Aryl-O-glucoside of Diclofop: A Detoxication Product in Wheat Shoots and Wild Oat Cell Suspension Culture

Richard H. Shimabukuro,* Wendy C. Walsh, and Andrew Jacobson[1]

The major detoxication product of diclofop-methyl [methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]-propanoate] was identified as the O-glucoside of aryl-hydroxylated diclofop [(hydroxylated 2,4-dichlorophenyl)diclofop]. This metabolite was isolated as the major metabolite from resistant excised wheat shoots (*Triticum aestivum* L.) and as a minor metabolite from suspension cell culture of susceptible wild oat (*Avena fatua* L.). Although this detoxication product was a minor metabolite in wild oat cells, it was the predominant component in the suspension cell medium after 10 days. The recycling of metabolism of diclofop-methyl and some of its metabolites in a cell suspension system probably resulted in the accumulation of the more stable detoxication product identified above.

Diclofop-methyl is one of the few postemergence grass herbicides that selectively controls a number of annual grass weeds in cereal crops such as wheat (Miller and Nalewaja, 1974; Andersen, 1976). The basis for herbicidal selectivity between susceptible wild oat or cultivated oat (*Avena sativa* L.) and resistant wheat is differential metabolism, resulting in the more rapid detoxication of diclofop-methyl in wheat than in wild oat or oat (Shimabukuro et al., 1979; Donald and Shimabukuro, 1980; Jacobson and Shimabukuro, 1984).

Both wheat and wild oat hydrolyze diclofop-methyl rapidly to its phytotoxic free acid, diclofop, which is metabolized subsequently to nonphytotoxic water-soluble polar conjugates. These conjugates have been detected in shoots, roots, cell suspension cultures, and callus cultures of both susceptible and resistant species (Shimabukuro et al., 1979; Jacobson and Shimabukuro, 1984; Boldt and Putnam, 1981; Dusky et al., 1980, 1982). The major, inactive bound form of diclofop-methyl in susceptible oat was identified by mass spectroscopy as the neutral glucose ester conjugate of the phytotoxic free acid, diclofop (Jacobson and Shimabukuro, 1984). In resistant wheat, diclofop is detoxified by aryl hydroxylation followed by conjugation to a phenolic water-soluble conjugate (Gorbach et al., 1977; Shimabukuro et al., 1979). This phenolic conjugate has not been characterized. The objective of this study was to isolate and characterize this major detoxication product of diclofop in resistant wheat by mass spectroscopy. This metabolite was also isolated and characterized from a suspension cell culture of susceptible wild oat.

MATERIALS AND METHODS

Chemicals. [^{14}C]Diclofop-methyl [[U-^{14}C]-2,4-dichlorophenoxy] was purchased from New England Nuclear (sp act. 2 mCi/mmol). Unlabeled diclofop-methyl and diclofop either were a gift from Hoechst Chemical Co. (Sommerville, NJ) or were obtained by extraction and purification from the emulsifiable concentrate (Lucas et al., 1984).

Analytical Methods. Thin-layer chromatograms (TLC) (silica gel HF, 250 μm) were developed, and radioactive compounds were detected and quantified as described previously (Shimabukuro et al., 1979). Chromatograms were developed in the following solvents: (A) chloroform–methanol–water–acetic acid (65:25:4:2); (B) methylene chloride–ethyl acetate (10:90); (C) toluene–acetic acid (50:8); and (D) butanol–acetic acid–water (120:30:50).

Radioactivity in plant extracts, column effluent, partitioning solvents, gel from TLC plates, and plant residues was detected and quantified as described previously (Shimabukuro et al., 1979).

Electron impact (70-eV) and chemical ionization (NH_3) mass spectra were obtained with a Varian MAT CH-5DF and a Varian MAT 112S mass spectrometer, respectively, using a solid sample probe.

