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Metabolism of *O,S*-Dimethyl Propionyl- and Hexanoylphosphoramidothioate in the House Fly and White Mouse[1]

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The metabolism of *O,S*-dimethyl propionyl- and hexanoylphosphoramidothioate was investigated in the white mouse and house flies. Compared to the hexanoylphosphoramidothioate, the propionyl analog is approximately 35-fold more toxic to house flies and is 10-fold less toxic to mice. On a percentage basis, substantially larger amounts of methamidophos were detected in house flies treated topically with the propionylphosphoramidothioate than in flies treated with the hexanoyl derivative. The reverse was evident in the case of the mouse where much larger amounts of methamidophos were formed after oral treatment with the hexanoylphosphoramidothioate. Minor amounts of other metabolic products also were detected, including an unknown from the hexanoylphosphoramidothioate. Metabolism of the *S*-methyl moiety to carbon dioxide appeared to be a major pathway for metabolic degradation of both compounds in both the white mouse and house fly. The difference in toxicity of the two acylphosphoramidothioates to the mouse and house fly is attributed to difference in the amounts of methamidophos formed in the animals.

O,S-Dimethyl phosphoramidothioate (methamidophos) is an outstanding insecticide which is currently being marketed as a broad spectrum insecticide. Although methamidophos has many virtues as an insecticide, unfortunately it is also somewhat toxic to warm-blooded animals (oral rat and mice LD_{50} are 20 and 14 mg/kg, respectively) (1). In contrast, the *N*-acetyl (acephate) and *N*-propionyl derivatives of methamidophos, while still retaining the good insecticidal and acaricidal properties of methamidophos, are of low mammalian toxicity (rat oral LD_{50} of 900 and >1000 mg/kg, respectively). Selectivity, however, is reversed when the acyl chain length is increased, and the hexanoyl derivative, with a mouse oral LD_{50} of 35 mg/kg, is slightly more toxic to the mouse than it is to house flies (1).

Owing to the interesting contrast in insecticidal activity and mammalian toxicity between the hexanoyl derivative and the smaller chain acyl derivatives, a study of the comparative metabolism of these compounds in a mammal and insect was undertaken to establish the basis for their toxicological properties. This report is concerned with the metabolism and mode of action of *O,S*-dimethyl propionyl- (I) and hexanoylphosphoramidothioate (II) in the white mouse and house fly.

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MATERIALS AND METHODS

In general, the procedures described by Magee (1) for the synthesis of acylphosphoramidothioates were used to prepare *O,S*-dimethyl propionyl- (I), hexanoylphosphoramidothioate (II), and related derivatives. They are described briefly as follows.

O,O-Dimethyl Propionylphosphoramidothioate

A mixture of 14.1 g of *O,O*-dimethyl phosphoramidothioate (2), 13.5 g of propionic acid anhydride, 0.25 ml of phosphoric acid (85%), and 30 ml of dry methylene chloride was stirred under nitrogen for 24 hr at room temperature. The mixture was added to 15 ml of saturated aqueous ammonium chloride containing 7 g of ice, and 15% ammonium hydroxide was added to neutralization (25 ml). The organic phase was separated and the aqueous phase was extracted twice with methylene chloride. The combined organic extract was washed with saturated ammonium chloride, dried over anhydrous magnesium sulfate, and the product, *O,O*-dimethyl propionylphosphoramidothioate, was distilled by falling-film distillation at a wall temperature of 60°C (0.05 mm), n_D^{25} 1.4998. The product gave the following pmr[2] signals (δ, 60 MHz, $CDCl_3$, TMS): triplet, 1.0–1.3 (3H, CH_3); quartet, 2.3–2.7 (2H, CH_2); doublet, 3.7–3.9 (6H, OCH_3, J = 12 Hz); and doublet, 7.8–8.1 (1H, NH).

O,O-Dimethyl Hexanoylphosphoramidothioate

An equimolar mixture of 7.0 g of *O,O*-dimethyl phosphoramidothioate and 6.7 g of hexanoyl chloride in 25 ml of methylene chloride was heated at reflux for 2 hr. The mixture was washed with water, with saturated aqueous sodium chloride, and dried over magnesium sulfate. Falling-film distillation at a wall temperature of 126–130°C (0.05 mm) gave 5.6 g of product, n_D^{25} 1.4822. The pmr spectrum showed a triplet at 0.9–1.1 (3H, CH_3), broad multiplet at 1.1–1.9 [6H, $(CH_2)_3$], triplet at 2.4–2.7 (2H, $OCCH_2$), and doublet at 3.7–4.0 (6H, OCH_3, J = 12 Hz).

