Efrotomycin Interaction with Soil Clay and Organic Matter Fractions

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Efrotomycin, a narrow-spectrum antibiotic in the elfamycin family, is readily bound to soil particulates. In Freehold sandy loam (0.45% organic matter, 18% clay), more than 90% of the efrotomycin was sorbed to soil particulates with approximately 66% associated with the clay fraction. In contrast, approximately 67% of the efrotomycin was bound by Pahokee muck (no clay, 84% organic matter). Aluminum chelation had no effect on efrotomycin binding to a variety of mineral soils, but it caused a slight increase (<2%) in sorption to Pahokee muck. Inclusion of pyridine in the adsorption mixture reduced efrotomycin binding to the mineral soils but had no effect with Pahokee muck. Similarly, pyridine increased desorption from some mineral soils. Sorption of efrotomycin to a mineral soil did not change the distribution among efrotomycin isomers whereas with the organic soil a decrease in the A_1 isomer and an increase in the polar and B isomer fractions occurred.

Efrotomycin, an *N*-methylhydroxypyridone glycoside (Figure 1), is a narrow-spectrum antibiotic intended for use in animals, such as poultry and swine (Frost et al., 1976; Maiese and Wax, 1977; Stutz, et al., 1983). This antibiotic is a disaccharide derivative of aurodox (Wax et al., 1976). Efrotomycin is most active in vitro against isolates of *Moraxella, Pasteurella, Yersinia, Haemophilus, Streptococcus*, and *Corynebacterium* species (Frost et al., 1976). Hence, it has potential as a growth-promoting agent and for control of infections. It is anticipated that efrotomycin will be included in feed mixes (2–16 μg/g) for starter through finisher hogs. Therefore, swine dosed at the maximum level would receive an average of 36.8 mg of efrotomycin/day, assuming an average consumption of 2.3 kg of feed by a 45-kg pig, which would be the approximate average weight of pigs to be treated.

Since essentially all of the efrotomycin consumed by the animal is voided in the feces (Merck Sharp & Dohme Research Laboratories, 1985, unpublished data), the fate of the antibiotic in the soil ecosystem becomes important. Once the antibiotic enters the soil profile, it may be biologically or chemically decomposed, complexed to inorganic soil colloids, or incorporated into soil organic matter fractions. In the latter situation, the antibiotic could be physically sorbed to the organic matter as occurs with clay minerals or it could be covalently linked to humic substances (Stevenson, 1982; Tate, 1987). Sorbed molecules are subject to desorption and subsequent biological decomposition or migration within the soil profile whereas those covalently bound to soil organic fractions are much more stable. Thus, the objectives of this project were (a) to evaluate the mechanism of sorption of efrotomycin to soils (i.e., to which soil fraction is it bound and what is the nature of the association), (b) to determine the effect of aluminum chelation on binding of efrotomycin to soil, and (c) to develop a means of extraction of soil-bound efrotomycin so that the changes in efrotomycin prior to or while sorbed to the soil can be assessed. Interest in the aluminum chelate is derived from the observation that efrotomycin can function as a bidentate ligand by coordinating to the metal via two oxygen donors (Kaplan et al., 1984). This modification of the molecular arrangement could alter the degree of association of the antibiotic with soil particulates and thereby increase or decrease its mobility and availability to soil microbial populations. These data will be useful in predicting the ultimate environmental fate of efrotomycin, the potential for its accumulation in soils and groundwaters, and the biological decomposition rates of this antibiotic.