High-performance liquid chromatography (HPLC) was performed with a Waters HPLC system (Model M45 pumps, Model 660 solvent programmer, U6K injector, Model 440 detector, 8 mm-i.d. RCM C-18 column). A radioactive flow monitor (CAI instruments) was used to detect ^{14}C activity. The HPLC column was eluted with a 20-min linear gradient of 40–90% CH_3CN in 1% acetic acid at a flow rate of 2 mL/min.

The purified water-soluble phenolic conjugate of diclofop-methyl was derivatized initially by acetylation in acetic anhydride and pyridine (4:1, v/v) at 45 °C for 16–24 h. The acetylated metabolite was then methylated with diazomethane as previously reported (Shimabukuro et al., 1979) before analysis by mass spectrometry.

Plant Treatment. Wheat plants seeded in vermiculite in perforated flats and grown in the greenhouse were excised at the vermiculite level in their 1.5–2-leaf stages (11 days) of growth and treated as previously described (Shimabukuro et al., 1979). Briefly, approximately 16 excised wheat shoots were placed in each 20-mL vial containing 15 mL of 10 μM [^{14}C]diclofop-methyl in 1% acetone (sp act. 0.273 mCi/mmol). After 24 h in a controlled-environment chamber (Shimabukuro et al., 1979), the shoots were rinsed, the lower shoot sections that had been immersed in the treating solution were separated from the remainder of the shoots (leaf blades), and the fresh weights of the separated parts were determined and frozen for subsequent extraction and analysis. The posttreatment solutions remaining in the vials were combined, concentrated, and counted for ^{14}C activity.

Cell suspensions of wild oat were treated with diclofop-methyl as described previously for similar treatments of cells from cultivated oat (Dusky et al., 1982). Wild oat cell suspensions were started and maintained in B-5 medium (Gamborg, 1975) containing 0.45 μM 2,4-D. Fresh cell suspension cultures were started by inoculating 50 mL of fresh medium with 10 mL of a suspension culture containing an equivalent of 20–40 mg (dry weight) of cells.

Metabolism and Radiation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota 58105.

[1]Permanent address: Research Laboratories, Rohm and Haas Co., Spring House, PA 19477.

