Initial Metabolism of Dimethenamid in Safened and Unsafened Wheat Shoots†

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The metabolic fate of the chloroacetamide herbicide dimethenamid was examined in excised shoots of unsafened and safened wheat seedlings. Compared to unsafened controls, fluxofenim treatment increased the rate of metabolism of dimethenamid to water-soluble compounds. Thin layer chromatography revealed that similar dimethenamid metabolites were present in extracts from both safened and unsafened wheat shoots. However, three metabolites were more abundant in extracts from fluxofenim-treated wheat relative to unsafened wheat. Reversed-phase high-performance liquid chromatography and mass spectrometry were used to isolate and confirm the identity of dimethenamid metabolites extracted from safened wheat shoots 1 h after treatment with dimethenamid. In addition to the presence of the glutathione conjugate of dimethenamid, γ-glutamylcysteine and cysteinylglycine dipeptide conjugates, as well as a cysteine conjugate and an oxygenated glutathione conjugate of dimethenamid, were detected. On the basis of time course analysis of dimethenamid metabolites by thin layer chromatography and structural identification by mass spectrometry, it appears that from 5 to 100 min after treatment with dimethenamid metabolism occurs exclusively via the glutathione conjugation pathway in wheat. Thus, the protection conferred to wheat by fluxofenim treatment is most likely due to increased glutathione-mediated detoxification of dimethenamid.

Keywords: *Dimethenamid; herbicide metabolism; fluxofenim; safener; glutathione; wheat*

INTRODUCTION

Dimethenamid is a soil-applied chloroacetamide herbicide used to selectively control grass and certain broadleaf weeds in corn and soybeans (Jordan and Woolley, 1993; Osborne et al., 1995). Wheat is sensitive to dimethenamid; however, the tolerance of wheat to dimethenamid and other chloroacetamide herbicides can be increased through the use of seed-applied herbicide safeners (Riechers et al., 1994a). Herbicide safeners have been used successfully to protect several monocot crop species from chloroacetamide herbicides (Hatzios, 1989). Safeners have been shown to increase the rate of metabolic detoxification of chloroacetamide herbicides, mainly through the induction of the glutathione conjugation pathway (Fuerst and Gronwald, 1986; Gronwald, 1989). Increases in the activity of glutathione *S*-transferase (GST, EC 2.5.1.18) isozymes capable of utilizing chloroacetamide herbicides as substrates (Dean et al., 1990; Fuerst et al., 1993), as well as increased levels of glutathione (GSH) (Lay and Casida, 1976; Breaux et al., 1987; Gronwald et al., 1987; Tal et al., 1995), in response to safener treatment have been correlated with increased crop tolerance to the herbicides (Kreuz, 1993). Safeners generally increase the activity of constitutively expressed detoxification enzymes in plants, rather than induce the expression of novel pathways of herbicide metabolism (Kreuz, 1993; Cole, 1994). Three such examples are GST-catalyzed GSH conjugation, hydroxylation by cytochrome P450-dependent monooxygenases, and glucose conjugation by glucosyl transferases (Kreuz, 1993; Cole, 1994).

Most research studying safener mode of action has used corn and grain sorghum as model plant systems. We have previously shown that the safener fluxofenim, normally used in grain sorghum to protect against chloroacetamide injury, is also active in wheat by increasing the rate of glutathione conjugation of dimethenamid (Riechers et al., 1994b). The objectives of the present study were to determine the effect of fluxofenim treatment on the rate of metabolism of dimethenamid in wheat, to compare dimethenamid metabolites from unsafened and fluxofenim-treated wheat extracts over time using thin layer chromatography (TLC), and to isolate and identify dimethenamid metabolites in fluxofenim-treated wheat 1 h after treatment using reversed-phase high-performance liquid chromatography (HPLC) and mass spectrometry (MS).

MATERIALS AND METHODS

Chemicals. Analytical grade dimethenamid [99.4% pure as determined by gas chromatography (GC) and radiolabeled (3-[^{14}C] *thiophene ring*) dimethenamid (50.6 mCi/mmol) were provided by Sandoz Agro Inc., Des Plaines, IL. The commercial formulation of fluxofenim (Concep III; 959 g of active ingredient/L) was supplied by Ciba Corp. (Greensboro, NC). HPLC and reagent-grade solvents were purchased from J. T. Baker Inc. (Phillipsburg, NJ). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Plant Material. Winter wheat (*Triticum aestivum* L. var. Madsen) seeds were treated with fluxofenim at a rate of 0.25 g/kg of seed (labeled rate in sorghum is 0.4 g/kg of seed) using the following protocol: Twenty-five milligrams of fluxofenim and 150 μL of seed binder (Gustafson Phase II Binder, McKinney, TX) were dissolved in 3 mL of acetone and sonicated for 30 s. Safener and binder solution was poured into a plastic beaker containing 100 g wheat seeds and stirred with a spatula for 20 s. A stream of air was then applied with continued stirring for about 30 s. Seeds were placed on blotter paper under a laboratory hood for at least 10 min to allow the acetone to completely evaporate. Seeds were then removed

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from the hood and left uncovered overnight. Control wheat seeds were treated with binder and acetone only.

