complete metabolism of permethrin in flooded soil was due to anaerobic transformations as oxygen was metabolized and lost from the soil. The accumulation of organic acids, especially the DCVA in flooded soil treated with [*carbonyl*-^{14}C]-*trans*-permethrin, is evidence of the fermentative character of the microorganisms responsible for permethrin metabolism in flooded soil.

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Registry No. CO_2, 124-38-9; DCVA, 55701-05-8; PBalc, 13826-35-2; PBacid, 3739-38-6; PBald, 39515-51-0; permethrin, 52645-53-1; *trans*-permethrin, 61949-77-7; *cis*-permethrin, 61949-76-6.

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Identification of the Initial Metabolites of Acetochlor in Corn and Soybean Seedlings

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The initial metabolism of acetochlor (2-chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide), a selective preemergent herbicide that is used to control problem grass and some broadleaf weeds, was examined in tolerant corn and soybean seedlings in order to delineate the detoxification pathway for this herbicide. Acetochlor was rapidly absorbed and metabolized by etiolated corn seedlings to the glutathione (GSH; glutamylcysteinylglycine) conjugate. Acetochlor was also rapidly absorbed and metabolized by etiolated soybean seedlings. However, in this case the initial metabolite was the homoglutathione (hGSH; glutamylcysteinyl-β-alanine) conjugate and not the glutathione conjugate. The two initial detoxification metabolites were isolated by high-performance liquid chromatography (HPLC), and identification was based upon mass spectral methods, especially fast atom bombardment mass spectrometry (FAB MS).

INTRODUCTION

Acetochlor (1) is a preemergent herbicide used to control grass weeds and some problem broadleaf weeds in a variety of crops such as corn and soybeans. This paper describes the isolation and identification of the initial metabolites of acetochlor in tolerant corn and soybean seedlings. These metabolites were identified in order to delineate the pathway used by tolerant plants to detoxify this herbicide.

The initial corn metabolite of the chloroacetanilide herbicide propachlor [2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide] was previously identified as the glutathione conjugate on the basis of chromatographic comparisons with synthetic standards (Lamoureux et al., 1971). It has recently been reported that propachlor (Lamoureux and Rusness, 1981) and a related chloroacetanilide [2-chloro-*N*-(2,3-dimethylphenyl)-*N*-(1-methylethyl)acetamide] are converted to similar conjugates in soybeans (Hussain et al, 1983). One of the objectives of the present study was to isolate the initial acetochlor metabolites and confirm the structural assignments by mass spectrometry. Peptide thiol conjugates have been difficult to analyze by mass spectrometry for several reasons (Hudson, 1976). However, the recent introduction of fast atom bombardment mass spectrometry (FAB MS) has greatly aided the mass spectral identification of these nonvolatile conjugates (Frear et al., 1985). The results of the identification of the initial metabolites of acetochlor in tolerant corn and soybean seedlings are detailed below.

EXPERIMENTAL SECTION

Materials. Isotopically labeled acetochlor [[U-^{14}C]-phenyl; specific act. 9.8 mCi/mmol; ^{13}C at the carbon containing the chlorine] was prepared and purified at Monsanto Co. The radiochemical purity was greater than

