Identification of Metabolites of *N*-(1,1-Dimethylpropynyl)-3,5-dichlorobenzamide in Soil and Alfalfa

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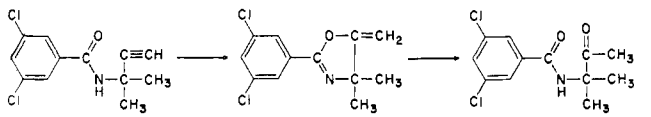
Results of a study of the metabolism of the new herbicide *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (Compound A) in soil and alfalfa using C^{14}-labeled compound are presented. In soil, Compound A is readily cyclized to 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methyleneoxazoline, which is subsequently hydrolyzed to *N*-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide. Several other metabolites are also formed, including 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-hydroxymethyloxazoline, *N*-(1,1-dimethyl-3-hydroxyacetonyl)-3,5-dichlorobenzamide, *N*-(1,1-dimethyl-3-hydroxypropyl)-3,5-dichlorobenzamide, *N*-(1,1-dimethyl-2,3-dihydroxypropyl)-3,5-dichlorobenzamide, β-(3,5-dichlorobenzamido)-β-methylbutyric acid, α-(3,5-dichlorobenzamido)isobutyric acid, and β-(3,5-dichlorobenzamido)-β-methyl-α-ketobutyric acid. When applied by foliar application to alfalfa, the rate of metabolism is slow, but most of the metabolites formed in the soil can be identified in the plant.

The compound, *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (Kerb, Rohm and Haas RH-315, hereafter designated Compound A), is a new herbicide with broad spectrum activity (Viste *et al.*, 1970). Metabolism studies with C^{14}-carbonyl labeled Compound A have shown that two major products, 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methyleneoxazoline (I) and *N*-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide (II), can be isolated from treated soil (Yih *et al.*, 1970).



COMPOUND A I II

This paper describes the isolation, identification, and synthesis of metabolites of Compound A in soil and alfalfa.

EXPERIMENTAL

Synthesis of Compounds and Metabolites. The preparation of unlabeled Compound A (Viste *et al.*, 1970) and C^{14}-carbonyl labeled Compound A (Yih *et al.*, 1970) have been described. Melting points are uncorrected. In all cases ir and nmr data support structural assignments. (A complete listing of the structures of the metabolites will be found in Table I.)

2-(3,5-DICHLOROPHENYL)-4,4-DIMETHYL-5-METHYLENEOXAZOLINE (COMPOUND I)

(This procedure has been previously described by Easton *et al.*, 1965.)

A solution of *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (10.0 g, 0.039 mole) in dimethylformamide (30 ml) was treated with a solution of silver nitrate (2.0 g, 0.011 mole) in dimethylformamide (3 ml), stirred at room temperature for 3 hr, diluted with ether (300 ml), washed with water, dried, and the solvent removed to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-methylene-oxazoline (8 g, 80%): b.p. 127–129° C/0.25 mm.

ANAL. Calcd. for $C_{12}H_{11}Cl_2NO$: C, 56.26; H, 4.33; N, 5.51. Found: C, 56.23; H, 4.36; N, 5.58.

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N-(1,1-DIMETHYLACETONYL)-3,5-DICHLOROBENZAMIDE (COMPOUND II)

A solution of concentrated hydrochloric acid (10 ml) in water (40 ml) was added to a solution of *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (100 g, 0.39 mole) in ethanol (500 ml), and the mixture heated under reflux for 8 hr. Water was added to the hot solution until it became cloudy, and on cooling crystals of *N*-(1,1-dimethylacetonyl)-3,5-dichlorobenzamide (80 g, 75%) separated: m.p. 141–142° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_2$: C, 52.59, H, 4.78; N, 5.11. Found: C, 52.58; H, 4.68; N, 5.15.

