Metabolism of *O*-Ethyl *S*,*S*-Dipropyl Phosphorodithioate (Mocap) in Bean and Corn Plants

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Methylene chloride extracts of bean and corn plants grown in soil treated with Mocap-ethyl-^{14}C or Mocap-propyl-^{14}C (*O*-ethyl *S*,*S*-dipropyl phosphorodithioate) contained ethyl propyl sulfide, ethyl propyl sulfoxide, ethyl propyl sulfone, and propyl disulfide. The major water-soluble metabolite isolated from plants was *O*-ethyl *S*-propyl phosphorothioic acid. Desethyl Mocap was not recovered from plants. In the metabolic scheme proposed, the propyl thiolate ion occupies an important place. It attacked and dealkylated the parent compound to form ethyl propyl sulfide, and it was also thiophilic in forming propyl disulfide. Only a small fraction of the labeled Mocap administered to soil was taken up by bean or corn plants, and only a portion of this material was extractable from beans or corn as Mocap or its metabolites. Most of the administered radioactivity remained in the soil, or, especially in later samples, was unextractable from plants.

Mocap, *O*-ethyl *S*,*S*-dipropyl phosphorodithioate, a nematocide-insecticide recently introduced by the Mobil Chemical Co., is a nonfumigant, contact material with good soil movement and a relatively short residual life (Mobil Chemical Co., 1969). Mocap has been shown to give excellent control of nematodes attacking nursery and ornamental plants (Heald and Self, 1967; Kerr, 1968; O'Bannon and Taylor, 1967; Taylor and O'Bannon, 1968), cotton (Birchfield, 1968), tobacco and potatoes (Mistric and Smith, 1969), sweet potatoes (Birchfield and Martin, 1968), and onions (Rhoades, 1969), among others (Wilson *et al.*, 1965). The material has shown effectiveness as a soil incorporation treatment, a root-dip, and a soil drench (Wilson *et al.*, 1965). It has been registered with the United States Department of Agriculture for use on tobacco for nematode and wireworm control, on commercial turf peanuts, soybeans, sweet potatoes, pineapples, and bananas for nematode control, and on corn for rootworm control.

Mocap will most frequently be applied to the soil in agricultural practice. Soil from Mocap-treated plots in the midwestern United States was analyzed to determine the persistence of Mocap in soils. At an application rate of 1 lb/acre, half-lives of 5 to 9 days were determined, and at 2 lb/acre, 10 to 12 days, when the material was applied as a 10% granular formulation on attaclay (Mobil Chemical Co., 1969). When Mocap was applied in Virginia as a technical grade liquid, the half-life was 3 days at 1 lb/acre and 4 days at 2 lb/acre.

Mocap, even though grouped with the phosphorodithioates, is structurally different from most of the phosphorodithioates used as pesticides. It has two alkyl-sulfur groups involved in ester linkages with phosphorus, and does not have P=S. Because of this important structural difference, Mocap is expected to behave differently in biological systems. Mocap is a newly introduced compound, and there are no previous reports on its metabolism.

MATERIALS AND METHODS

Radiochemicals. Mocap, *O*-ethyl *S*,*S*-dipropyl phosphorodithioate-ethyl-α-^{14}C (specific activity 1.25 mCi per mmol) and Mocap-propyl-α-^{14}C (specific activity 2.8 mCi per mmol) were obtained from Nuclear Research Chemicals, Orlando, Fla. The radiochemicals were chromatographed on silicic acid columns before use on which they each gave a single homogeneous peak.

Synthesis of Ethyl Propyl Sulfoxide. Ethyl propyl sulfide (2.25 g, 25 mmol) was placed in a round-bottomed flask, and concentrated nitric acid (1.15 g, 12.5 mmol) was added slowly at 0° C with continuous stirring over a period of 15 min (Reid, 1960). After cooling, a red solution resulted. Ten ml of petroleum ether (Skellysolve F) was then added with continuous stirring. The solution changed from red to pale green while the two layers were separating. The upper layer was separated and discarded. The acidic lower layer left behind was neutralized with solid sodium bicarbonate. Then 15–20 ml of 95% ethanol was added, resulting in the precipitation of excess sodium bicarbonate which was filtered out. The ethanol was evaporated under vacuum at 50° C. Some solid contaminants left behind were slurried with an additional 10 ml of ethanol and filtered out. The ethanol was evaporated under vacuum at 60° C. This purification step was carried out until no solid contaminants were found in the ethyl propyl sulfoxide. The sulfoxide synthesized (1.56 g, 69% yield) was soluble in chloroform, benzene, ethanol, and acetone. An infrared spectrum showed a distinct sulfoxide peak at 1010 cm^{-1}. The purity was checked on thin-layer, R_f = 0.13 (hexane:chloroform 3:2).