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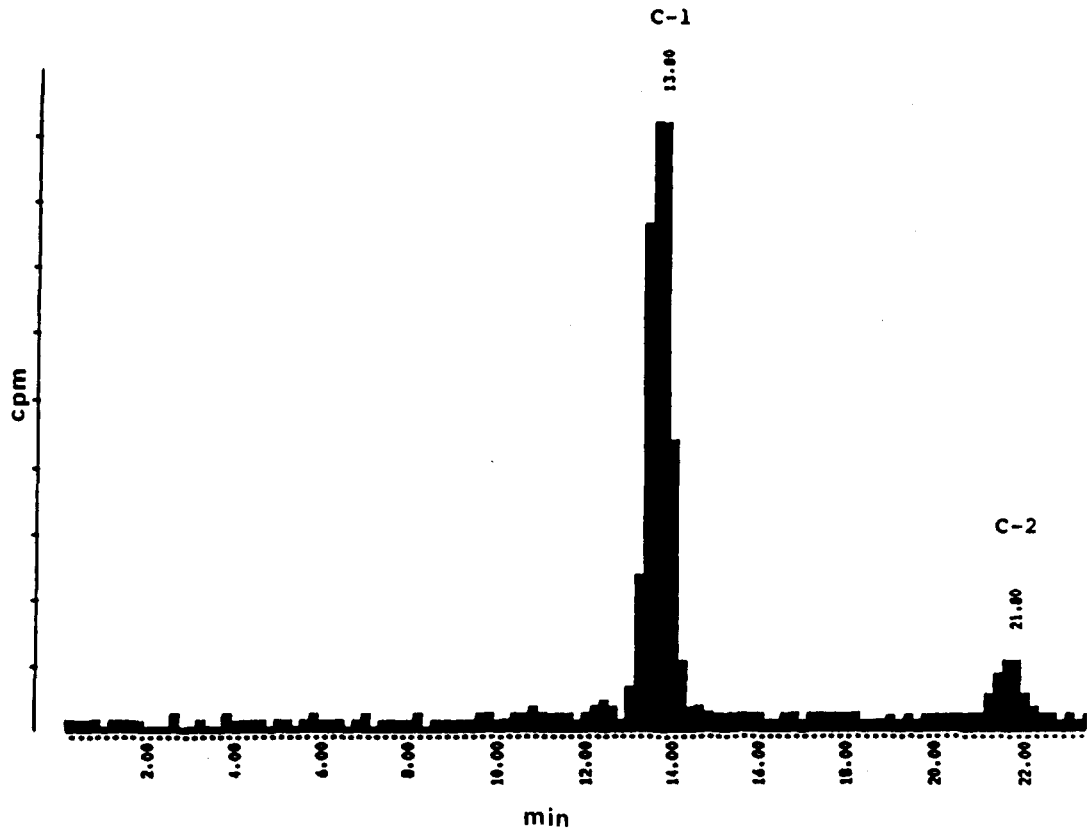

Figure 1. HPLC analysis of the corn shoot extract 12 h after acetochlor application.

98% as determined by HPLC and GC analysis. Synthetic samples of the radiochemically labeled glutathione and cysteine conjugates were prepared for use as mass spectral and chromatographic standards in the manner described by Lamoureux and Davidson (1975). All reagents were obtained from commercial sources and were of the highest available purity.

Crop seeds were obtained from commercial sources and germinated on paper towels or in vermiculite. The seedlings were grown in the dark for 3–5 days before use. The herbicide was applied to the shoots of etiolated corn seedlings and the hypocotyls of etiolated soybean seedlings.

Analytical Methods. The isotopically labeled herbicide was applied to the shoots, and uptake and metabolism were monitored with time. The seedlings were harvested 2, 4, 8, 12, and 24 h after treatment, the uptake was determined, and the extracts were (80% methanol) analyzed by reversed-phase HPLC. On the basis of these studies the optimum harvest time for corn seedlings was 12 h and soybeans 24 h after treatment.

The HPLC system used was a gradient system consisting of two Waters Model 6000A pumps, a Waters 660 solvent programmer, a Model U6K injector, and a Model 450 absorbance detector. In addition, a Packard RAM 7500 radioactivity detector was used to monitor the HPLC eluent. A Waters C-18 μBondapak column (7.8 mm × 30 cm) was used for this study.