Wheat seeds were planted in vermiculite in plastic pots, watered, and covered with aluminum foil. Plants were grown at 30°C and 100% relative humidity in a growth incubator without light for 4 days. Wheat shoots (2−3 cm) were excised and used for metabolism studies immediately after harvest.

Rate of Dimethenamid Metabolism. Excised wheat shoots (0.5 g) were washed with water and then vacuum infiltrated for 1 min in 2.5 mL of 4 μM [^{14}C]dimethenamid (0.2 μCi/mL). The radiolabeled solution was subsequently removed, and the shoots were washed twice with 3 mL of water and incubated at 25 °C without an external solution. After 3, 10, 30, and 100 min of incubation, the shoots were frozen in liquid nitrogen and stored at −20 °C until further analysis.

Radioactive dimethenamid and metabolites were extracted from wheat shoots by homogenization in 5 volumes of 80% (v/v) methanol using a glass mortar and pestle. The resulting crude homogenate was centrifuged in a microfuge at 12000*g* for 5 min. The supernatant was removed, and the pellet was reextracted with 1 mL of 80% methanol as previously described. The supernatant was decanted and combined with the initial supernatant. Radioactivity in an aliquot was quantified by liquid scintillation spectrometry (LSS) to determine dimethenamid uptake.

Methanol in the crude extract was removed under vacuum with a rotary evaporator at 35 °C, followed by the addition of water to adjust the final volume to 5 mL. The resulting aqueous extract was partitioned twice against 1 volume of chloroform to separate water-soluble metabolites from unmetabolized dimethenamid. The volumes of the aqueous and chloroform phases were determined after partitioning, and radioactivity in each phase was quantified by LSS. Radioactivity in the aqueous phase was plotted as a percentage of the total radioactivity recovered from both phases after partitioning. The average recovery of total radioactivity was 94% following partitioning of the crude extract. Each treatment was repeated once, and the results are expressed as the mean ± standard error.

TLC of Dimethenamid Metabolites. Excised wheat shoots (0.5 g) were washed with water and then vacuum infiltrated for 2 min in 2.5 mL of 10 μM [^{14}C]dimethenamid (0.5 μCi/mL). The radiolabeled solution was subsequently removed, and the shoots were washed twice with 3 mL of water and incubated at 25 °C without an external solution. After 5, 30, and 100 min of incubation, the shoots were removed and frozen as previously described.

Crude extracts were prepared as previously described and uptake was quantified by LSS. Aliquots containing 3000 dpm from the 80% methanol crude extracts were spotted directly onto the preadsorbent zone of a channeled TLC plate (Analtech Uniplate silica gel HLF, 250 μm thick, Newark, DE). Synthetic standards of the glutathione and cysteine conjugates of [^{14}C]dimethenamid (synthesis protocol described below) and parent [^{14}C]dimethenamid were spotted for reference. The plate was developed twice in the same dimension using a chloroform/methanol/formic acid/water solvent system (first 75:25:4:2 and then 60:40:4:2 v/v/v/v) (Miller et al., 1996). The solvent front was allowed to migrate to the top of the plate during each run (20 cm). Radioactive compounds were visualized using a radioanalytic imaging system (Ambis, Ambis Systems, San Diego, CA) or by exposing autoradiography film (Reflection, DuPont, Wilmington, DE) for 10 days at −80 °C after the plate was sprayed with a surface fluorography reagent (ENHANCE, DuPont).

HPLC and Mass Spectral Analysis of Dimethenamid Metabolities. Only fluxofenim-treated wheat seed was used for isolation of dimethenamid metabolites by HPLC. The purification procedure is briefly summarized in Figure 1. Metabolites from unsafened wheat were not analyzed by HPLC or mass spectrometry due to the lower levels of metabolites formed. Excised wheat shoots (1 g) were washed with water, then incubated continuously for 30 min in 3 mL of 200 μM [^{14}C]dimethenamid (specific activity = 1.0 mCi/mmol). The uptake solution was subsequently removed, and the shoots were washed twice with 3 mL of water and incubated at 25°C for an additional 30 min without an external solution (60 min total). The shoots were then frozen in liquid nitrogen and stored at −80°C until metabolite extraction. This procedure was repeated 10 times for a total of 11 1-g samples of dimethenamid-treated wheat shoots.