2-(3,5-DICHLOROPHENYL)-4,4-DIMETHYL-5-HYDROXYMETHYLOXAZOLINE (COMPOUND III) AND *N*-(1,1-DIMETHYL-2,3-DIHYDROXYPROPYL)-3,5-DICHLOROBENZAMIDE (COMPOUND VI)

2-(3,5-DICHLOROPHENYL)-4,4-DIMETHYL-5-BROMOMETHYLENEOXAZOLINE. A hot solution of *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (154 g, 0.6 mole) in ethyl acetate (1000 ml) was cooled rapidly to 20° C to give a fine suspension. A solution of bromine (96 g, 9.6 mole) in carbon tetrachloride (50 ml) was added dropwise to the stirred suspension, maintaining the temperature at 20° C by mild cooling. The suspension was stirred for 1 hr and filtered to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-bromomethyleneoxazoline hydrobromide (241 g, 92%): m.p. 213–215° C (dec). An ether suspension of the hydrobromide was treated with triethylamine and washed with water to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-bromomethyleneoxazoline: m.p. 118–121° C.

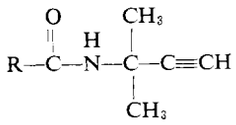
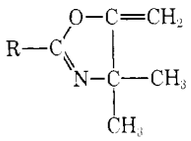
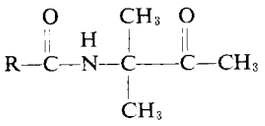
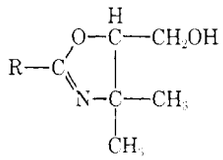
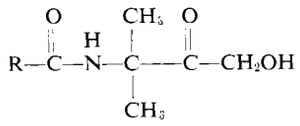
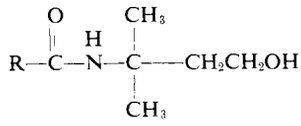
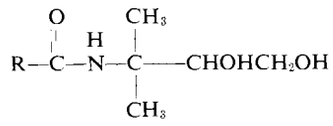
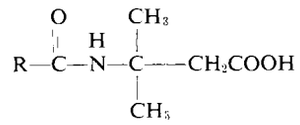
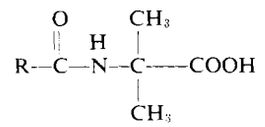
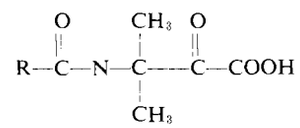
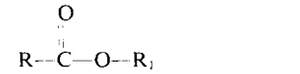
ANAL. Calcd. for $C_{12}H_{10}BrCl_2NO$: C, 43.02; H, 3.01; N, 4.18. Found: C, 43.34; H, 3.11; N, 4.24.

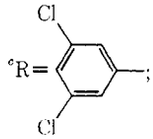
N-(1,1-DIMETHYL-3-BROMOACETONYL)-3,5-DICHLOROBENZAMIDE. A solution of 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-bromomethyleneoxazoline (35 g, 0.11 mole) in concentrated sulfuric acid (80 ml) was heated overnight at 100° C in a steam bath, then cooled and poured onto ice. The precipitated solid was taken up in ether, the solution dried and stripped, and the residue triturated with hexane to give *N*-(1,1-dimethyl-3-bromoacetonyl)-3,5-dichlorobenzamide (30.5 g, 87%): m.p. 153–155° C.

ANAL. Calcd. for $C_{12}H_{12}BrCl_2NO_2$: C, 40.81; H, 3.43; N, 3.97. Found: C, 40.50; H, 3.48; N, 3.92.

2-(3,5-DICHLOROPHENYL)-4,4-DIMETHYL-5-HYDROXYMETHYLOXAZOLINE. A solution of *N*-(1,1-dimethyl-3-bromoace-

Table I. Structures, Thin-Layer Chromatographic R_f Values[a] and Amounts of Compound A and Its Soil Metabolites Found 90 Days After Soil Treatment[b]

Metabolite Number	Structure[c]	% Total Activity Found	R_f A	R_f B	R_f C
Compound A[d]		10.0	0.62	S_f	S_f
I		9.2	0.82	S_f	S_f
II		76.9	0.42	0.79	S_f
III		1.6	0.23	0.69	S_f
IV		0.1	0.13	0.61	S_f
V		0.4	0.0	0.49	S_f
VI		0.1	0.0	0.46	S_f
VII		0.6	0.0	0.0	0.71
VIII		0.5[e]	0.0	0.0	0.49
IX					
X[f]		...	0.0	0.0	0.63

[a] R_f values obtained using the following solvent system: (A) Acetone:benzene (5:95); (B) Acetone:benzene (25:75); and (C) Isopropyl alcohol:ethyl acetate:water (25:65:10). [b] Soil was incorporated with C^{14}-carbonyl labeled Compound A stored in a sealed glass jar at 26° C for 90 days. [c]  ; R_1 is unknown. [d] C^{14} labeled on the carbonyl carbon. [e] Same R_f value for both metabolites VIII and IX, see text. [f] Present in alfalfa, not in soil.

