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PLANT METABOLISM OF INSECTICIDES

Metabolism of the Systemic Insecticide *O,O*-Diethyl *S*-Ethylthiomethyl Phosphorodithioate (Thimet) in Plants

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O,O-diethyl *S*-ethylthiomethyl phosphorodithioate (Thimet) is metabolized by plants to form very potent anticholinesterase agents. When used as a systemic insecticide for seed treatment of cotton, the metabolites within the plant consist of O,O-diethyl *S*-ethylsulfinylmethyl phosphorodithioate, O,O-diethyl *S*-ethylsulfonylmethyl phosphorodithioate, O,O-diethyl *S*-ethylsulfinylmethyl phosphorothiolate, and O,O-diethyl *S*-ethylsulfonylmethyl phosphorothiolate. The last of these metabolites is the most active cholinesterase inhibitor and provides a method of residue analysis. Cotton seeds treated with Thimet on charcoal at concentrations as high as 32 pounds of Thimet per 100 pounds of seed showed less than 0.03 p. p. m. of Thimet or metabolites in the seeds maturing from the treated plants. The residual persistence following soil and foliage application was studied with six vegetable crops and radioactive Thimet.

MANY *O,O*-DIALKYL *S*-ALKYLTHIOALKYL phosphates and thiophosphates are very active contact and systemic insecticides (*1, 3, 9, 12, 17*). The metabolism in plants of demeton (mixture of *O,O*-diethyl *S*-ethylthioethyl phosphorothiolate and *O,O*-diethyl *O*-ethylthioethyl phosphorothioate) has been studied extensively (*4–7, 10, 11, 13, 15, 16, 18*). Fukuto and coworkers (*6, 7*) have shown clearly that, in plants, these isomers are oxidized to their corresponding sulfoxides and sulfones. *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate (American Cyanamid 3911 or Thimet) was the most active systemic insecticide of a large series of phosphorodithioates (*3*). Thimet has shown economic promise in the treatment of cotton seed for protection of young plants against early season pests and as a systemic insecticide for control of chewing and sucking insects following soil or foliage application (*3*). Registration has been received recently from the Food and Drug Administration for the use of Thimet as a cotton seed treatment.

This study concerns the nature and rate of plant metabolism of Thimet in relation to the persistence of toxic residues.

Methods

Synthesis of Radioactive *O,O*-Diethyl *S*-Ethylthiomethyl Phosphorodithioate. Phosphorus-32 pentasulfide was prepared from 1.25 grams of red phosphorus-32 (service irradiation at Atomic Energy Commission, Oak Ridge, Tenn.), 3.22 grams of sulfur, and 0.54 gram of carrier phosphorus pentasulfide. The mixture was reacted in a nitrogen atmosphere by slowly raising the temperature to 270° C. and maintaining it at 270° to 300° for 4 hours. *O,O*-diethyl phosphorodithioic acid was formed by dropwise addition of 5.75 ml. of absolute ethyl alcohol to the phosphorus pentasulfide dissolved in 6 ml. of toluene and the reaction mixture was held at 90° to 95° C. for 1 hour. The final toluene solution was added dropwise to a mixture of 2.64 grams of potassium hydroxide, 8.28 ml. of absolute ethyl alcohol, and 4.14 ml. of benzene at 25° to 35° and the reaction mixture held at 80° C. for 1 hour. Potassium diethyl phosphorodithioate was recov-

Table I. Characterization of Thimet Oxidation Products

Structural Formula[a]	Chemical Name	Properties: % Phosphorus Calcd.	% Phosphorus Found	% Sulfur Calcd.	% Sulfur Found	n_D^{25}	d_4^{25}
$(C_2H_5O)_2P(S)SCH_2S(O)C_2H_5$	*O,O*-diethyl *S*-ethylsulfinylmethyl phosphorodithioate[b]	11.2	11.64	34.8	34.84	1.5408	1.230
$(C_2H_5O)_2P(S)SCH_2S(O)_2C_2H_5$	*O,O*-diethyl *S*-ethylsulfonylmethyl phosphorodithioate[c]	10.6	10.87	32.9	33.04	1.5317	1.257
$(C_2H_5O)_2P(O)SCH_2SC_2H_5$	*O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate[b]	12.7	12.46	26.3	26.45	1.4944	1.154
$(C_2H_5O)_2P(O)SCH_2S(O)C_2H_5$	*O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate[d]	11.9	11.97	24.6	24.62	1.5022	1.225
$(C_2H_5O)_2P(O)SCH_2S(O)_2C_2H_5$	*O,O*-diethyl *S*-ethylsulfonylmethyl phosphorothiolate[e]	11.2	10.25	23.2	23.97	1.4919	1.281