O-Methyl S-Sodio Propionylphosphoramidothioate

A mixture of 7.88 g of *O,O*-dimethyl propionylphosphoramidothioate, 3.6 g of *n*-butanethiol, 2 g of sodium hydroxide, and 25 ml of methanol was heated at reflux under nitrogen for 3 hr and allowed to stand overnight. The product which separated was collected and recrystallized from acetonitrile, mp 116–118°C. The pmr spectrum taken in D_2O gave a triplet at 1.0–1.3 (3H, CH_3), quartet at 2.3–2.7 (2H, CH_2), and doublet at 3.6–3.8 (3H, OCH_3, J = 12 Hz).

O-Methyl S-Sodio Hexanoylphosphoramidothioate

This compound was made in the same manner as the propionyl analog, except sodium ethyl xanthate was used as the dealkylating agent and methyl ketone as the solvent. The product was recrystallized from acetonitrile, mp 92–94°C. The pmr spectrum (D_2O) showed a triplet at 0.9–1.1 (3H, CH_3), broad multiplet at 1.1–1.9 [6H, $(CH_2)_3$], triplet at 2.4–2.7 (2H, $OCCH_2$), and doublet at 3.1–4.0 (3H, OCH_3).

O,S-Dimethyl Propionylphosphoramidothioate (I)

A mixture of 0.28 g of methyl methanesulfonate, 0.6 g of *O*-methyl *S*-sodio propionylphosphoramidothioate, and 10 ml

[2] Abbreviations used: pmr, proton magnetic resonance; TMS, tetramethylsilane; J, spin-coupling constant; tlc, thin-layer chromatography; AChE, acetyl cholinesterase.

of acetonitrile was heated at reflux for 3 hr. The mixture was filtered, concentrated, and the residual product was recrystallized from ether-hexane, mp 47–49°C. *Elemental analysis.* $C_5H_{12}NO_3PS$ requires (%): C, 30.46; H, 6.09; found: C, 31.12; H, 6.27. The pmr spectrum ($CDCl_3$) showed a triplet at 1.0–1.3 (3H, CH_3), doublet at 2.3–2.5 (3H, SCH_3, J = 14 Hz), quartet at 2.3–2.7 (2H, CH_2), and doublet at 3.7–3.9 (3H, OCH_3, J = 12 Hz). The product showed tlc properties identical to a sample obtained from the Chevron Chemical Company, Ortho Division, Richmond, Calif.

O,S-Dimethyl Hexanoylphosphoramidothioate (II)

This compound was prepared similarly to the propionyl analog. Purification was achieved by column chromatography using SilicAR CC 7 Special (Mallinckrodt, St. Louis, Mo.) and acetone–hexane (2:1, v/v) as the eluting solvent. The product, an oil (n_D^{25} 1.4835), gave the following pmr signals: triplet, 0.8–1.1 (3H, CH_3); broad multiplet, 1.2–1.8 [6H, $(CH_2)_3$]; doublet, 2.3–2.5 (3H, SCH_3, J = 14 Hz); triplet, 2.2–2.6 (2H, $OCCH_2$); and doublet, 3.8–4.0 (3H, OCH_3, J = 12 Hz). *Elemental analysis.* Calcd for $C_8H_{18}NO_3PS$: C, 40.17; H, 7.53; found: C, 40.88; H, 7.46.

S-Methyl O-Sodio Propionylphosphoramidothioate

A mixture of 0.2 g of *O*-methyl *S*-sodio propionylphosphoramidothioate, 0.17 g of methyl iodide, and 10 ml of acetone was stirred at room temperature for 24 hr. Removal of the solvent gave the solid product which was recrystallized from acetonitrile, mp 130–132°C. The pmr spectrum (D_2O) showed a triplet at 1.0–1.3 (3H, CH_3), doublet at 2.1–2.6 (3H, SCH_3, J = 14 Hz), and quartet at 2.3–2.7 (2H, CH_2). *Elemental analysis.* Calcd for $C_4H_9NNaO_3PS$: C, 23.41; H, 4.39; found: C, 23.72; H, 4.38.

S-Methyl O-Sodio Hexanoylphosphoramidothioate

This compound was prepared in the same manner as the propionyl derivative. The product, recrystallized from acetonitrile, mp 129–130°C, gave the following pmr signals: triplet, 0.8–1.2 (3H, CH_3); broad multiplet, 1.2–1.8 [6H, $(CH_2)_3$]; triplet, 2.3–2.7 (2H, $OCCH_2$); and doublet, 2.1–2.4 (3H, SCH_3, J = 14 Hz). *Elemental analysis.* Calcd for $C_7H_{15}NNaO_3PS$: C, 34.01; H, 6.07; found: C, 33.40; H, 6.32.

O,S-dimethyl phosphoramidothioate (methamidophos) (3), *O,S*-dimethyl phosphorothioic acid (4), and *S*-methyl *O*-sodio phosphoramidothioate (5) were prepared by literature methods.