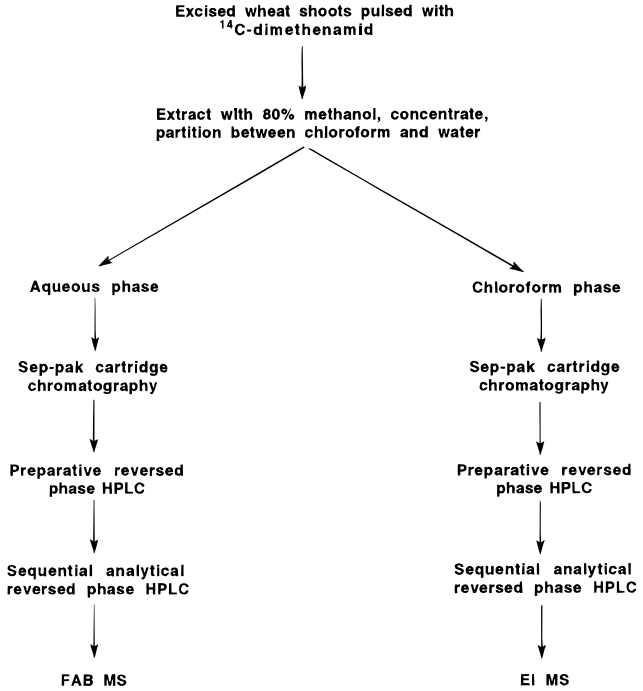

Figure 1. Purification flow diagram for dimethenamid metabolites extracted from fluxofenim-safened excised wheat shoots pulsed with [^{14}C]dimethenamid.

Frozen shoots were pooled (11 g), pulverized with a chilled mortar and pestle, and then homogenized in 60 mL of 80% (v/v) methanol. The resulting crude homogenate was centrifuged at 3000*g* for 15 min, the supernatants were decanted, and the pellets were reextracted with 10 mL of 80% methanol and centrifuged at 3000*g* for 10 min. The resulting supernatants were decanted and combined with the initial supernatants. Radioactivity in an aliquot of the crude extract was quantified by LSS to determine dimethenamid uptake. Approximately 20% of the supplied radioactivity was absorbed during the 30 min incubation period. The crude extract was concentrated by removing the methanol under vacuum in a rotary evaporator at 35 °C. The resulting aqueous extract was partitioned once against 1 volume of chloroform, and radioactivity in each phase was quantified by LSS. Recovery of radioactivity was 87% of the initial amount absorbed following partitioning the crude extract. Each phase was stored at −20 °C until further analysis.

The aqueous phase was adjusted to 0.15% (v/v) trifluoroacetic acid (TFA) and loaded onto a preparative C_{18} cartridge (Sep-Pak, Waters, Milford, MA) previously equilibrated in 0.02% (v/v) TFA. After the cartridge was washed with 5 volumes of 0.02% (v/v) TFA, radioactivity was eluted with 5 volumes of 90% acetonitrile/0.02% (v/v) TFA. Cartridge eluates were concentrated by vacuum centrifugation (SpeedVac, Savant Instruments, Inc., Farmingdale, NY) and stored at −20 °C until HPLC analysis.

The chloroform phase was evaporated to dryness by vacuum centrifugation, reconstituted in 25% (v/v) acetonitrile, loaded onto the preparative C_{18} cartridge, washed, and eluted as described above, except that TFA was not included in the mobile phase. Cartridge eluates were concentrated and stored as previously described above. Recovery of radioactivity after preparative C_{18} cartridge chromatography was >99% from the aqueous phase and 89% from the chloroform phase.

Preparative HPLC and analytical HPLC were performed using the instrumentation and columns as described by Miller et al. (1996). For preparative HPLC, samples previously subjected to preparative C_{18} cartridge chromatography were

Table 1. Analytical HPLC Gradients Used To Purify Dimethenamid and Metabolites from Safened Wheat Shoot Extracts

gradient	conditions[a]
A	0−3 min, 100% A; 3−33 min, 0−100% B; 33−43 min, 100% B
B	0−3 min, 100% A; 3−13 min, 0−50% B; hold at 50% B
C	0−3 min, 100% A; 3−13 min, 0−40% B; hold at 40% B
D	0−3 min, 100% A; 3−13 min, 0−30% B; hold at 30% B
E	0−3 min, 100% A; 3−13 min, 0−25% B; hold at 25% B
F	0−3 min, 100% A; 3−13 min, 0−20% B; hold at 20% B
G	0−3 min, 100% A; 3−13 min, 0−15% B; hold at 15% B

[a] Solvent A = 0.02% (v/v) TFA; solvent B = acetonitrile + 0.02% (v/v) TFA.

adjusted to 0.02% (v/v) TFA and injected onto the column [equilibrated in 0.02% (v/v) TFA] in 2 mL aliquots. The column was washed with 0.02% (v/v) TFA for 5 min, and then radioactivity was eluted from the column using a linear gradient from 0 to 100% acetonitrile/0.02% (v/v) TFA in 50 min. This gradient resolved the water-soluble and chloroform-soluble dimethenamid metabolites from dimethenamid. The flow rate was 3 mL/min, and column eluate was collected in 3 mL fractions. An aliquot was analyzed by LSS to quantify radioactivity in each fraction. Recovery of radioactivity following preparative HPLC was >99% from the aqueous phase and about 63% from the chloroform phase.