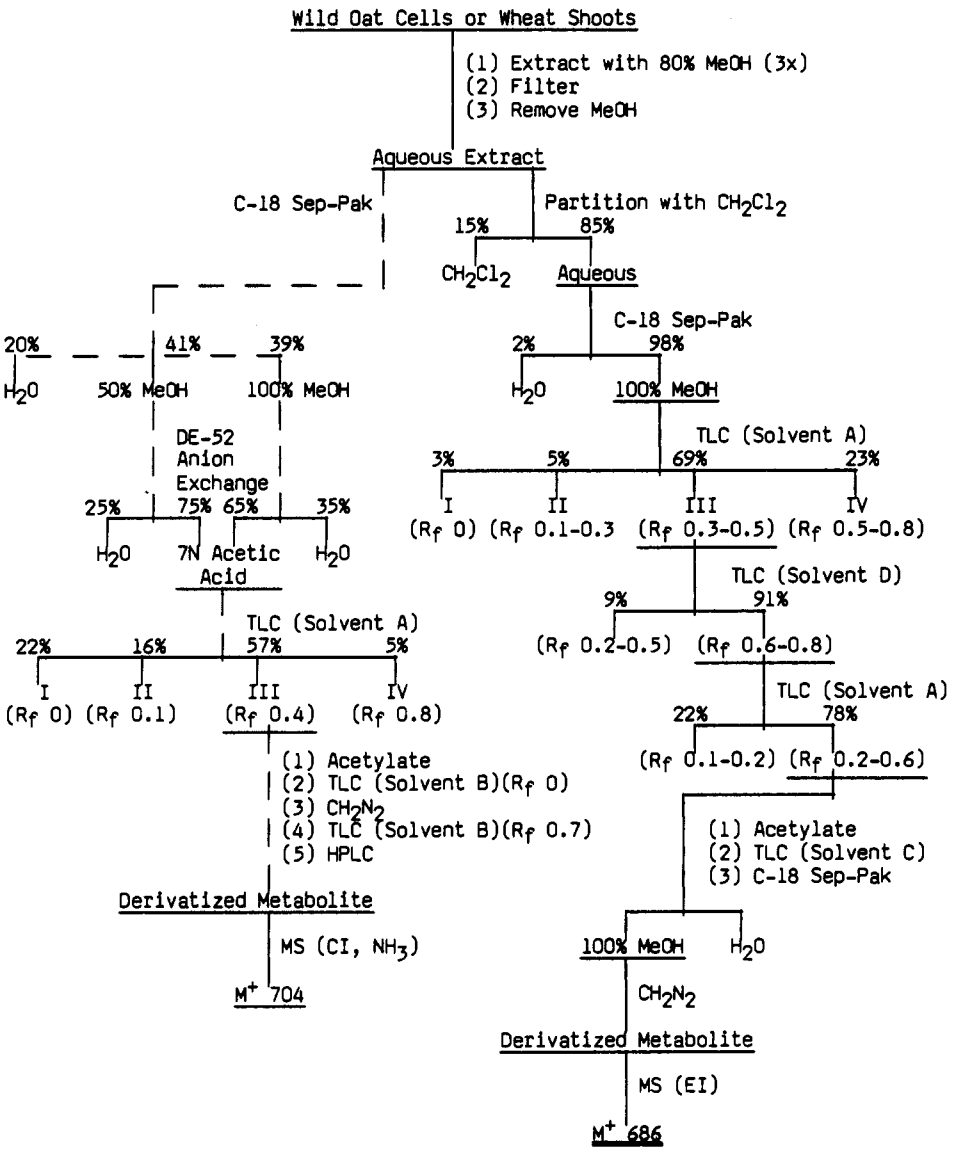

Figure 1. Purification of the water-soluble conjugate of diclofop-methyl from excised wheat shoots (solid line) and wild oat cell suspension culture (broken line).

After 4 days, a stock solution of radioactive and nonradioactive diclofop-methyl in acetone was added to each culture flask to give a final [^{14}C]diclofop-methyl concentration of 5 μM (sp act. 0.69 mCi/mmol) in 0.5% acetone. Twelve flasks of cells were treated and kept in the dark until cells were harvested 10 days after treatment. The cells were separated from the medium by filtration through Miracloth, and both parts were saved for subsequent analysis.

Extraction and Purification. The extraction and purification procedures for wheat shoots and wild oat cells are shown in Figure 1. The procedures are similar to those reported previously (Shimabukuro et al., 1979; Jacobson and Shimabukuro, 1984). The flow diagram outlined in broken lines represents the procedure for wild oat cell suspensions, and the diagram outlined in solid lines represents wheat shoots following the initial extraction to obtain an aqueous extract. Only the lower sections of wheat shoots that had been immersed in the treating solution were extracted for metabolite identification. At least 97% of the absorbed ^{14}C from [^{14}C]diclofop-methyl is expected to remain in the lower sections (Shimabukuro et al., 1979). Both wheat shoots and wild oat cells were extracted by homogenization in 10 mL of 80% methanol/g (fresh weight).

Diclofop-methyl and its nonpolar metabolites in wheat shoots were separated from the water-soluble conjugates by partitioning with CH_2Cl_2. The water-soluble conjugates were separated further and purified by C-18 Sep-Pak chromatography and TLC for identification.

In wild oat cell suspensions, at least 97–98% of the radioactivity was present as polar, water-soluble conjugates (R_f 0.0, solvent C). Therefore, partitioning of the aqueous extract with CH_2Cl_2 was not performed. The polar, water-soluble conjugate of interest was separated and purified by C-18 Sep-Pak and DE-52 anion-exchange chromatography, TLC, and HPLC for identification.

