Metabolism of Alachlor by the Fungus *Cunninghamella elegans*

Jairaj V. Pothuluri,† James P. Freeman,† Frederick E. Evans,† Thomas B. Moorman,‡ and Carl E. Cerniglia*,†

National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079, and National Soil Tilth Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Ames, Iowa 50011

The fungus *Cunninghamella elegans* ATCC 36112 transformed 98.6% of [^{14}C]alachlor [2-chloro-*N*-methoxymethyl-*N*-(2,6-diethylphenyl)acetamide] added to Sabouraud's dextrose broth to four metabolites within 96 h. Metabolism occurred predominantly by benzylic hydroxylation of one of the arylethyl side chains. Metabolites were separated by reversed-phase high-performance liquid chromatography and identified by ^{1}H nuclear magnetic resonance, UV, and mass spectral techniques. Two major metabolites were isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)-phenyl]acetamide and another was 2-chloro-*N*-(2,6-diethylphenyl)acetamide; the minor metabolite was 2-chloro-*N*-(methoxymethyl)-*N*-(2-vinyl-6-ethylphenyl)acetamide. The fungal transformations appear to be similar to those of mammalian microsomal oxidation since *C. elegans* oxidized alachlor at the benzylic positions and N-dealkylation occurred.

INTRODUCTION

Alachlor [2-chloro-*N*-(methoxymethyl)-*N*-(2,6-diethylphenyl)acetamide], an important acetanilide herbicide, is used widely for control of weeds in corn, soybeans, and many other crops (Chesters et al., 1989). Even though alachlor and related acetanilides are degraded rapidly by soil microorganisms, alachlor and de(methoxymethyl)-alachlor have been detected in groundwater samples (Cohen et al., 1986; Novick et al., 1986). Alachlor is degraded to polar products in groundwater; however, it is not mineralized completely (Novick et al., 1986). Under anaerobic conditions, alachlor may persist for long periods with estimated half-lives ranging from 2.5 weeks in anaerobic stream sediments (Bollag et al., 1986) to 79 weeks in aquifer samples (Pothuluri et al., 1990).

The U.S. Environmental Protection Agency (EPA) has classified alachlor has a probable human carcinogen on the basis of oncogenic effects in rats and mice (U.S. EPA, 1986a, 1987). A single study on hepatocyte DNA repair assays in rats has shown that alachlor is weakly genotoxic and causes stomach, thyroid, and nasal turbinate tumors in rats; it also causes lung tumors in mice (U.S. EPA, 1984, 1986b). However, there is a lack of sufficient evidence on the oncogenic effect in mice. Jacobsen et al. (1991) studied alachlor and its analogues with mouse liver microsomal mixed-function oxidase system and found these to be formaldehyde progenitors. Their oncogenic effect was based on the formation of DNA–protein cross-links which bind to DNA as reported by other researchers (Le Botlan, 1989; McGhee and von Hippel, 1977; Wilkins and MacLeod, 1976; Zimmerman, 1988).

The genotoxic potential of alachlor (Georgian et al., 1983) has been questioned in the wake of recent negative findings obtained from the in vivo rat bone marrow cytogenic assays (Kier et al., 1992). Another in vivo study on mouse and rat liver DNA-damaging activity of alachlor and its major metabolite 2,6-diethylaniline also reports negative results, since the assays did not indicate liver as the possible target for the carcinogenic action of alachlor (Taningher et al., 1992). Previous metabolic studies with alachlor in rat liver mixed-function oxidase systems indicated formation of 2,6-diethylaniline as an intermediate, which is converted to 2,6-diethylnitrosobenzene (DENB) (Kimmel et al., 1986). DENB has been implicated as a bacterial mutagen in the *Salmonella* assay. However, similar studies by Feng and Wratten (1987) indicated that the DENB formed was extremely unstable and 4-amino-3,5-diethylphenol was formed as the major product of oxidation of 2,6-diethylaniline by rat liver microsomes. Wratten et al. (1987) have further substantiated that the positive bacterial mutagenicity tests were from the decomposition products rather than from the DENB itself.