Synthesis of Ethyl Propyl Sulfone. Ethyl propyl sulfide (0.61 g, 5 mmol) was placed in a round-bottomed flask with 6.25 ml of glacial acetic acid. Potassium permanganate (1.975 g) was dissolved in 25 ml of 2 *N* sulfuric acid and the solution was added dropwise to the flask with continuous shaking (Reid, 1960). The last 3 to 4 ml of potassium permanganate solution turned the mixture dull brown. The mixture was left overnight and then extracted three times with 20 ml of chloroform each time and evaporated under vacuum at 50° C. The resulting clear solution was redissolved in 25 ml of chloroform, washed twice with sodium bicarbonate, once with distilled water, and filtered. The residue left after evaporation under vacuum at 60° C was ethyl propyl sulfone (0.42 g, 75% yield). The infrared spectrum of the purified sulfone was compared with that of ethyl propyl sulfide and had, in addition, distinct sulfone peaks at 1010 cm^{-1} and 1300 cm^{-1}. On thin-layer it produced a spot at R_f = 0.31 (hexane:chloroform 3:2).

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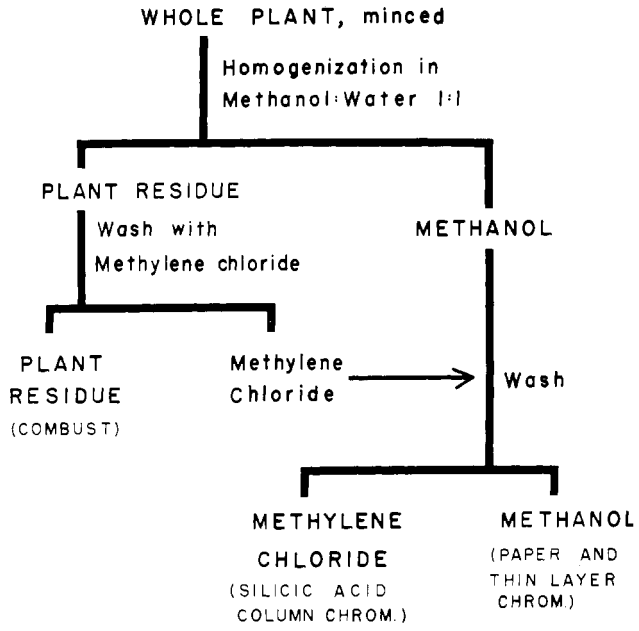

Figure 1. Representation of the procedure followed in the extraction of bean and corn plants

Ethyl Propyl Sulfide Synthesis by Sodium Ethoxide-Ethanol Cleavage of Mocap. An experiment was conducted to determine whether ethyl propyl sulfide could be produced by an intramolecular rearrangement of Mocap. Bacon and LeSeur (1954) were able to synthesize mixed alkyl sulfides by the cleavage of *O,O,S*-trialkyl phosphoric esters in a sodium alkoxide-alcohol system.

Sodium ethoxide was prepared by the addition of 0.23 g (10 mmol) of metallic sodium to 3 ml of 95% ethanol. Then 1.21 g (5 mmol) Mocap with about 200,000 cpm of Mocap-ethyl-^{14}C added was introduced dropwise with continuous stirring over a period of 20 min. The mixture was then refluxed for about 6 hr. After cooling, 15 ml distilled water was added and the solution was saturated with NaCl and extracted with diethyl ether. The ether fraction was dried with magnesium sulfate and evaporated under vacuum at 50° C. The residue was purified by silicic acid column and thin-layer chromatography.

Other Chemicals. The following potential metabolites of Mocap were synthesized in the Organic Synthesis Laboratory of Mobil Chemical Co., Ashland, Va., using standard techniques: *O*-ethyl phosphoric acid; *O*-ethyl *S*-propyl phosphorothioic acid; *S,S*-dipropyl phosphorodithioic acid (desethyl Mocap); *S,S,S,S*-tetrapropyl tetrathiopyrophosphate; other pyrophosphates; propyl mercaptan; dipropyl disulfide; dipropyl sulfide; and ethyl propyl sulfide.

Formulation of Mocap Granules. Mocap was administered as 10% granules to the soil in which snap beans (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.) were planted. To prepare a 10% granular formulation of Mocap, heavy aromatic naphtha (Standard Oil of New Jersey, Linden, N.J.), 1 g and Mocap, 1 g, were dissolved in approximately 10 ml of petroleum ether (30–60° C). Attaclay 30/60 mesh LVM (Engelhard Minerals and Chemicals, Inc., Menlo Park, N.J.), 4.66 g, was added and the solvent was evaporated, giving a homogeneous concentration of Mocap on the granules. The formulation was then divided into two parts of 2.25 g each. To one was added Mocap-ethyl-^{14}C and to the other Mocap-propyl-^{14}C, such that 50 mg of the formulation contained 992,600 cpm and 1,270,800 cpm, respectively.