RESULTS

Isolation and Identification of the Major Water-Soluble Conjugate of Diclofop-methyl from Wheat Shoots. The [^{14}C]diclofop-methyl treatment solution was absorbed within 24 h by resistant wheat shoots. The lower shoot sections immersed in the treatment solution weighed 39 g (fresh weight) while the upper portions of the shoots weighed 76 g (fresh weight). About 76% of the ^{14}C activity applied in the treatment solution was recovered in the extract of the lower shoot sections, post-treatment solution and insoluble residue. This means that 24% of the applied radioactivity was probably translocated to the upper

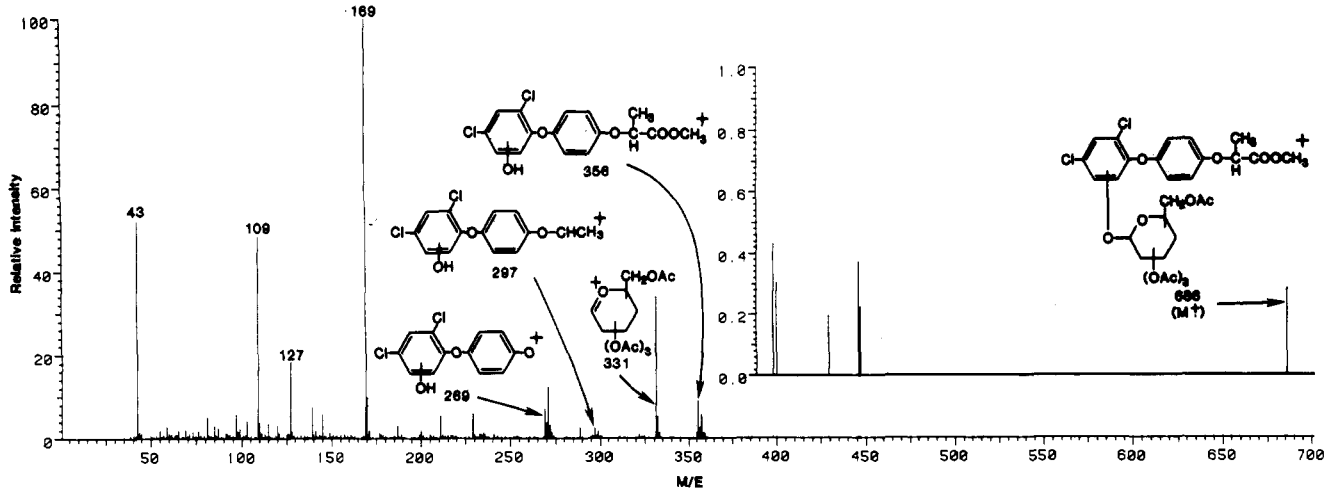

Figure 2. Mass spectrum (EI) of major water-soluble conjugate of diclofop-methyl in excised wheat shoots.

portions of the excised shoots that were not extracted for this study. The distribution of the ^{14}C activity recovered indicated that 80% was in the soluble plant extract, 3% was in the insoluble residue, and only 17% remained in the posttreatment solution.

The radioactive components in the soluble plant extract were separated and quantified prior to isolation and purification of the major metabolite (conjugated aryl hydroxylated diclofop). It was determined after TLC in solvent C that 85% of the radioactivity was present as polar, water-soluble conjugates, 9% as the acid diclofop, 1% as parent diclofop-methyl, and 5% as aryl-hydroxylated diclofop (2,4-dichlorophenyl ring-hydroxylated diclofop) and unknowns.

The purification and derivatization procedure for the water-soluble conjugate isolated from resistant wheat shoots is shown in Figure 1. All purification steps from partitioning of the aqueous extract with dichloromethane to acetylation resulted in recoveries of ^{14}C activity between 80 and 92% for each step. About 98% of the radioactivity in the aqueous fraction following partitioning with CH_2Cl_2 eluted with 100% methanol when chromatographed on C-18 Sep-Pak cartridges. Zone III from TLC in solvent A (R_f 0.3–0.5) accounted for 69% of the radioactivity on the plate. The components in zone III hydrolyzed in 6 N HCl predominantly to ring-hydroxylated diclofop (77%; R_f 0.4, solvent C).