Chesters et al. (1989) have listed the microorganisms capable of metabolizing alachlor and the degradation pathways for both pure and mixed cultures. Smith and Phillips (1975) studied the degradation of alachlor by *Rhizoctonia solani* and found that the growth of cultures and degradation of alachlor were proportional to the content of sugar in the medium, since *R. solani* was unable to utilize alachlor as a sole source of carbon. Lee (1986) found that *R. solani* degrades alachlor by forming 8-ethyl-2-hydroxy-1-(methoxymethyl)-1,2,3,4-tetrahydroquinoline, *N*-(hydroxyacetyl)-2,3-dihydro-7-ethylindole, and 9-ethyl-1,5-dihydro-1-(methoxymethyl)-5-methyl-4,1-benzoxazepin-2(3*H*)-one as water-soluble products in pure culture. *Streptomyces lavendulae* produces 2-hydroxy-2′,6-diethyl-*N*-(methoxymethyl)acetamide as the major product of alachlor metabolism, whereas other microorganisms, including *Bacillus brevis*, *Bacillus cruciviae*, and *Pseudomonas putida*, did not produce this metabolite (Lee, 1984).

Microbial metabolism of alachlor and a related herbicide metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide] involves ring cleavage by aryl acylamidases to form aniline derivatives (Blake and Kaufman, 1975; Engelhardt et al., 1973; Hammond et al., 1983). However, aryl acylamidase cleavage of alachlor and metolachlor is not the major pathway of microbial metabolism (Bollag et al., 1978; Liu et al., 1987; McGahen and Tiedje, 1978).

* Author to whom correspondence should be addressed [(501) 543-7341].
† National Center for Toxicological Research.
‡ National Soil Tilth Laboratory.

The soil fungus *Chaetomium globosum* metabolizes ring-labeled [^{14}C]alachlor to six organic-soluble products and releases chloride ion without producing $^{14}CO_2$ (Tiedje and Hagedorn, 1975). *Fusarium oxysporum* releases chloride from alachlor but does not produce aniline intermediates (Kaufman and Blake, 1973).

We studied the metabolism of alachlor by *Cunninghamella elegans* because of its potential to detoxify environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs). In this study, we report in detail on the metabolism of alachlor by *C. elegans* and on the isolation and identification of the fungal metabolites of alachlor.

MATERIALS AND METHODS

Chemicals. Unlabeled alachlor was purchased from Chem Service Inc., West Chester, PA. Alachlor metabolite standard de(methoxymethyl)alachlor and ring-labeled [^{14}C]alachlor were gifts from Dr. W. R. Purdum, Monsanto Co., St. Louis, MO. The specific activity of the [^{14}C]alachlor was 10.0 mCi/mmol, and the radiochemical purity was >98% (Monsanto Chemical Co.). High-performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Chemical Co., Pittsburgh, PA. All other chemicals were of reagent grade and of the highest purity available.

Microorganism and Culture Conditions. Stock cultures of *C. elegans* ATCC 36112 were maintained on Sabouraud dextrose agar plates and stored at 4 °C. Spores and mycelia from several plates were used to inoculate 125-mL Erlenmeyer flasks, each flask containing 30 mL of Sabouraud dextrose broth (Difco Laboratories, Detroit, MI). The flasks were incubated at 24 °C on a rotary shaker at 140 rpm. After 48 h, 20 mg of alachlor dissolved in 0.5 mL of dimethyl sulfoxide was added to each culture containing approximately 3 g (dry weight) of mycelium. Sterile control flasks were prepared by autoclaving similar cultures before alachlor was added.

All cultures were incubated for an additional 48 h and the contents (50 mL) pooled and filtered to separate the broth from the mycelia. The broth samples were then extracted with three equal volumes (150 mL total volume) of ethyl acetate. The extract was dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure at 38 °C. The residue was dissolved in methanol and analyzed by HPLC.