Analytical HPLC was used to purify samples previously subjected to preparative HPLC. The flow rate was 1 mL/min, and column eluate was collected in 1 mL fractions. An aliquot was analyzed by LSS to quantify radioactivity in each fraction. Metabolites were purified by analytical HPLC using the gradients listed in Table 1. Specific gradients used to purify each water-soluble metabolite are listed in Table 2. Analytical HPLC gradients A−C (C twice) were used to purify the most abundant chloroform-soluble compound (Figure 5, peak F).

The purified water-soluble metabolites were analyzed by positive ion fast atom bombardment mass spectrometry (FAB MS) using glycerol/acetic acid/water (100:1:1 v/v/v) as the matrix. Chloroform-soluble radioactive compounds were analyzed by electron impact mass spectrometry (EI MS) using an ionization energy of 50 eV. Mass spectrometry was performed using a VG 7070EHF mass spectrometer operating in the positive ion mode, and samples were introduced with a solid-sample probe.

Preparation of Synthetic Standards. The glutathione, cysteinylglycine, and cysteine conjugates of dimethenamid were synthesized *in vitro* for use as reference standards. The reaction was carried out in 5 mL of water and contained 60 mM TAPS buffer (final unadjusted pH was 8.6), 20 mM GSH, L-cysteinylglycine or L-cysteine, and 0.8−2 mM dimethenamid. The reaction mixture was incubated at 40 °C for 8 h, and then the reaction was terminated by partitioning against 1 volume of methylene chloride to remove unreacted dimethenamid. Synthetic conjugates were purified from the aqueous phase as previously described using preparative C_{18} cartridge chromatography and HPLC prior to use as analytical standards in TLC, HPLC, and MS.

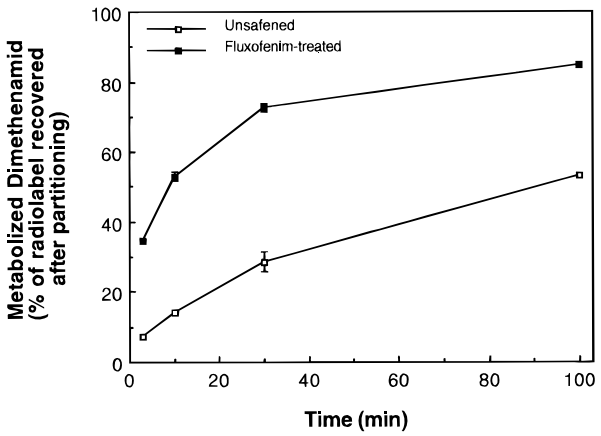

Figure 2. Dimethenamid metabolism to water-soluble compounds in excised wheat shoots. Safened wheat was treated with seed-applied fluxofenim. Metabolized dimethenamid is plotted as the amount of radioactivity in the aqueous phase as a percentage of the total radioactivity recovered from both phases after partitioning with chloroform.

RESULTS AND DISCUSSION

Rate of Dimethenamid Metabolism. Excised wheat shoots grown from seed treated with fluxofenim metabolized dimethenamid to water-soluble metabolites more rapidly than unsafened wheat (Figure 2). The half-life of dimethenamid in fluxofenim-treated wheat was about 9 min, compared to about 90 min in unsafened wheat. In addition, the initial rate of metabolism was enhanced in fluxofenim-treated wheat (3−10 min after treatment) compared to that in unsafened wheat. From 10 to 100 min, the rate of metabolism was similar between safened and unsafened wheat, although the absolute amount of dimethenamid metabolized was greater in fluxofenim-treated wheat. Similar results have been reported for chloroacetamide herbicides in sorghum and corn shoots (Fuerst and Gronwald, 1986; Fuerst and Lamoureux, 1992). Under our experimental conditions, the greatest difference in metabolism occurred during the first 10 min, where 53% of dimethenamid was metabolized in fluxofenim-treated wheat, while only 14% was metabolized in unsafened wheat.

The reasons for the similar rates of metabolism observed when safened and unsafened wheat are compared from 10 to 100 min after treatment are unclear. One possible explanation could be that the GST substrate dimethenamid is acting as an inducer of GSTs capable of metabolizing dimethenamid in the unsafened control and that this induction process is occurring during the initial 10 min period. Metolachlor, a similar

Table 2. HPLC Isolation and Positive Ion FAB MS Analysis of Water-Soluble Metabolites of Dimethenamid from Safened Wheat Shoot Extracts

peak from Figure 4	retention time[a] (min)	purification gradients[b]	% abundance[c]	molecular ion $(M + H)^+$	dimethenamid metabolite[d]
A	27.8	A−G (A and G twice)	3	563	oxygenated GSH
B	37.1	A−C (A twice)	36	547	GSH
C	39.0	A−C	53	490	γ-glutamylcysteine
D[e]	44.3	A−C	8	361	cysteine
	44.3	A−C (C twice)		418	cysteinylglycine

[a] Retention times during preparative HPLC. For the gradient used, see Materials and Methods. [b] Analytical HPLC gradients used for metabolite purification, listed in Table 1. [c] Abundance of radioactivity in each peak in Figure 4, as a percentage of the total radioactivity recovered following preparative HPLC. [d] Structures are shown in Figure 6. [e] Two radioactive metabolites cochromatographed as peak D during preparative HPLC but were resolved by analytical HPLC.

