per cent. An infrared spectrum showed a strong sulfoxide peak at 1010 cm^{-1}, and the synthetic material gave a single spot on four thin-layer systems. *Elemental Anal.* Calcd. for $C_4H_{10}SO$: C, 45·25; H, 9·49. Found: C, 45·27; H, 8·55. The yield of methyl propyl sulfone was 75 per cent and the material showed the characteristic sulfone peaks in the infrared spectrum at 1010 and 1300 cm^{-1}. It gave a single spot on four thin-layer systems.[4] *Elemental Anal.* Calcd. for $C_4H_{10}SO_2$: C, 39·32; H, 8·25. Found: C, 39·40; H, 8·41.

Synthesis of S,S-dipropyl phosphorodithioic acid. *O*-ethyl S,S-dipropyl phosphorodithioate (2·42 g, 10 m-moles) was dissolved in 3 ml of 0·1 N HCl in a water bath at 50° and shaken for 1 hr. The mixture was allowed to cool, extracted three times with

chloroform, and evaporated under vacuum at 55°, leaving a clear solution which was purified on thin-layer to give a spot at R_f = 0·69 (acetonitrile–water–ammonia, 40:9:1). The product was easily separated from and was not *O*-ethyl S,S-dipropyl phosphorodithioate, S-propyl phosphorothiolic acid, *O*-ethyl S-propyl phosphorothiolic acid, or *O*-ethyl phosphoric acid. The infrared spectrum was consistent with the structure of S,S-dipropyl phosphorodithioic acid. There was insufficient material available for further characterization.

Synthesis of S-propyl phosphorothiolic acid. *O*-ethyl S-propyl phosphorothiolic acid (0·184 g, 1·0 m-mole) was dissolved in 1 ml of 0·1 N HCl at 50° and shaken for 2 hr in a Dubnoff metabolic shaking incubator. At the end of 2 hr the mixture was heated to 85° in a water bath for about 5 min. Solid sodium bicarbonate was added to neutralize excess acid. Ethanol was then added to precipitate excess bicarbonate, which was filtered out. The ethanol was evaporated, leaving behind a clear solution which was further purified on thin-layer to give a light grey spot (Table 1) after exposure to iodine and spraying with palladium chloride. The product was insoluble in chloroform, but was readily soluble in acetone, ethanol and water. The product was neither *O*-ethyl S-propyl phosphorothiolic acid nor *O*-ethyl phosphoric acid and was therefore tentatively concluded to be S-propyl phosphorothiolic acid. There was insufficient material available for further characterization.

Synthesis of S-ethylglutathione. S-ethylglutathione was prepared by the procedure of Martin and Edsall[12] with modifications. Sodium hydroxide (0·8 g, 20 m-moles) in 5 ml water was slowly added in the dark to silver nitrate (3·4 g, 20 m-moles) with continuous stirring. In about 20 min, a black precipitate of silver oxide appeared, which was filtered out. Reduced monosodium glutathione (Nutritional Biochemicals Company; 0·5 g, 1·63 m-moles) was dissolved in 10 ml of deionized, deoxygenated water, 0·38 g (1·63 m-moles) of freshly prepared silver oxide was added in the dark, and the stoppered flask was magnetically stirred for 90 min. During that time the black silver oxide was replaced by the white precipitate of silver glutathionate. To this suspension ethyl iodide (0·75 ml, 5 m-moles) was slowly added and the mixture was stirred in the dark for about 20 hr. The white precipitate of silver iodide formed was filtered out. The solvent was evaporated under vacuum. The residual liquid which contained S-ethylglutathione was redissolved in 3 ml water and about 10 ml acetone was added to precipitate the white, sticky, solid S-ethylglutathione. The acetone was evaporated and the solid S-ethylglutathione dried (yield, 65 per cent; m.p., 212–213°). *Anal. Calcd.* for $C_{11}H_{19}N_3O_6S$: C, 41·11, H, 5·96. Found: C, 40·06; H, 5·98.

The dinitrophenyl (DNP) derivative of S-ethylglutathione was prepared according to the Sanger procedure.[13] It was recrystallized from aqueous methanol (m.p. 92–95°).

Other chemicals. The following potential metabolites of *O*-ethyl S,S-dipropyl phosphorodithioate were supplied by Mobil Chemical Company: *O*-ethyl S-propyl phosphorothiolic acid, propyl sulfide, propyl disulfide and ethyl propyl sulfide.