The three sets of HPLC conditions that were used in this study are as follows. A: eluent 1 = 1% acetic acid in 40% aqueous acetonitrile, eluent 2 = 1% acetic acid in 80% aqueous acetonitrile; gradient conditions, eluent 1 to eluent 2 in 10 min, linear gradient; flow rate, 2 mL/min; retention times of standards, (glutathione conjugate), 9.6 min, (cysteine conjugate) 13.8 min, (acetochlor) 19.2 min. B: eluent 1 = 0.001 M ammonium dihydrogen phosphate in 20% aqueous acetonitrile, eluent 2 = 0.001 M ammonium dihydrogen phosphate in 80% aqueous acetonitrile; gradient conditions, eluent 1 to eluent 2 in 30 min, linear gradient; flow rate, 2 mL/min; retention times of standards, (glutathione conjugate) 13.8 min, (cysteine conjugate) 21.8 min, (acetochlor) 32.0 min. C: eluent 1 = 1% acetic acid in 30% aqueous acetonitrile, eluent 2 = 1% acetic acid in 50% acetonitrile; gradient conditions, eluent 1 to eluent 2 in 30 min, curve 7 on the Waters 660 programmer; flow rate, 2 mL/min; retention times of standards, (glutathione conjugate) 17.0 min, (cysteine conjugate) 18.2 min. The first set of conditions (A) were used to rapidly monitor metabolism in order to determine the optimum harvest time for metabolite identification. The second set of conditions (B), which utilized a long linear gradient to separate the metabolites, were used compare the metabolites to synthetic standards and for the coinjection studies. The third set of conditions (C) were used to purify the metabolites for mass spectral analysis.

The metabolites were extracted and analyzed by HPLC to follow the disappearance of the parent herbicide. The metabolites were isolated and purified by reversed-phase HPLC using conditions B and C described above. The Raney nickel reductive desulfurization method used followed the general procedure of Diesperger and Sandermann (1979).

The purified metabolites were analyzed by FAB mass spectrometry using either a Varian 311 or a VG 70E mass spectrometer. The VG data system was used to record the spectra. Glycerol was normally used as the FAB matrix. However, it was found that the use of diethanolamine gave superior results for the soybean major metabolite, SB-1.

RESULTS AND DISCUSSION

Analytical Methods. In order to identify the initial metabolites of acetochlor a combination of ^{14}C- and ^{13}C-labeled herbicide was used. The acetochlor treatment solution contained a 1:1 ^{12}C:^{13}C ratio. The use of this mixture ensured that the metabolite generated doublet ions of equal intensity upon mass spectral analysis. These