tonyl)-3,5-dichlorobenzamide (17.5 g, 0.05 mole) in methanol (500 ml) was treated with sodium borohydride (2.5 g, 0.092 mole) in small portions. The reaction mixture was heated on a steam bath overnight, the solvent removed under reduced pressure, and the residue taken up in ether. The ethereal solution was washed with water, dried, and the solvent removed to give a concentrate (13 g, 95%), which was chromatographed on 80–200 mesh 10% deactivated alumina (1200 g). Elution with 5% ether/benzene gave a forerun which, on recrystallization from octane, gave 2-(3,5-dichlorophenyl)-4,4-dimethyl-5,6-dihydro-(4*H*)-1,3-oxazin-5-ol (2.1 g, 15%): m.p. 117–118° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_2$: C, 52.52; H, 4.78; N, 5.13. Found: C, 52.14; H, 4.89; N, 4.89.

Continued elution with 5% ether/benzene yielded fractions which, after recrystallization from octane, gave 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-hydroxymethyloxazoline (4.0 g, 29%): m.p. 88.5–90.5° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_2$: C, 52.52; H, 4.78; N, 5.13. Found: C, 52.54; H, 4.41; N, 5.10.

N-(1,1-DIMETHYL-2,3-DIHYDROXYPROPYL)-3,5-DICHLOROBENZAMIDE. A 3:2 mixture of the oxazinol and oxazoline from the above reaction (16.5 g, 0.06 mole) was heated under reflux for 7 hr in a mixture of ethanol (100 ml), concentrated hydrochloric acid (5 ml), and water (10 ml). The ethanol was removed under reduced pressure and water removed by azeotroping with benzene. The benzene was removed under reduced pressure and the residue dissolved in hot acetone (150 ml). The volume of the solution was reduced to 75 ml and, on cooling, a mixture (11.5 g, 58%) of 2-hydroxy-3-amino-3-methylbutyl)-3,5-dichlorobenzoate hydrochloride and 2-(1-hydroxy-3-amino-3-methylbutyl)-3,5-dichlorobenzoate hydrochloride precipitated as a white solid. Treatment of an aqueous solution of the mixture with excess sodium hydroxide followed by extraction with ether gave *N*-(1,1-dimethyl-2,3-dihydroxypropyl)-3,5-dichlorobenzamide (7 g, 40%): m.p. 95–99° C.

ANAL. Calcd. for $C_{12}H_{15}Cl_2NO_3$: C, 49.32; H, 5.18; N, 4.79. Found: C, 49.02; H, 5.28; N, 4.64.

N-(1,1-DIMETHYL-3-HYDROXYACETONYL)-3,5-DICHLOROBENZAMIDE (COMPOUND IV)

N-(1,1-DIMETHYL-3-ACETOXYACETONYL)-3,5-DICHLOROBENZAMIDE. A solution of *N*-(1,1-dimethyl-3-bromoacetonyl)-3,5-dichlorobenzamide (17.6 g, 0.05 mole) and potassium acetate (4.9 g, 0.05 mole) in acetic acid (250 ml) was heated at 90° C for 4 days. The solution was cooled, potassium bromide (4.3 g, 72%) was filtered off, and the solvent removed under reduced pressure. The residue was slurried with ether (30 ml) and *N*-(1,1-dimethyl-3-acetoxyacetonyl)-3,5-dichlorobenzamide (4.0 g, 24%) filtered off. A sample was recrystallized from acetone: m.p. 185–189° C.

ANAL. Calcd. for $C_{14}H_{15}Cl_2NO_4$: C, 50.61; H, 4.55; N, 4.22. Found: C, 50.80; H, 4.74; N, 4.29.

N-(1,1-DIMETHYL-3-HYDROXYACETONYL)-3,5-DICHLOROBENZAMIDE. A slurry of *N*-(1,1-dimethyl-3-acetoxyacetonyl)-3,5-dichlorobenzamide (7.5 g, 0.022 mole) and anhydrous potassium carbonate (0.3 g) in methanol (300 ml) was stirred at 25° C for 24 hr, during which time the mixture became homogeneous. The solvent was removed under reduced pressure and the residue triturated with water to give *N*-(1,1-dimethyl-3-hydroxyacetonyl)-3,5-dichlorobenzamide (6.1 g, 94%). A sample was recrystallized from hexane/acetone: m.p. 108–109° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_3$: C, 49.67; H, 4 52; N, 4.83. Found: C, 49.74; H, 4.59; N, 4.92.