Zone III was purified further by TLC in solvents D (R_f 0.6–0.8) and A (R_f 0.2–0.6) and eluted from the gel with methanol. The purified water–soluble conjugate remained at the origin upon TLC in solvent C. The conjugate was then acetylated and further purified by TLC in solvent C. The conjugate migrated to R_f 0.28 in solvent C following acetylation. The acetylated conjugate was further purified by C-18 Sep-Pak chromatography and methylated with diazomethane. Methylation caused an increase in the R_f of the conjugate to 0.36 in solvent C. The changes in the R_f in solvent C indicated successive derivatization by acetylation and methylation of the conjugate.

The electron impact mass spectrum of the acetylated and methylated metabolite of diclofop-methyl isolated from wheat shoots is shown in Figure 2. The conjugated metabolite was identified as the O-glucoside of ring-hydroxylated diclofop. Three possible isomers of ring-hydroxylated diclofop have been reported (Gorbach et al., 1977). However, the position of hydroxyl substitution on the 2,4-dichlorophenyl ring of diclofop-methyl was not determined in this study. The molecular ion M^+ 686 agreed with the mass for the methylated tetra-O-acetyl-glucoside of ring-hydroxylated diclofop. The major ions

Table I. Thin-Layer Chromatography of Acid-Hydrolyzed Water-Soluble Conjugates of Diclofop-methyl from Cell Extract and Culture Medium of Wild Oat Cell Suspension

components[a]	% ^{14}C act. in ea fraction	
	cell extr[b]	medium[c]
polar unhydrolyzed	17	20
diclofop-methyl	6	1
diclofop	44	18
ring-hydroxylated diclofop	29	60
unknowns	4	1

[a] TLC in solvent C after hydrolysis. [b] Of total ^{14}C activity 52% was recovered from the cells (96% polar metabolites). [c] Of total ^{14}C activity 43% was recovered from the medium (94% polar metabolites).

m/z 331, 169, 109, and 43 are characteristic fragments from the acetylated glucosyl moiety (Paulson et al., 1973). The ions m/z 356 (M – glucosetetraacetate), 297 (m/z 356 – $COOCH_3$), and 269 were characteristic fragments observed in the fragmentation of methylated ring-hydroxylated diclofop (Shimabukuro et al., 1979).

Isolation and Identification of the Water-Soluble Conjugate of Diclofop-methyl from Cell Suspension Culture of Wild Oat. Wild oat cell suspension cultures were filtered and the cells and medium separated. The total ^{14}C activity recovered in the cell extract, medium, and insoluble residue was 94.5% of the initial radioactivity added to the cell suspension cultures. Of the total ^{14}C activity recovered, 52% was in the cell extract, 43% was in the medium, and 5% was in the insoluble residue. These results were similar to an earlier report on the metabolism of diclofop-methyl in callus cultures of wild oat where 42% and 45% of the total ^{14}C activity recovered were detected in the cells and medium, respectively, 10 days after initial treatment (Dusky et al., 1982).

The extraction and purification procedure for the minor water-soluble conjugate of diclofop-methyl in wild oat cell suspension cultures is shown in broken lines in Figure 1. The radioactive components in the crude aqueous cell extract and medium were separated by TLC (solvent C) and quantified. About 96% and 94% of the radioactivity were present as polar water-soluble components (R_f 0) in the extract and medium, respectively. Aliquots of the aqueous extract and medium were hydrolyzed in 6 N HCl, and the hydrolyzed components were separated by TLC (solvent C). The hydrolyzed radioactive components were quantified (Table I). The major component (44%) in the hydrolyzed cell extract was the free acid, diclofop, indicating that the major water-soluble conjugate in wild oat

cells was the previously identified glucose ester conjugate of diclofop (Jacobson and Shimabukuro, 1984). Of the radioactivity 29% was in ring-hydroxylated diclofop with 17% in unhydrolyzed polar products.

The radioactive components found in the medium differed quantitatively from the components in the cell extract. The major component (60%) in the hydrolyzed medium was ring-hydroxylated diclofop (Table I), indicating that the major water-soluble conjugate in the medium was a phenolic conjugate of ring-hydroxylated diclofop. The glucose ester conjugate of diclofop accounted for 18% of the radioactivity in the medium.