[a] Prepared by oxidation of corresponding ethylthiomethyl phosphorothioate derivatives with perphthalic acid. Oxygen analog of Thimet prepared by reaction of monothio acid salt with ethyl thiomethyl chloride. Samples and analytical figures supplied by G. A. Johnson, American Cyanamid Co., Stamford, Conn.
[b] No impurities detectable by chromatographic or infrared analysis.
[c] 5% Thimet impurity based on chromatography and infrared analysis.
[d] 15% *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate based on chromatography and infrared analysis.
[e] 1.8% *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate based on chromatography and infrared analysis.

ered by filtration and separated from unreacted acid by several washes with benzene and from unreacted potassium hydroxide by dissolving the potassium phosphate salt in acetone. Five grams of ethylthiomethyl chloride (96.7% pure) was added to this acetone solution and held for 12 hours at 30° C. The product was dissolved in ether, washed with 5% sodium carbonate solution, and chromatographed on the Celite column.

Two materials were eluted from the column, weighing 2.07 grams and 0.26 gram in the order of elution. The first fraction formed in 17.6% yield, based on total phosphorus, gave the infrared spectrum shown in Figure 2, which is identical to that for known *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate. This radioactive material was further shown to be *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate because it eluted from the column in a similar position to known material, and was the same as the known Thimet based on anticholinesterase activity and partitioning between hexane and acetone-water. The second fraction eluted from the Celite column was similar to *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate in the position of elution from the column (Figure 1) and in its anticholinesterase activity and partitioning between hexane and acetone-water. An infrared spectrum was similar to that for known *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate, indicating that this impurity might be either this material or *O,S*-diethyl *S*-ethylthiomethyl phosphorothiolate.

Preparation and Properties of Thimet Derivatives. Oxidized derivatives of Thimet which were considered as possible metabolites were synthesized in the Stamford Laboratories of the American Cyanamid Co. Thimet and its oxygen analog were prepared by reacting the di- or monothio acid salt with ethylthiomethyl chloride. The sulfoxides and