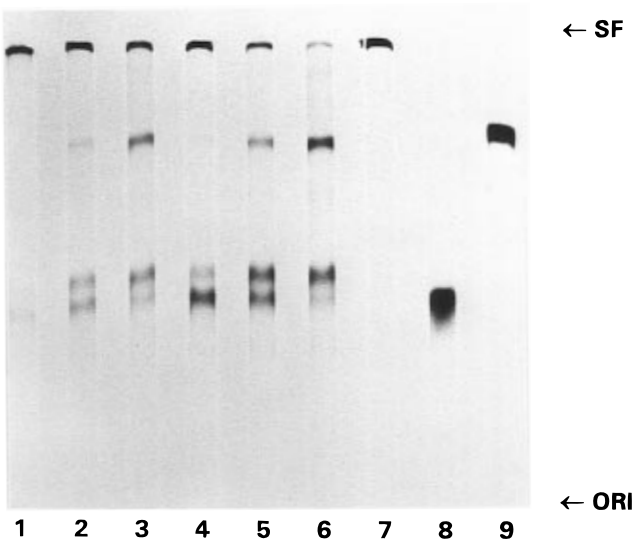

Figure 3. TLC analysis of dimethenamid metabolites extracted from excised wheat shoots during a 100 min time course. Aliquots from crude extracts were spotted on a silica gel TLC plate (3000 dpm/lane), and the plate was developed as described under Materials and Methods. (Lane 1) unsafened wheat, 5 min incubation period; (lane 2) unsafened wheat, 30 min incubation period; (lane 3) unsafened wheat, 100 min incubation period; (lane 4) fluxofenim-treated wheat, 5 min incubation period; (lane 5) fluxofenim-treated wheat, 30 min incubation period; (lane 6) fluxofenim-treated wheat, 100 min incubation period; (lane 7) dimethenamid standard; (lane 8) synthetic glutathione−dimethenamid conjugate standard; (lane 9) synthetic cysteine−dimethenamid conjugate standard; (ORI) origin; (SF) solvent front.

chloroacetamide herbicide, induces specific GST isozymes in sorghum that utilize the herbicide as a substrate (Dean et al., 1990). Other possibilities should not be precluded, however. The increased initial rate of metabolism (from 3 to 10 min after treatment) in fluxofenim-treated wheat, however, is consistent with previously reported abilities of safeners to increase the GST and/or GSH pools (Gronwald, 1989; Kreuz, 1993, and references cited therein), thereby enabling safener-treated plants to rapidly metabolize the herbicide.

TLC of Dimethenamid Metabolites. Crude extracts from safened and unsafened wheat shoots treated with [^{14}C]dimethenamid were analyzed by TLC to compare the relative abundance of dimethenamid metabolites at 5, 30, and 100 min after treatment. Qualitatively, the same dimethenamid metabolites were extracted from both safened and unsafened wheat. However, an increase in the quantity of three metabolites was evident in fluxofenim-treated wheat relative to unsafened wheat (Figure 3). A concomitant decrease in the amount of unmetabolized dimethenamid at each time point was also noted. The most abundant metabolite extracted from safened and unsafened wheat at 5 min after treatment cochromatographed with the synthetic GSH conjugate of dimethenamid. After 30 min, a second unknown metabolite with a slightly increased R_f value relative to that of the GSH conjugate was detected. This second metabolite did not cochromatograph with either standard. A third minor metabolite was also detected that cochromatographed with the synthetic cysteine conjugate of dimethenamid. After 100 min, the GSH−dimethenamid conjugate was detected at low levels, the abundance of the unknown metabolite increased in unsafened wheat or was similar to that at 30 min after treatment in fluxofenim-treated wheat, and the cysteine conjugate increased relative to its abundance at earlier time points. Because the GSH conjugate of dimethenamid was the major metabolite present after 5 min, it appears to be the initial metabolite formed in wheat. The abundance of the unknown metabolite increased from 5 to 30 min and then remained constant (fluxofenim-treated wheat) or increased (unsafened wheat) from 30 to 100 min. The abundance of the cysteine-dimethenamid conjugate increased from 30 to 100 min after treatment. The steady increase of the cysteine−dimethenamid conjugate during the time course and the concomitant decrease in the abundance of the GSH−dimethenamid conjugate at 100 min after treatment suggest that the initial GSH−dimethenamid conjugate is catabolized to the cysteine−dimethenamid conjugate and that the unknown metabolite is probably an intermediate dipeptide−dimethenamid conjugate.