The aqueous cell extract was purified initially by C-18 Sep-Pak chromatography (Figure 1). The recovery of ^{14}C activity was 87% in this step with a distribution of 20%, 41%, and 39% of the recovered radioactivity in the water, 50% methanol, and 100% methanol fractions, respectively. Methanol was removed under vacuum from the 50% and 100% methanol fractions after addition of small quantities of water. The remaining aqueous fractions were chromatographed separately by DE-52 anion-exchange chromatography. Of the radioactivity derived from the 50% and 100% methanol fractions, 75% and 65%, respectively, were retained on the anion-exchange column and eluted with 7 N acetic acid. The acetic acid fractions were combined, acetic acid was removed under vacuum, and the metabolites were purified further by TLC in solvent A. At least four zones on the TLC plates with R_f values from 0.0 to 0.8 were detected (Figure 1). Quantitatively, zones I–IV accounted for 22%, 16%, and 57%, and 5% of the radioactivity recovered from the plate, respectively.

The water-soluble conjugate of diclofop-methyl was purified further by removing the gel from zone III and eluting the radioactive component with methanol. An aliquot of the radioactive solution was taken to dryness, and the conjugate was acetylated in acetic anhydride as described previously. The acetylated conjugate had R_f 0.0 upon TLC in solvent B. The acetylated conjugate was then methylated with diazomethane. The completely derivatized conjugate had R_f 0.7 upon TLC in solvent B. Final purification was accomplished by HPLC of the acetylated and methylated conjugate by the method described previously. The derivatized metabolite had a retention time of 16 min.

The chemical ionization (NH_3) mass spectrum of the metabolite gave a molecular ion of M^+ 704 (M + 18). This agreed with the EI mass spectrum of the derivatized water-soluble conjugate from excised wheat shoots that gave a molecular ion of M^+ 686 (Figure 2). The base peak in the CI mass spectrum was m/z 331 (tetraacetylglucose) with a significant molecular ion (M^+ 704) at 54% relative abundance. A significant peak (22% relative abundance) at m/z 374 was also detected. This ion was equivalent to the ion from the methyl ester of aryl-hydroxylated diclofop at m/z 356 in Figure 2. Other ions at m/z 169 and 109 from acetylated glucose fragmentation were also detected.

DISCUSSION

The major water-soluble metabolite of diclofop-methyl in wheat shoots was identified as the *O*-glucoside conjugate of (hydroxylated 2,4-dichlorophenyl)diclofop, confirming previous reports that aryl hydroxylation and subsequent conjugation was the detoxication mechanism in resistant species (Gorbach et al., 1977; Shimabukuro et al., 1979). Ring-hydroxylated diclofop alone was inactive when applied to roots of susceptible oat (Jacobson et al., 1985). Very little of the free ring-hydroxylated diclofop is normally present in plant tissues. Therefore, the absence of phytotoxic activity in sensitive tissues treated with ring-hydroxylated diclofop may be due to its rapid conjugation to the water-soluble *O*-glucoside in plant tissues.