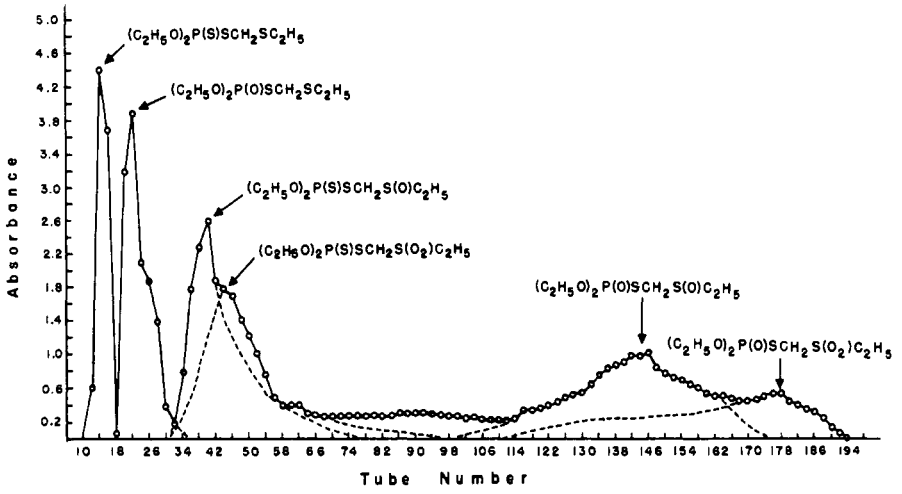


Figure 1. Chromatogram of Thimet and oxidation products

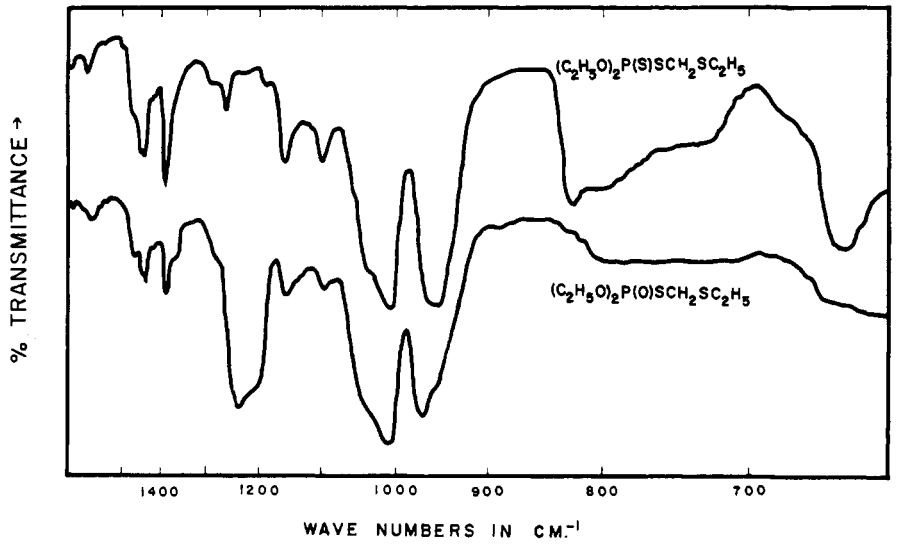


Figure 2. Infrared spectra of radioactive O,O-diethyl S-ethylthiomethyl phosphorodithioate and O,O-diethyl S-ethylthiomethyl phosphorothiolate

sulfones were prepared by oxidizing Thimet or its oxygen analog with monoperphthalic acid. Characterization data for those oxidation products are given in Table I. These materials were column purified before use and yielded the infrared spectra shown in Figures 2, 3, and 4. *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorodithioate was also prepared with hydrogen peroxide in acetone and peracetic acid in chloroform. Exposure of Thimet in thin films to sunlight or ultraviolet light formed more polar derivatives which were more active anticholinesterase agents than either Thimet or its sulfoxide. Thimet is apparently subject to slow oxidation on charcoal, as about 2% of the Thimet recovered on a column from a chloroform extract of cotton seeds, treated with chromatographically pure Thimet on charcoal and held for 1 month at 30° C., appeared in a more polar chromatographic fraction.

Thimet is stable to thermal degradation, but heating for 24 hours at 150° resulted in considerable decomposition. The heated product was increased in over-all anticholinesterase activity but when chromatographed only one major component was recovered, this eluting from the column at the position of Thimet, but possessing no anticholinesterase activity and yielding infrared absorption, peaks at 680, 967, 1053, 1250, 1375, 1450, and 2940 cm^{-1}.

Column Chromatography and Partition Coefficients. The technique of column preparation was based on that of Gardner and Heath (*8*). One hundred grams of Celite (Hyflo Supercel of Johns-Manville) was mixed with 60 ml. of absolute methanol, which was two-thirds saturated with iso-octane. This mixture was slurried in iso-octane four-fifths saturated with methanol and packed to form a column of 3.6 × 26 cm., which allowed a flow rate of one drop per second with a positive pressure of about 2 pounds. The insecticide was placed on the top of the column, washed in with three small volumes of the mobile phase, and about 1 gram of Celite preparation was added to the top of the column and packed to minimize backwashing of the insecticide. Fractions of 400 drops or about 10 ml. were collected and the organophosphate peaks located by total phosphorus, radioassay, or anticholinesterase methods. A column of this size was suitable for amounts up to about 1-gram of insecticide.

The chromatogram shown in Figure 1 was made with a mixture of 200 mg. each of Thimet and its oxidation products. The poor resolution with this excessive amount of material on the column necessitated the use of infrared spectra to locate the position of the individual components. The total 2.33-gram yield from the radioactive synthesis fractionated well on a column of