Previous studies examining chloroacetamide and chloro-*s*-triazine herbicide metabolism have shown that glutathione conjugates are catabolized to γ-glutamylcysteine and cysteine conjugates in monocot crop species, where first glycine and then glutamate are sequentially removed from GSH by peptidases or related enzymes (Lamoureux and Rusness, 1989, and references cited therein). It appears that the GSH conjugate of dimethenamid is catabolized in both unsafened and fluxofenim-treated wheat between 5 and 100 min after dimethenamid treatment. TLC analysis of dimethenamid metabolites extracted from wheat shoots supports the results of the rate of metabolism study, in which similar increases in the formation of water-soluble metabolites of dimethenamid were detected in response to fluxofenim treatment (Figures 2 and 3). It appears that glutathione conjugation, followed by catabolism of the GSH conjugate, is the major route of dimethenamid metabolism in wheat and that the safener fluxofenim enhances the initial flux through this metabolic route in wheat. These results are similar to those reported by Breaux et al. (1989), who found that the pathway of metabolism of the chloroacetamide herbicide acetochlor in flurazole-safened corn seedlings was not different from that in the unsafened control, although the rate of metabolism was increased.

Isolation and Identification of Initial Dimethenamid Metabolites. Preparative HPLC and analytical HPLC were used to purify the initial metabolites of dimethenamid extracted from safened wheat shoots 1 h after treatment, and FAB MS was used to identify their structures. FAB MS was used by Breaux and colleagues to successfully identify water-soluble metabolites of the chloroacetamide herbicide acetochlor in corn and soybean seedlings (Breaux, 1986) and the GSH conjugate of the safener flurazole in corn and grain sorghum seedlings (Breaux et al., 1989). FAB MS was also used by Polge and Barrett (1995) to identify a polar metabolite of chlorimuron ethyl produced by naphthalic anhydride-induced maize microsomes and by Lamoureux et al. (1991) to identify metabolites of chlorimuron ethyl produced by corn roots.

The preparative HPLC separations of water-soluble metabolites and chloroform-soluble compounds are presented in Figures 4 and 5, respectively. Following partitioning of the concentrated aqueous crude extract, 90% of the radioactivity partitioned into the aqueous phase and 10% of the radioactivity partitioned into the chloroform phase. Four radiolabeled metabolites were resolved from the aqueous phase of the crude extract partitioning step (Figure 4). Two major metabolites,

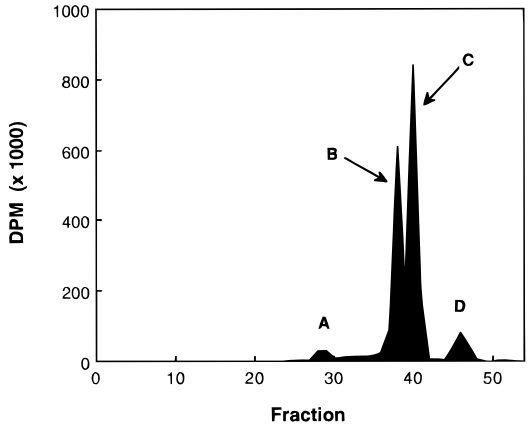

Figure 4. Radiochromatogram of preparative reversed-phase HPLC analysis of water-soluble extracts from safened wheat shoots pulse-labeled with [^{14}C]dimethenamid. A linear gradient of 0−100% acetonitrile/0.02% (v/v) TFA in 50 min was used to resolve dimethenamid metabolites. The flow rate was 3 mL/min, and 3 mL fractions were collected. Radioactivity in each fraction was determined by LSS. (Peak A) oxygenated GSH−dimethenamid conjugate; (peak B) GSH−dimethenamid conjugate; (peak C) γ-glutamylcysteine−dimethenamid conjugate; (peak D) cysteine− and cysteinylglycine−dimethenamid conjugates.

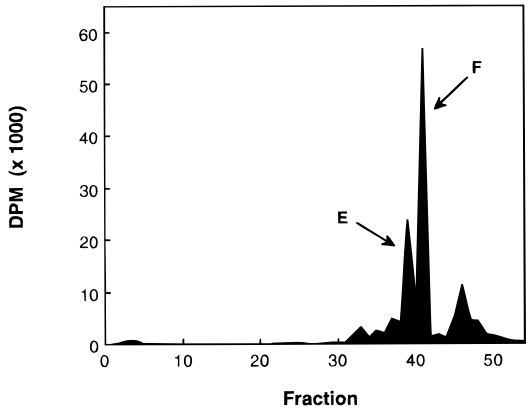

Figure 5. Radiochromatogram of preparative reversed-phase HPLC analysis of chloroform-soluble extracts from safened wheat shoots pulse-labeled with [^{14}C]dimethenamid. A linear gradient of 0−100% acetonitrile/0.02% (v/v) TFA in 50 min was used to resolve dimethenamid from dimethenamid metabolites. The flow rate was 3 mL/min, and 3 mL fractions were collected. Radioactivity in each fraction was determined by LSS. (Peak E) unidentified compound; (peak F) dimethenamid.