N-(1,1-DIMETHYL-3-HYDROXYPROPYL)-3,5-DICHLOROBENZAMIDE (COMPOUND V)

A solution of 3,5-dichlorobenzoyl chloride (17.3 g, 0.0825 mole) in ether (75 ml) was slowly added to a well stirred solution of 3-amino-3-methyl-butanol (8.50 g, 0.0825 mole) (Soday, 1946) in ether (75 ml), and 10% sodium hydroxide solution (33.0 g, 0.0825 mole) at 15° C. After stirring for 1 hr, the ethereal phase was separated, washed, and dried, and the solvent removed. The residue (19 g, 83%) was recrystallized from 3:2 hexane/acetone to give *N*-(1,1-dimethyl-3-hydroxypropyl)-3,5-dichlorobenzamide: m.p. 108–110° C.

ANAL. Calcd. for $C_{12}H_{15}Cl_2NO_2$: C, 52.18; H, 5.47; N, 5.07: Found: C, 52.34; H, 5.70; N, 5.25.

β-(3,5-DICHLOROBENZAMIDO)-β-METHYLBUTYRIC ACID (COMPOUND VII)

A solution of 3,5-dichlorobenzoyl chloride (6.8 g, 0.033 mole) in octane (10 ml) was added slowly to a well stirred solution of 3-amino-3-methylbutyric acid hydrochloride (5.0 g, 0.033 mole) (Crook *et al.*, 1966) and sodium hydroxide (3.9 g, 0.098 mole) in water (25 ml) at 10° C. Stirring was continued overnight, maintaining the temperature at 10° C for the first half hour. The aqueous phase was separated, acidified, and extracted with ether, and the extract washed with water, dried, and the solvent removed to give β-(3,5-dichlorobenzamido)-β-methylbutyric acid (8.7 g, 92%) which was recrystallized twice from benzene: m.p. 113–117° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_3$: C, 49.75; H, 4.52; N, 4.83: Found: C, 49.82; H, 4.75; N, 4.75.

METHYL β-(3,5-DICHLOROBENZAMIDO)-β-METHYLBUTYRATE. A solution of the acid (13.5 g, 0.0466 mole) in methanol (100 ml) containing a catalytic quantity of anhydrous hydrogen chloride was allowed to stand at room temperature overnight. The solvent was removed under reduced pressure and the residue taken up in ether, washed with dilute base and water, dried, and the solvent removed. Methyl-3,5-dichlorobenzoate impurity (2.0 g) was removed from the crude product by sublimation, and recrystallization of the nonvolatile residue from hexane gave methyl β-(3,5-dichlorobenzamido)-β-methylbutyrate (9.6 g, 68%): m.p. 61–63° C.

ANAL. Calcd. for $C_{13}H_{15}Cl_2NO_3$: C, 51.32; H, 4.97; N, 4.61. Found: C, 51.39; H, 5.16; N, 4.57.

α-(3,5-DICHLOROBENZAMIDO)ISOBUTYRIC ACID (COMPOUND VIII)

A solution of 3,5-dichlorobenzoyl chloride (160 g, 0.767 mole) in octane (200 ml) was slowly added to a well stirred solution of α-methyl-α-alanine (79.0 g, 0.767 mole) and 50% sodium hydroxide (122.7 g, 1.534 moles) in water (550 ml) at 25–30° C. When the aqueous phase became neutral (pH 7–8), it was separated and acidified. The resulting precipitate was recrystallized from aqueous methanol to give α-(3,5-dichlorobenzamido)isobutyric acid (159 g, 75%): m.p. 190–192° C.

ANAL. Calcd. for $C_{11}H_{11}Cl_2NO_3$: C, 47.95; H, 4.02; N, 5.07. Found: C, 47.91; H, 4.15; N, 4.88.