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Table I. Mechanical Analysis, pH, and Organic Matter Contents of Soils Used in this Study

soil	mechanical anal., % sand	silt	clay	organic matter, %	pH
Freehold sandy loam	44	38	18	0.45	5.6
Trinchera	36	47	17	4.1	4.6
Pahokee muck	0	0	0	84	5.6
Riverside loam	50	36	15	2.5	6.7
Iowa clay loam	26	36	28	4.6	5.0

MATERIALS AND METHODS

Soils. Pertinent chemical and physical properties of the soils used in this study are listed in Table I. Soils were classified as follows: Freehold sandy loam (a fine, loamy, mixed, mesic typic hapludult); Trinchera (a mixed isoperthermic typic tropohumult); Pahokee muck (a euic, hyperthermic lithic medisaprist); Iowa clay loam (a fine, silty, mixed, mesic aquic argiudoll). Along with these soils, an unclassified loam soil from Riverside, CA, was used for a portion of this study. Geographical sources of the soils were as follows: Freehold sandy loam, Adelphia, NJ; Pahokee muck, Everglades Agricultural Area of south Florida; Trinchera, Panama; Iowa clay loam, Newton, IA. Organic matter contents ranged from a minimum of 0.45% in the Freehold sandy loam to a maximum of 84% (Pahokee muck), with clay contents ranging from zero (Pahokee muck) to approximately 28% in the clay loam from Iowa. Soil pH values ranged from 4.6 for the Trinchera soil collected in Panama to 6.7 for the loam from Riverside, CA. All soils (except the Freehold sandy loam and the Pahokee muck) were sampled from the soil surface (top 20 cm), sieved to pass 2 mm, air-dried, and stored at room temperature. The Pahokee muck and Freehold soils were sampled and processed similarly, but they were stored at field moisture at room temperature. Sterile soil samples were prepared by adjusting the soil moisture to approximately 0.03 mPa and autoclaving on 2 consecutive days.

Efrotomycin. Unlabeled efrotomycin was provided by the Chemical Data Department, Merck Sharp & Dohme Research Laboratories (MSDRL). Carbon-14-labeled efrotomycin A_1 was prepared, purified, and supplied by the Labeled Compound Synthesis Group of Animal Drug Metabolism MSDRL. The label was incorporated by inclusion of [1-^{14}C]proprionate in growth media of *Nocardia lactamdurans* (Mertel et al., 1984). The purified labeled material had a specific activity of 46.8 μCi/mg and was >98% radiochemically pure by HPLC analysis.

Sorption Studies. A 5-mL portion of [7-^{14}C]efrotomycin solution (24.8 or 124 μg/mL containing approximately 3000 dpm/mL) in 0.01 M $CaCl_2$ was mixed with 1.0 g of dry soil. Samples were mixed 16 h by end over end tumbling at room

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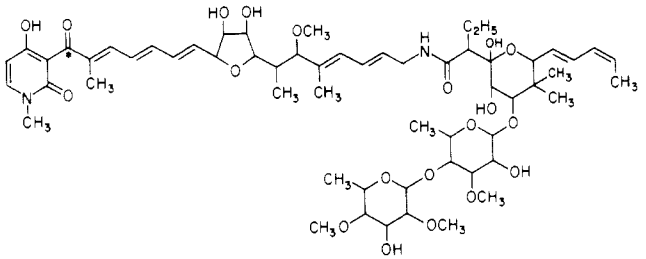

Figure 1. Structure of efrotomycin. The site of carbon-14 labeling is C-7 (*). The A_1 isomer is shown.

temperature. Soils were removed by centrifugation at 10000*g* for 10 min. ^{14}C label in the supernatant fraction was measured by mixing 1 mL of supernatant with 20 mL of Aquasol (New England Nuclear) scintillation cocktail. These assays were conducted in triplicate. Quantities bound in the soil were determined by combustion in a Packard TriCarb Model B306 sample oxidizer. Blank and control oxidizer standards were assayed to determine background counts and oxidizer efficiency. All samples were combusted for 40 s. Trapped $^{14}CO_2$ and scintillation cocktail were delivered by the oxidizer to the scintillation vials.

To evaluate the effect of aluminum chelation on antibiotic sorption to soil, 4 mL of 1.06×10^{-3} M $AlK(SO_4)_2$ was added to 50 mL of 24.8 μg/mL efrotomycin (Kaplan et al., 1984). This solution was mixed for 1 h at room temperature before being amended to the soil. Sorption was measured as indicated above.