Kinetic experiments were also conducted as described above but with [^{14}C]alachlor (0.058 μCi) plus 20 mg of unlabeled alachlor, dissolved in 0.5 mL of dimethyl sulfoxide added to duplicate flasks. After incubation for various periods, the flask contents were extracted and analyzed for metabolites.

Physical and Chemical Analyses. The metabolites of alachlor were separated using a Perkin-Elmer Series 10 HPLC, equipped with an LC-95 UV–visible absorbance detector (Perkin-Elmer, Norwalk, CT) set at 254 nm. The HPLC was fitted with a Zorbax ODS column (25 cm by 4.6 mm, i.d.; Du Pont, Wilmington, DE). A 40-min linear gradient of acetonitrile/water (30:70 to 95:5 v/v) at a flow rate of 1.0 mL/min eluted the metabolites. Compounds were collected from repeated injections of the culture extracts, and fractions with similar HPLC retention times were pooled and concentrated using a Speed-Vac concentrator (Savant Instruments, Hicksville, NY). Two metabolites (compounds I and II) were further purified by HPLC.

In experiments with [^{14}C]alachlor, 0.5-mL fractions were collected every 0.5 min and added to scintillation vials containing 7.0 mL of Scintisol scintillation fluid (Isolab, Akron, OH). The radioactivity was determined on a Packard 2000CA Tri-Carb liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

UV–visible absorption spectra of the metabolites were determined in methanol on a Beckman DU-7 spectrophotometer (Beckman Instruments, Fullerton, CA). Mass spectral (MS) analyses were performed on a Finnigan MAT 4023 mass spectrometer (Finnigan, San Jose, CA), employing a platinum-wire direct-exposure probe (DEP) and a Vacumetrics desorption current programmer (Vacumetrics, Ventura, CA). The mass spectrometer was operated in the electron-impact (EI) mode with a source temperature of 270 °C and an electron energy of 70 V. The linear scan program was from m/z 50 to 650 in 1.0 s. The DEP probe current was programmed linearly from 0 to 3 amp in 120 s. The mass spectrometer was operated in both positive-ion chemical ionization (PICI) and negative-ion chemical ionization (NICI) modes with a source temperature of 220 °C. The chemical ionization reagent gases were 10% NH_3 plus 90% N_2 at 0.15 Torr (3×10^{-5} Torr analyzer pressure). The fast atom bombardment (FAB) analyses were done on a Kratos MS50 mass spectrometer, using xenon at 10 kV and a thioglycerol matrix.

The ^{1}H nuclear magnetic resonance (NMR) measurements were carried out at 500 MHz and 29 °C on a Bruker AM500 spectrometer (Bruker Instruments, Billerica, MA). Metabolites were dissolved in 0.6 mL of deuterated acetone. Chemical shifts are reported in parts per million (ppm) by assigning the acetone resonance to 2.05 ppm. Typical spectral acquisition conditions were as follows: data size, 32K; flip angle, 80°; sweep width, 7 kHz; and relaxation delay, 0 s, except for spectra recorded under semiquantitative conditions, for which a 10-s delay was used. The number of scans for the alachlor metabolites varied from 40 to 240; for metabolite IV, 650–1000 scans were used due to its low concentration.

RESULTS

Identification of Alachlor Metabolites. The reversed-phase HPLC elution profile of the ethyl acetate-extractable metabolites formed during incubation of [^{14}C]alachlor with *C. elegans* for 72 h is shown in Figure 1. Alachlor was metabolized by *C. elegans* to four principal compounds, referred to as metabolites I–IV, which eluted at 13.1, 14.8, 16.3, and 21.6 min. Alachlor eluted at 22.7 min.