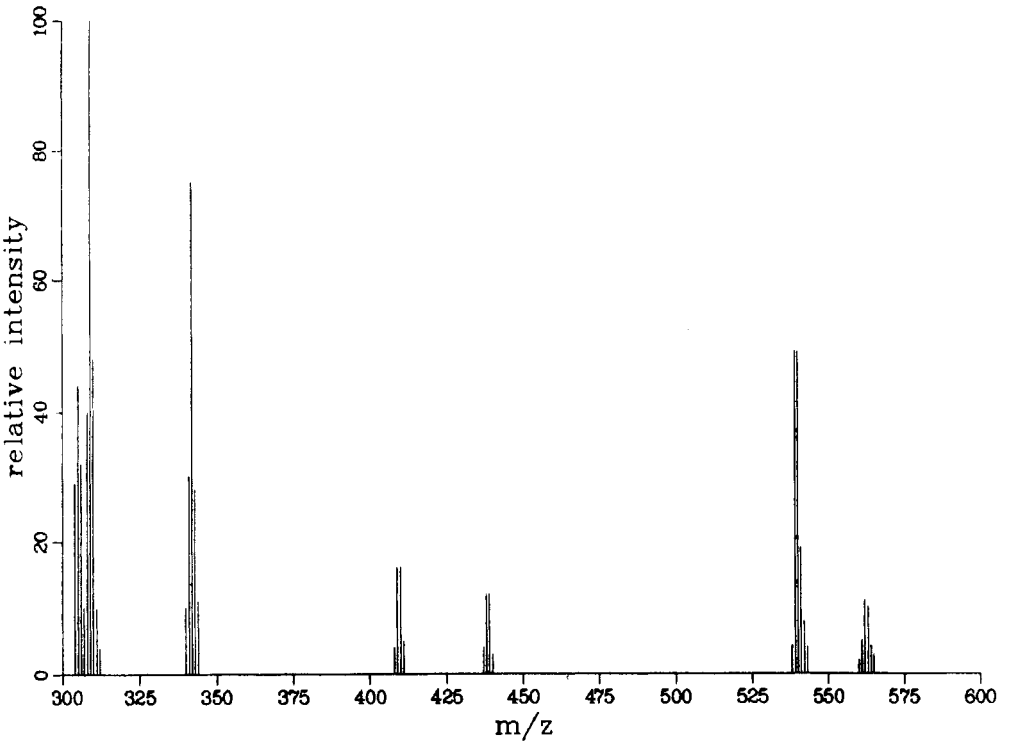

Figure 2. Negative-ion FAB mass spectrum of the major corn shoot acetochlor metabolite.