Antibiotic Extraction. A variety of solvents were evaluated for extraction of efrotomycin bound to soil particulates. In all cases, the soil pellet from the sorption study was suspended in 5 mL of the indicated solvent or solution and mixed for 1 h with end over end mixing. Particulates were removed by centrifugation, and ^{14}C label was quantified. Generally, the labeled material was quantified by mixing 1 mL of the solution with 20 mL of Aquasol as described above. Where quenching was a problem, the solvent was evaporated and the residue suspended in Aquasol for counting.

Analytical Analysis. Indentity of efrotomycin and distribution of ^{14}C label in supernatant liquids and extracts were determined by separation and coelution with spiked, unlabeled efrotomycin by high-pressure liquid chromatography (HPLC). These analyses were performed on a Spectra-Physics Model 3500 B liquid chromatograph. Peaks were detected by UV absorbance on a Schoeffel Model 770 spectroflow monitor at a wavelength of 233 nm and attenuation setting of 0.04 AUFS. Separation was on a DuPont Zorbax octadecylsilane reversed-phase analytical column (4.6 × 250 mm) with a particle size of 5–6 μm. The analytical column was preceded by a guard column (2 × 70 mm) packed with Whatman Co:Pell ODS (30–38-μm) packing material. The mobile phase consisted of 63% (v/v) methanol–37% (v/v) water containing 0.15% (v/v) phosphoric acid (adjusted to pH 6.0 with NaOH) and was delivered at 1.0 mL/min. Sample injection was conducted on a Waters WISP Model 710 B autosampler. Efrotomycin A_1 isomer eluted at 16–19 min.

Confirmation of the identity of the A_1 isomer in unspiked $CaCl_2$ extracts from whole soil and soil–clay fractions was achieved with a Hewlett-Packard HP1090 liquid chromatograph with diode array detection and Raytest GmbH Ramona-LS flow monitor equipped with a 0.6 mL of calcium floride solid cell. Column and mobile phases were similar to those described above. The maximum UV absorbance (230 nm) of the putative A_1 isomer coincided with a peak of ^{14}C activity on the flow monitor. The diode array spectrum of this peak matched a standard efrotomycin A_1 spectrum. The intensities of the UV peaks where the B isomers were expected to elute were too low for positive identification.

Separation of Soil–Clay Fraction. Following sorption of efrotomycin to the soil, the soil was collected by centrifugation at 10000*g* for 10 min. The resultant pellet was resuspended in 25 mL of distilled water plus 2.0 mL of sodium metaphosphate (10 g/L). The sample was mixed and the suspension centrifuged at 500*g* for 2 min. The clay particles remained suspended in the liquid phase. The pellet was resuspended in distilled water and again centrifuged at 500*g*. The two supernatant liquid fractions were combined. Clay was removed from the supernatant liquid by centrifugation at 10000*g* for 10 min. [7-^{14}C]Efrotomycin in the clay fraction was quantified by combustion as described above for whole soil.

Table II. Variation of Efrotomycin Concentration and Binding to Native and Sterile Freehold Sandy Loam and Pahokee Muck

soil treatment	concn, μg/mL	sorption μg[a]	sorption %[b]
native			
Freehold sandy loam	24.8	23.2 ± 0.4	93.4 A
	124	114.3 ± 0.9	92.3 A
Pahokee muck	24.8	16.5 ± 0.5	66.8 B
	124	80.8 ± 0.5	65.2 B
sterile			
Freehold sandy loam	24.8	23.1 ± 0.3	93.1 A
	124	112.4 ± 0.7	90.7 A
Pahokee muck	24.8	11.4 ± 0.2	46.0 D
	124	65.3 ± 5.0	52.6 C

[a] Mean ± SD (n = 3). [b] Values followed by a different letter are significantly different (p = 0.05) by Duncan's new multiple-range test.

Data were statistically analyzed with the MSTAT program (Nissen et al., 1985). A minimum of three replicates was used for all studies.