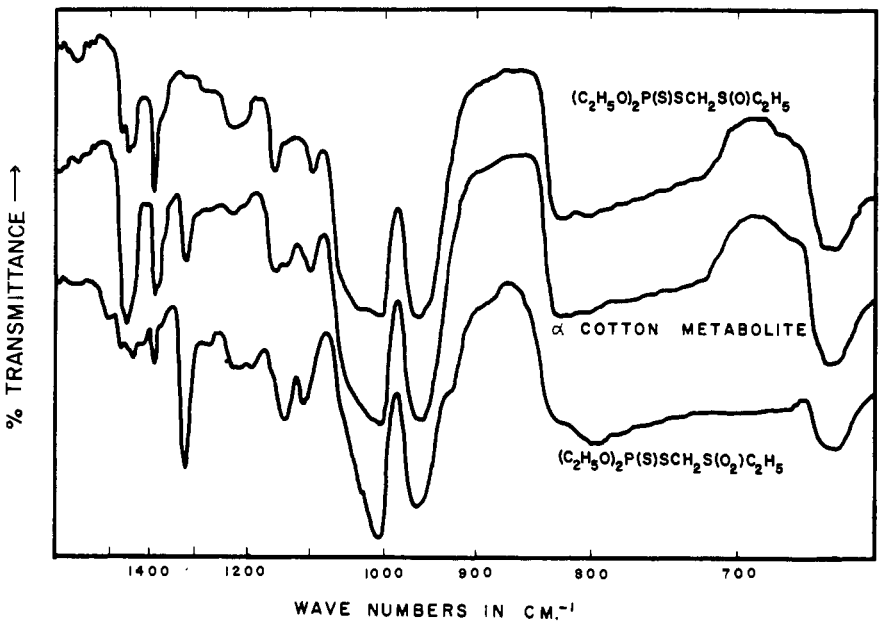


Figure 3. Comparison of infrared spectra of "alpha" metabolite fraction from cotton with O,O-diethyl S-ethylsulfinylmethyl phosphorodithioate and O,O-diethyl S-ethylsulfonylmethyl phosphorodithioate

Table II. Comparison of Thimet Oxidation Products and Plant Metabolites

Compound[a]	Anti ChE Activity, pI_{50} ChE	Solubility Properties[b] $CHCl_3/H_2O$	Solubility Properties[b] Hexane/acetone-H_2O
$(RO)_2P(S)SCH_2SR$	3.17	100/0	100/0
$(RO)_2P(S)SCH_2S(O)R$	3.35	100/0	30/70
$(RO)_2P(S)SCH_2S(O)_2R$	5.00	100/0	57/43
$(RO)_2P(O)SCH_2SR$	5.87	100/0	62/38
$(RO)_2P(O)SCH_2S(O)R$	6.76	100/0	2/98
$(RO)_2P(O)SCH_2S(O)_2R$	7.02	99/1	4/96
Metabolites			
Pea-alpha	3.93	100/0	38/62
Cotton-alpha	4.53	100/0	27/73
Cotton-beta	6.98	100/0	3/97

[a] All materials purified on Celite column. R = C_2H_5.
[b] Solubility properties expressed as % recovery of total phosphorus in each layer.

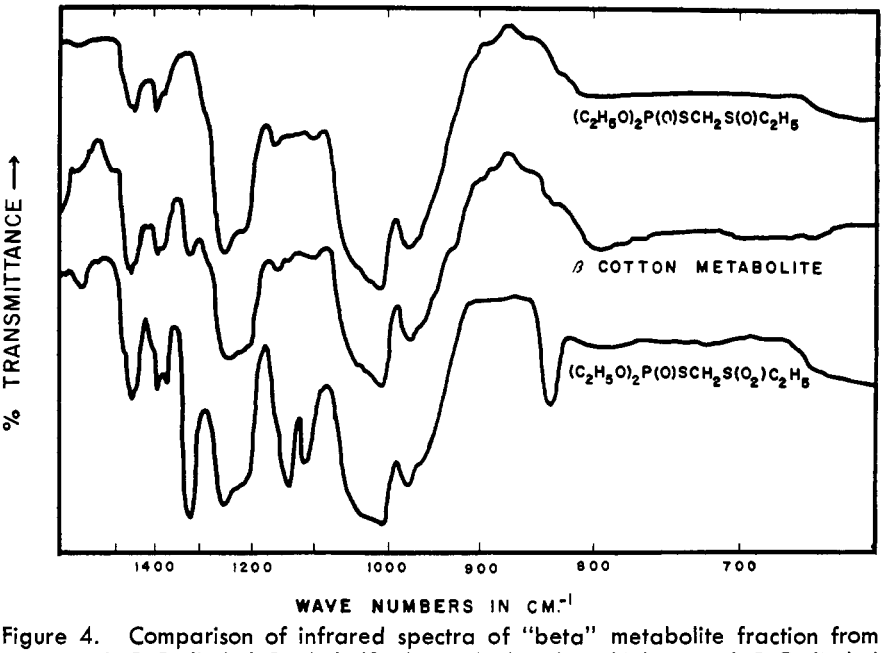
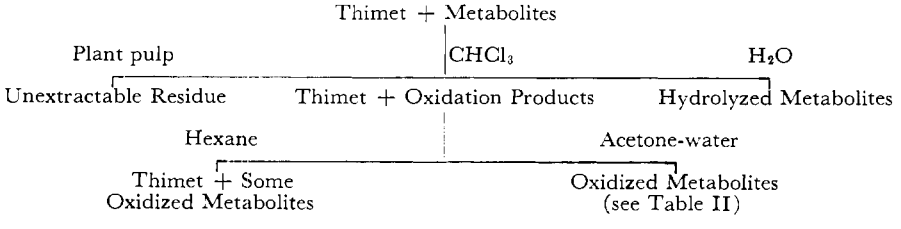