METHYL α-(3,5-DICHLOROBENZAMIDO) ISOBUTYRATE. A solution of the acid (5.0 g, 0.018 mole) in methanol (150 ml) containing a catalytic quantity of anhydrous hydrogen chloride was allowed to stand at room temperature overnight. Excess solvent was removed under reduced pressure, and the residue taken up in ether and washed with dilute base and water, dried,

and the solvent removed to give methyl α-(3,5-dichlorobenzamido) isobutyrate (3.5 g, 67%) m.p. 110–111° C.

ANAL. Calcd. for $C_{12}H_{13}Cl_2NO_3$: C, 49.67; H, 4.52; N, 4.83. Found: C, 49.53; H, 4.69; N, 4.80.

β-(3,5-DICHLOROBENZAMIDO)-β-METHYL-α-KETOBUTYRIC ACID (COMPOUND IX)

N-(1,1-DIMETHYL-3,3-DICHLOROACETONYL)-3,5-DICHLOROBENZAMIDE. Excess chlorine was passed into a solution of *N*-(1,1-dimethylpropynyl)-3,5-dichlorobenzamide (250 g, 0.956 mole) in carbon tetrachloride (2600 ml) heated under reflux. The solution was then cooled and seeded, and the precipitate (44.5 g) filtered off and slurried in ether to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-chloro-5-dichloromethyloxazoline hydrochloride (19 g, 5%) m.p. 112–114° C (dec). The free base was prepared by treating 9 g of the hydrochloride in ether suspension with bicarbonate solution, drying, and stripping the ether phase to give 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-chloro-5-dichloromethyloxazoline (7 g, 86%). A sample was recrystallized from hexane: m.p. 94–95° C.

ANAL. Calcd. for $C_{12}H_{10}Cl_5NO$: C, 39.86; H, 2.79; N, 3.87. Found: C, 40.53; H, 2.56; N, 3.85.

The ether and carbon tetrachloride filtrates from the above reaction were combined and the solvents removed. The residue (348 g) of crude oxazoline free base was heated under reflux in ethanol (1800 ml), water (110 ml), and concentrated hydrochloric acid (17 ml) for 1 hr, then water (600 ml) was added and the solution allowed to cool overnight. The crystals which separated were filtered off and recrystallized from ethyl acetate to give *N*-(1,1-dimethyl-3,3-dichloroacetonyl)-3,5-dichlorobenzamide (67.2 g, 20%): m.p. 156–158° C.

ANAL. Calcd. for $C_{12}H_{11}Cl_4NO_2$: C, 42.02; H, 3.23; N, 4.08. Found: C, 42.36; H, 3.37; N, 4.03.

2-(3,5-DICHLOROPHENYL)-4,4-DIMETHYL-5-FORMYL-5-METHOXYOXAZOLINE. *N*-(1,1-Dimethyl-3,3-dichloroacetonyl)-3,5-dichlorobenzamide (34.3 g, 0.1 mole) was added to a freshly prepared sodium methoxide solution [sodium metal (4.6 g, 0.2 mole) in methanol (180 ml)] at 50° C. The reaction mixture was stirred for 2 hr at 50° C, during which time sodium chloride separated and the pH dropped to 7. The reaction mixture was cooled and filtered. The filtrate was concentrated, slurried in benzene, and filtered to remove unreacted starting material (3.5 g). The filtrate was concentrated, then taken up in ether and, on treatment with dry hydrogen chloride, 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-formyl-5-methoxyoxazoline hydrochloride precipitated as a tacky solid and was dissolved in water. The aqueous solution was washed with ether and then basified with sodium hydroxide; the precipitated oil was extracted with ether, and the extract was dried and concentrated to an oil (6.5 g) which slowly crystallized. Recrystallization from hexane gave 2-(3,5-dichlorophenyl)-4,4-dimethyl-5-formyl-5-methoxyoxazoline (5.3 g, 17.6%): m.p. 76.5–81.0° C.

ANAL. Calcd. for $C_{13}H_{13}Cl_2NO_3$: C, 51.67; H, 4.33; N, 4.64. Found: C, 51.66; H, 4.44; N, 4.61.