Treatment of rats

Three male and three female white rats, Sprague–Dawley derived (Flow Laboratories, Inc., Bethesda, Md.), approximately 150 g, were treated through a stomach tube with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate (1·28 × 10^6 counts/min/rat) and in a separate experiment with *O*-ethyl S,S-dipropyl-^{14}C phosphorodithioate

($3{\cdot}0 \times 10^6$ counts/min/rat) in 0·25 ml distilled water. Rats were placed in metabolism cages designed to collect urine and faeces separately. Urine was sampled at 6, 12, 24, 36, 48 and 72 hr after treatment with the *O*-ethyl-^{14}C-labeled compound and at 8, 24, 48 and 72 hr after treatment with the propyl-^{14}C-labeled compound. The faeces were collected only at the end of the experiment.

Extraction procedures. Rat urine from each sampling time was extracted three times with equal volumes of chloroform. The combined extracts were assayed for radioactivity.

In certain cases the water fraction of rat urine that remained after chloroform extraction was concentrated to approximately 1 ml at 55° with a gentle stream of air. The concentrated water fraction was then added to 10 ml of 0·1 N HCl, and extracted with equal amounts of ether and ethyl acetate. The organosoluble radioactivity thus obtained and the remaining water fractions were further analyzed by thin-layer chromatography. Some unacidified water fractions of urine that were not further extracted were concentrated to about 2 ml and mixed with 0·01 N HCl for anion-exchange chromatography.

Enzymatic studies with rat and rabbit

Liver microsomes and supernatant. Rabbit and rat liver microsomes were prepared according to a described procedure.[14] The rats were killed by a blow on the head, and rabbits were killed by exsanguination. The final supernatant solution was decanted and saved for further incubation studies, and the final microsomal pellet was re-suspended in about 5 ml of fresh ice-cold 0·15 M KCl to give a protein concentration of approximately 5 mg/ml.

A typical incubation flask of rat liver microsomes or supernatant contained approximately 250,000 counts/min of either labeled compound or about 750,000 counts/min with rabbit liver microsomes or supernatant. Microsomal incubations contained 2 ml of microsomal preparation and 2 μmoles NADPH in tris buffer. Supernatant incubations included 5 ml supernatant from the rabbit microsomes or 2·6 ml from rat microsomes and 1 ml of 5 mM reduced monosodium glutathione (Nutritional Biochemicals Company) or 0·4 ml of 3·3 mM glutathione respectively. Flasks were incubated at 37·5° for 4 hr. Equal volumes of chloroform were added to stop the reaction. The incubation mixture was then extracted three times with chloroform. Both the aqueous and chloroform fractions were assayed for radioactivity. Chloroform fractions were chromatographed on silicic acid columns, whereas the water fractions were chromatographed on anion-exchange columns. In some experiments both the rats and rabbits were pretreated for 2 days with 75 mg/kg of sodium phenobarbital prior to killing. The second injection was given 12 hr prior to excision of the liver.

Unlabeled ethyl propyl sulfide was also incubated with rat and rabbit liver microsomes to ascertain whether it would be oxidized by the microsomal system to ethyl propyl sulfoxide. With 2 ml of rabbit or rat liver microsomes and 1 ml NADPH (2 μM), 0·25 mole ethyl propyl sulfide per flask was incubated for 4 hr at 37·5°. The incubation mixtures were extracted with chloroform as above. The protein concentration of the microsomes was determined by the biuret procedure,[15] using bovine serum albumin as the standard.

Chromatography

The preparation of silicic acid columns has been previously described.[4]

Anion-exchange column chromatography with Dowex 1-X8, 100–200 mesh, ionic form Cl^-, was employed to resolve metabolites in the water fractions of rat urine, liver microsomes and supernatant. The procedure followed was essentially the same as that described by Plapp and Casida.[16] About 50 g resin was slurried with distilled water and poured into a column (19 mm i.d.) to a height of 25 cm. The column was washed with distilled water until the eluate was above pH 3. Gradient elution was used with 0·01 N HCl, 0·1 N HCl, 1 N HCl and concentrated HCl as eluents, with redistilled acetone and methanol as co-solvents.

The sample to be chromatographed was first concentrated to about 2 ml at 55°, if necessary, and then was mixed with an equal volume of 0·01 N HCl before adding it to the column. Fractions of 20 ml were collected. Aliquots of 1 ml were counted for radioactivity using the Triton X-100 scintillation solution.

Detection and identification of metabolites

Metabolites of *O*-ethyl S,S-dipropyl phosphorodithioate were identified by co-chromatography with synthetic, unlabeled, purified standards with radioactive extracts or pooled peaks from anion-exchange columns or radioactive spots scraped off thin-layer plates; by chemical degradation and fragment analysis; and by utilizing the differently labeled samples of *O*-ethyl S,S-dipropyl phosphorodithioate to evaluate the loss of either the ethyl or propyl moiety from the molecule. After resolution of the metabolites on thin-layer plates, the location of the unlabeled standards was determined by exposing the plates to iodine vapors and then spraying palladium chloride reagent prepared by diluting 5 ml of a 5% palladium chloride solution in water with 1 ml HCl and 94 ml of 95% ethanol. *O*-ethyl S,S-dipropyl phosphorodithioate and dithioate derivatives gave deep brownish-yellow spots on a white background, the derivatives with one S—P bond gave lighter spots, while the compounds which lacked S—P bond gave grey spots of varying shades. R_f values in the different solvent systems developed for water-soluble standards and metabolites on Silica gel thin-layer plates are noted in Table 1. Values for organo-extractable materials have been previously reported.[4]