Figure 4. Comparison of infrared spectra of "beta" metabolite fraction from cotton with O,O-diethyl S-ethylsulfinylmethyl phosphorothiolate and O,O-diethyl S-ethylsulfonylmethyl phosphorothiolate

5 × 40 cm., and the small amounts of plant metabolites gave good resolution on columns of 2.3 × 26 cm.

Partitioning studies were made with either chloroform and water in equal volumes or hexane, acetone, and water in volume ratios of 5:4:7. Each phase was analyzed for total phosphorus and the results were expressed as the per cent of total phosphorus appearing in each layer. The solubility properties of the Thimet oxidation products are given in Table II.

Fractionation of Plant Samples. Thimet and its oxidation products were recovered from plant brei greater than 99% by extraction with an equal volume of chloroform. When Thimet and its various oxidation products were hydrolyzed with alkali, the hydrolysis products remained completely in the aqueous layer when extracted with chloroform. Unfortunately, chloroform also extracts many pigments from the plant material. Hexane, acetone, and water in the ratios of 5:4:7 fractionated the chloroform-extractable materials from Thimet-treated plants so that all of the Thimet and the majority of the plant pigments and oils appeared in the hexane layer along with smaller proportions of the various oxidation products. Most of the Thimet oxidation products and only small amounts of plant extractives appeared in the acetone-water layer. These solubility properties were used in the following fractionation system:



For residue analysis the plant samples were withdrawn from field plots at various intervals after treatment and representative 100-gram subsamples were stored at −10° C. until analyzed. Each 100-gram sample was macerated with 200 ml. of chloroform in a Waring Blendor, and the residue of insoluble plant materials was removed by straining through cheesecloth. The chloroform and aqueous layers were separated by salting out with saturated sodium chloride, and the radioactive phosphorus was determined in appropriate aliquots of the aqueous plant juice and the solid residue. The chloroform was filtered through anhydrous sodium sulfate and the sulfate was washed with chloroform until the soluble plant pigments were removed. This chloroform extract was assayed for total radioactivity and anticholinesterase activity, evaporated on a steam bath to a small volume, and the remaining solvent was removed under reduced pressure at room temperature.

Table III. Persistence of Thimet- P^{32} and Metabolites in Vegetable Crops[a]

	Days Following Thimet-P^{32} Application						
	0.1	1	2	4	8	17	32
Foliage Application							
Thimet equivalent[b], p. p. m.							
Hexane frac.	6.75	2.84	2.49	1.69	0.90	0.44	0.040
Acetone-water frac.	2.60	2.39	1.66	1.45	0.59	0.067	0.003
Hydrolysis products[c]	0.28	0.37	0.62	0.85	1.33	0.43	0.27
Unextr. residue[d]	1.44	1.03	0.81	1.62	1.54	1.09	2.28
AntiChE activity, 50% inhib.[e]							
Plant tissue, g.	1.3	0.47	0.59	0.54	1.6	>2.2	>2.2
pI_{50} metabolites	5.44	6.14	6.29	6.62	6.11	<7.22	<7.42
Soil Application							
Thimet equivalent, p. p. m.							
Hexane frac.	0.82	0.34	0.40	0.26	0.15	0.049	0.026
Acetone-water frac.	0.12	0.31	0.56	0.36	0.18	0.047	0.005
Hydrolysis products	0.052	0.12	0.13	0.24	0.25	0.21	0.110
Unextr. residue	0.15	0.16	0.42	1.20	0.46	0.11	0.078
AntiChE activity, 50% inhib.							
Plant tissue, g.	1.8	0.94	1.3	1.2	1.3	>2.9	>3.0
pI_{50} metabolites	6.89	7.26	6.66	6.72	7.00	7.15	7.22

[a] Results are average of figures from beans, beets, cabbage, carrots, lettuce and peas in same field plot.
[b] P.p.m. of Thimet or metabolites appearing in fraction indicated with calculations based on P^{32} and molecular weight of Thimet.
[c] Water-soluble products not extracted into chloroform.
[d] P^{32} remaining in solid portion after both water and chloroform removed.
[e] Results based on chloroform-soluble metabolites. Amount of plant material containing enough metabolites for 50% inhibition and pI_{50} (negative logarithm of molar concentration effecting 50% inhibition) of these metabolites are reported.