β-(3,5-DICHLOROBENZAMIDO)-β-METHYL-α-KETOBUTYRIC ACID. 2-(3,5-Dichlorophenyl)-4,4-dimethyl-5-formyl-5-methoxyoxazoline (3.3 g, 0.01 mole) was added in small portions to a vigorously stirred suspension of freshly precipitated silver oxide prepared by the addition of a solution of silver nitrate (3.8 g, 0.022 mole) in deionized water (10 ml) to a solution of sodium hydroxide (1.8 g, 0.045 mole) in deionized water (10 ml). After 20 min the precipitated silver was removed by filtration, and the filtrate was acidified. The resulting white precipitate was filtered off, dried, and recrystallized from benzene to give β-(3,5-dichlorobenzamido)-β-methyl-α-ketobutyric acid (2.0 g, 61%): m.p. 154–157° C (dec).

ANAL. Calcd. for $C_{12}H_{11}Cl_2NO_4$: C, 47.38; H, 3.65; N, 4.61. Found: C, 47.17; H, 3.47; N, 4.30.

METHYL β-(3,5-DICHLOROBENZAMIDO)-β-METHYL-α-KETOBUTYRATE. A solution of β-(3,5-dichlorobenzamido)-β-methyl-α-ketobutyric acid (1.0 g, 0.0036 mole) in methanol (100 ml) containing a catalytic amount of hydrogen chloride was allowed to stand overnight at 25° C. The methanol was removed and the residue recrystallized from benzene/hexane (1:1, v/v) to give methyl β-(3,5-dichlorobenzamido)-β-methyl-α-ketobutyrate (0.7 g, 66%): m.p. 130–132° C.

ANAL. Calcd. for $C_{13}H_{13}Cl_2NO_4$: C, 49.07; 4.12; N, 4.40. Found: C, 49.25; H, 4.08; N, 4.30.

Treatment of Soil. A known amount of loam soil with a moisture level of 14% was treated with a 1:1 mixture of unlabeled and C^{14}-carbonyl labeled Compound A to give a final concentration of 20 ppm Compound A.

Treatment of Alfalfa. A 20 ft^2 field plot of alfalfa was sprayed with a solution of 120 mg C^{14}-carbonyl labeled Compound A (sp. act. 6.94 mc per g) and 297 mg unlabeled Compound A in 43 ml acetone using a hand sprayer with a Tee Jet 8002 E nozzle. The sprayer was powered by a pressure of 20 psi, and the nozzle was held at a height to give a spray coverage of 25 gal/A. This application was equivalent to 2 lb/A.

Method of Sampling. Field soil samples were obtained from the above C^{14}-labeled Compound A alfalfa plot, 115 days after treatment. The samples taken were a 3-in. diameter × 1 in. deep cake of soil, from the surface inch and each lower inch, to a total depth of 6 in. Each sample was mixed thoroughly and stored at 0° C before analysis. Based on our previous studies, most of the C^{14} radioactivity was in the top inch section. This was all that was subjected to analysis for metabolites. For alfalfa samples, above-ground parts of the treated plants were collected 17, 50, and 112 days after treatment for analysis. During this period, a total of 14.67 in. rainfall was accumulated. The temperature ranged from 4° C to 36° C.

Extraction of Compound A and Its Metabolites from Soil and Alfalfa. At treatment, the dense alfalfa stand resulted in only small amounts of the compound being sprayed onto the soil. For confirmation of the presence of the metabolites in the soil, six different soil types, including silty clay loam, sandy loam, sand, loam, clay, and sandy clay loam, soil samples treated with C^{14}-labeled Compound A in the laboratory were used. Duplicate 50 g samples of soil from each soil type at various time intervals were extracted with 250 ml of methanol for 20 hr, using a Soxhlet extractor. Alfalfa plants were cut above the ground level and the entire shoot was used for analysis of C^{14} content. Duplicate 10–20 g of alfalfa were cut into 5–10 mm pieces, and ground in methanol in a VirTis homogenizer. The filtered residue was extracted in the Soxhlet using the same solvent for a period of 20 hr. The extracts were evaporated under reduced pressure while cooled in a dry ice and acetone bath, and the dried samples diluted to 5 ml with methanol for further analysis.

Separation of Compound A and Its Metabolites. Compound separations were achieved on commercially available thin-layer chromatograms (Eastman Chromatogram Sheet, Type K, 301R, Silica Gel or Brinkmann thin-layer chromatographic plates Silica Gel F-254) using: (A) acetone:benzene (5:95, v/v); (B) acetone:benzene (25:75 v/v); and (C) isopropyl alcohol:ethyl acetate:water (25:65:10, v/v). Thin-layer chromatographic plates were radioautographed.

Radioassay. Radioassay techniques have been described (Yih *et al.*, 1970).