TABLE 1. R_f VALUES FOR *O*-ETHYL S,S-DIPROPYL PHOSPHORODITHIOATE AND ITS PROPOSED WATER-SOLUBLE METABOLITES ON THREE THIN-LAYER CHROMATOGRAPHIC SYSTEMS

Compound	Metabolite designation	R_f in System*		
		I	II	III
O-ethyl S,S-dipropyl phosphorodithioate	MO	0·87		
O-ethyl phosphoric acid	C	0·08	0·23†	0·42†
O-ethyl S-propyl phosphorothioic acid	F,G	0·43	0·66	0·53
S,S-dipropyl phosphorodithioic acid	H	0·69	0·93	0·98
S-propyl phosphorothiolic acid	D	0·22	0·15	0·05
S-ethylglutathione	B	0·24		

* Thin-layer chromatography on Silica gel G using acetonitrile–water–ammonia (40:9:1) in system I; ethyl acetate–ethanol–acetic acid (9:9:1) in system II and *n*-butanol–acetic acid–water (35:3:10) in system III.

† Serious tailing was observed.

Glutathione and S-ethylglutathione were detected as purple spots on thin-layer plates by spraying with ninhydrin reagent (95 ml of 0·2% triketohydrindene hydrate in *n*-butanol plus 5 ml of 10% aqueous acetic acid) and heating to 110° for about 10 min. Dinitrophenyl derivatives of S-ethylglutathione and the radioactive metabolite were also synthesized and co-chromatographed.

Acetylation of standards and metabolites. As a further step in characterization, acetylation of certain water-soluble standards was carried out[17] by mixing the individual standards (0·25 g) with 0·5 ml of acetylating reagent (acetic anhydride–concentrated sulfuric acid, 40:1) at 0°. The mixture was heated at 85° in a water bath for 30 min and then was cooled to zero. Ice equivalent to about 0·5 ml water was added, and the mixture was extracted three times with benzene. Benzene fractions were combined and purified on thin-layer plates. The polar urinary metabolites were similarly acetylated for co-chromatography with acetylated standards.

Radioassay procedures. Procedures used for the determination of radioactivity have been previously described.[4]

RESULTS

Identification and chemical nature of metabolites. When rats were treated with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate, no organo-extractable radioactivity resulted in the urine, but after treatment with *O*-ethyl S,S-dipropyl-^{14}C phosphorodithioate, three peaks were separated on silicic acid columns after the urine was extracted with chloroform. Anion-exchange column chromatography was utilized to separate water-soluble metabolites. The methanol washes of silicic acid columns and the water fractions of rat urine after extraction with chloroform were chromatographed on Dowex 1-X8 anion-exchange columns. Of the total of ten different metabolites, designated A-K, eluted from the column, five contained both ethyl and propyl-^{14}C labels, three contained only the ethyl label, and two contained only the propyl label

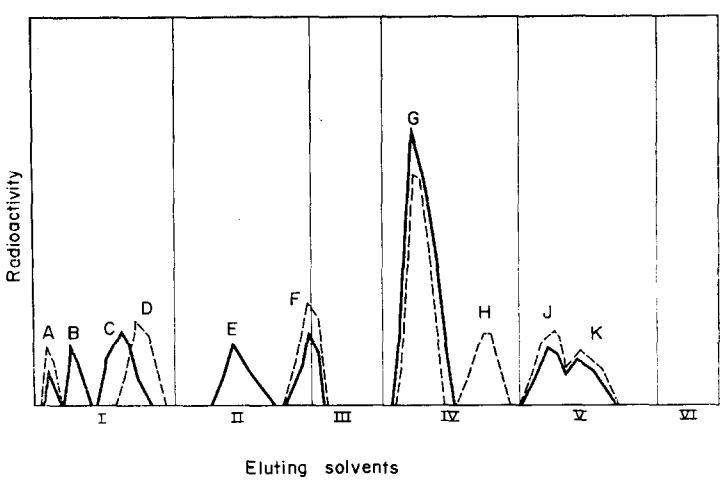

FIG. 1. Representation of the separation of water-soluble metabolites of *O*-ethyl S,S-dipropyl phosphorodithioate achieved on Dowex 1-X8 anion-exchange column. Eluting solvents indicated are: (I) elution gradient 0·01–0·1 N HCl (200 ml each); (II) elution gradient 0·1–1 N HCl (200 ml each); (III) water (200 ml); (IV) elution gradient 0·1 N HCl–methanol (1:3) to 1 N HCl–methanol (1:3) (200 ml each); (V) elution gradient 1 N HCl–methanol (1:3) to conc. HCl–water–acetone (1:1:6) (200 ml each); (VI) conc. HCl–water–methanol (1:1:6) (200 ml). Ethyl-^{14}C, ——; propyl-^{14}C, – – –.