Treatment of Bean and Corn Plants. Green beans, Contender variety, were planted in vermiculite flats in plant growth chambers with 16 hr of daylight and 80° F minimum and 90° F maximum temperatures. Approximately 2 weeks later individual plants were transplanted to clay pots containing steam-sterilized soil (approximately 350 g) with 14.3 ppm of either Mocap-ethyl-^{14}C or Mocap-propyl-^{14}C applied as a granular formulation. Samples of soil and bean plants were taken at weekly intervals. Five individual plants per sample were cut off at the soil level for analysis. Soil and roots from the five pots were pooled and thoroughly mixed.

Corn was planted directly in the clay pots in the steam-sterilized soil (350 g per pot) in the greenhouse. Germination took place in about 10 days. The first sample was taken on the 18th day after planting and the rest of the samples were taken at 10-day intervals. Again, five individual plants per sample were cut off at the soil level for analysis, and the soil and roots from the same five pots were pooled and thoroughly mixed for analysis.

Extraction of Plants. The five-plant sample was chopped into small pieces and homogenized with 50% methanol in a Lourdes Multi-mix homogenizer. The homogenate was filtered through a Buchner funnel and the plant pulp was re-extracted with methylene chloride. The methylene chloride and methanol fractions were combined, shaken well, and the phases were allowed to separate. A small quantity of silicic acid was added to the methylene chloride fraction, which was then evaporated to dryness under a gentle stream of air. The fraction was then chromatographed on a silicic acid column. The methanol fraction from some samples was chromatographed on a Dowex anion exchange column (Iqbal and Menzer, 1971).

The plant pulp remaining after the extractions was dried, ground in a mortar, and then combusted in an oxygen atmosphere in a closed system using a modification of the method of Gutenmann and Lisk (1960) and Gutenmann *et al.* (1961). Monoethanolamine was used to absorb $^{14}CO_2$ evolved from the labeled Mocap derivatives. An aliquot of monoethanolamine was counted in a liquid scintillation spectrometer.

The plant extraction method is diagrammed in Figure 1.

Extraction of Soil. Two aliquots of 100 g each of the combined soil and remaining roots from five pots were extracted by mixing with 200 ml of methanol in a Waring Blendor. The suspension was filtered and the soil residue was reextracted twice with 150 ml of methanol. The extracts were combined, reduced in volume on a rotary evaporator, and assayed for radioactivity. The residual soil was further extracted with methanol in a Soxhlet apparatus for approximately 16 hr and the extracts were assayed in the same manner as above.

Chromatography. The methylene chloride extracts of bean and corn plants and the methanol extracts of soil were chromatographed on silicic acid columns. Approximately 50 g of silicic acid were slurried in hexane and poured into a 19 mm i.d. column with a fiberglass plug. The silicic acid settled to a height of about 25 cm. The methylene chloride plant extract was mixed with a small amount of silicic acid and added carefully to the top of the column. The column was eluted with solvent mixtures in the following order: 100 ml of hexane; 200 ml of hexane:ether (4:1); 200 ml of hexane:ether (1:1); 200 ml of hexane:ether (1:4); and 200 ml of methanol. Following elution, the column contents were further extracted with methanol to recover any remaining radioactivity. Aliquots of the 10 ml fractions collected were assayed for radioactivity.

Table I. R_f Values for Potential Mocap Organosoluble Metabolites on Four Thin-Layer Chromatographic Systems

Compound	Metabolite Number on Silicic Acid Column	R_f in System[a] A	B	C	D
Mocap	MO	0.53	0.17	0.55	0.84
Ethyl propyl sulfide	I	0.85	0.55	0.99	0.93
Ethyl propyl sulfoxide	V	0.13	0.08	0.21	0.75
Ethyl propyl sulfone	IV	0.39	0.12	0.43	0.88
Methyl propyl sulfide	II	0.82	0.62	0.90	0.90
Methyl propyl sulfoxide	V	0.21	0.13	0.25	0.90
Methyl propyl sulfone	IV	0.47	0.31	0.40	0.93
Propyl disulfide	III	0.78	0.68	0.88	0.85

[a] Thin-layer chromatography on Silica gel G using hexane:acetone 2:1 in system A; hexane:chloroform 3:2 in system B; benzene:acetone 10:1 in system C; and acetonitrile:water:ammonia 40:9:1 in system D.