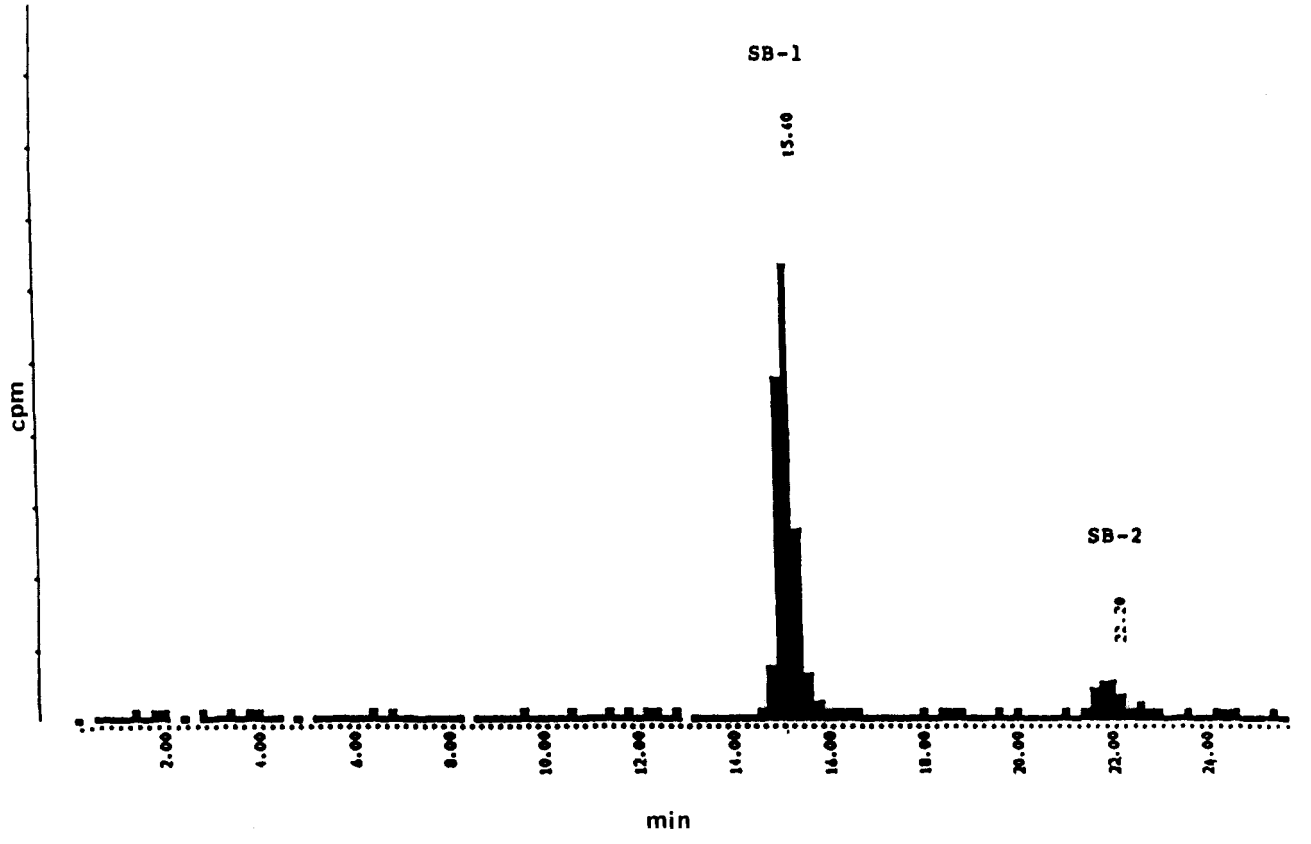

Figure 3. HPLC analysis of the soybean seedling extract 24 h after acetochlor application.

doublet ions greatly facilitated the detection of metabolite ions in the presence of ubiquitous ions from the plant matrix.

Identification of Corn Seedling Metabolites. Acetochlor was rapidly absorbed and metabolized by corn seedlings. The optimum application rate was found to be 20 μg/shoot while the optimum harvest time was 12 h. After 12 h, no acetochlor was present in the extracts and only one major polar metabolite (C-1, Figure 1; 95.4% of the seedling extract) and a minor less polar metabolite (C-2, Figure 1) were detected. All of the activity remained in the shoot application zone. HPLC comparisons of the corn seedling metabolites with synthetic standards indicated that the polar metabolite (C-1) was the glutathione conjugate while the less polar metabolite (C-2) was the cysteine conjugate. The corn metabolite mixture was

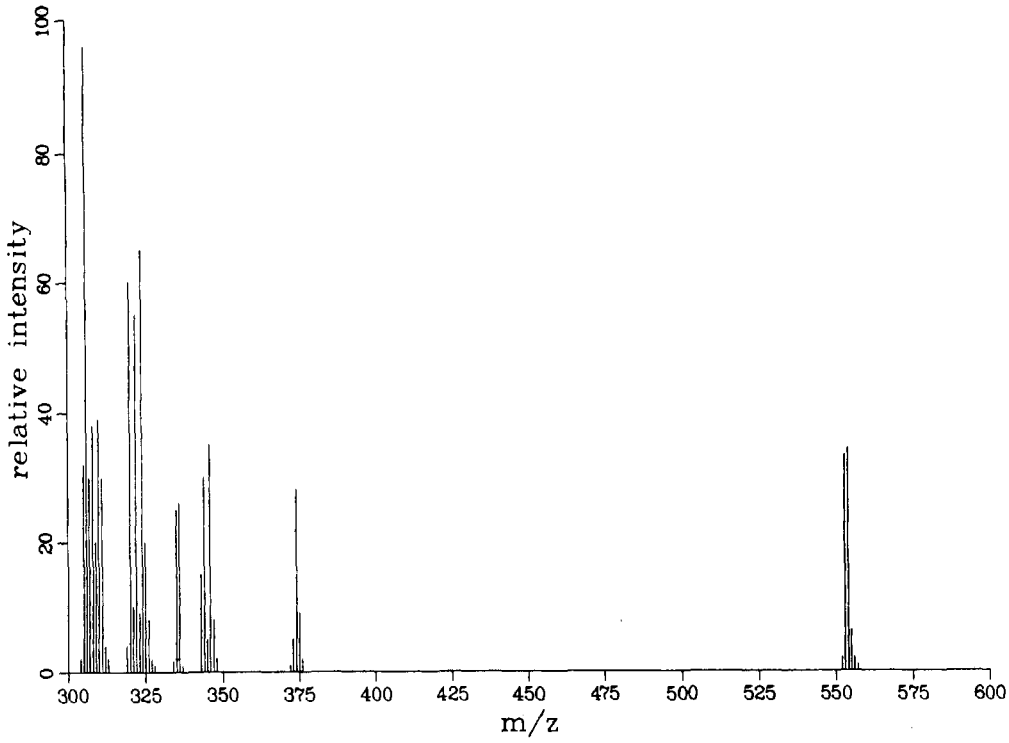

Figure 4. Negative-ion FAB mass spectrum of the major soybean seedling acetochlor metabolite.