Radiolabels

S-[^{14}C]Methyl *O*-methyl propionylphosphoramidothioate was prepared in the same manner as the nonradioactive preparation using 5.6 mg (0.5 mCi) of [^{14}C]methyl methanesulfonate, and 10.3 mg *O*-methyl *S*-sodio propionylphosphoramidothioate. Because of the sample size, the product was purified by preparative tlc using PQ-1-1000 plates (Quantum Industries, Fairfield, N. J.) and acetonitrile–methylene chloride (9:1, v/v) as the developing solvent. The purified product was >99% radiochemically pure and had a specific activity of 10 mCi/mmol.

S-[^{14}C]Methyl *O*-methyl hexanoylphosphoramidothioate was prepared and purified in the same manner. It was >99% radiochemically pure and had a specific activity of 6.2 mCi/mmol.

Thin-layer Chromatography

Separation and identification of the metabolites of I and II were accomplished by tlc using 20 × 20-cm Q1F Silica Gel plates (0.25-mm thickness) purchased from Quantum Industries, Fairfield, N. J. R_f values and the identification of the various model metabolites in two solvent systems,

TABLE 1

The tlc Properties of O,S-Dimethyl Propionyl- (I) and Hexanoylphosphoramidothioate (II) and Possible Metabolites

Number	Structure	R_f	
		Acetone–hexane (2:1)	CH_3CN–CH_2OH–H_2O (6:3:1)
I	$(CH_3O)(CH_3S)P(O)NHCOC_2H_5$	0.58	0.72
II	$(CH_3O)(CH_3S)P(O)NHCOC_5H_{11}$-*n*	0.70	0.74
III	$(CH_3O)(CH_3S)P(O)NH_2$ (methamidophos)	0.39	0.49
IV	$(HO)(CH_3S)P(O)NHCOC_2H_5$	0	0.59
V	$(HO)(CH_3S)P(O)NHCOC_5H_{11}$-*n*	0	0.60
VI	*O,S*-dimethyl phosphorothioic acid	0	0.67
VII	$(HO)(CH_3S)P(O)NH_2$	0	0.49
VIII	Unknown	0.26	0.74

A, acetone–hexane (2:1), and B, acetonitrile–methanol–water (6:3:1), are given in Table 1. All tlc solvents were AR grade.

Radioactive spots were detected by means of a Berthold (Varian Aerograph) thin-layer radioscanner (Model LB 2723) equipped with a dot printer. Results from the radioscanner were confirmed by exposing the tlc plates to Kodak Blue Brand No-Screen X-ray film. Radioactive spots on tlc plates were scraped into counting vials and radioactivity was quantified in a Beckman Model LS-230 liquid scintillation spectrometer, using scintillation fluid prepared from 1000 ml of dioxane, 100 g of naphthalene, 7 g of PPO, and 0.3 g of POPOP. Radioactivity was corrected to actual disintegrations per minute by use of an external standard and converted to unit weight (μg) from the known specific activity of the parent compound.

Metabolism in House Flies

Radioactive I or II was applied to the thorax of 3-day-old female susceptible house flies (S_{NAIDM} strain) as previously described (6). Eighty flies were used for each penetration experiment and 200 flies were used for each metabolism study. After treatment, the flies were placed in a 500-ml conical filter flask connected with tubing for the passage of air. Air from the affluent side was passed through potassium hydroxide and ascarite traps, and the effluent air was passed through a sintered glass bubbler into a carbon dioxide absorption tower containing 45 ml of β-phenethylamine, 40 ml of methanol, and 20 ml of toluene. Flies were sacrificed at 1, 4, 8, and 12 hr for the penetration study and 6 hr for the metabolism study by placing them in the dry ice chest for 10 min. Analysis for penetration and metabolism was conducted immediately upon removal from the chest.

The frozen flies were analyzed as previously described (7). The flies and holding flask were separately rinsed three times each with 10 ml of acetone–methanol (1:1, 30 ml total). Radioactivity recovered by rinsing the holding vessel is referred to as the *cage-rinse*, and that obtained by rinsing the flies as *body-rinse*. The rinsed flies were homogenized and extracted three times each with 10 ml of acetone–methanol solvent. The radioactivity in the combined extracts is referred to as the *internal extract*. The individual rinses and extracts were reduced to near dryness with the aid of an Evapo-Mix concentrator (Buchler Instruments), and to ultimate dryness under a gentle stream of dry nitrogen. The residue was dissolved in a known volume of methanol and aliquots were taken for

scintillation counting to estimate recovered radioactivity. The remaining sample was applied to tlc plates, predeveloped with *n*-hexane to remove lipids, and then developed with solvent system A, in the vertical direction, and solvent system B, in the horizontal direction.

The residual house fly material remaining after solvent extraction was air dried, and the radioactivity present was estimated after combustion of the residue in a Packard Model 306 Tri-Carb Sample Oxidizer.