The detoxication mechanism (aryl hydroxylation, and conjugation) is also present in susceptible wild oat as a minor pathway as evidenced by the isolation and identification of the *O*-glucoside conjugate from wild oat cells in suspension culture. This confirmed previous reports that early metabolism of diclofop-methyl between resistant and susceptible species was similar qualitatively but differed quantitatively (Jacobson and Shimabukuro, 1984; Shimabukuro et al., 1979). However, the *O*-glucoside conjugate was the predominant component in the culture medium (Table I). The higher concentration of the *O*-glucoside conjugate over that of the glucose ester conjugate in the culture medium indicates the greater stability of the *O*-glucoside conjugate. Glucose ester conjugation is probably a reversible reaction, and the inactive ester conjugate may be in equilibrium with the phytotoxic acid diclofop in susceptible tissues. This was suggested clearly in studies of susceptible oat roots treated with the acetylated glucose ester conjugate. It is expected that the derivatized ester conjugate will behave similarly to the underivatized metabolite in vivo. The glucose tetraacetate ester conjugate demonstrated phytotoxicity nearly equal to that of the parent diclofop-methyl and its hydrolyzed acid diclofop in oat roots (Jacobson et al., 1985). Therefore, the in vivo hydrolysis of the derivatized glucose ester conjugate must regenerate phytotoxic diclofop that is metabolized subsequently in susceptible oat tissues by either the aryl hydroxylation and conjugation pathway (minor) or the ester conjugation pathway (major). Upon cell death, the major metabolite in the susceptible cells (glucose ester conjugate) is released into the medium and recycled in its metabolism by absorption into living cells in suspension culture. The end result of the recycling activity after 10 days is the increased concentration of the more stable conjugate (*O*-glucoside conjugate) in the medium of the susceptible wild oat cell suspension culture. This is not expected to occur in intact plant metabolism.

Our results indicate that wild oat cells in suspension cell culture formed primary hydroxylated metabolites and secondary glycoside conjugates similarly to intact wild oat plants (Shimabukuro et al., 1979). However, this may not be always true when comparing metabolism of xenobiotics between whole plant tissues and their cells in tissue culture. Carrot and cotton cells in suspension cell cultures oxidized readily the herbicide cisanilide (*cis*-2,5-dimethylpyrrolidine-1-carboxanilide) to its alcohol metabolite. However, aryl hydroxylation to the phenol metabolite was limited as compared to whole plant tissue. Whole plant tissues formed rapidly the secondary glycoside conjugates, but glycoside conjugation was very limited in suspension cell cultures, resulting in the accumulation of primary oxidation products (Frear and Swanson, 1975). The results suggest that generalizations on xenobiotic metabolism between whole plant tissues and their cells in tissue culture may not hold true in all cases. Similarities or differences between whole plant tissues and their cells in tissue culture appear to depend on the plant species and the specific xenobiotic involved.

ACKNOWLEDGMENT

We thank Dr. David G. Davis and Rosa Stolzenberg for the wild oat cell suspension cultures, Jeanette Kraft and Jeraldine Schmidt for their help in the preparation of the manuscript, and Thomas Hlavaty for the preparation of the figures. We also thank Richard Zaylskie for the mass spectral analyses.

Registry No. Diclofop-methyl, 51338-27-3.

LITERATURE CITED
Andersen, R. N. *Weed Sci.* **1976**, *24*, 266–269.
Boldt, P. F.; Putnam, A. R. *Weed Sci.* **1981**, 29, 237–241.
Donald, W. W.; Shimabukuro, R. H. *Physiol. Plant.* **1980**, *49*, 459–464.
Dusky, J. A.; Davis, D. G.; Shimabukuro, R. H. *Physiol. Plant.* **1980**, 49, 151–156.
Dusky, J. A.; Davis, D. G.; Shimabukuro, R. H. *Physiol. Plant.* **1982**, *54*, 490–494.
Frear, D. S.; Swanson, H. R. *Pestic. Biochem. Physiol.* **1975**, *5*, 73–80.
Gamborg, O. L. In *Plant Tissue Culture Methods*; Gamborg, O. L., Wetter, L. R., Eds.; National Research Council of Canada: Saskatoon, Canada, 1975; pp 1–10.
Gorbach, S. G.; Kuenzler, K.; Asshauer, J. J. *J. Agric. Food Chem.* **1977**, *25*, 507–511.
Jacobson, A.; Shimabukuro, R. H. *J. Agric. Food Chem.* **1984**, *32*, 742–746.
Jacobson, A.; Shimabukuro, R. H.; McMichael, C. *Pestic. Biochem. Physiol.* **1985**, *24*, 61–67.
Lucas, W. J.; Wilson, C.; Wright, J. P. *Plant Physiol.* **1984**, *74*, 61–66.
Miller, S. D.; Nalewaja, J. D. *Proc.—North Cent. Weed Control Conf.* **1974**, *29*, 38–39.
Paulson, G. D.; Zaylskie, R. G.; Dockter, M. M. *Anal. Chem.* **1973**, *45*, 21–27.
Shimabukuro, R. H.; Walsh, W. C.; Hoerauf, R. A. *J. Agric. Food Chem.* **1979**, *27*, 615–623.