Pyridine Complexation to Soil and Efrotomycin. Duplicate 5-mL portions of pyridine solution (1.7 μg/mL) in 0.01 M $CaCl_2$ were mixed with 1.0 g of dry Riverside or Iowa soil, tumbled 16 h, and centrifuged. Standards of pyridine at 1.2–2.2 μg/mL were prepared. A 100-μL sample of each solution was diluted with 5.0 mL of water and scanned from 320 to 200 nm in a Perkin-Elmer 559 UV–visible spectrophotometer. Concentrations of pyridine in the samples equilibrated with soil were determined by comparing the absorption maxima to the maxima for the standards.

To investigate pyridine complexation to efrotomycin in solution, 1 mL of efrotomycin (12 μg/mL, 0.01 μmol) in 0.01 M $CaCl_2$ was placed into the sample and reference cuvettes of the spectrophotometer. The absorbance difference spectrum was monitored from 500 to 200 nm before and after three successive additions of 10 μL of 0.9 μL/mL of pyridine (0.01 μmol/10 μL) in 0.01 M $CaCl_2$ to the standard cuvette and 10 μL of $CaCl_2$ solution to the reference cuvette.

RESULTS

A mineral soil and an organic soil, Freehold sandy loam and Pahokee muck, respectively, were used to evaluate efrotomycin binding to soil clay or colloidal organic matter fractions. Freehold sandy loam contained 18% clay and 0.45% organic matter whereas Pahokee muck was comprised of 0% clay and 84% organic matter. Greatest retention by the particulate fraction was noted with the Freehold sandy loam where more than 90% of the antibiotic was retained by the soil pellet (Table II). This binding of the antibiotic by the mineral soil was independent of antibiotic concentration or soil sterilization. With the organic soil, sorption ranged from 46 to 67% with reduced binding with the steam-sterilized soil samples. This suggests either that the binding to the organic soil was biologically mediated, in part, or that the sterilization procedure resulted in modification or even volatilization of a soil organic fraction complexing with the efrotomycin. Note that this organic soil contained no clay. Thus, all of the sorption resulted from interactions with colloidal organic matter.

Dilution of the Freehold soil with native soil organic matter (Pahokee muck) resulted in a proportional decline in efrotomycin sorption (Table III). The quantity of efrotomycin bound in the 50:50% mixture and the 10:90% mixtures was essentially that predicted from the results with pure Freehold sandy loam and Pahokee muck. For example, with the 50:50% mixture, the 82.2% retention observed was essentially equivalent to half of that bound to the Freehold soil alone (47.4%) plus half of that bound

Table III. Effect of Variation of Percent Organic Matter on Efrotomycin Binding to Freehold Sandy Loam

soil mixture	sorption: % retained actual[a]	theoretical
Freehold sandy loam	94.8 A	
0.5 Freehold/0.5 Pahokee	82.2 B	82.0
0.1 Freehold/0.9 Pahokee	72.7 C	71.8
Pahokee muck	69.2 D	

[a] Values followed by a different letter are significantly different ($p = 0.05$) by Duncan's new multiple-range test ($n = 3$).

Table IV. Association of Efrotomycin with the Clay Fraction of Freehold Sandy Loam

soil fraction	sorption: % retained
whole soil	100
soil (clay removed)	47.6
clay fraction	66.4

Table V. Effect of Al^{3+} Chelation by Efrotomycin on Antibiotic Binding to Soil

soil	sorption: % retained absence[a]	presence
Freehold sandy loam	94.9 A	95.4 A
Pahokee muck	69.8 D	71.6 C
Trinchera	91.3 B	91.2 B

[a] Values followed by a different letter are significantly different ($p = 0.05$) by Duncan's new multiple-range test ($n = 3$).

to the Pahokee muck (34.6%).

Efrotomycin enrichment by the clay fraction was shown with differential centrifugation separation of soil particulates (Table IV). The clay fraction, which comprised 18% of the Freehold sandy loam, accounted for approximately 66% of the efrotomycin bound to that soil.