The UV–visible absorption spectra for metabolites I and II showed absorption maxima at 218, 270, 360, and 433, and 226, 271, 360, and 433 nm, respectively. The EI mass spectra for metabolites I and II were similar (Figure 2). Metabolites I and II showed very weak molecular ions [MH^+] at m/z 285 with single chlorine isotope peaks at m/z 287 and base peaks at m/z 146. Characteristic fragment ions at m/z 267 and 269 of both metabolites exhibited an apparent +2 isotope ion with intensities indicating one chlorine atom in each species. These fragment ions were probably formed by the loss of a water molecule from the molecular ions. The fragment ions at m/z 77 and 79 also exhibit a single chlorine content and probably arise from the N-substituent $[C(O)CH_2Cl]^+$. Also, the base peaks at m/z 146 for both metabolites appear to contain one chlorine atom. Rearrangement of the molecular ion, involving ring closure of the nitrogen atom and its substituents, may have been responsible for the complicated mass spectra (Figure 2). Positive- and negative-ion chemical ionization mass spectrometry also produced approximately the same mass spectra for both metabolites and substantiated the molecular weight as 285 by strong peaks indicative of water loss [$M^+ - H_2O$] in both modes. The most intense ion that appeared to contain a chlorine atom was m/z 268. Fast atom bombardment (FAB) MS confirmed the presence of the chlorine-containing ion at m/z 268. The mass spectra indicated hydroxylation of alachlor occurred in these two metabolites. Metabolite I, an isomer of metabolite II separable by HPLC, showed a mass spectral fragmentation pattern similar to that of metabolite II except that the intensities of the peaks were different. We concluded that these two metabolites were the same as described previously in studies of the mammalian oxidation of alachlor (Feng and Patanella, 1989). Feng and Patanella (1989) suggest that rotation at the aromatic nitrogen bond becomes restricted in the presence of an adjacent benzylic hydroxyl group, leading to the formation of isolable

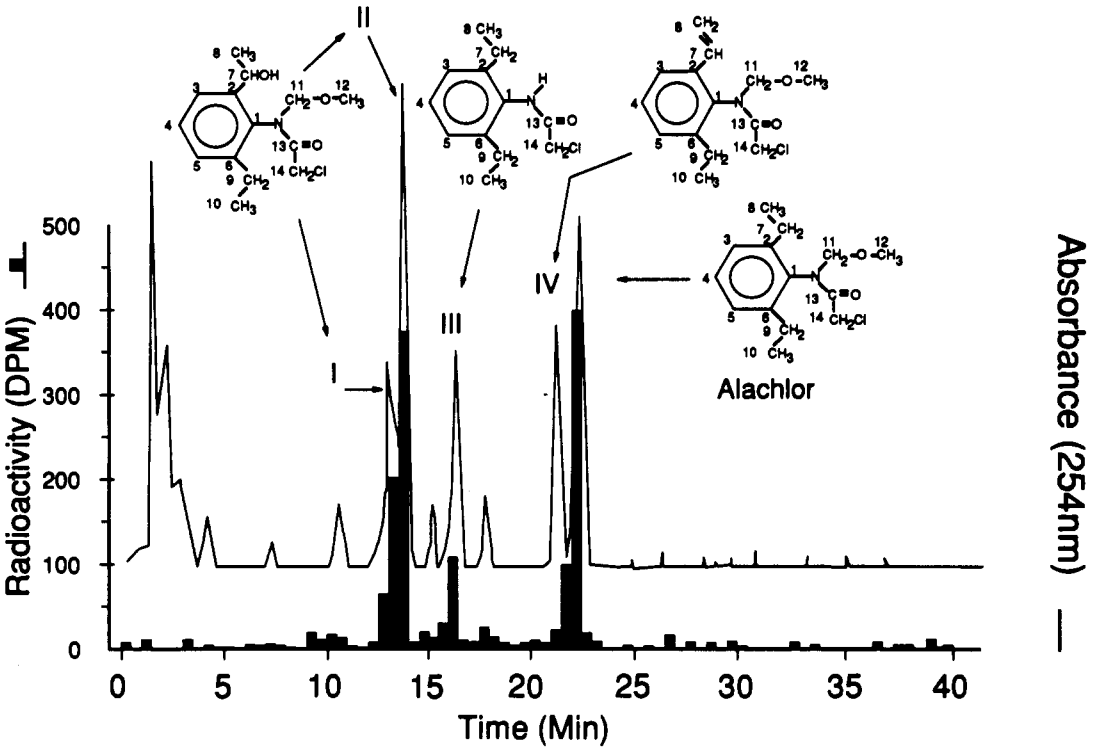

Figure 1. HPLC elution profile of the ethyl acetate-soluble metabolites formed from [^{14}C]alachlor by *C. elegans*. Fractions eluting from the column were collected at 0.5-min intervals, and their radioactivity was measured by liquid scintillation.