representing about 89% of the radioactivity recovered after preparative HPLC, and three minor metabolites, representing about 11% of the radioactivity recovered after preparative HPLC, were isolated and further purified using analytical HPLC (Table 2). The compound in peak B (Figure 4) was tentatively identified as the GSH conjugate of dimethenamid on the basis of cochromatography with a synthetic standard by HPLC and was subsequently confirmed by structural analysis via FAB MS (Table 2). A protonated molecular ion was present at m/z 547 $(M + H)^+$, and fragment ions were present at m/z 418 and 272. The fragment ion at m/z 418 $(MH - 129)^+$ is due to the loss of the γ-L-glutamyl moiety from GSH, while the fragment ion m/z 272 $(MH - 275)^+$ is due to the loss of the GSH moiety (minus the sulfur atom). The spectrum was similar to the spectrum of the synthetic GSH conjugate also analyzed by FAB MS. The most abundant water-soluble metabolite (peak C, Figure 4) was the γ-glutamylcysteine conjugate of dimethenamid on the basis of FAB MS. A protonated molecular ion produced from the compound in peak C (Figure 4) was present at m/z 490 $(M + H)^+$, but no fragment ions were detected. This ion was most likely derived from the corresponding unknown metabolite that chromatographed by TLC with a slightly increased R_f value relative to that of the GSH conjugate (Figure 3). Further purification of peak D (Figure 4) by analytical HPLC resolved two radioactive metabolites that were coeluting during preparative HPLC. The two metabolites were determined by FAB MS to be the cysteine and the cysteinylglycine conjugates of dimethenamid. The metabolite that initially eluted during analytical HPLC was the cysteine conjugate, which yielded a protonated molecular ion in FAB at m/z 361 $(M + H)^+$, while the second metabolite was the cysteinylglycine conjugate, which yielded a protonated molecular ion at m/z 418 $(M + H)^+$. Neither metabolite yielded detectable fragment ions. The spectra of both the cysteine and cysteinylglycine conjugates of dimethenamid were similar to the spectra of the synthetic standards also analyzed by FAB MS.

Cysteinylglycine conjugates are commonly detected in animals but not in plants; however, γ-glutamylcysteine conjugates are the most common dipeptide catabolites of GSH conjugates detected in plants (Rennenberg, 1982; Lamoureux and Rusness, 1989). Plants may lack the appropriate peptidase enzyme(s) to catabolize GSH conjugates to cysteinylglycine conjugates, or cysteinylglycine conjugates may be more rapidly catabolized to cysteine conjugates so that they do not accumulate at detectable levels. Wheat apparently contains the peptidase enzyme(s) necessary to catabolize the GSH−dimethenamid conjugate to both dipeptide conjugates, but in the predominant metabolic pathway the γ-glutamylcysteine conjugate forms first, followed by the cysteine conjugate.

The metabolite with the shortest retention time during preparative HPLC, peak A (Figure 4), was determined to be an oxygenated form of the GSH−dimethenamid conjugate, on the basis of the spectrum obtained by FAB MS. A protonated molecular ion was present at m/z 563 $(M + H)^+$, which corresponds to the GSH−dimethenamid conjugate plus an oxygen atom or a hydroxyl group $(MH + 16)^+$. Sulfoxides of GSH conjugates have been reported previously in plants (Lamoureux and Rusness, 1989). Whether or not this metabolite was enzymatically produced *in vivo* or is an oxidation product that occurred during sample handling and storage is not known.

Radioactive compounds isolated by preparative and analytical HPLC from the chloroform phase of the crude extract partitioning step were analyzed by EI MS. The most abundant chloroform-soluble compound (Figure 5, peak F) cochromatographed with the analytical dimethenamid standard during HPLC and was subsequently confirmed by EI MS to be unmetabolized dimethenamid. The spectra of the extracted compound in peak F and the analytical dimethenamid standard were identical. The molecular ion was present at m/z 275, and several fragment ions were present at m/z 230, 203, 154 (base peak), 138, 126, and 111. The identity of the compound in peak E (Figure 5) could not be determined by EI MS or GC/MS due to insufficient quantity following purification by analytical HPLC. Although spectra were obtained, metabolite identification could not be deduced from the available information. The compounds with the longest retention time during preparative HPLC (fractions 46−48, Figure 5) were not analyzed due to