The residue was dissolved in 40 ml. of acetone, filtered into a separatory funnel and an aliquot was removed, and the total radioactivity was determined to confirm that no Thimet derivatives had been lost in the evaporation process. To the remaining acetone solution, *n*-hexane and distilled water were added in a ratio of 4 parts of acetone, 5 parts of hexane and 7 parts of water. Following extraction, the two layers were separated and analyzed for total radioactivity. Thimet–phosphorus-32 added to a plant brei at 1 p.p.m. gave essentially 100% recovery in the hexane layer when fractionated through this scheme. The chemical nature of the unextractable residues (Tables III and IV) has not been determined.

Cotton seeds were freed of adhering fibers, cracked, and ground in a mortar. Then 200-gram portions were extracted with 800 ml. of ethyl ether in a Soxhlet for 8 hours and the solvent was removed by evaporation and vacuum-stripping. About 18% of the weight of the seed was recovered as oil with ethyl ether and a subsequent extraction of the cottonseed meal with chloroform recovered an additional 0.5% oil. The cottonseed oil was fractionated with hexane and acetone-water, both layers were analyzed by radioactive determinations, and the acetone-water layer was assayed for anticholinesterase agents (Table IV).

Cotton and pea plants were fractionated in an attempt to isolate sufficient amounts of Thimet metabolites to obtain an infrared spectrum. Cotton plants were grown 2 weeks, from seed treated with chromatographically pure Thimet at 4 pounds per 100 pounds of seed, 2100 grams of foliage were fractionated as indicated above, and the acetone-water fraction was chromatographed on Celite. In the position of elution of the phosphorodithioate sulfoxide and sulfone, 40 mg. of an α-metabolite fraction was recovered from the column, and 8 mg. of a β-metabolite fraction was recovered in the position of the phosphorothiolate sulfoxide and sulfone. Pea plants were similarly fractionated 14 days after soil treatment and an α-metabolite fraction was recovered.

Field Studies. The persistence of Thimet or its metabolites in plants was studied following field application of the radioactive insecticide to beans, beets, cabbage, carrots, lettuce, and peas (Table III). Foliage and soil applications were made to 24-foot rows of each vegetable with similar sized, untreated buffer strips to minimize contamination. A 10% radioactive Thimet emulsifiable concentrate was prepared by dissolving 6 grams of Thimet–phosphorus-32 and 6 ml. of Triton X-155 in 48 ml. of xylene. The radioactivity at the time of application was 155 c.p.m. per γ of Thimet. For foliage application the spray volume for each vegetable was calculated so that the foliage would be wetted to the point of run-off and applications

were made with a hand sprayer at the rate of 1 pound per acre or 0.5 gram per 24-foot row. Soil applications to the 24-foot rows were made by evenly distributing 300 ml. of emulsion containing 0.5 gram of actual insecticide. This was poured around the base of the plants with care to minimize surface contamination. The vegetable foliage was randomly sampled at 0, 1, 2, 4, 8, 17, and 32 days after treatment and the samples were stored in polyethylene bags at −10° C. until analyzed. Initial samples were taken about 2 hours after treatment to allow the foliage to dry.

Cotton seeds treated with radioactive Thimet on charcoal were grown in both Wisconsin and Oklahoma (Table IV). Equal weights of charcoal and radioactive Thimet were thoroughly mixed and this mixture was coated on seeds wetted with 2% methylcellulose. Insecticide loss through handling was minimized by spraying and drying with 5% methylcellulose several times to form a hard coat, a treatment which did not reduce seed germination. Seeds planted in Wisconsin were treated at 16 pounds of Thimet per 100 pounds of seed or about 16 mg. or 4,000,000 c.p.m. of Thimet per seed. The Wisconsin cotton was sampled at 3.9, 7.4, and 10.7 weeks after planting. Seeds treated with 32 pounds of Thimet per 100 pounds of seed or 8,000,000 c.p.m. of Thimet per seed at planting time were grown in Stillwater, Okla., and the residues determined after 16 weeks.