Metabolism in the White Mouse

Mouse metabolism studies were carried out as previously described (8). Female Swiss white mice were treated orally with a solution of radioactive I or II contained in 0.1 ml of corn oil. The treated mouse was held in a closed all-glass metabolism chamber which allowed for the separate collection of expired carbon dioxide, urine, and feces. The total urine samples from each mouse were reduced in volume by means of an Evapo-Mix concentrator, and to dryness under a gentle stream of nitrogen, and the residual radioactivity was dissolved in 1 ml of methanol. Aliquots were taken for assessment of radioactivity and for tlc analysis. Feces were homogenized in acetone–methanol solvent in an all-glass homogenizer, centrifuged, and the supernatant was concentrated for radioassay and tlc analysis. The mouse was anesthetized with ether and the liver, kidney, stomach, and other organs were removed. The various organs were homogenized in the same solvent mixture and assayed for radioactivity in the same manner as for the feces.

Metabolism by Mouse Liver Microsomes

Mouse livers taken from three freshly sacrificed mice were rinsed, weighed, and homogenized in cold 1.15% potassium chloride solution (20%, w/v). The homogenate was centrifuged at 10,000*g* for 30 min at 5°C in a Servall RC-2B refrigerated centrifuge. The supernatant was decanted and further centrifuged at 100,000*g* for 1 hr in a Beckman L2-50 ultracentrifuge to provide the microsomal pellet. The pellet was suspended in 1.15% potassium chloride as needed (9).

Radioactive I or II (50 μg) was added to 25 ml of reaction mixture made up of 2 ml of microsomal enzyme preparation (0.445 g of liver/ml), 5×10^{-2} *M* phosphate buffer (pH 7.4), 10^{-3} *M* glucose-6-phosphate, 10^{-4} *M* NADP, 2.5×10^{-3} *M* potassium chloride, and glucose-6-phosphate dehydrogenase (2–4 enzyme units). The mixture was shaken in an open

TABLE 2

Toxicological Properties of O,S-Dimethyl Propionyl- (I), Hexanoylphosphoramidothioate (II), and Methamidophos (III)

	I	II	III (Methamidophos)
Mouse oral LD_{50} (mg/kg)[a]	350	35	14.0
Mouse i.p. LD_{50} (mg/kg)	92[b]	25[c]	5.3
House fly LD_{50}, S_{NAIDM} LD_{50} (μg/g)	1.1	38	1.20
AChE inhibition, bovine erythrocyte, k_i (M^{-1} min^{-1})	<1	<1	1.8×10^3
AChE inhibition, house fly-head, k_i (M^{-1} min^{-1})	1.3	2.4	2.5×10^3

[a] Corn oil was used as the carrier.

[b] Compound was dissolved in saline (0.95% sodium chloride).

[c] Compound was dissolved in propylene glycol–saline (1:1, v/v).

Erlenmyer flask for 1 hr at 37.5°C, and the reaction was terminated by the addition of 2 ml of 5% aqueous trichloroacetic acid. Metabolic products were extracted into ethyl acetate and the extract was concentrated and examined by tlc in the usual manner.

Bioassay

Toxicity of I and II to S_{NAIDM} susceptible house flies (*Musca domestica*) was determined by topical application as previously described (10). Test compounds were dissolved in corn oil for the determination of mouse oral toxicity, and in saline for intraperitoneal toxicity. Five mice, 3–4 months old, were used at each dosage level and mice were treated as previously described (11). LD_{50} values were estimated from log-probit plots. Methods to determine anticholinesterase activity have been described (12).

RESULTS

Toxicology

Toxicological data for *O,S*-dimethyl propionyl- (I) and hexanoylphosphoramidothioate (II) are presented in Table 2. Results for mouse and house fly toxicity are in general agreement with those reported by Magee (1) for the rat and house fly. Compared to II, I is approximately 35-fold more toxic to S_{NAIDM} house flies and is 10-fold less toxic to mice (oral). Results reported by Magee showed that I was >7-fold more toxic to house flies and 16- to 25-fold less toxic to rats (LD_{50} >1000 mg/kg). Evidently I is about 3-fold more toxic to mice than to rats.

Anticholinesterase data in Table 1 show that both I and II are very poor inhibitors of house fly-head (HFAChE) and bovine erythrocyte acetylcholinesterase (BAChE). Concentrations of I and II as high as 1.0 *M* were required to obtain measurable rates of AChE inhibition. For all practical purposes I and II may be considered inert to these enzymes. Methamidophos (III) is substantially more effective as an anticholinesterase than I or II, but is relatively poor compared to other organophosphorus esters such as paraoxon and tetraethyl pyrophosphate (13).