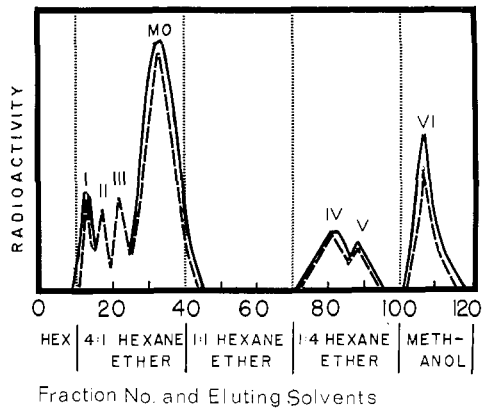

Figure 2. Separation of organosoluble metabolites of Mocap achieved on silicic acid columns

———— Mocap-ethyl-^{14}C
– – – – – – Mocap-propyl-^{14}C

Silica gel G was used for thin-layer chromatography. Normally thin-layers of 0.25 or 0.5 mm thickness were used except when purifying the standards, in which case layers of 1 mm thickness were found to be essential.

Gas chromatographic analysis of certain column fractions was accomplished using an F & M Model 700 instrument modified to accommodate a dual flame photometric detector which allowed the simultaneous detection of phosphorus and sulfur compounds (Bowman and Beroza, 1968). A 6-ft $^1/_4$-in. glass column packed with 20% UC-W-98 on Chromosorb W (60- to 80-mesh) was used. The injection temperature was 225° C and the column oven temperature was programmed from 100° to 200° C at 5° per min. Nitrogen was used as a carrier gas at a flow rate of 50 ml per min.

Radioassay Procedures. For counting nonaqueous samples, a scintillation mixture containing 5 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzene (dimethyl POPOP) per l. of toluene was used. For aqueous samples, 5.5 g of PPO and 0.2 g of dimethyl POPOP per l. of a 2:1 mixture of toluene:Triton X-100 was used. For monoethanolamine samples in which $^{14}CO_2$ was absorbed, a counting solution consisting of 0.55% PPO in a 2:1 toluene:2-methoxyethanol was used (Menzer and Casida, 1965).

Radioautography was used to detect radioactive spots on thin-layer chromatographic plates. The plates were exposed to Kodak No-Screen Medical X-ray film for a 1–2 week period, depending on the amount applied. Radioactive areas appeared as dark spots on the transparent film after development.

Detection and Identification of Metabolites. Metabolites of Mocap were identified by: cochromatography with known, unlabeled purified standards with radioactive extracts or pooled peaks from silicic acid, and/or 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 Mocap to evaluate the loss of either the ethyl or propyl moiety from the molecule. After the resolution of the metabolites, the location of the unlabeled standards was determined by exposing the plates to iodine vapors and then spraying a palladium chloride reagent prepared by diluting 5 ml of a 5% palladium chloride solution in water with 1 ml of HCl and 94 ml of 95% ethanol. Mocap and phosphorodithioate derivatives gave deep brownish-yellow spots on a white background, the derivatives with one S—P bond gave lighter spots, but the compounds which lacked an S—P bond gave grey spots of varying shades.

Identification was confirmed if both the radioactive spot and the unlabeled, known standard spot migrated together in three different solvent systems. The different solvent systems developed for the organoextractable standards and metabolites on silica gel thin-layer plates are noted in Table I.

Acid and Base Hydrolysis. Mocap-ethyl-^{14}C and Mocap-propyl-^{14}C (approximately 250,000 cpm) were incubated with 0.05 *N* NaOH and 0.1 *N* HCl at 25° C in separate experiments to determine the rates of hydrolysis for comparison with those of unknown metabolites. One-milliliter aliquots were sampled at intervals, partitioned with equal amounts of chloroform, and the fractions were assayed for radioactivity. The chloroform and water fractions were each subjected to thin-layer chromatography to determine the nature of the hydrolysis products.

RESULTS

Chemical Stability of Mocap. In the studies designed to evaluate the stability of Mocap to acid and base hydrolysis, it was observed that Mocap was more stable in acid than in base. When incubated with 0.05 *N* NaOH, the propyl thiolate ion was very easily liberated. The half-life of Mocap-ethyl-^{14}C under these conditions was 35 min. The hydrolytic product isolated was *O*-ethyl *S*-propyl phosphorothioic acid, indicating the greater susceptibility of the P—S bond to hydrolysis over the P—O bond. The half-life of Mocap when incubated in 0.1 *N* HCl was greater than 60 min. Very little hydrolysis had taken place during the 60-min period of incubation.

Chemical Nature of Metabolites. When chloroform extracts of bean and corn plants were chromatographed on silicic acid columns, five peaks resulted after treatment with Mocap-ethyl-^{14}C and six peaks after treatment with Mocap-propyl-^{14}C (Figure 2). Further analysis of the peaks was accomplished on a number of thin-layer chromatographic systems and the R_f values are listed in Table I.