Identification of the Metabolites. Two-dimensional thin-layer chromatography was used to identify the metabolites by comparison with reference compounds of known structure. Additional confirmation of the identity of metabolites II and III was obtained by the reverse isotope dilution technique. Structural confirmation of metabolite VIII was obtained by isotope dilution of the methyl ester of the metabolite.

RESULTS AND DISCUSSION

Metabolism in Soil. Figure 1 shows a typical chromatogram from a 90-day treatment in six different soils. The identification of the two major metabolites I and II has been previously described (Yih *et al.*, 1970). The radioactivity remaining at the origin in Figure 1 can be eluted, using more polar solvent mixtures. As shown in Figure 2A, four more metabolites (metabolites III, IV, V, and VI) are separated using acetone:benzene (25:75) in a one-dimensional system, and two further metabolites (metabolites VII and VIII or IX) using isopropyl alcohol:ethyl acetate:water (25:65:10) (Figure 2C), numbered I through IX in order of increasing polarity. The amounts of the metabolites were too small for identification by standard chemical methods, and the technique adopted in this study has been to compare each metabolite with a series of authentic synthetic compounds by thin-layer chromatography, as described in the experimental section. Choosing which compounds to synthesize was based on conjecture from the polarity of the metabolites, as indicated by the tlc R_f values, and the observation that increasing knowledge of the system showed that metabolism involved almost exclusively the two terminal carbon atoms of the side chain. By this method, metabolites III to VII have been identified as the compounds listed in Table I. The identity of the metabolites was confirmed by cochromatography (Figures 2B and 2D).

As indicated in Table I, metabolites VIII and IX give the same R_f value and were hence originally considered to be one compound. It was subsequently found that the methyl esters of these acids could be separated using 10% acetone in hexane (R_f's 0.29 and 0.24, respectively), and methylation of the crude metabolite mixture with methanol/1 *N* hydrochloric acid at 60° C for 1 hr prior to chromatography demonstrated the presence of both metabolites VIII and IX in Compound A treated soil.

No evidence has been found for the presence of 3,5-dichlorobenzoic acid among the metabolites; however, metabolite X may be a simple "conjugate" or derivative of this acid.

Metabolism in Alfalfa. Methanolic extraction of vigorously growing alfalfa sampled at various times after foliar application of high levels of C^{14}-carbonyl labeled Compound A produced a methanol soluble fraction and plant residue fraction (Table II). The total recovery of radioactivity decreased rapidly as the interval between Compound A application and sampling increased. Root absorption studies showed that alfalfa readily absorbed Compound A and translocated it into the plant, but it was absorbed only slowly by foliage. The decrease in C^{14} residue can be accounted for by dilution from the growth as well as by surface residue washed off by rain. The radioactivity remaining in the residue which was not extracted by methanol increased as the interval between Compound A application and sampling increased to 112 days after treatment. These results indicate that with time the C^{14} radioactive compound(s) is converted from a methanol soluble form to bound complexes.

Chromatography of the methanol soluble fraction showed that Compound A was relatively slowly metabolized in the

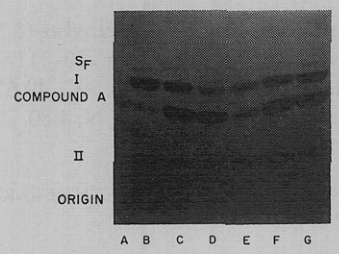


Figure 1. Typical tlc radioautogram of a chromatogram of methanol extracts of six different soils 90 days after C^{14} Compound A treatment. A. Standard C^{14} Compound A. B. Silty clay loam. C. Sandy loam. D. Sand. E. Loam. F. Clay. G. Sandy clay loam. System, acetone:benzene (5:95)