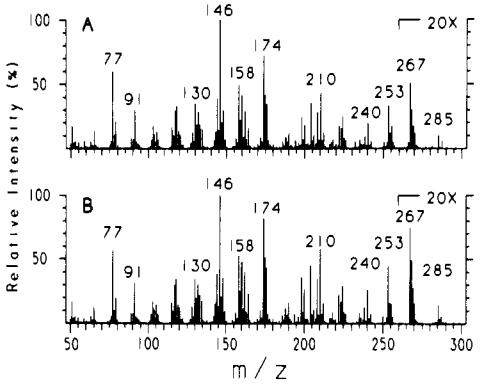

Figure 2. Electron-impact mass spectra of the alachlor metabolites I (A) and II (B) produced from *C. elegans* cultures exposed to alachlor.

rotational isomers. Apparently, the two metabolites of the present study are rotational isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)phenyl]-acetamide.

We have analyzed the ^{1}H NMR spectra of metabolites I and II (Table I). The data are consistent with the interpretation of rotational isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)phenyl]acetamide. Further NMR studies are underway in an attempt to assign the two rotational isomers.

The UV–visible absorption spectrum of metabolite III showed absorption maxima at 219, 257, 265, and 345 nm. The mass spectrum obtained by EI direct-probe analysis of metabolite III (Figure 3A) exhibited a molecular ion at *m/z* 225 with a single chlorine atom isotope peak at *m/z* 227. Ions at *m/z* 210 and 196, with corresponding monochlorine isotope peaks, probably resulted from the loss of methyl and ethyl groups from the molecular ion. The base peak (*m/z* 176), with no chlorine isotope, probably was formed by loss of the CH_2Cl group from the molecular ion. Additionally, the HPLC retention times, UV–visible spectra, and EI mass spectra of metabolite III (Figure 3A)

Table I. ^{1}H NMR Chemical Shifts (δ) and Coupling Constants (J) of Alachlor and Alachlor Metabolites[a]

	alachlor	I	II	III	IV
δ					
3	7.29	7.55	7.55	7.12	7.59
4	7.39	7.46	7.46	7.20	7.43
5	7.29	7.38	7.38	7.12	7.41
7	2.58, 2.63	4.90	4.96	2.50	6.87
8	1.24	1.44	1.47	1.15	5.40,[c] 5.82[b]
9	2.58, 2.63	2.59, 2.64	2.60	2.50	2.62, 2.64
10	1.24	1.25	1.24	1.15	1.25
11	4.93	4.96, 5.06	4.68, 5.15		4.93, 4.95
12	3.42	3.44	3.42		3.40
14	3.78	3.76, 3.82	3.76, 4.10	4.30	3.68, 3.75
J, Hz					
J_{3-4}	7.7	7.8	7.8		
J_{3-5}		1.7	1.7		
J_{4-5}	7.7	7.8	7.8		
J_{7-8}		6.4	6.4	6.4	17.8,[b] 11.0[c]
J_{8-8}		1.3			
J_{9-10}	7.5	7.5	7.5	7.6	7.5
J_{11-11}		9.9	9.9		9.9
J_{14-14}		13.9	14.6		14.2

[a] Spectral parameters are for the major subspectrum (amide oxygen trans to the aryl ring). The numbering system is shown in Figure 4. [b] H_8 trans to H_7. [c] H_8 cis to H_7. The numbering system followed is not related to nomenclature but is merely a system of flags to uniformly identify the carbon atoms reported in the table and figures.

and the *N*-de(methoxymethyl)alachlor standard (Figure 3B) were identical. ^{1}H NMR analyses confirmed the identity of metabolite III (Table I), including the loss of the CH_2OCH_3 group. Metabolite III was found to be 2-chloro-*N*-(2,6-diethylphenyl)acetamide.