Other Methods. The techniques used for radioactive measurement, anticholinesterase analysis, and total phosphorus determination have been described (*2*). Infrared absorption spectra were made with 10% solutions in chloroform in a sodium chloride prism with a Baird spectrophotometer. Activation experiments were carried out with rat liver slices according to the methods of Metcalf and March (*14*).

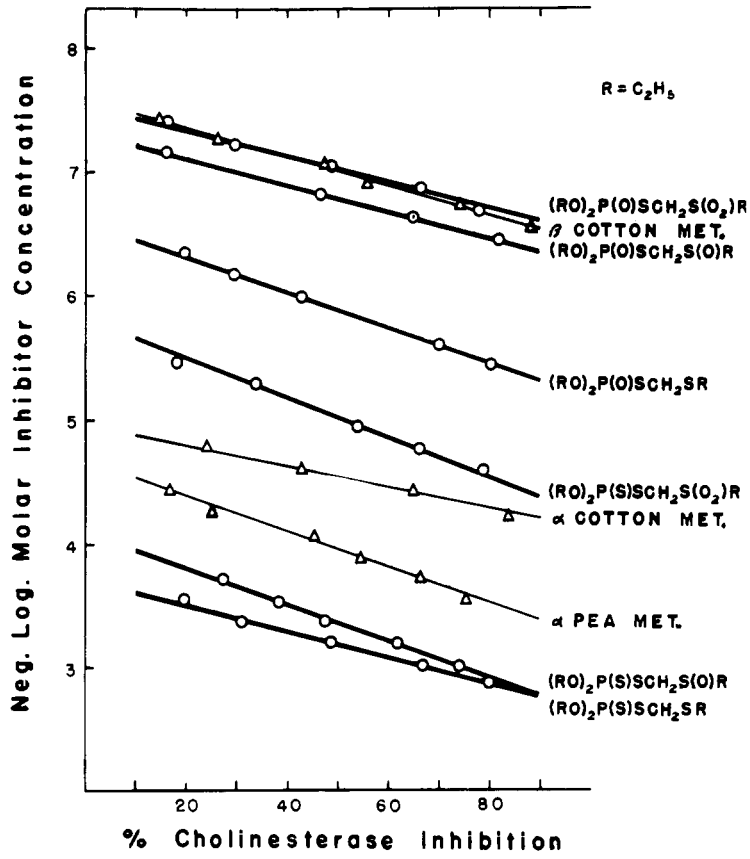


Figure 5. Comparison of anticholinesterase curves for Thimet oxidation products and plant metabolites

Discussion

Metabolic Pathway for *O,O*-Diethyl *S*-Ethylthiomethyl Phosphorodithioate. *O,O*-diethyl *S*-ethylthiomethyl phosphorodithioate is metabolized in plants to form more active anticholinesterase agents. The total anticholinesterase activity of greenhouse pea plants sprayed with Thimet increased for about the first 4 days and then declined, but inhibitors still persisted for 20 to 30 days. Radioactive Thimet applied in the field also increased in anticholinesterase activity and became

Table IV. Residues of Thimet-P^{32} and Metabolites in Cotton Following Seed Treatment

Lb. Thimet/ 100 Lb. Seed	Location	Weeks	Plant Part	P. P. M. Thimet Equiv. (P^{32}) Present: Hexane frac.	Acetone-water frac.	Hyd. prod.	Unextr. residue	P. P. M. Phosphorothiolate Sulfone Equiv.[a]
16	Wis.	3.9	Foliage	0.27	0.29	0.54	0.93	0.42
16	Wis.	7.4	Foliage	<0.0006[b]	0.0036	0.019	0.035	.....
16	Wis.	10.7	Leaves	<0.0006	<0.0006	<0.0006	<0.0006	0.0084
16	Wis.	10.7	Squares	<0.006	<0.006	<0.006	<0.006	0.063
16	Okla.[c]	16	Cottonseed[d]	.....	<0.025[a]	.....	.....	.....
32	Okla.	16	Cottonseed[d]	<0.030	<0.030	.....	.....	<0.025
32	Okla.	16	Leaves	<0.005	<0.005	.....	.....	.....