Metabolite I was present after the plants were treated with both Mocap-ethyl-^{14}C and Mocap-propyl-^{14}C. The peak was concentrated and analyzed for phosphorus and sulfur content by flame photometric gas chromatography (Bowman and Beroza, 1968). The metabolite contained sulfur but no phosphorus, and gave a retention time the same as that of ethyl propyl sulfide on a W-98 column. The metabolite

also cochromatographed with pure samples of ethyl propyl sulfide in four different thin-layer solvent systems. The sodium ethoxide-ethanol cleavage of Mocap-ethyl-^{14}C indicated the production of ethyl propyl sulfide-ethyl-^{14}C (yield less than 1%). When the reaction product was chromatographed on a silicic acid column, the radioactive peak cochromatographed with pure samples of ethyl propyl sulfide, and its infrared spectrum was identical with that of ethyl propyl sulfide. Metabolite I was therefore designated ethyl propyl sulfide.

A peak was isolated on columns from some plant extracts (metabolite II) which cochromatographed with a purified sample of methyl propyl sulfide in four different thin-layer chromatographic solvent systems. It may have been produced as a result of *S*-methylation of propyl mercaptan which was very easily liberated by the hydrolysis of Mocap under slightly alkaline conditions. Metabolite II was therefore designated methyl propyl sulfide. In many cases, it was very hard to distinguish between metabolites I and II on the silicic acid column. There was no evidence that both metabolites I and II were present simultaneously in the same plants.

Metabolite III was observed only in extracts of Mocap-propyl-^{14}C-treated-plants, not in extracts of Mocap-ethyl-^{14}C-treated plants. Flame photometric analysis indicated the presence of sulfur and absence of phosphorus and the same retention time on a W-98 column as that of propyl disulfide. It also cochromatographed with a purified sample of propyl disulfide on four different thin-layer chromatographic systems.

The peak designated MO on the silicic acid column (Figure 2) was always the largest one when plant extracts were chromatographed. By flame photometric gas chromatography it was determined that this material contained two sulfur atoms and one phosphorus atom per molecule, and that its retention time under a variety of conditions was the same as that of Mocap itself. It also cochromatographed with Mocap in four different thin-layer chromatographic systems. It was designated, therefore, unchanged Mocap.

Metabolites IV and V could not be isolated in sufficient quantities from the bean and corn plants for definitive characterization studies. Both the peaks were present in Mocap-ethyl-^{14}C and Mocap-propyl-^{14}C plant extracts. Peak IV cochromatographed on thin-layer plates and silicic acid columns with ethyl propyl sulfone and Peak V with ethyl propyl sulfoxide. These peaks also cochromatographed with methyl propyl sulfoxide and sulfone, respectively. The presence of these materials in plant extracts could not be definitely established, however, because they could not be separated from ethyl propyl sulfoxide and sulfone on thin-layer chromatograms.

Recovery of Administered Radioactivity. Good recovery of administered radioactivity was achieved from the plant treatments, including soil, considering the nature of the radiolabeled materials administered. Recoveries were calculated as Mocap-ethyl-^{14}C- and Mocap-propyl-^{14}C-equivalents. The half-life of Mocap in the soil when applied as a 10% granular formulation to the soil was about 30 days when beans were grown in that soil in plant growth chambers (Table II). Determination of a meaningful half-life in the soil when corn was grown was not possible because of the large errors introduced in the analyses by the rapid growth of the plants (Table II). Recoveries of extractable radioactivity from bean and corn plants grown in treated soil reached a maximum of 3.59% and 3.86%, respectively, of the administered dose to soil. A significant portion of this activity was present in the methanol-water extract in the form of hydrolytic products. A considerable amount of radioactivity was recovered, especially in the later sampling times, from the plant residue after methylene chloride and methanol extractions (maximum of 11.50% from beans and 71.13% from corn). The total radioactivity recovered in some samples exceeded 100%. This may be partially explained by the difficulty of obtaining a homogeneous sample for analysis from the plant residue and the soil and the large volume of the pulp resulting after extraction of the later corn samples. Recovery after administration of propyl-labeled Mocap is generally lower because of the probability of the release of the extremely volatile, labeled propyl mercaptan.

The methylene chloride extracts of bean and corn plants were chromatographed on silicic acid columns in order to separate the various organoextractable metabolites (Table III). In nearly all cases the major compound present was Mocap itself. Ethyl propyl sulfide was consistently present in moderate amounts, while there were minor amounts of other metabolites present in some sampling periods. In order to ascertain the composition of the methanol column wash, one sample each from ethyl and propyl labels was concentrated and chromatographed on the anion exchange column and was found to consist of hydrolytic metabolites of Mocap that were apparently at least partially extracted from the plants by methylene chloride. The major hydrolytic product in both cases was despropyl Mocap.

All the radioactivity extracted with methanol-water from the sterilized soil was due to unchanged Mocap. However, it is apparent from Table II that there was a loss of radioactivity from the soil that is not accounted for in the plant extracts. This must result from volatilization of Mocap from the soil and possibly metabolism of Mocap to volatile radioactive components which are also lost from the soil.