The UV–visible absorption spectrum of metabolite IV showed absorption maxima at 212, 253, 293, and 322 nm. Direct-probe mass spectral and GC/MS analysis of metabolite IV produced similar mass spectra with some changes in the relative intensities of the ions. However, GC/MS analysis showed one major peak to have a molecular ion at *m/z* 267 with one Cl and major fragment ions at *m/z* 222 (one Cl) and 146 (no Cl) (spectrum not

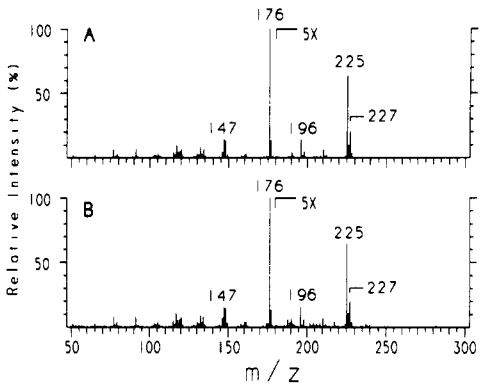

Figure 3. Electron-impact mass spectra of metabolite III produced from *C. elegans* cultures exposed to alachlor (A) and the authentic de(methoxymethyl)alachlor (B).

shown). Additionally, fragment ions at m/z 236, 222, 186, and 158 indicated 2 Da less than those observed in the alachlor spectrum (not shown). The apparent molecular weight of 267 therefore indicated a possible vinyl substituent formed by loss of water from the hydroxyethyl group of metabolite III.

The structural identification of metabolite IV was determined by 500-MHz ^{1}H NMR spectroscopy (Figure 4). The interpretation was aided by comparison with the NMR spectral parameters of the parent compound alachlor. Resonances for three aromatic protons with an ABM coupling pattern establish that the aromatic ring has not been further substituted but rather that the side chains at C-2 and C-6 of the aromatic ring differ as a result of metabolism (Figure 4). This is supported by the appearance of resonances for a three-proton fragment in the downfield region (δ 5.2–7.0) of the spectrum. All chemical shifts and coupling constants associated with this fragment are in accord with NMR spectral parameters reported for vinyl groups attached to an aromatic ring

Table II. Percent [^{14}C]Alachlor Remaining and Formation of Alachlor Metabolites with *C. elegans*

	incubation time				
compd	0[a] h	24 h	48 h	72 h	96 h
I	0	27.8	11.5	23.8	39.6
II	0	9.2	26.2	35.1	53.5
III	0	7.7	8.7	12.5	3.6
IV	0	3.3	15.1	10.5	1.9
alachlor	100	52.0	38.5	18.1	1.4

[a] Values of percent recoveries have been corrected for ethyl acetate extraction efficiency.

(Chamberlain, 1974). All aliphatic protons of metabolite IV are accounted for, with the methylene protons on carbons 11 and 14 being nonequivalent. The NMR spectrum also contains minor resonances that can be attributed to slow cis–trans isomerism about the amide bond, which is similar to that previously reported for alachlor (Jacobson et al., 1991) and several N-substituted arylamides (Evans and Miller, 1983). ^{1}H NMR chemical shifts for the major isomer of IV (amide oxygen trans to the aryl ring) are given in Table I.

Although the NMR spectrum is not clean, the assignments were judged to be complete because they were in accord with the molecular weight subsequently determined by mass spectrometry. On the basis of these spectroscopic analyses, metabolite IV was identified as 2-chloro-*N*-(methoxymethyl)-*N*-(2-vinyl-6-ethylphenyl)acetamide (Figure 4).