(Fig. 1). Further characterization of the metabolites was accomplished by thin-layer chromatography.

The three organo-extractable metabolites of *O*-ethyl S,S-dipropyl-^{14}C phosphorodithioate isolated from rat urine are designated metabolites II, IV and V, using the nomenclature and chromatographic conditions previously described.[4]

Metabolite II co-chromatographed with a purified sample of methyl propyl sulfide in four different thin-layer chromatographic systems. It may have been produced as a result of S-methylation of propyl mercaptan, which was very easily liberated by the hydrolysis of *O*-ethyl S,S-dipropyl phosphorodithioate under slightly alkaline conditions and which was indirectly shown to occur in plants.[4] Metabolite II was therefore designated methyl propyl sulfide. Ethyl propyl sulfide can be differentiated from methyl propyl sulfide by gas, thin-layer and column chromatography and was not present in rat urine after treatment with either ethyl-^{14}C or propyl-^{14}C-labeled *O*-ethyl S,S-dipropyl phosphorodithioate, although it was present in plant extracts.[4]

The two peaks designated metabolites IV and V were detected only when the propyl-^{14}C-labeled compound was administered to rats, and, therefore, could not be the ethyl propyl sulfoxide and ethyl propyl sulfone found in plants.[4] These peaks co-chromatographed with methyl propyl sulfoxide and methyl propyl sulfone, respectively, and are believed to have been formed as a result of the oxidation of the S-methylated propyl mercaptan (metabolite II) released from *O*-ethyl S,S-dipropyl phosphorodithioate.

Metabolite A was eluted from the column with the solvent front and was observed in both ethyl and propyl label. It was a minor peak and did not co-chromatograph with any of the known compounds on hand. It is possible that this peak may represent incorporation of the ethyl or propyl groups, or both, from *O*-ethyl S,S-dipropyl phosphorodithioate into certain naturally occurring compounds. Metabolite A, therefore, remains unknown.

Metabolite B was observed only in the water fraction of rat and rabbit liver supernatant incubated with the ethyl-^{14}C label in the presence of reduced glutathione. It co-chromatographed with a purified sample of S-ethylglutathione. For further confirmation, the 2,4-dinitrophenol derivative of a portion of the peak co-chromatographed on a two-dimensional thin-layer system (direction 1, *n*-propanol–ammonia, 40:1; direction 2, chloroform–ethyl acetate–formic acid, 9:9:2) with the dinitrophenol derivative of known S-ethylglutathione. Metabolite B is therefore, designated S-ethylglutathione.

Metabolite C was observed in rat urine and in liver microsomes. It contained only the ethyl label, and was too nonvolatile to give any peaks upon gas chromatographic analysis using a W-98 column.[4]

The peak was acidified with gaseous hydrogen chloride and allowed to react with dicyclohexylcarbodiimide (DCC) in the presence of methanol before gas chromatographic analysis. A peak was observed, indicating the formation of a volatile compound, whose retention time was identical to that formed when *O*-ethyl phosphoric acid was reacted with DCC–methanol and chromatographed. When the metabolite solution was treated with DCC–methanol without prior acidification, no ester was formed, indicating that the phosphate was present as a salt rather than as a free acid. Metabolite C isolated from rat urine co-chromatographed with known, standard ethyl dihydrogen phosphate in two polar thin-layer chromatographic systems. When the

metabolite was made less polar by acetylating with acetic anhydride in the presence of concentrated sulfuric acid, it co-chromatographed with acetylated ethyl dihydrogen phosphate. Metabolite C is, on the basis of evidence cited, designated *O*-ethyl phosphoric acid.

Metabolite D was present in rat urine and rat and rabbit liver microsomes after animals were treated with the propyl-^{14}C label, but was not isolated after rabbit liver supernatant incubation. It was a very polar metabolite and co-chromatographed with a sample of S-propyl phosphorothiolic acid in three different thin-layer chromatographic systems. When acetylated with acetic anhydride, it co-chromatographed with an acetylated sample of S-propyl phosphorothiolic acid. Metabolite D, therefore, was designated S-propyl phosphorothiolic acid.

Metabolite E was a minor peak present after animals were treated with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate and did not co-chromatograph with any of the known standards. When the peak was concentrated by evaporation and conditions were made more acidic, it produced a radioactive spot at $R_f = 0{\cdot}49$ in thin-layer chromatography system I (Table 1). It was not subjected to any further analysis, and its identity remains unknown.