Aluminum chelation had a minimal effect on antibiotic sorption by Freehold sandy loam, Pahokee muck, or a high-aluminum soil (Trinchera) from Panama (Table V). The latter soil had a 67% aluminum saturation and contained 3.86 mequiv of extractable aluminum/100 g of dry soil. No effect of the aluminum was noted with the Freehold sandy loam and the Trinchera soil whereas sorption was increased by less than 2% with the organic soil. The increase in adsorption to the Pahokee muck was statistically significant ($p = 0.05$), but due to natural variation in native systems probably of little real environmental concern.

The nature of the association of efrotomycin with the soil organic or clay fraction was evaluated by observing the effect of competition of pyridine with antibiotic sorption. Examination of the antibiotic structure (Figure 1) suggests that the most likely association of the molecule with clay particles and perhaps with organic matter would be through the *N*-methylhydroxypyridone ring. Thus, 0.2% (v/v) pyridine was added to the standard efrotomycin solution used in sorption studies to determine whether the pyridine would compete for binding sites with the antibiotic. No significant effect with binding to the organic soil was detected (Table VI), but with the three mineral soils, reductions in sorption ranged from less than 10% to nearly 70% (Iowa clay loam and Riverside loam, respectively). This suggests that the aromatic ring structure plays an important role in association of the antibiotic to soil mineral matter. For the organic soil, there are two logical explanations of the failure of pyridine to affect the antibiotic sorption: I.e., either binding involves interaction of more than just the pyridone ring of the efrotomycin structure with soil particulates or sufficient binding sites

Table VI. Effect of Pyridine on Sorption of Efrotomycin to Several Soil Types

soil	treatment	sorption,[a] % added
Pahokee muck	+ pyridine	68.2 CD
	no pyridine	65.8 D
Freehold sandy loam	+ pyridine	71.5 C
	no pyridine	94.9 A
Riverside loam	+ pyridine	16.7 F
	no pyridine	54.8 E
Iowa clay loam	+ pyridine	87.1 B
	no pyridine	95.5 A

[a] Values followed by a different letter are significantly different ($p = 0.05$) by Duncan's new multiple-range test ($n = 3$).

Table VII. Extraction of Bound Efrotomycin with Na_2CO_3 or $Na_4P_2O_7$

soil mixture	extractant	efrotomycin,[a,b] %
Pahokee muck	Na_2CO_3	20.6 C
	$Na_4P_2O_7$	34.4 B
0.1 Freehold/0.9 Pahokee	Na_2CO_3	21.6 C
	$Na_4P_2O_7$	29.4 BC
0.5 Freehold/0.5 Pahokee	Na_2CO_3	26.8 BC
	$Na_4P_2O_7$	42.4 A
Freehold sandy loam	Na_2CO_3	33.1 A
	$Na_4P_2O_7$	38.6 A

[a] As percent of efrotomycin bound to soil particulates. [b] Values followed by a different letter are significantly different ($p = 0.05$) by Duncan's new multiple-range test ($n = 3$).

exist in the soil to accommodate both the pyridine and the antibiotic sorption.

No association was observed by UV difference spectra between efrotomycin and pyridine in $CaCl_2$ solution at respectively 1:1 to 1:3 molar concentrations. Pyridine at 1.7 μg/mL bound to 1-g portions of Riverside and Iowa soils to the extent of 9 and 24%, respectively. An increase in the solution pH caused by the pyridine would increase the ionization of the efrotomycin and might reduce binding to soil organic matter but might conversely increase binding to clay particles. The effects were just the opposite, based on the lack of an effect on the binding of efrotomycin to Pahokee muck and a decrease in binding to mineral soils. For these reasons the effects pyridine has on the binding of efrotomycin to soil are thought to occur at the sorption sites, not in solution.

The question of whether the antibiotic was modified chemically or biologically during or prior to binding to soil particulates was asked. Since the radiolabeled carbon of efrotomycin A_1 used for these studies resided close to the pyridine ring, the potential exists that a portion of the molecule could be cleaved prior to binding. Such reactions would not be detected by the assay used above. To answer this question, several extraction procedures were evaluated with the ultimate objective of developing a technique that would allow for identification of the product bound to and subsequently extracted from soil.