Received for review May 19, 1986. Revised manuscript received October 21, 1986. Accepted January 15, 1987. Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Adsorption of Butachlor to Soils

Tatsuo Sato,* Shigeru Kohnosu, and John F. Hartwig[1]

The adsorption of butachlor [α-chloro-2,6-diethyl-*N*-(butoxymethyl)acetanilide] from water to rice paddy soils in Japan was studied with use of high-pressure liquid chromatography in order to better understand its behavior. The adsorption to most of the soils was better described by the Freundlich isotherm than by the Langmuir isotherm. The adsorption increased with butachlor concentration, soil organic carbon, and temperature. The positive temperature dependence of adsorption implies that the adsorption is endothermic and the heat of adsorption is positive. The heat of adsorption, ΔH, calculated from the Clausius–Clapeyron equation was 5–7 kcal/mol, indicating that the adsorption is physical and reversible. The kinetics of adsorption and the effect of molecular weight of the herbicide on adsorption were also studied. The adsorption of alachlor (α-chloro-2,6-diethyl-*N*-(methoxymethyl)acetanilide) was measured to study the molecular weight effect.

Adsorption of herbicides to soil from water is an important factor affecting their fate, biological activity, and persistence in soil. Better understanding of these processes will permit more effective weed control with minimum residues.

Adsorption of herbicides to soils can be described either by the Freundlich equation, where A = amount adsorbed, C = equilibrium concentration, and K, $1/n$ = constants

$$A = KC^{1/n} \quad (1)$$

$$\log A = 1/n \log C + \log K \quad (2)$$

or by the Langmuir equation (Bailey and White, 1970), where A_∞ = adsorption maximum and K_1, K_2 = constants.

$$A/A_\infty = K_1C/(K_2C + 1) \quad (3)$$

$$C/A = K_2C/K_1A_\infty + 1/K_1A_\infty \quad (4)$$

The Freundlich equation is purely empirical and imposes no limiting adsorption, while the Langmuir equation is based on theoretical consideration and shows maximum adsorption when a monolayer of molecules has covered all surfaces. Conformity of isotherms to the Freundlich or the Langmuir equation can be tested by measuring the linearity of plotting log A vs. log C for the Freundlich or plotting C/A vs. C for the Langmuir, respectively.

Monsanto Agricultural Research Station, Kawachi, Ibaraki, Japan.
[1]Present address: Princeton University, Princeton, NJ.

Table I. Properties of Herbicides

common name	butachlor	alachlor
chemical name	α-chloro-2,6-diethyl-*N*-(butoxymethyl) acetanilide	α-chloro-2,6-diethyl-*N*-(methoxymethyl) acetanilide
mol wt	311.9	269.8
solubility in water, ppm		
10 °C	22	164
20 °C	23	212
35 °C	34	338

Adsorption of butachlor has been studied by Brightwell and Rueppel (1978). They found that the adsorption of butachlor followed the Freundlich isotherm and the Freundlich coefficient K value ranged from 3.5 to 20.0. They concluded that butachlor adsorption is reversible but butachlor would not be readily desorbed into water.

The purpose of this work was to study the butachlor adsorption in more detail to better understand the behavior of butachlor in a rice paddy field. Parameters studied were butachlor concentration, rate of adsorption, temperature effect, and molecular weight effect. Correlations with the Freundlich and the Langmuir isotherms were also determined.

MATERIALS AND METHODS

Herbicides. Technical-grade butachlor (93.1%) and alachlor (93.6%) produced by Monsanto were used as received. The molecular weight and the solubility in water of butachlor and alachlor at various temperature are shown

0021-8561/87/1435-0397$01.50/0