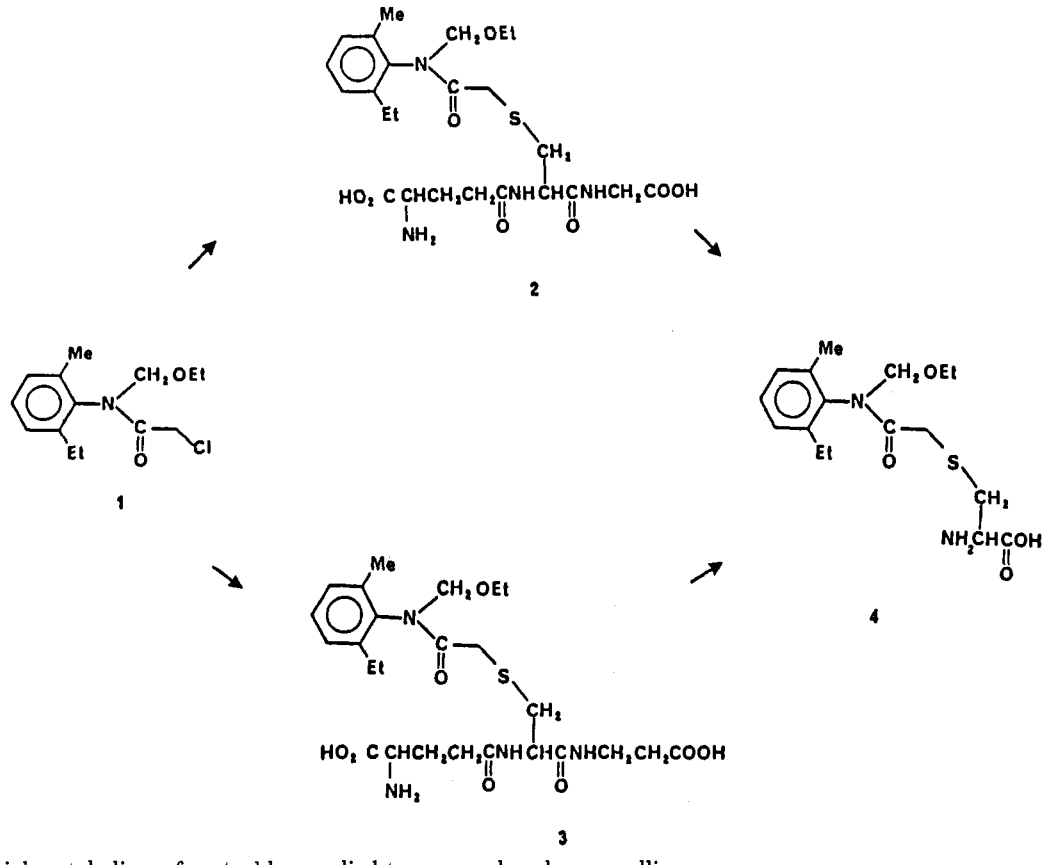

Figure 5. Initial metabolism of acetochlor applied to corn and soybean seedlings.

treated with the Raney nickel desulfurization catalyst and only one reduction product, *N*-(ethoxymethyl)-[2-ethyl-6-methylphenyl]acetamide, was detected. The conversion of the metabolites to the acetamide was further evidence that these metabolites were sulfur-containing conjugates.