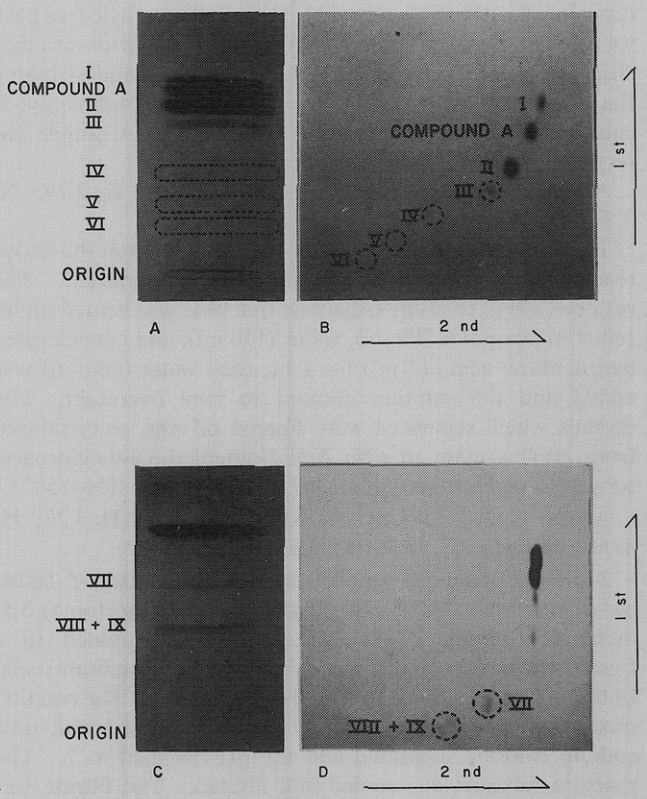


Figure 2. One- and two-dimensional thin-layer chromatograms showing C^{14}-carbonyl labeled metabolites of Compound A from C^{14} Compound A treated soil 90 days after treatment. A. Radioautogram of a thin-layer chromatogram, system, acetone:benzene (5:95). B. Radioautogram of a two-dimensional thin-layer chromatogram, systems, (1) methanol:benzene (10:90) and (2) acetone:benzene (25:75). C. Radioautogram of a thin-layer chromatogram, system, isopropyl alcohol:ethyl acetate:water (25:65:10). D. Radioautogram of a two-dimensional thin-layer chromatogram, systems, (1) acetone:benzene (25:75) and (2) isopropyl alcohol:ethyl acetate:water (25:65:10)

Table II. Radioactive Residue (μg per g of Alfalfa) in Field Alfalfa Sprayed with Compound A (2 lb/A)

Days after Treatment	Total Radioactive Residue (μg/g)	Methanol Extract		Plant Residue	
		%	μg/g	%	μg/g
0	167.7	...	...	...	...
17	25.5	96.1	24.5	3.9	1.0
50	1.8	95.1	1.7	4.9	0.1
112	0.5	54.4	0.27	45.6	0.23

Table III. Distribution of C^{14} Activity in Methanol Extracts of Alfalfa Sampled at Intervals After Treatment

Metabolite Number	Radioactivity					
	17 days		50 days		112 days	
	%	μg/g	%	μg/g	%	μg/g
Compound A	89.2	21.86	60.9	1.03	28.0	0.075
I	0.9	0.23	1.0	0.02	3.3	0.009
II	3.4	0.83	1.3	0.02	4.1	0.011
III	0.3	0.06	0.5	0.01	0.9	0.002
IV	0.6	0.14	1.7	0.03	6.3	0.017
V	1.1	0.28	1.7	0.03	3.2	0.009
VI	0.5	0.12	3.4	0.06	9.7	0.026
VII	2.4	0.60	16.4	0.27	27.4	0.073
VIII	0.1	0.03	2.2	0.04	5.9	0.015
IX	1.0	0.23	4.1	0.07	4.7	0.013
X	0.5	0.12	6.8	0.12	6.5	0.017
	100.0	24.50	100.0	1.70	100.0	0.27

plant, and that all the metabolites present in soil were also identifiable in the plant (Table III). Note metabolite X was found in alfalfa, but it was not found in soil. Since Table III data were obtained from field treated alfalfa in which no attempt was made to keep Compound A off the soil at the time of spraying, the experiment does not exclude the possibility that compounds found in alfalfa came from the soil. To establish that the indicated metabolism did occur in alfalfa, an independent study was carried out in the greenhouse under conditions in which alfalfa foliage was treated to ensure there was no Compound A on the soil. Under these conditions, similar results were obtained.

Metabolite X found in alfalfa but not found in soil was not identified. However, the methyl ester of 3,5-dichlorobenzoic acid was obtained after vigorous treatment with methanol/HCl, and this is the reason for showing metabolite X in Table I as a derivative of 3,5-dichlorobenzoic acid.

Possible metabolic pathways in soil and alfalfa are discussed in the following paper.

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