When metabolites F and G from the column were mixed together, only one radioactive spot was produced in three different thin-layer chromatographic systems. Both peaks co-chromatographed with *O*-ethyl S-propyl phosphorothioic acid. It is possible that metabolite F did not elute completely in the elution gradient of pH 1 to 1 N HCl of the ion-exchange column, but when the pH was raised with 200 ml water and methanol was added as co-solvent, it eluted immediately. Metabolite G was a major metabolite in rat urine, rat and rabbit liver microsomes and supernatant. It contained both labels, and in addition to co-chromatographing with *O*-ethyl S-propyl phosphorothioic acid on thin-layer and ion-exchange columns, its acetylated derivative co-chromatographed with the acetylated standard.

Metabolite H was a minor peak eluting soon after metabolite G that contained only the propyl label. It was present in rat urine, but was more pronounced in the rabbit liver supernatant. It co-chromatographed with the synthetic compound tentatively identified as S,S-dipropyl phosphorodithioic acid on three different thin-layer chromatographic systems. It was also extractable with chloroform from urine upon acidification to pH 1. Metabolite H, therefore, is designated S,S-dipropyl phosphorodithioic acid.

Metabolites J and K were rather heterogeneous peaks present after both ethyl-^{14}C and propyl-^{14}C label treatments. They did not co-chromatograph with any of the known standards. They were unstable under acidic conditions and did not give consistent R_f values on thin-layer chromatography. They were present only in rat urine. They were not analyzed further and their identities remain unknown.

Recovery of administered radioactivity. Treatment of rats with *O*-ethyl S,S-dipropyl phosphorodithioate resulted in the recovery in the urine of only about 50 per cent of the administered radioactivity from the ethyl-^{14}C label (Table 2) and about 65 per cent from the propyl-^{14}C label (Table 3).

Additional radioactivity, 0·32 and 0·24 per cent of the administered dose, was recovered when the feces from the male and female rats treated with the ethyl-^{14}C-labeled compound were extracted with 50% methanol and then acetone. Similarly, 0·68 and 0·56 per cent of the administered dose was recovered from the faeces

TABLE 2. URINARY METABOLITES OF *O*-ETHYL-[^{14}C]S,S-DIPROPYL PHOSPHORODITHIOATE*

Compounds in extracts	Per cent of administered dose found in extracts of urine									
	Male rats					Female rats				
	(0–6 hr)	(6–12 hr)	(12–24 hr)	(24–48 hr)	(48–72 hr)	(0–6 hr)	(6–12 hr)	(12–24 hr)	(24–48 hr)	(48–72 hr)
Water fraction										
Unknown A		0·18	0·07	0·04	0·002		0·23	0·05	0·001	
O-ethyl phosphoric acid	3·49	0·24	0·12	0·41	0·19	4·26	0·40	0·21	0·45	0·11
Unknown E	5·77					3·79				
O-ethyl S-propyl phosphorothioic acid	33·71	0·88	0·78	0·34	0·01	41·54	0·97	0·93	1·17	0·22
Unknown J	2·78					2·02				
Unknown K	1·25					1·35				
Chloroform fraction	<0·001					<0·001				
Total excreted	47·09	1·30	0·96	0·79	0·20	52·95	1·58	1·19	1·63	0·33

* Percentages of administered dose found in the extracts of urine collected at various times after dosing of male and female rats.

TABLE 3. URINARY METABOLITES OF *O*-ETHYL S,S-DIPROPYL-[^{14}C]PHOSPHORODITHIOATE*

Compounds in extracts	Per cent of administered dose found in extracts of urine								
	Male rats					Female rats			
	(0–6 hr)	(6–12 hr)	(12–24 hr)	(24–48 hr)	(48–72 hr)	(0–8 hr)	(8–24 hr)	(24–48 hr)	(48–72 hr)
Water fraction									
Unknown A	3·19	1·17	0·94	0·03		0·92	0·13	0·09	
S-propyl phosphorothiolic acid	7·55	3·15	2·62	3·00	0·15	3·65	5·88	3·20	0·51
O-ethyl S-propyl phosphorothioic acid	23·06	5·29	5·79	0·81	0·11	20·79	22·32	2·65	0·57
S,S-dipropyl phosphorodithioic acid	1·40	1·01	0·97	0·07		1·12	0·83	0·10	
Unknown J	2·82	0·98	0·06			0·92	0·42		
Unknown K						0·01	0·01		
Chloroform fraction									
Methyl propyl sulfide	0·10					0·01			
Methyl propyl sulfoxide	0·94	0·23				0·18	0·10		
Methyl propyl sulfone		0·46	0·40				0·73	0·31	0·71
Total excreted	38·39	12·29	10·77	3·19	0·25	27·60	30·42	6·35	1·79

* Percentages of administered dose found in the extracts of urine collected at various times after dosing of male and female rats.