TABLE 3

Distribution of Radioactivity in House Flies (S_{NAIDM}) after Topical Application of 0.535 μg/g of S-[^{14}C]Methyl O-Methyl Propionylphosphoramidothioate (I) and 20.78 μg/g of S-[^{14}C]Methyl O-Methyl Hexanoylphosphoramidothioate (II)

Distribution	Recovery (%) of applied radioactivity at indicated time interval (hr)			
	1.0	4.0	8.0	12.0
Compound I				
CO_2	0.62	4.1	6.7	17.8
External	75.3	64.5	42.2	33.7
Internal	19.6	23.5	39.6	33.8
Total	95.5	92.1	88.5	85.3
Compound II				
CO_2	0.54	7.4	16.4	11.2
External	90.6	60.5	39.5	40.6
Internal	13.2	29.8	28.9	36.1
Total	104.3	97.7	84.8	87.9

Metabolism in House Flies

For the penetration, distribution, and metabolism studies, house flies were treated topically with approximately one-half the LD_{50} values of I or II. Radioactive I was applied at a dosage of 0.535 μg/g and II at 20.78 μg/g. At these dosages the flies showed signs of hyperexcitability but no mortality was observed at 6 hr. Table 3 gives data for the distribution of radioactivity after different time intervals (up to 12 hr) following topical treatment with I and II. In gross aspects, little difference is observed in the distribution of radioactivity between the two compounds. Penetration, as estimated by the amount of recovered external radioactivity, appeared to be slightly faster initially (1 hr) for I, but was virtually the same as II after longer time intervals. Based on the data, it is unlikely that differences in toxicity

TABLE 4

Metabolic Products Recovered from House Flies 6 hr after Topical Application of 0.535 μg/g S-[^{14}C]Methyl O-Methyl Propionylphosphoramidothioate (I)

Metabolite	Recovery		
	Equivalents of I (μg/g)	Recovered radioactivity (%)	Applied radioactivity (%)
Cage rinse			
I Parent compound	0.023	4.6	4.3
IV *O*-Demethyl-I	0.004	0.8	0.7
Body rinse			
I Parent compound	0.170	34.1	31.7
III Methamidophos	0.018	3.7	3.4
IV *O*-Demethyl-I	0.011	2.2	2.0
Internal extract			
I Parent compound	0.064	12.9	12.0
III Methamidophos	0.116	23.3	21.6
IV *O*-Demethyl-I	0.005	1.0	0.9
Unextractable	0.050	10.1	9.4
CO_2	0.036	7.3	6.8
Total	0.497	100	92.8

may be attributed to differences in penetration rates. Total recovery of applied radioactivity in these studies ranged from 85–104%.

Results for the metabolism of I in S_{NAIDM} flies are presented in Table 4 and those for II in Table 5. The parent compound and only two metabolites were isolated from flies treated with I. These were methamidophos (III, *O,S*-dimethyl phosphoramidothioate) and the *O*-demethylated derivative of I (IV, hydrogen *S*-methyl propionylphosphoramidothioate). Of the three compounds found in the *internal extract*, methamidophos (III) was by far the most prominent, representing approximately 23% of the recovered or 22% of the applied radioactivity. Owing to the substantially higher anticholinesterase activity of III compared to I, it is probable that III is the agent responsible for intoxication.

Significant amounts of what appeared to be $^{14}CO_2$ were retained in the phenethylamine trap attached to the effluent side of the fly metabolism chamber. In a separate experiment with [^{14}C]acephate (*O,S*-dimethyl acetylphosphoramidothioate) the effluent air from the chamber was passed into a saturated solution of barium hydroxide (14). Barium carbonate which precipitated was collected, dried, and counted for radioactivity. The radioactivity trapped by this procedure corresponded with that obtained using the phenethylamine trap. Evidently, the methyl group attached to the sulfur atom in acephate, and presumably in I and II, is metabolized to carbon dioxide.

Compared to I, the metabolism of II in the house fly is more complex and at least five metabolites, in addition to the parent compound, were isolated. The metabolites were methamidophos (III), the *O*-demethylated derivative of II (V, hydrogen *S*-methyl hexanoylphosphoramidothioate), *O*-demethylated methamidophos (VI, hydrogen *S*-methyl phosphoramidothioate), *O,S*-dimethyl phosphorothioic acid (VII), and an unknown (VIII) of similar but slightly different chromatographic proper-

ties as methamidophos (R_f: solvent system A, 0.26; solvent system B, 0.74). A minor amount of a highly polar material, which remained at the origin after development with both solvent systems, was detected and this is labeled as a conjugate in the table. All six compounds, including the parent material II, were detected in the *internal extract* and, as in the case of I, methamidophos was most prominent although on a percentage basis less was found than with I (4.55% of recovered radioactivity compared to 23.3% for I). However, owing to the 39-fold larger dosage of II compared to I, 5.7-fold more methamidophos was detected in the *internal fraction* of flies treated with II (after correction for molecular weights).