[a] Results based on anticholinesterase activity of chloroform-soluble materials, except in cottonseed oil which was further fractionated to recover acetone-water fraction for analysis. Addition of phosphorothiolate sulfone to untreated seed at 0.025 p. p. m. yielded 15% cholinesterase inhibition in acetone-water fraction and at 0.05 p. p. m. yielded 33% inhibition.

[b] Sensitivity limit of method: no radioactivity or anticholinesterase activity detectable.

[c] Cotton grown in Oklahoma through generous cooperation and assistance of R. M. Chatters and H. E. Howell, Oklahoma A & M College.

[d] Mature cottonseed. Figures for p. p. m. and anticholinesterase activity based on materials extractable by both ether and chloroform in a Soxhlet and expressed on basis of whole seed weight.

distinctly more polar in its solubility properties (Table III). However, some Thimet per se persisted in vegetable crops for 17 and 32 days after soil and foliage applications, based on the proportion of radioactivity appearing in the hexane compared with the acetone-water fraction (Table III). However, cotton contained little if any Thimet compared with oxidation products in the foliage of plants grown for 52 days from Thimet-treated seed (Table IV). Soil-treated pea plants and seed-treated cotton after 2 weeks' metabolism were fractionated for possible characterization of the metabolites. Residual Thimet in the foliage of these plants was not characterized because of interference from plant pigments. No *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate, a possible metabolite, was found in either case. An α-metabolite fraction was recovered in each case and eluted from the column in the position of the mixed sulfoxide and sulfone of the phosphorodithioate.

The anticholinesterase activity and partitioning characteristics of these metabolite fractions (Table II, Figure 5) were also indicative of a mixture of the phosphorodithioate sulfoxide and sulfone. Infrared spectra (Figure 3) confirmed this assignment of structure and showed the "alpha" fraction of peas to be 80% sulfoxide and 20% sulfone, and that of cotton 61% sulfoxide and 39% sulfone. From the cotton a β-metabolite fraction was recovered in the chromatographing position of the phosphorothiolate sulfoxide and sulfone. Anticholinesterase activity, partitioning characteristics, and infrared spectra (Table II, Figures 4 and 5) showed this "beta" fraction to be 70% sulfoxide of the phosphorothiolate and 30% sulfone of the phosphorothiolate.

The young cotton plants grown 2 weeks from Thimet-treated seed contained no *O,O*-diethyl *S*-ethylthiomethyl phosphorothiolate, 11.6 p.p.m. of *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorodithioate, 7.4 p.p.m. of *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorodithioate, 1.7 p.p.m. of *O,O*-diethyl *S*-ethylsulfinylmethyl phosphorothiolate, and 1.1 p.p.m. of *O,O*-diethyl *S*-ethylsulfonylmethyl phosphorothiolate. The metabolic pathway for Thimet in cotton is as follows:

O,O - diethyl *S* - isopropylthiomethyl phosphorodithioate (American Cyanamid 12008) appeared to follow a similar metabolism route to Thimet. Oxidation of 12008 with 2.4 moles of peracetic acid yielded a mixture of phosphorodithioate and phosphorothiolate sulfoxides and sulfones based on column chromatography and infrared spectra. Exposure of 12008 in thin films to light also formed more polar derivatives. Pea plants grown from seeds treated with 12008 on charcoal formed a very active anticholinesterase fraction that could not be increased in anticholinesterase activity by incubation with rat liver slices. Foliage application of 12008 to peas, carrots, cabbage, and potatoes resulted in a marked increase in its anticholinesterase activity. The anticholinesterase metabolites in pea foliage appeared within one day and persisted in high concentration for at least 9 days and in detectable amounts for 21 days.

Residues and Residue Analysis of Thimet-Treated Plants. Anticholinesterase analysis appears applicable for the determination of toxic residues in Thimet-treated plants. Thimet per se is too weak an inhibitor to be detected in micro amounts, but when applied to plants, the Thimet is very rapidly converted to potent anticholinesterase agents (Tables II and III) and the final unhydrolyzed metabolite in the oxidation series is the most active inhibitor. This active phosphorothiolate sulfone can be detected at 0.01 p.p.m. or less in plant tissue.

The metabolic pathway of Thimet (Table III) is similar to that already established with demeton (*6*, *7*). Following seed treatment of cotton at 16 pounds per 100 pounds of seed, the residues are detectable in the foliage after 7.4 weeks, but less than 0.030 p.p.m. of Thimet or metabolites are present in the cotton seed harvested after 16 weeks (Table IV).

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