DISCUSSION

A number of organoextractable and water-soluble metabolites of Mocap have been detected in bean and corn plants. The metabolites formed are the result of dealkylation of the Mocap molecule. The resulting fragments were then able to react with or be incorporated into other constituents of the plants. A proposed scheme for the degradation of Mocap in plants is depicted in Figure 3.

In the proposed metabolic scheme the status of the propyl thiolate ion is of critical importance. The *S*-propyl group has been found to be the favored leaving group in a displacement reaction with hydroxide ion. The propyl thiolate ion thus liberated is strongly nucleophilic and can attack a phosphorothioic ester in an analogous fashion to the reaction mechanism formulated by Harvey and Jacobson (1963):

$$(RO)_2P(O)SR' + R'S^- \rightarrow (RO)(R'S)P(O)O^- + R'SR$$

A similar reaction mechanism may be involved in the production of ethyl propyl sulfide in plants, which was observed to be an important metabolite of Mocap. Ethyl propyl sulfide was present in significant quantities in the methylene chloride extracts of both bean and corn plants, although it was actually only a very small percentage of the administered dose. The mechanism for formation of ethyl propyl sulfide may involve the prior formation of the propyl thiolate ion and subsequent attack on an intact Mocap molecule, or it may involve an intramolecular reaction which would yield ethyl propyl sulfide. It has been demonstrated that

such a mixed sulfide can result from the sodium ethoxide-ethanol catalyzed cleavage of Mocap, even though the yield was less than 1%. Pilgram (1966) reported that higher mercaptans react with alkyl esters of phosphoric, phosphorothiolic, and phosphinic acids at elevated temperatures to produce alkyl aryl sulfides. In all cases it was found that the alkoxy group was the alkyl donor, not the alkylthio group. Although on the basis of the current information a decision between these two mechanisms is difficult, one would tend to favor the prior formation of the propyl thiolate ion since other products are formed whose presence would also be explained by a reaction involving the propyl thiolate ion.

The propyl thiolate ion would itself be strongly thiophilic (Kirby and Warren, 1967) and would be expected to react further with another thiol to form a disulfide. Propyl disulfide was in fact isolated from the methylene chloride extracts of bean and corn plants treated with Mocap-propyl-

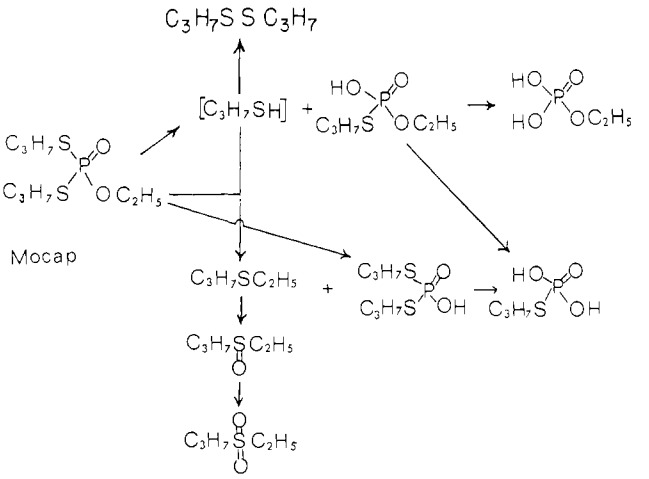

Figure 3. Proposed metabolic degradation scheme of Mocap in bean and corn plants

Table II. Recovery of Mocap-Equivalents Calculated as Percent of Administered Dose Present in Various Fractions of Bean and Corn Plants and Soil

Days After Treatment	% Administered Dose Recovered in				
	CH_2Cl_2	CH_3OH: Water	Plant Residue	Soil Extract	Total
Bean Plants					
Mocap-ethyl-^{14}C Administered to Soil					
7	1.20	0.60	0.41	110.75	112.96
21	0.96	0.55	2.71	72.78	77.78
28	...[a]	...[a]	4.13	58.29	...
35	1.50	0.68	6.81	62.26	71.25
42	1.66	0.23	7.09	52.93	61.91
49	2.49	1.10	7.36	44.96	55.91
56	1.87	0.98	11.50	37.01	51.36
63	1.91	1.44	9.17	23.84	36.36
Mocap-propyl-^{14}C Administered to Soil					
7	0.17	0.18	0.23	88.65	89.23
21	0.43	0.50	1.25	75.72	77.90
28	0.50	0.88	4.08	59.50	64.96
35	0.67	0.51	4.90	56.63	61.71
42	1.09	0.19	4.59	60.71	66.59
49	0.79	0.54	6.77	38.91	47.01
56	0.89	0.32	7.08	31.97	40.24
63	0.84	0.52	6.91	25.86	34.13
Corn Plants					
Mocap-ethyl-^{14}C Administered to Soil					
18	0.46	0.44	0.06	65.27	66.23
28	0.91	0.18	1.32	75.06	77.47
38	0.96	0.29	7.14	59.01	67.40
48	1.23	...	4.36	90.43	96.02
58	2.27	0.33	8.28	109.10	119.98
68	2.52	1.34	17.53	103.53	124.92
78	1.72	0.90	38.71	64.27	105.60
88	1.52	0.45	56.97	70.28	129.22
100	1.53	1.11	71.13	49.43	123.20
Mocap-propyl-^{14}C Administered to Soil					
18	0.11	0.08	0.07	44.49	44.75
28	0.84	0.67	0.08	55.08	56.67
38	0.47	0.20	1.35	43.21	45.23
48	0.49	0.26	2.14	40.66	43.55
58	0.44	0.17	7.66	59.21	67.48
68	0.60	0.55	9.15	49.12	59.42
78	0.46	0.18	11.24	45.56	57.44
88	0.40	0.39	27.97	36.84	65.60
100	0.41	0.38	34.09	26.93	61.81