Metabolism in the White Mouse

Results for the metabolism of I in a single white mouse 6 hr after oral treatment at a dosage of 50.16 mg/kg are presented in Table 6. At this time, about two-thirds of the administered radioactivity was eliminated from the mouse as urinary, fecal, and respiratory (presumably as car-

TABLE 5

Metabolic Products Recovered from House Flies 6 hr after Topical Application of 20.78 μg/g S-[^{14}C]Methyl O-Methyl Hexanoylphosphoramidothioate (II)

Metabolite		Recovery		
		Equivalents of II (μg/g)	Recovered radioactivity (%)	Applied radioactivity (%)
Cage rinse				
II	Parent compound	0.99	5.7	4.8
III	Methamidophos	0.07	0.4	0.3
V	*O*-Demethyl-II	0.15	0.9	0.8
VI	$(CH_3O)(CH_3S)P(O)OH$	0.02	0.1	0.1
VII	$(HO)(CH_3S)P(O)NH_2$	0.04	0.2	0.2
VIII	Unknown	0.06	0.4	0.3
	Conjugates	0.01	<0.1	<0.1
Body rinse				
II	Parent compound	8.65	49.6	41.7
III	Methamidophos	0.15	0.9	0.8
V	*O*-Demethyl-II	0.61	3.5	3.0
VI	$(CH_3O)(CH_3S)P(O)OH$	0.16	0.9	0.8
VII	$(HO)(CH_3S)P(O)NH_2$	0.14	0.8	0.7
VIII	Unknown	0.16	0.9	0.8
	Conjugates	0.03	0.1	0.1
Internal extract				
II	Parent compound	2.54	14.5	12.2
III	Methamidophos	0.79	4.6	3.8
V	*O*-Demethyl-II	0.34	1.9	1.6
VI	$(CH_3O)(CH_3S)P(O)OH$	0.07	0.4	0.3
VII	$(HO)(CH_3S)P(O)NH_2$	0.04	0.2	0.2
VIII	Unknown	0.39	2.3	1.9
	Conjugates	0.03	0.1	0.1
	Unextractable	0.36	2.1	1.7
	CO_2	1.65	9.4	7.9
Total		17.45	100.0	84.2

TABLE 6

Metabolic Products Recovered from the White Mouse 6 hr after Oral Administration of 50.16 mg/kg S-[^{14}C]Methyl O-Methyl Propionylphosphoramidothioate (I)

Metabolite	Recovery		
	Equivalents of I (μg/g)	Recovered radioactivity (%)	Applied radioactivity (%)
Urine			
I Parent compound	13.05	30.5	26.0
III Methamidophos	0.94	2.2	1.9
IV *O*-Demethyl-I	1.24	2.9	2.5
Feces			
I Parent compound	6.10	14.2	12.1
III Methamidophos	0.35	0.8	0.7
IV *O*-Demethyl-I	0.43	1.0	0.9
CO_2	11.31	26.5	22.6
Remaining in mouse tissue[a]	9.36	21.9	18.7
Total	42.78	100	85.4

[a] See Table 7 for identity of tissues.

bon dioxide) products. Another 18.7% of applied radioactivity was present in various mouse tissues indicated in Table 7. Total recovery of radioactivity was 85.4%.

As in the case of house flies, I and only two metabolites were recovered from the mouse after treatment with I. These were present as both urinary and fecal products and were methamidophos and IV. Of the radioactivity isolated, I was present in greatest abundance with a combined total of more than 38% of the applied dosage present in urine and feces. Methamidophos was the least abundant metabolite, totaling about 2.5% in urine and feces. Thus, it appears that I is quite stable to metabolism and over a third of the applied dosage passed through the mouse unchanged. This may be attributable to the high water solubility of I. The most significant pathway for the metabolism of I appears to be through a process leading to the generation of carbon dioxide (approximately 23% of the applied dosage).

Relatively large amounts of radioactivity (18.7%) remained in different tissues of the mouse (Table 7). Of the radioactivity detected in these tissues, the largest amounts were present in the gut. However, distribution of radioactivity was widespread and significant amounts were present in the blood and liver. Owing to the low levels of radioactivity in the various tissues, tlc analysis of the tissue extracts was not carried out.