Kinetics of Alachlor Metabolite Formation. The rates of disappearance of alachlor and the production of ethyl acetate-extractable metabolites I–IV by *C. elegans* at 24-h intervals during 96 h of incubation are illustrated in Table II. Initially about 40.5% of the radioactivity was recovered in the organic phase at zero time of incubation. The remainder was bound in or to the mycelia. At zero time 78% of the recovered radioactivity in the organic

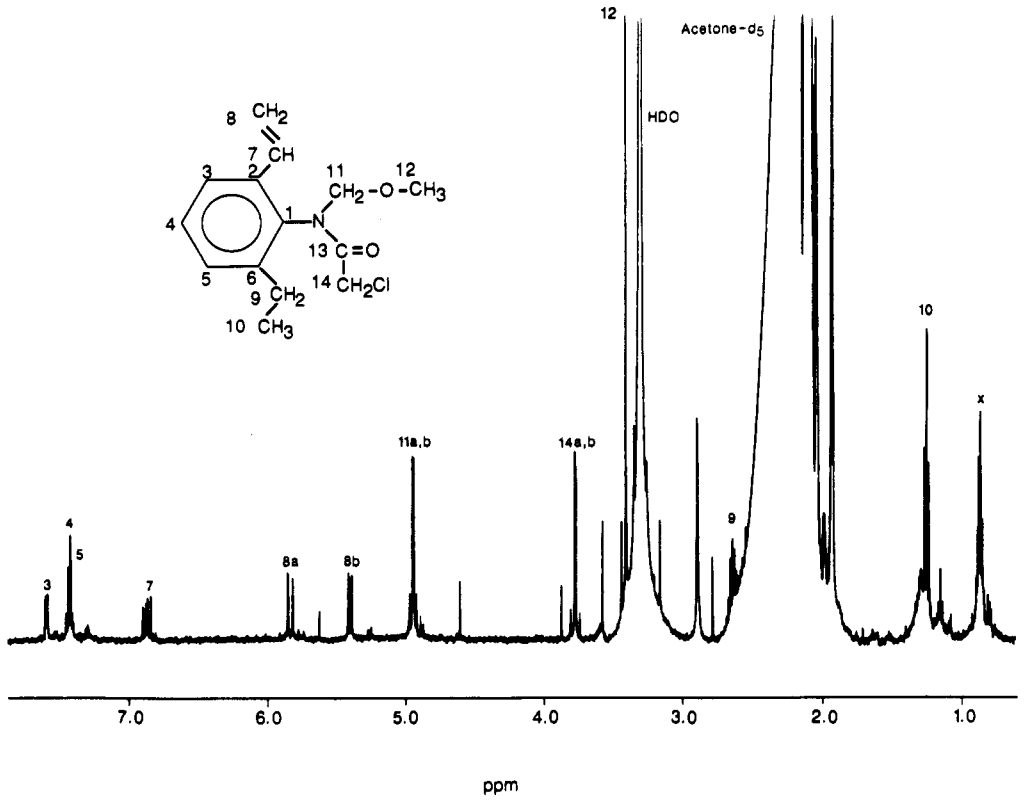

Figure 4. 500-MHz ^{1}H NMR spectra of 2-chloro-*N*-(methoxymethyl)-*N*-(2-vinyl-6-ethylphenyl)acetamide (metabolite IV) with the structure and resonance assignments.

phase was from the alachlor peak. The percent recoveries of alachlor and metabolites reported in Table II have been corrected for extraction efficiency. Alachlor decreased to about 18% during 72 h of incubation, with less than 2% remaining at 96 h. Metabolites I and II, isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)phenyl]acetamide, accumulated to maximum levels at 96 h of incubation, accounting for about 40 and 54%, respectively, of the total radioactivity recovered in ethyl acetate extracts. However, the extractable fraction of metabolite III, 2-chloro-*N*-(2,6-diethylphenyl)acetamide, peaked at 72 h (more than 12% of the total recovered radioactivity). The extractable fraction of metabolite IV, 2-chloro-*N*-(methoxymethyl)-*N*-(2-vinyl-6-ethylphenyl)-acetamide, reached a maximum level at 48 h (15% of the total recovered radioactivity). Recovered radioactivities of the organic-soluble and water-soluble metabolites were 55.8 and 34%, respectively, at 120 h of incubation (data not shown).