[a] Sample lost due to contamination

Table III. Percentages of Compounds (Corrected for Recovery) Recovered from Methylene Chloride Fraction of Bean and Corn Plants Grown in Mocap-Treated Soil

Days After Treatment	Metabolite					Methanol Col. Wash
	EtSPr	PrSSPr	Mocap	EtSOPr	$EtSO_2Pr$	
Bean Plants						
Treatment with Mocap-ethyl-^{14}C						
7	2.56	...	15.36	...	...	7.68
21	13.95	...	38.89	5.90	...	22.10
28	24.43[a]	...	40.54[a]	...	...	...
35	15.83	...	36.44	7.35	3.37	23.22
42	...[b]	...	68.05[b]	2.70	22.65	5.88
49	28.40	...	4.52	...	...	41.57
56	42.83	...	21.29	...	...	18.63
63	60.36	...	20.03	...	5.88	11.12
Treatment with Mocap-propyl-^{14}C						
7	...	...	45.59	...	...	13.34
21	...	...	62.60	4.61	...	15.94
28	2.41	...	50.95	...	5.73	40.78
35	...	...	48.66	1.72	6.23	28.08
42	5.73	...	56.13	...	...	17.69
49	16.09	5.39	15.77	...	...	46.11
56	11.44[c]	4.18	27.85	...	...	37.75
63	12.22	2.15	37.58	...	...	36.98
Corn Plants						
Treatment with Mocap-ethyl-^{14}C						
18	2.95	...	82.75	...	...	11.04
28	7.36	...	73.50	...	...	16.28
38	32.90	...	27.00	3.03	...	32.17
48	8.80	...	43.80	1.80	...	43.90
58	36.40	...	19.20	...	7.80	36.60
68	17.50	...	36.20	...	...	46.30
78	30.80	...	22.50	...	19.20	27.50
88	34.70	...	18.50	...	7.90	38.90
100	6.30	...	57.20	...	14.60	21.90
Treatment with Mocap-propyl-^{14}C						
18	...	...	31.60	...	...	68.40
28	1.70	1.80	77.90	4.60	...	14.00
38	...	...	81.10	...	...	18.90
48	5.00[c]	...	56.60	16.70	...	21.70
58	...	...	61.70	...	...	38.30
68	3.40	...	53.60	...	...	43.00
78	0.20	...	48.20	...	10.90	40.70
88	8.95	5.30	22.30	...	...	63.45
100	26.10[c]	9.10	23.80	...	10.00	31.00

[a] Not corrected for recovery. [b] Combined percentages of EtSPr and Mocap. [c] May be at least partly MeSPr.

^{14}C. Of course, it was not detected from plants which had been treated with Mocap-ethyl-^{14}C.

An additional point in favor of the formation of the propyl thiolate ion is the fact that *O*-ethyl *S*-propyl phosphorothioic acid was a major component of the methanol-water fraction of corn plants. *O*-Ethyl *S*-propyl phosphorothioic acid must release a second molecule of propyl mercaptan since *O*-ethyl phosphoric acid was also found in the methanol-water extract of plants. Both of these materials have also been detected in the urine of rats treated with Mocap (Iqbal and Menzer, 1971).

Because of the central place of the propyl thiolate ion in the proposed route of metabolism of Mocap and the proposal that it deethylates Mocap, it is of interest that desethyl Mocap was not found in plants, although it was present in rat urine (Iqbal and Menzer, 1971). However, desethyl Mocap was found to be rather unstable, and it probably loses propyl mercaptan to give *S*-propyl phosphorothioic acid, which was found in plants.