The major metabolite was isolated and purified by HPLC and then analyzed by mass spectrometry. The initial attempts using field desorption and direct-probe chemical ionization techniques were not successful, however. The polar major corn metabolite of acetochlor (C-1) was then analyzed by positive- and negative-ion FAB mass spectrometry. The spectra obtained for this metabolite were similar to those obtained for the synthetic glutathione conjugate. The negative-ion spectrum for the metabolite is shown in Figure 2. Parent ions were observed for the metabolite (M − H, m/z 539, 540) and the synthetic

standard. In addition, sodium adduct ions (M − M + Na, m/z = 562, 563) and doublet ions resulting from the characteristic loss of pyroglutamic acid (m/z 410, 411) were also detected. On the basis of chromatographic and mass spectral comparisons with synthetic standards and the Raney nickel derivatization the major corn acetochlor metabolite was identified as the glutathione conjugate (2, Figure 5).

The second and minor metabolite, C-2, was tentatively identified as the cysteine conjugate on the basis of comparison with the synthetic standard under several different sets of chromatographic conditions. Insufficient metabolite was available for mass spectral analysis, unfortunately.

Identification of the Soybean and Mung Bean Seedling Metabolites. Isotopically labeled acetochlor was also rapidly absorbed and metabolized by soybean seedlings. In this case the optimum harvest time was 24 h after herbicide application. After 24 h no unmetabolized acetochlor was detected in the seedling extract (Figure 3). Although most of the activity remained in the treated hypocotyls, a significant portion (28.3%) migrated to the dicotyledons. Only two metabolites, a major polar metabolite (SB-1, 91.7% of the shoot extract) and a minor less polar metabolite (SB-2) were detected after 24 h. Raney nickel reduction of the soybean acetochlor metabolite mixture yielded only one product. This product was the same acetamide produced by the Raney nickel reduction of the corn metabolite mixture which indicated that the soybean metabolites were also thioether conjugates. HPLC comparison using synthetic standards and coinjection studies revealed that the minor metabolite was the cysteine conjugate but the major metabolite was not the glutathione conjugate although it was chromatographically very similar to the glutathione conjugate. HPLC conditions were developed (conditions A), which separated the synthetic glutathione conjugate and the soybean major metabolite (SB-1). Under these conditions the soybean metabolite was found to have a longer retention time (15.4 min) than the synthetic glutathione conjugate or the corn seedling major metabolite (13.8 min).

The major soybean metabolite was isolated and purified by reversed-phase HPLC and then analyzed by mass spectrometry. The negative-ion spectrum is shown in Figure 4. The metabolite pseudomolecular ion doublet (M − H, m/z 553, 554) was 14 mass units higher than that observed for the corn seedling metabolite, which was identified as the glutathione conjugate. On the basis of amino acid analysis Carnegie reported that mung beans contain a tripeptide thiol homologue of glutathione in which glycine has been replaced by β-alanine (1963). Mung bean hypocotyls were treated with isotopically labeled acetochlor, and it was found that the mung bean metabolite was chromatographically identical with the soybean polar metabolite SB-2 using coinjection techniques and a variety of HPLC conditions. On the basis of these studies it was concluded that the soybean major acetochlor metabolite was the homoglutathione conjugate **3**. While this work was in progress Frear and co-workers reported that the herbicides metribuzin (1985) and acifluorifen (1983) were also converted to the homoglutathione conjugates by soybean seedlings. Homoglutathione was subsequently synthesized and used to confirm the structure of the soybean and mung bean peptide thiol as glutamylcysteinyl-β-alanine (Breaux et al., 1986).

The minor metabolite SB-2 was tentatively identified as the cysteine conjugate (**3**) on the basis of chromatographic comparisons with standards under a variety of conditions. As was the case with the corn minor metabolite, insufficient material was available for mass spectral analysis.

In summary, acetochlor was rapidly absorbed and metabolized by both corn and soybean seedlings. In both cases one major polar metabolite was formed initially. The major corn metabolite was the glutathione conjugate of acetochlor while the major initial soybean acetochlor metabolite was identified as the homoglutathione conjugate. In both cases a second and minor metabolite was detected that was tentatively identified as the cysteine conjugate. The initial metabolism of the herbicide acetochlor in tolerant corn and soybean seedlings is outlined Figure 5. Studies are under way to identify the initial metabolites of acetochlor in susceptible plant seedlings.

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