TABLE 4. MICROSOMAL METABOLISM OF *O*-ETHYL S,S-DIPROPYL PHOSPHORODITHIOATE*

Compounds in extracts	Ethyl-[^{14}C] substrate				Propyl-[^{14}C]substrate			
	Rat		Rabbit		Rat		Rabbit	
	Pretreated†	Control	Pretreated†	Control	Pretreated†	Control	Pretreated†	Control
Chloroform fraction								
O-ethyl S,S-dipropyl phosphorodithioate	35·58	80·40	44·57	35·69	45·86	52·10	2·61	1·30
Unknown	1·68	0·86	1·80	0·95				
Water fraction								
Unknown A	7·59		2·38	0·45	2·71	0·25	2·16	
O-ethyl phosphoric acid	14·56	0·13	8·80	0·86				
S-propyl phosphorothiolic acid					9·45		5·20	
Unknown E	8·14		2·72	0·03				
O-ethyl S-propyl phosphorothioic acid	26·43	0·64	27·38	2·11	20·75	0·96	33·83	1·80
Total	93·98	82·03	87·65	40·09	78·75	53·31	43·80	3·20

* Percentages of metabolites from microsomes after incubation for 4 hr at 37·5° with ethyl-[^{14}C] and propyl-[^{14}C] substrate.
† Pretreated with 75 mg/kg sodium phenobarbital per day for 2 days before removal of livers.

after treatment with the propyl-^{14}C-labeled compound to male and female rats respectively. The radioactivity in the urine resulting from treatment with ethyl-^{14}C was not extractable with chloroform. All of the activity was present in the water phase as hydrolytic products. However, about 2·43 per cent of the administered radioactivity resulting from treatment with propyl-^{14}C was extracted by chloroform. When the urine was acidified to pH 1 and extracted with chloroform, an additional 15 per cent of the radioactivity was released. By successive equal-volume extractions with ether and ethyl acetate, about 30–35 per cent more radioactivity was released, leaving about 55 per cent of the activity still in the water in the form of nonorgano-extractable products.

Rats were able to degrade *O*-ethyl S,S-dipropyl phosphorodithioate most efficiently, and most of the radioactivity in the form of hydrolytic products was excreted in the urine in the first 6 hr. The major metabolite in rat urine was *O*-ethyl S-propyl phosphorothioic acid (Tables 2 and 3), with several other metabolites being present in small but significant quantities.

Metabolism in vitro *of O-ethyl S,S-dipropyl phosphorodithioate.* When rat and rabbit liver microsomes and supernatant preparations were incubated with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate and *O*-ethyl S,S-dipropyl-^{14}C phosphorodithioate, most of the organo-extractable radioactivity was due to the unchanged parent compound, as determined by silicic acid column chromatography and co-chromatography on thin-layer plates with known samples of *O*-ethyl S,S-dipropyl phosphorodithioate. Another organo-extractable metabolite containing only ethyl-^{14}C was observed which co-chromatographed on thin-layer plates with ethyl propyl sulfoxide, but not on the silicic acid column. Furthermore, it was doubtful that it was ethyl propyl sulfoxide, since microsomes were not able to oxidize ethyl propyl sulfide to any other product during 4 hr of incubation. It was not separated in sufficient amounts to be subjected to detailed characterization studies, but it may be an organo-extractable constituent of the microsomal preparation which incorporated the labeled carbon from the ethyl-^{14}C moiety.

TABLE 5. METABOLISM OF *O*-ETHYL S,S-DIPROPYL PHOSPHORODITHIOATE IN RAT AND RABBIT LIVER SUPERNATANT PREPARATIONS*

Compounds in extracts	Ethyl-[^{14}C]substrate		Propyl-[^{14}C]substrate	
	Rat	Rabbit	Rat	Rabbit
Chloroform fraction				
O-ethyl S,S-dipropyl phosphorodithioate	93·90	78·42	40·96	20·97
Water fraction				
S-ethylglutathione	1·36	3·84		
O-ethyl S-propyl phosphorothioic acid	2·31	9·32	1·06	2·07
S,S-dipropyl phosphorodithioic acid			0·86	2·67
Total recovered	96·97	91·58	42·88	25·71

* Percentages of metabolites recovered after incubation for 4 hr at 37·5° with ethyl-[^{14}C] and propyl-[^{14}C]substrate in the presence of reduced glutathione. The incubation medium contained 3 ml supernatant and 1 ml of 5×10^{-3} M glutathione (rabbit) and 2·6 ml supernatant and 0·4 ml of $3·3 \times 10^{-3}$ M glutathione (rat). Rat and rabbit were pretreated with 75 mg/kg of sodium phenobarbital/day for 2 days before removal of livers.