The question of the possible formation of methyl propyl sulfide by methylation of the propyl thiolate ion was not resolved by this investigation. Although ethyl propyl sulfide and methyl propyl sulfide could be readily separated by gas chromatography using a column of 20% UC-W-98 on Chromosorb W, it was not always possible to distinguish between them on silicic acid columns. There were a number of instances in which the presence of methyl propyl sulfide was suspected in plant extracts, but the amounts were too small for confirmatory analyses by gas chromatography. However, methyl propyl sulfide was definitely formed as a metabolite of Mocap in rats (Iqbal and Menzer, 1971).

The ethyl propyl sulfide formed in plants was oxidized to form its sulfoxide and sulfone. The separation on silicic acid columns of methylene chloride extracts of plant samples at different intervals indicated that ethyl propyl sulfoxide appeared first, then the sulfoxide and sulfone appeared together, and finally, in later samples, only ethyl propyl sulfone was found.

The recovery of administered radioactivity from plants was quite adequate, since the whole plants were taken for analysis and the soil in which they were grown was also considered. Recoveries of greater than 100% in the later plant samples probably resulted from the errors involved in calculating the activity in the plant residue and the soil based on the rather small aliquots of each that could be conveniently analyzed.

In considering the recovery of radioactivity from plants, including the treated soil, it must also be realized that the soil used had been steam-sterilized before application of the chemical. This was done with the idea of reducing the adsorptive capacity of the soil so that the maximum amount of Mocap would be available for uptake by the plants. Konrad and Chester (1969) found that twice as much Ciodrin was bound to nonsterile soil (pH 7.2) as to sterile soil, and that its half-life was longer in sterile soil. In addition, degradation in sterile soil would be slower than in nonsterile soil, both because of decreased adsorption and decreased microbial activity (Konrad *et al.*, 1967; Matsumura and Bousch, 1966). In fact, no metabolism of Mocap was observed in the sterile soil used.

This study of Mocap metabolism in plants has shown that the compound is rapidly converted to metabolites that are not likely to be considered toxic in any way. Uptake of Mocap from treated soil is slow and the amounts of Mocap likely to be present in the plant tissues at any time are low. Likewise, there is no buildup of Mocap metabolites that will have undesirable effects on the plant or on the consumer of the plant. Even though Mocap is grouped with the phosphorodithioates, it is different from most of these compounds in that it does not require oxidation to the oxygen analog for its anticholinesterase activity. Mocap should be considered an important insecticide-nematocide because of its reported effectiveness, and the simplicity of its metabolic products.

LITERATURE CITED

Bacon, W. E., LeSeur, W. M., *J. Amer. Chem. Soc.* **76,** 610 (1954).
Birchfield, W., *Plant Dis. Rep.* **52,** 786 (1968).
Birchfield, W., Martin, W. J., *Plant Dis. Rep.* **52,** 127 (1968).
Bowman, M. C., Beroza, M., *Anal. Chem.* **40,** 1448 (1968).
Gutenmann, W. H., Lisk, D. J., J. AGR. FOOD CHEM. **8,** 306 (1960).
Gutenmann, W. H., St. John, L. E., Barry, D. L., Jones, E. D., Lisk, D. J., J. AGR. FOOD CHEM. **9,** 50 (1961).
Harvey, R. G., Jacobson, H. I., *J. Amer. Chem. Soc.* **85,** 1618 (1963).
Heald, C. M., Self, R. L., *Plant Dis. Rep.* **51,** 1035 (1967).
Iqbal, M. Z., Menzer, R. E., unpublished data (1971).
Kerr, S. H., *J. Econ. Entomol.* **61,** 523 (1968).
Kirby, A. J., Warren, S. G., "The Organic Chemistry of Phosphorus," p. 98, Elsevier Pub. Co., New York, 1967.
Konrad, J. G., Chester, G., J. AGR. FOOD CHEM. **17,** 226 (1969).
Konrad, J. G., Armstrong, D. E., Chester, G., *Agron. J.* **59,** 591 (1967).
Matsumura, F., Bousch, G. M., *Science* **153,** 1278 (1966).
Menzer, R. E., Casida, J. E., J. AGR. FOOD CHEM. **13,** 102 (1965).
Mistric, W. J., Jr., Smith, F. D., *J. Econ. Entomol.* **62,** 712 (1969).
Mobil Chemical Co., Technical Bulletin (1969).
O'Bannon, J. H., Taylor, A. L., *Plant Dis. Rep.* **51,** 995 (1967).
Pilgram, K., *Tetrahedron* **22,** 1241 (1966).
Reid, E. E., "Organic Chemistry of Bivalent Sulfur," Vol. II, Chem. Publ. Co., pp. 64–66, New York, 1960.
Rhoades, H. L., *Plant Dis. Rep.* **53,** 728 (1969).
Taylor, A. L., O'Bannon, J. H., *Plant Dis. Rep.* **52,** 218 (1968).
Wilson, J. H., Jr., Melton, T. M., Downing, C. R., *Va. J. Sci.* **16,** 314 (1965).

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