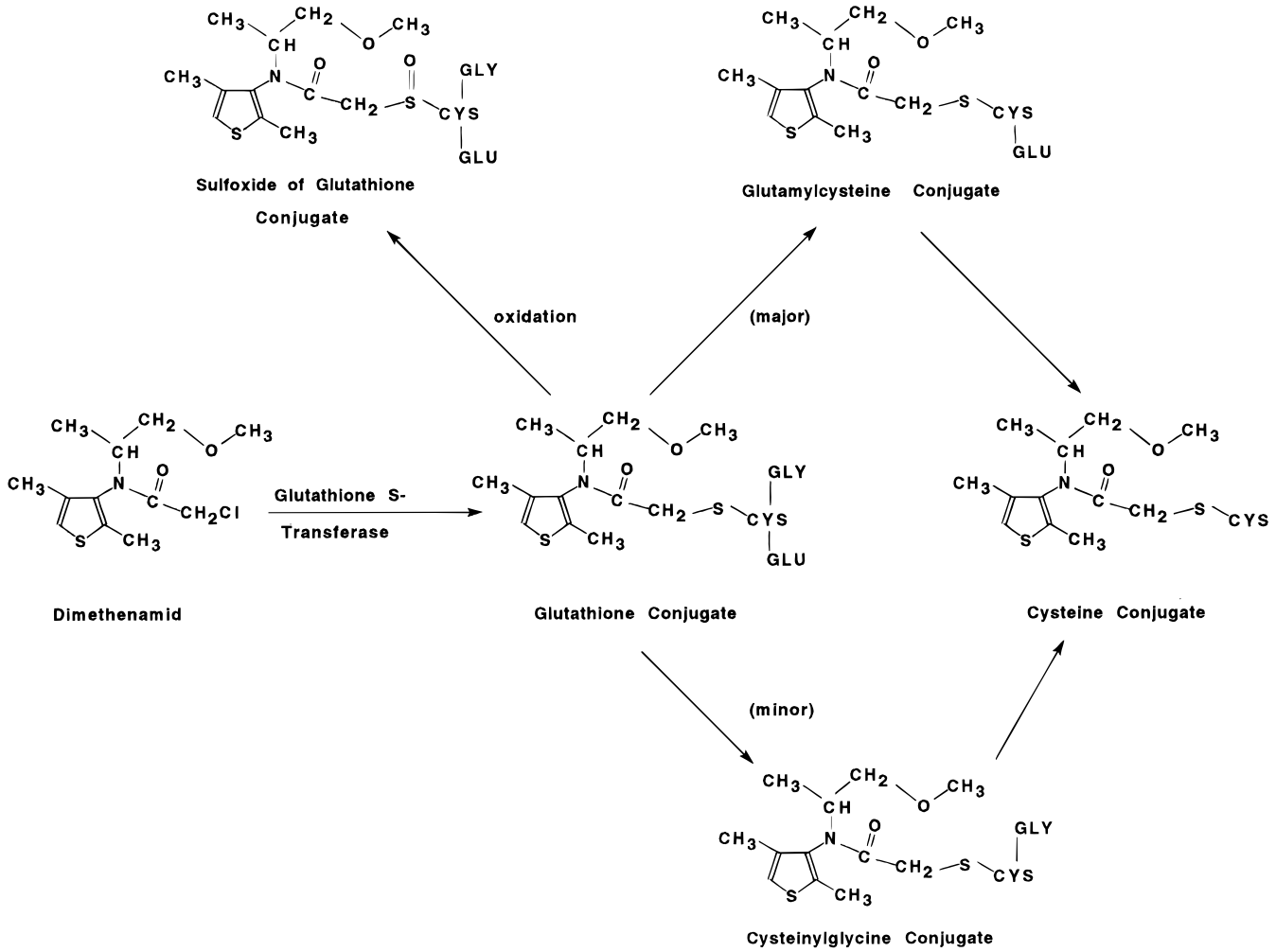

Figure 6. Proposed metabolism pathway of dimethenamid in fluxofenim-treated wheat. The sulfoxide of the GSH−dimethenamid conjugate is shown as an example of a possible oxygenated metabolite.

their low abundance. They may be trace amounts of the cysteine− or cysteinylglycine−dimethenamid conjugates that did not completely partition into the aqueous phase (compare Figures 4 and 5).

In summary, dimethenamid is initially metabolized in excised wheat shoots via the glutathione conjugation pathway. The seed-applied safener fluxofenim enhanced the rate of metabolism of dimethenamid to water-soluble conjugates, which include the GSH conjugate, catabolites of the GSH conjugate, and an oxidation product. The proposed pathway of dimethenamid metabolism in fluxofenim-treated wheat is outlined in Figure 6. Future studies will continue to evaluate the role of GST-mediated metabolism of dimethenamid in fluxofenim-treated wheat, including identification and isolation of GST isozymes that are highly induced by safener treatment and that utilize dimethenamid as a substrate.

ABBREVIATIONS USED

Dimethenamid, 2-chloro-*N*-[(1-methyl-2-methoxy)-ethyl]-*N*-(2,4-dimethylthien-3-yl)acetamide; EI MS, electron impact mass spectrometry; FAB MS, fast atom bombardment mass spectrometry; fluxofenim, 2,2,2-trifluoro-4′-chloroacetophenone *O*-(1,3-dioxolan-2-ylmethyl)oxime; GC/MS, gas chromatography/mass spectrometry; GSH, reduced glutathione; GST, glutathione *S*-transferase; HPLC, high-performance liquid chromatography; LSS, liquid scintillation spectrometry; TAPS, *N*-tris[hydroxymethyl]methyl-3-aminopropanesulfonic acid; TFA, trifluoroacetic acid; TLC, thin layer chromatography.

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