The most common soil organic matter extraction procedure involves use of strong NaOH solutions (Stevenson, 1982). Due to the potential instability of efrotomycin under alkaline conditions, milder methods were evaluated. Two such procedures involve use of sodium carbonate, a mildly alkaline extractant believed to solubilize colloidal organic matter through conversion of acidic components to ions, and sodium pyrophosphate, a neutral extractant that forms insoluble precipitates with calcium and other polyvalent cations that may be involved with linking organic matter to soil particulates (Stevenson, 1982). Thus, recovery of bound efrotomycin by extraction of the soil pellet with 0.5 M Na_2CO_3 or 0.1 M $Na_4P_2O_7$ was examined

Table VIII. Extraction of Efrotomycin from Soil Particulates with 0.2% (v/v) Pyridine in 0.01 M $CaCl_2$

soil	% extracted[a]
Pahokee muck	42.8 B
Freehold sandy loam	22.0 C
Riverside loam	74.0 A
Iowa clay loam	10.0 C

[a] Values followed by a different letter are significantly different ($p = 0.05$) by Duncan's new multiple-range test ($n = 3$).

Table IX. Extraction of Efrotomycin (%) from Soil with Acetone, Acidic Chloroform, or 0.01 M $CaCl_2$ (See Text for Details of the Extraction Procedures)[a]

	extraction fraction				
procedure	acetone	citrate	$CHCl_3$	$CaCl_2$	soil
Pahokee muck					
acetone/$CHCl_3$	55.7	2.0	17.0		25.3
$CHCl_3$		2.8	39.6		57.6
0.01 M $CaCl_2$				23.2	76.8
Freehold sandy loam					
acetone/$CHCl_3$	45.0	0.4	13.6		41.0
$CHCl_3$		0.8	25.4		73.8
0.01 M $CaCl_2$				2.8	97.2

[a] Quantity of efrotomycin in extractant as percent of that efrotomycin originally bound by the soil.

(Table VII). Extraction efficiencies ranged from a low of 20.6% with the Pahokee muck to a maximum of 42.2% with the 50:50 mixture of muck and Freehold sandy loam. From the variable recoveries, it appears that the quantities extracted with these general extractants did not relate to the organic matter or clay contents of the soil.

As was demonstrated above, the *N*-methylhydroxypyridone ring appears to be active in binding of the antibiotic. Thus, the potential exists that soil-bound antibiotic could be exchanged with pyridine in an extraction solution. This was found to occur to varying degrees with a variety of mineral soils plus Pahokee muck (Table VIII). Extraction efficiency ranged from a low of 10% with the Iowa clay loam to 74% with the Riverside loam. The efficiency did not appear to relate to clay or organic matter contents, but the pyridine solution was shown to be a reasonably good extractant for some soils.

Acetone and chloroform have been used to extract efrotomycin from *Nocardia lactamdurans* NRRL 3802 mycelia (Wax et al., 1976). Accordingly, these solvents were evaluated with efrotomycin sorbed to Pahokee muck and Freehold sandy loam. With the complete procedure, the soil pellet following sorption was extracted with acetone. This was followed by a mixture of 5 mL of 0.01 M citrate buffer, pH 5.0, plus 5 mL of chloroform. For comparison, the acetone extraction step was omitted with the citrate/chloroform procedure, or soil pellets were extracted with 0.01 M $CaCl_2$ (Table IX). Resuspension of the soil pellet in 0.01 M $CaCl_2$ removed 23.2 and 2.8% of the efrotomycin bound to the Pahokee muck or Freehold sandy loam, respectively. Highest antibiotic recovery was achieved when Pahokee muck was extracted with acetone followed by the citrate buffer plus chloroform mixture. Nearly 75% of the bound antibiotic was recovered. This procedure yielded approximately a 60% recovery with the mineral soil. Exclusion of the acetone extraction step greatly reduced the extraction efficiency.