TABLE 7

Distribution of Radioactivity in Various Tissues of the White Mouse 6 hr after Oral Administration of 50.16 mg/kg S-[^{14}C]Methyl O-Methyl Propionylphosphoramidothioate (I)

Tissue	Recovery		
	Equivalents of I (μg/g)	Recovered radioactivity (%)	Applied radioactivity (%)
Brain	0.36	3.9	0.7
Blood	1.07	11.4	2.1
Liver	1.77	18.9	3.6
Heart	0.61	6.5	1.2
Lung	0.20	2.1	0.4
Stomach	0.82	8.8	1.6
Large-intestine	1.40	15.0	2.8
Small intestine	2.18	23.3	4.4
Kidney	0.46	4.9	0.9
Bladder	0.19	2.0	0.4
Spleen	0.30	3.2	0.6
Total	9.36	100	18.7

TABLE 8

Metabolic Products Recovered from the White Mouse 6 and 24 hr after Oral Administration of 18.50 and 14.88 mg/kg, respectively, of S-[^{14}C]Methyl O-Methyl Hexanoylphosphoramidothioate (II)

Metabolite		Recovery		
		Equivalents of II (μg/g)	Recovered radioactivity (%)	Applied radioactivity (%)
6 hr after treatment at 18.5 mg/kg				
Urine				
II	Parent Compound	0.19	1.2	1.0
III	Methamidophos	2.53	15.8	13.7
V	*O*-Demethyl-II	0.19	1.2	1.0
VI	$(CH_3O)(CH_3S)P(O)OH$	0.09	0.5	0.5
VII	$(HO)(CH_3S)P(O)NH_2$	0.20	1.3	1.1
VIII	Unknown	0.61	3.8	3.3
Stomach				
II	Parent compound	4.99	31.1	27.0
III	Methamidophos	0.31	1.9	1.7
V	*O*-Demethyl-II	0.44	2.8	2.4
	CO_2	4.56	28.5	24.7
Remaining in mouse tissue		1.91	11.9	10.3
Total		16.02	100	86.7
24 hr after treatment at 14.88 mg/kg				
Urine				
II	Parent compound	0.07	0.5	0.5
III	Methamidophos	2.72	21.1	18.3
V	*O*-Demethyl-II	0.19	1.5	1.3
VII	$(HO)(CH_3S)P(O)NH_2$	1.03	8.0	6.9
VIII	Unknown	0.76	5.9	5.1
	Conjugate	0.01	0.1	0.1
Feces				
II	Parent compound	0.05	0.4	0.3
III	Methamidophos	1.11	8.6	7.4
V	*O*-Demethyl-II	0.10	0.8	0.7
VII	$(HO)(CH_3S)P(O)NH_2$	0.03	0.3	0.2
VIII	Unknown	0.65	5.0	4.4
	Conjugate	0.31	2.4	2.1
	CO_2	5.85	45.4	39.3
Total		12.88	100	86.6

Data for the metabolism of II in white mice treated at dosages of 18.50 and 14.88 mg/kg are presented in Table 8. Compared to I, elimination of radioactivity after treatment with II was slow, and substantial amounts of radioactivity remained in the mouse stomach 6 hr after treatment with 18.50 mg/kg of II. Because of this, another mouse was dosed (14.9 mg/kg) and analysis of metabolic products was conducted 24 hr after treatment. At 6 hr, only 20% of the applied radioactivity was eliminated via the urine and, surprisingly, no radioactivity was present in the feces. However, 31% of the applied radioactivity still remained in the stomach and almost 25% was expired as carbon dioxide. At 24 hr following treatment with 14.88 mg/kg II, about 87% of

the applied radioactivity was eliminated in the excreta or as carbon dioxide.

At the 6-hr period the metabolism of II in the mouse was qualitatively similar to that in house flies. The same five metabolites, methamidophos, V, VI, VII, and the unknown VIII, were isolated as urinary products. In contrast to I, where methamidophos was a minor metabolite, methamidophos was the major metabolite present in urine after treatment with II and amounted to 13.7% of the applied dosage. In terms of actual quantity, the amount of methamidophos isolated from the mouse (urine and stomach) treated with II was 1.68 mg/kg. This is substantially more than 0.92 mg/kg, the amount of methamidophos isolated from the mouse treated with 50.16 mg/kg of I. Thus, even though the dosage of II on a weight basis was 2.7-fold less than I, almost 2-fold more methamidophos was recovered from the mouse treated with II.

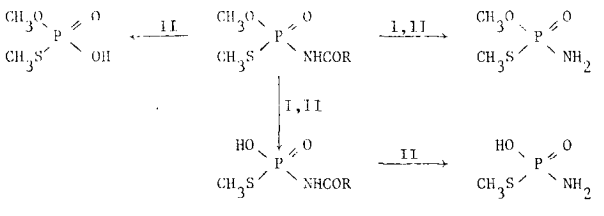
The 24-hr data largely supported the 6-hr results, although there were minor differences. Metabolite VI (*O,S*-dimethyl phosphorothioic acid) was not detected but this was of minor significance even in the 6-hr analysis. Metabolism to carbon dioxide, accounting for 39% of the applied radioactivity, was by far the most important pathway of degradation. Substantially more methamidophos, amounting to 25.7% of the applied radioactivity, was detected in the 24-hr urine and feces. In actual amounts this corresponds to 2.28 mg/kg of methamidophos.