When the water fraction of the microsomes after extraction with chloroform was chromatographed on an anion-exchange column, four radioactive peaks were separated after incubation with *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate and three after incubation with *O*-ethyl S,S-dipropyl-^{14}C phosphorodithioate. The peaks were further analyzed by thin-layer chromatography. The major metabolite was *O*-ethyl S-propyl phosphorothioic acid (Table 4). *O*-ethyl phosphoric acid and S-propyl phosphorothiolic acid were also present along with the unknown metabolites A and E. S,S-dipropyl phosphorodithioic acid was not produced in the microsomal incubations.

When the water fraction of rat and rabbit liver supernatant incubated with ethyl-^{14}C-labeled and propyl-^{14}C-labeled *O*-ethyl S,S-dipropyl phosphorodithioate was chromatographed on an anion-exchange column, the largest radioactive peaks were *O*-ethyl S-propyl phosphorothioic acid and S,S-dipropyl phosphorodithioic acid (Table 5). Another radioactive peak, metabolite B, was separated only from ethyl-^{14}C and not from propyl-^{14}C incubation, and was identified as S-ethylglutathione. This material was apparently produced in this system as a result of ethyl-transfer from *O*-ethyl S,S-dipropyl phosphorodithioate to the glutathione which had been added to the liver supernatant preparation, indicating the presence of a glutathione-dependent *O*-dealkylase. No other radioactive materials were isolated from the supernatant incubations.

Rabbit liver microsomes metabolized *O*-ethyl S,S-dipropyl phosphorodithioate in greater amounts than did rat liver microsomes. Pretreatment of rats and rabbits with sodium phenobarbital prior to removal of livers resulted in greater metabolism of the substrate. The poor recovery from the propyl-^{14}C-labeled compound, which was noticed frequently in these studies, was probably due to the production of the volatile labeled propyl mercaptan.

DISCUSSION

A number of organo-extractable and water-soluble metabolites of *O*-ethyl S,S-dipropyl phosphorodithioate have been detected in rat urine and in liver microsomal systems. The metabolites formed were largely the result of deethylation and depropylation of the molecule. The resulting fragments were then able to react with or be incorporated into other constituents of the rats. A proposed scheme for the degradation of *O*-ethyl S,S-dipropyl phosphorodithioate in rats is depicted in Fig. 2.

It can be observed from Fig. 2 that the formation of the propyl thiolate ion is postulated as the mechanism for the initial metabolism of the compound. Other metabolites are proposed to result after the formation of this initial product. Chloroform extracts of urine resulting from the treatment of rats with the propyl-^{14}C-labeled compound revealed the presence of methyl propyl sulfide. This identification is supported by the fact that no organo-extractable radioactivity resulted in urine after the treatment of rats with the ethyl-^{14}C label. The fact that methyl propyl sulfide is formed also supports the theory of prior formation of the propyl thiolate ion which, in this case, could be methylated by S-methyl transferase systems.

There is no possibility that the metabolite identified as methyl propyl sulfide in rat urine is the same as the compound earlier identified as ethyl propyl sulfide in plants.[4] The two compounds can be readily separated by gas chromatography using a column of 20% W-98 on Chromosorb W (60–80 mesh). In addition, ethyl propyl sulfide would

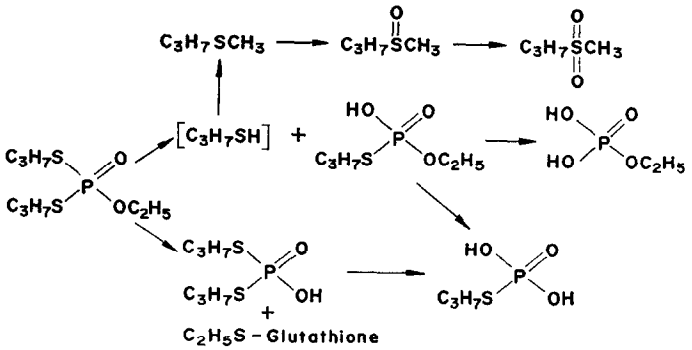

FIG. 2. Proposed metabolic degradation scheme of *O*-ethyl S,S-dipropyl phosphorodithioate in rats and in rat and rabbit liver microsomes and supernatant preparations.

result from treatment with *O*-ethyl S,S-dipropyl phosphorodithioate labeled in either position, while methyl propyl sulfide would result only from the propyl-^{14}C label, which was observed to be the case.

The methyl propyl sulfide formed in rats was oxidized to form its sulfoxide and sulfone. The separation on Silica gel columns of chloroform extracts of rat urine at different intervals indicated that only trace amounts of methyl propyl sulfide were found in the earliest samples. Within 12 hr after treatment, methyl propyl sulfoxide and sulfone were found, but by 24 hr after treatment and thereafter, only methyl propyl sulfone could be detected.