The most efficient extraction procedures were combined with the soil types with which they were most effective to examine the identity of the labeled product bound to and subsequently extracted from the soil. The labeled products were separated into four peaks with the HPLC procedure: polar and nonpolar compounds plus the A_1 and B isomers

Table X. Identification of ^{14}C-Labeled Soil-Bound Efrotomycin Products (%) following Solubilization with the Acetone/$CHCl_3$ or Pyridine Extraction Procedures

	HPLC fraction			
fraction	polar	A_1	B isomer region	nonpolar
stock solution	3	75	18	1
Pahokee muck				
unadsorbed	42	44	15	2
acetone	17	48	27	1
$CHCl_3$	38	32	23	3
Riverside loam				
unadsorbed	10	77	13	1
pyridine	9	72	17	2

of efrotomycin (Table X). The B isomers are cyclic Michael adducts of the major isomer A_1 (Fink and Strong, 1984). The structure of the A_1 isomer is shown in Figure 1. Little change in the distribution of label between the various fractions following sorption and extraction from the Riverside loam was noted. That is, within the normal variation of the extraction procedure, the antibiotic was unchanged by binding to the mineral soil. In contrast, there was a significant decrease in the A_1 isomer and an increase in the polar and B isomer fractions following binding to the Pahokee muck. Further analyses will be necessary to determine whether this is an isomeric modification of the molecule or some chemical and or biological degradation has occurred.

DISCUSSION

Efrotomycin was found to be strongly sorbed to all soils examined. The percent bound ranged from approximately 55% in the Riverside loam to greater than 90% in the remaining mineral soils. Comparison of efrotomycin sorption in the mineral soils with that in the clay-free organic soil suggests that the bound antibiotic is distributed between the clay and the organic fractions. Approximately 66% of the antibiotic was bound by the Pahokee muck, and an essentially equivalent quantity was detected in the clay-enriched fraction of the Freehold sandy loam. The capacity to displace bound efrotomycin from the mineral soils with pyridine extraction suggests that the *N*-methylhydroxypyridone portion of the molecule contributes to the sorption. But, alteration of the molecular configuration of this portion of the molecule through aluminum chelation had no effect on the sorption in mineral soils. In these soils, sorption is essentially a chemical process in that soil sterilization had no effect on the binding and product distribution among efrotomycin isomers following binding resembled that of the added stock solution. In contrast, some structural modification of the efrotomycin molecule by association with the Pahokee muck was detected. Because of the decreased binding in sterile Pahokee muck and this structural change in the molecule, biological catalysis or modification of the molecule in the organic soil cannot be ruled out.

The strong association of efrotomycin with both the soil clay and colloidal organic matter fractions has major implications on the antibiotic mobility within the soil ecosystem. These data plus the Fruendlich and mean sorption distribution coefficients (MSDRL, 1985, unpublished data) support the conclusion that there is little potential for movement of efrotomycin to groundwaters. Disposal of efrotomycin-containing animal wastes in clay, loamy, or organic soils appears to pose little threat to groundwaters. Similarly, altering the molecular configuration through aluminum chelation either in the feed materials or subsequent to soil incorporation (into high-aluminum soils) would have little effect on the mobility.

Registry No. Efrotomycin A_1, 56592-32-6; aluminum, 7429-90-5; pyridine, 110-86-1; efrotomycin B, 96956-38-6.

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Fate of Dicrotophos in the Soil Environment

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The fate of dicrotophos (Bidrin insecticide), labeled with carbon-14 at the crotonamide moiety, in the aqueous and soil environment was examined. Hydrolysis rates are pH-dependent and follow first-order kinetics. The half-lives of dicrotophos in pH 5, 7, and 9 buffer solutions at 25 °C are 117, 72, and 28 days, respectively. *N,N*-Dimethylacetoacetamide and *O*-desmethyldicrotophos were the major hydrolytic degradation products detected. Aquatic and soil surface photolysis study showed the degradation of dicrotophos was not induced by light exposure. Soil metabolism studies conducted under aerobic and anaerobic conditions indicated the rapid and extensive decomposition of dicrotophos and its soil metabolite(s) to $^{14}CO_2$ and unextractable residues. The soil half-life of dicrotophos in a sandy loam soil was 3 days. *N,N*-Dimethylacetoacetamide and 3-hydroxy-*N,N*-dimethylbutyramide were detected as the major soil degradation products. Soil TLC data showed that dicrotophos has intermediate soil mobility. In view of the rapid and extensive degradation in the soil, results from this study indicated dicrotophos and its degradation products do not persist in the environment.