DISCUSSION

Alachlor was readily hydroxylated by *C. elegans* at the benzylic position, resulting in the formation of two isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)phenyl]acetamide (metabolites I and II, Figure 1). The formation of metabolites I and II constituted the major initial transformation products of alachlor by *C. elegans*, since these two metabolites together accounted for 93% of the total metabolism at 96 h of incubation. Studies of rat liver mixed-function oxidase systems with alachlor (Feng and Patanella, 1989) also indicate benzylic hydroxylation at one or both of the arylethyl side chains of alachlor and the formation of rotational isomers of 2-chloro-*N*-(methoxymethyl)-*N*-[2-ethyl-6-(1-hydroxyethyl)phenyl]-acetamide.

Also, deacetylation and dechlorination of alachlor with formation of 2,6-diethyl-*N*-(methoxymethyl)aniline (Fang, 1977; Tiedje and Hagedorn, 1975; Lee, 1986) and 2-hydroxy-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide (Lee, 1984, 1986) as major microbial metabolites have been previously reported. However, in this study, *C. elegans* hydroxylated the ethyl side chains of alachlor rather than the *N*-alkyl group, as in bacteria (Lee, 1984, 1986). McGahen and Tiedje (1978) reported transformation products of metolachlor by *C. globosum* to have occurred via dehalogenation with hydroxylation; dehydrogenation of the 6′-ethyl; dealkylation; demethoxylation, leaving hydroxyl; deethoxylation, leaving hydroxyl; and dealkylation and ring formation (indoline formation). Krause et al. (1985) found that metolachlor metabolites formed by an actinomycete contained chlorine, indicating the absence of deacylation. No evidence of ring cleavage was reported.

The N-dealkylation of alachlor resulted in the formation of de(methoxymethyl)alachlor (metabolite III, Figure 1). Feng and Patanella (1989) also reported the N-dealkylation of alachlor, via an initial O-demethylation to form an unstable carbinolamide, by rat liver microsomes in the presence of NADPH. Other microbial studies also report the dealkylation and formation of 2-chloro-*N*-(2,6-diethylphenyl)acetamide in the degradation of alachlor (Chou, 1977; Fang, 1977; Hargrove and Merkle, 1971; Sethi and Chopra, 1975; Tiedje and Hagedorn, 1975).

Oxidation via one arylethyl side chain of alachlor by *C. elegans* also occurred to form 2-chloro-*N*-(methoxymethyl)-*N*-(2-vinyl-6-ethylphenyl)acetamide (metabolite IV, Figure 1). In alachlor degradation by *C. globosum*, this intermediate is postulated by Tiedje and Hagedorn (1975). Since indoline intermediate was formed when incubated with de(methoxymethyl)alachlor, the authors postulated that the oxidation of alachlor via an intermediate was likely since the bond formation between a secondary amide and a carbon atom β to an aromatic ring is not favorable, while the oxidation of the ethyl group prior to cyclization to the indoline is possible. We therefore postulate that this intermediate (metabolite IV) was formed from metabolite III, de(methoxymethyl)alachlor, by *C. elegans*.

Our study demonstrates that the oxidation of alachlor in a microbial system is quite similar to that in mammalian systems. The mammalian metabolites of alachlor result from glutathione (GSH) conjugation, cytochrome P-450 oxidation, and subsequent glucuronidation of alachlor and its metabolites in the liver (Sharp, 1988). The main reaction involved in the transformation of alachlor by *C. elegans* is the hydroxylation of the arylethyl side chain. Apparently, benzylic oxidation appears to have occurred via cytochrome P-450 monooxygenase since this fungus is known to oxidize PAHs via NIH shift mechanism (Cerniglia and Gibson, 1978). Since hydroxylated compounds have higher solubility in water, they are degraded more easily than compounds which are less water-soluble. Therefore, the formation of hydroxylated products from alachlor by *C. elegans* may be an important step toward detoxification.

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