The formation of a propyl thiolate ion is also supported by the fact that *O*-ethyl S-propyl phosphorothioic acid was the major metabolite isolated from rat urine, and, in addition, was the major component of the methanol–water extract of corn plants.[4] *O*-ethyl S-propyl phosphorothioic acid must release a second molecule of propyl mercaptan, since *O*-ethyl phosphoric acid is an important metabolite which accumulates in later samples of rat urine.

In light of the central place of the propyl thiolate ion in the proposed route of metabolism of *O*-ethyl S,S-dipropyl phosphorodithioate, the presence of S,S-dipropyl phosphorodithioic acid is of interest. This metabolite was found only in rat urine, not in plants.[4] However, S,S-dipropyl phosphorodithioic acid was found to be rather unstable, and it probably loses propyl mercaptan to give S-propyl phosphorothiolic acid, which was found in plants. The fact that S,S-dipropyl phosphorodithioic acid was found only in rat urine may indicate that it was formed by another route in rats. It may have been produced by the direct deethylation of *O*-ethyl S,S-dipropyl phosphorodithioate not involving propyl thiolate ion attack. The fact that no ethyl propyl sulfide was isolated from rat urine supports this metabolic route. S-propyl phosphorothiolic acid was also found in significant quantities in rat urine.

The striking difference between these two routes of metabolism in plants and animals may be partially explained by the fact that *O*-ethyl S,S-dipropyl phosphorodithioate was completely metabolized in rats within 6 hr, but, on the other hand, unmetabolized *O*-ethyl S,S-dipropyl phosphorodithioate was still present in significant quantities in both beans and corn at harvest time. It is likely that the S-methyl transferase system in rats is more important than that in plants, and that it dominates the

metabolism of the compound in rats, while the system responsible for the formation of ethyl propyl sulfide in plants is relatively more important there. The propyl thiolate ion, if formed, was probably present for a very short time in rats before being methylated, while in plants there was time for it to react in a number of other ways.

The recovery of radioactivity from rat urine was not as high as might have been expected. However, it must be noted that the ^{14}C-labeled carbon atoms on both samples of *O*-ethyl S,S-dipropyl phosphorodithioate were located in the alpha-carbons of side chains, not in the central atom of the molecule, as in previous studies of organophosphates which have utilized ^{32}P-labeled materials. When an ethoxide ion or a propyl thiolate ion is released from *O*-ethyl S,S-dipropyl phosphorodithioate in a biological system, it becomes difficult to trace. It is incorporated into biological constituents, conjugated for excretion, or may even become a component of the waste gases expired by the animal. In a number of instances, radioactive compounds were detected on chromatograms that could not be related directly to the metabolic products of *O*-ethyl S,S-dipropyl phosphorodithioate. These may have been the result of the incorporation of the ^{14}C label from the compound into small molecules which were extracted and chromatographed along with metabolites retaining a greater portion of the parent compound. Therefore, the recovery data presented represent only one component of the elimination process of the animal.

The liver microsomal, NADPH-dependent enzymes were able to metabolize *O*-ethyl S,S-dipropyl phosphorodithioate to form *O*-ethyl S-propyl phosphorothioic acid, S-propyl phosphorothiolic acid, and *O*-ethyl phosphoric acid. Rabbit liver microsomes were more active than rat liver microsomes when both were induced by phenobarbital. Surprisingly, however, when ethyl propyl sulfide was incubated with liver microsomes, neither ethyl propyl sulfoxide nor sulfone was formed. Gillette and Kamm[18] with mammalian liver microsomes and Tsukamoto and Casida[19] with housefly abdomen microsomes have shown that the microsomal enzymes were able to carry out the sulfoxidation of 4,4′-diaminodiphenyl sulfide and 3,5-dimethyl-4-(methylthio) phenyl-*N*-methylcarbamate respectively.

Rat and rabbit liver supernatant enzymes, which require reduced glutathione, successfully *O*-deethylated *O*-ethyl S,S-dipropyl phosphorodithioate. A number of workers[20–23] have shown that in the process of *O*-dealkylation of certain compounds a methyl group is transferred to glutathione and that *O*-deethylation and *O*-depropylation were much slower than *O*-demethylation. *O*-ethyl-^{14}C S,S-dipropyl phosphorodithioate, when incubated with liver supernatant enzymes in the presence of glutathione, produced S,S-dipropyl phosphorodithioic acid and S-ethylglutathione-ethyl-^{14}C.

This study has shown that the metabolism of *O*-ethyl S,S-dipropyl phosphorodithioate in rats is extremely rapid and results only in metabolites which would not be expected to present a toxic hazard to man.

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