Dicrotophos (1, 3-hydroxy-*N,N*-dimethyl-*cis*-crotonamide, dimethyl phosphate) is the active ingredient for Bidrin insecticide. Dicrotophos is a contact organophosphorus insecticide, active against a wide spectrum of phytophagous insects (Corey, 1965). Studies of the metabolic fate of dicrotophos have been conducted in plants (Menzer and Casida, 1965; Bull and Lindquist, 1964), insects, and mammals (Menzer and Casida, 1965; Bull and Lindquist, 1964; Hall and Sun, 1965). However, there have been no published reports on the fate of dicrotophos in the environment. This report presents the studies of the hydrolytic, photolytic, and soil degradation of dicrotophos. The soil leaching potentials of dicrotophos and its soil degradation products are also discussed.

EXPERIMENTAL SECTION

Radiosynthesis. Dicrotophos (1), labeled with carbon-14 at the C3 position of crotonamide, was synthesized from *N,N*-dimethyl[3-^{14}C]acetoacetamide (2).

To a stirred solution of **2** [296 mg, 2.3 mmol, 31 mCi (Amersham Corp., Arlington Heights, IL)] in dry methylene chloride (4.6 mL) was added sulfuryl chloride (247 mg, 1.8 mmol; Aldrich Chemical Co.) dissolved in dry methylene chloride (3.7 mL) dropwise at room temperature. After the addition was complete, the reaction mixture was heated to reflux for 1 h. An additional amount of sulfuryl chloride (25 mg, 0.18 mmol) was added, and the solution was refluxed for an additional 15 min. The reaction mixture was cooled and was washed with 10% aqueous sodium bicarbonate followed by water. The organic phase was dried and concentrated to provide 2-chloro-*N,N*-dimethyl[3-^{14}C]acetoacetamide (3; 283 mg, 24 mCi). Trimethyl phosphite (455 mg, 3.7 mmol) was added dropwise at room temperature to **3** (283 mg, 1.7 mmol, 24 mCi) in a 10-mL round-bottom flask. Acetic acid (16 μL), used as an isomer enhancer, was added, and the mixture was heated to 95 °C, while stirring. The reaction was maintained between 95 and 110 °C for 4 h. At the end of this period, the reaction solution was cooled and the excess trimethyl phosphite was removed under vacuum. The residue was diluted with nonradioactive dicrotophos (60 mg) and was purified by preparative thin-layer chromatography (TLC; silica gel F-254, 0.5 mm, E. Merck) using hexane–acetone (1:3, v/v) as the developing solvent. The radiochemical purity and the specific activity of [^{14}C]dicrotophos were 98.3% and 52 μCi/mg (12.4 mCi/mmol), respectively. Mass spectral and infrared data of [^{14}C]dicrotophos were consistent with those of the unlabeled reference standard.

Other reference standards were synthesized at the Biological Sciences Research Center (BSRC), Shell Agricultural Chemical Co. These standards included unlabeled *N,N*-dimethylacetoacetamide (2), *O*-desmethyldicrotophos (4, 3-hydroxy-*N,N*-dimethylcrotonamide, methyl hydrogen phosphate), and 3-hydroxy-*N,N*-dimethylbutyramide (5). Chemical structures of these compounds are presented in Figure 1.

Chromatography and Radioassay. Radioactivity was quantitated in 15 mL of Aquasol-2 scintillation solution (New England Nuclear) on a Packard Model Tri-Carb 300 liquid

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