While I passed through the mouse rapidly and large amounts were recovered unchanged in the urine, this was not the case with II. This difference in behavior between I and II may be attributable to differences in their water solubility and lipid–water partitioning properties. The partition constants for I and II between octanol–water are 0.53 and 6.45, respectively.

The greater susceptibility of II to metabolism is also evident in their *in vitro* metabolism in the presence of mouse liver microsomes (Table 9). Although recovery was poor, the bulk of the radioactivity recovered after incubation of I with liver microsomes was unchanged I, but significant amounts of II were converted to methamidophos and *O*-demethylated II (V) under the same conditions.

DISCUSSION

Based on the data in Tables 4, 5, 6, and 8, it is apparent that the metabolism of *O,S*-dimethyl propionylphosphoramidothioate (I) in the white mouse and house fly is qualitatively and quantitatively different from that of the hexanoyl analog (II). Qualitative behavior of I and II in the mouse and house fly is summarized in the metabolic scheme below. According to the



scheme, methamidophos and the *O*-demethyl derivatives are obtained as metabolites from both I (R = C_2H_5) and II (R = C_5H_{11}), but *O,S*-dimethyl phosphorothioic acid and *O*-demethyl methamidophos are obtained only from II. Further, since I and II are both metabolized to methamidophos, it is assumed that *O,S*-dimethyl

TABLE 9

Metabolic Products Obtained from O,S-Dimethyl Propionyl- (I) and Hexanoylphosphoramidothioate (II) Incubated in the Presence of Mouse Liver Microsomes for 1 hr at 37.5°C

Metabolite	Recovered radioactivity (%)
I (Total recovery = 57.4%)	
I Parent compound	98.5
IV *O*-Demethyl-I	1.5
II (Total recovery = 54.5%)	
II Parent compound	72.0
III Methamidophos	17.5
V *O*-Demethyl-II	10.5

phosphorothioic acid (VI) and *O*-demethyl methamidophos (VII) are generated from II and *O*-demethyl-II, respectively, and not from methamidophos. This difference in the metabolic behavior between I and II is probably attributable to their contrasting lipophilic or hydrophilic properties, with I favoring water over octanol and II favoring octanol over water. This is evident in the large amounts of I (26% of applied dosage in the urine) which passed through the mouse unchanged, compared to substantially lesser amounts for II (1% of applied dosage). In contrast to most organophosphorus and other organic insecticides, I, owing to its high water solubility, may pass through the mouse unchanged.

The major difference of significance in the metabolism of I and II in the mouse appears to be in the larger amounts of methamidophos formed from II compared to I. Methamidophos is highly toxic to the mouse (LD_{50} 14 mg/kg), and probably is the agent responsible for intoxication when the mouse is treated with either I or II. The small amounts of methamidophos formed from I readily account for the safety of I to mice. On the same basis, the relatively high toxicity of II to mice may be attributed to the substantial quantity of methamidophos formed in the mouse treated with II.

The same reasoning may be used to account for the greater toxicity of I over II to house flies, although in this case the results are not as clear-cut as in the mouse. While a much smaller percentage of applied II was converted to methamidophos compared to I, actually larger amounts of methamidophos (5.7-fold) were present in the fly owing to the 39-fold greater dosage of II. Nevertheless, there is no doubt that I has a substantially greater tendency to be converted to methamidophos in the house fly than II, in line with the greater toxicity of I to house flies.

It is difficult to assess the role of the unknown metabolite in the mode of action of II since it was not identified. Based on its R_f value using the acetone–hexane solvent system, it was slightly more polar than methamidophos. Attempts to generate larger amounts required for structure determination by incubation of II with mouse liver microsomes in the usual manner failed to produce any of the unknown. Other oxidizing agents, such an *m*-chloroperbenzoic acid, peroxytrifluoroacetic acid, and Udenfriend system (15), also failed to produce the unknown.

Owing to the relatively poor anticholinesterase activity of methamidophos and acephate, the statement has been made (16) that both of these compounds require metabolic activation to a more potent cholinesterase inhibitor. The results from this study show that the propionyl (I) and hexanoyl (II) analogs of acephate are activated in the house fly and white mouse to methamidophos, but at different rates to account for their difference in toxicity. This activation process involves the hydrolysis of an amide linkage and it is likely that an amidase is involved. The presence of an amidase in mammalian liver, associated primarily with the microsomal fraction, which cleaves the amide linkage of dimethoate has been demonstrated (17). Insects also are known to effect this hydrolysis (18).

Evidence was not obtained for further activation of methamidophos, even though numerous attempts were made to demonstrate the formation of an activated product by incubating methamidophos with a variety of oxidizing agents; e.g., *m*-chloroperbenzoic acid, peroxytrifluoroacetic acid, mouse liver microsomal oxidase, and related model chemical oxidation systems. If an activated product of methamidophos is formed, it evidently is highly transitory in nature.

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