Metabolism of Metsulfuron Methyl in Wheat and Barley

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[*phenyl*-U-^{14}C]Metsulfuron methyl was metabolized in immature field-grown wheat and barley to [^{14}C]methyl 4-hydroxy-2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-benzoate and its carbohydrate conjugate. In addition to the above metabolites, [^{14}C]methyl 2-[[[[[4-(hydroxymethyl)-6-methoxy-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate was detected in greenhouse-grown wheat treated with [*triazine*-2-^{14}C]metsulfuron methyl. Other metabolites of metsulfuron methyl detected in plant tissue could be attributed to hydrolysis of the parent compound. In wheat treated with [*phenyl*-U-^{14}C]metsulfuron methyl, the total radiolabeled residues in the plant tissue decreased from 1.87 and 2.29 ppm in wheat and barley, respectively, immediately after foliar application (35 g/ha), to 0.07 and 0.22 ppm in the straw and <0.1 and 0.03 ppm in the grain at maturity.

Metsulfuron methyl (I, methyl [[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]-sulfonyl]benzoate) is an effective weed killer for use against broadleaf weeds and some grasses but is safe for use on wheat [see Beyer et al. (1987) for an excellent general review of sulfonylureas, including metabolism and environmental fate]. Plants metabolize sulfonylureas by oxidative mechanisms (chlorsulfuron: Sweetser et al., 1982; Hutchinson et al., 1984) with subsequent conjugation to glucose or by direct conjugation to glutathione (chlorimuron ethyl: Hutchinson et al., 1984). In the present study, the nature (and also the rate) of [^{14}C]metsulfuron metabolism in wheat and barley was investigated. Major metabolites were identified, and total residues present in the straw and grain at the time of harvest were determined. A preliminary report of these data appears in the review by Beyer et al. (1987).

This study was conducted as a requirement for registration of metsulfuron methyl, a terrestrial food crop pesticide (*CFR*, 1986), with the U.S. EPA. As such, the study was conducted primarily to determine the composition of the terminal residue(s) so that appropriate detection methodology and residue quantification data could be developed as mandated by the EPA (Schmidt, 1982). Such data are needed to aid in assessing the toxicological significance of residues resulting from the use of metsulfuron methyl on food-producing plants.

EXPERIMENTAL SECTION

Materials. [*phenyl*-U-^{14}C]Metsulfuron methyl, specific activity 24.8 μCi/mg, 98% radiochemical purity (New England Nuclear custom synthesis), and [*triazine*-2-^{14}C]metsulfuron methyl, specific activity 35.4 μCi/mg, >98% radiochemical purity (New England Nuclear custom synthesis), were used in these studies. When necessary, unlabeled metsulfuron methyl was added to the radiolabeled metsulfuron methyl to reduce the specific activity. Unlabeled analytical standard grade metsulfuron methyl and suggested metabolites and hydrolysis products (Figure 1) were prepared in the Agricultural Chemicals Department of E. I. du Pont de Nemours & Co., Inc., Wilmington, DE. Other chemicals and solvents were reagent grade or better. Solvents for HPLC analyses were glass distilled (Burdick & Jackson Laboratories).

Methods. *Application of [phenyl-U-^{14}C]Metsulfuron Methyl to Field-Grown Wheat.* On April 26, 1983, [^{14}C]metsulfuron methyl was foliarly applied at a nominal rate of 35 g/ha ($^1/_2$ oz/acre) to Stevens winter wheat (planted at Newark, DE, in Oct 1982) in the tiller stage. [^{14}C]Metsulfuron methyl (3.4 mg, 29.3 μCi) was dissolved in a spray solution [water/methanol/Surfactant WK spray additive (Du Pont), 90/10/0.25, v/v/v) and applied with a hand-held sprayer to a 1-m^2 plot of the wheat. When the spray solution had dried on the leaf surfaces, a representative plant sample was removed for analysis of radiolabeled materials. Thereafter, wheat was removed for analysis of radiolabeled residues at 1, 2, 4, and 9 weeks (maturity).

Quantification of Radiolabeled Residues in Wheat Tissue. Fresh wheat foliage was lyophilized on a Labconco Model 8 freeze-drier before being finely divided in a Waring blender. Grain was separated from the straw of mature wheat samples before pulverizing in a Waring blender. Samples of the freeze-dried plant tissues (10–100 mg) were combusted in a Model 306 Packard Tricarb sample oxidizer. Radiolabeled carbon dioxide, generated in the sample oxidizer, was trapped in Oxisorb–CO_2 (New England Nuclear, Boston, MA) and Oxiprep-2 scintillation cocktail (New England Nuclear) was added. Quantification of radioactivity was by liquid scintillation counting (LSC) as previously described (Harvey et al., 1985).

Analysis of Radiolabeled Residues in Wheat Tissue. Freeze-dried plant tissue was extracted three times with cold (4 °C) 80% acetone/20% H_2O on a Polytron tissue homogenizer (Brinkmann). Extraction solvent was separated from the solid material (straw) by vacuum filtration and set aside. Extracts were combined prior to LSC in Formula-947 scintillation cocktail (New England Nuclear). The acetone/H_2O-extracted straw was suspended in 0.01 M potassium phosphate buffer (pH 7.0) and stirred overnight at ambient conditions. After stirring, the supernatant was separated from the straw by filtration and analyzed by LSC in Formula-947 scintillation cocktail. The extracted straw was assayed for residual radioactivity by combustion analysis as described above.

The combined acetone/H_2O extract was concentrated to near-dryness by rotary evaporation at 45 °C and diluted to 100 mL with distilled H_2O. After adjustment to pH 3 with 1.0 M phosphoric acid, the combined extract was sequentially extracted (three times each) with methylene chloride and 1-butanol; the three methylene chloride extracts were combined, and the three 1-butanol extracts were combined. The aqueous phase was adjusted to pH 7.0 with 1.0 N NaOH after the final 1-butanol extraction. These three fractions (i.e., methylene chloride, 1-butanol, water) were evaporated to dryness by rotary evaporation at 45 °C. Residues were redissolved in either methylene chloride (for the methylene chloride fraction) or methanol (1-butanol and water fractions) and analyzed by HPLC system 1 (Table III). A second chromatographic method for analysis of the 1-butanol and water fractions used HPLC system 2 (Table III). Radiolabeled compounds in the extracts were identified by comparison of retention times with authentic reference standards.

Field-Grown Barley. Application of radiolabeled metsulfuron methyl to barley, sampling of plant tissue, quantification of radiolabel in the barley tissue, and the extraction of radiolabeled residues with acetone/water from the plant tissue were exactly the same as described for field wheat. The acetone/water barley extracts were also concentrated and partitioned as described for the wheat extracts.

The concentrated extracts were analyzed by thin-layer chromatography (TLC) with use of a solvent composed of methylene chloride/methanol/28% NH_4OH (144/50/6, TLC system 1). Radioactive compounds on the TLC plates were located by au-

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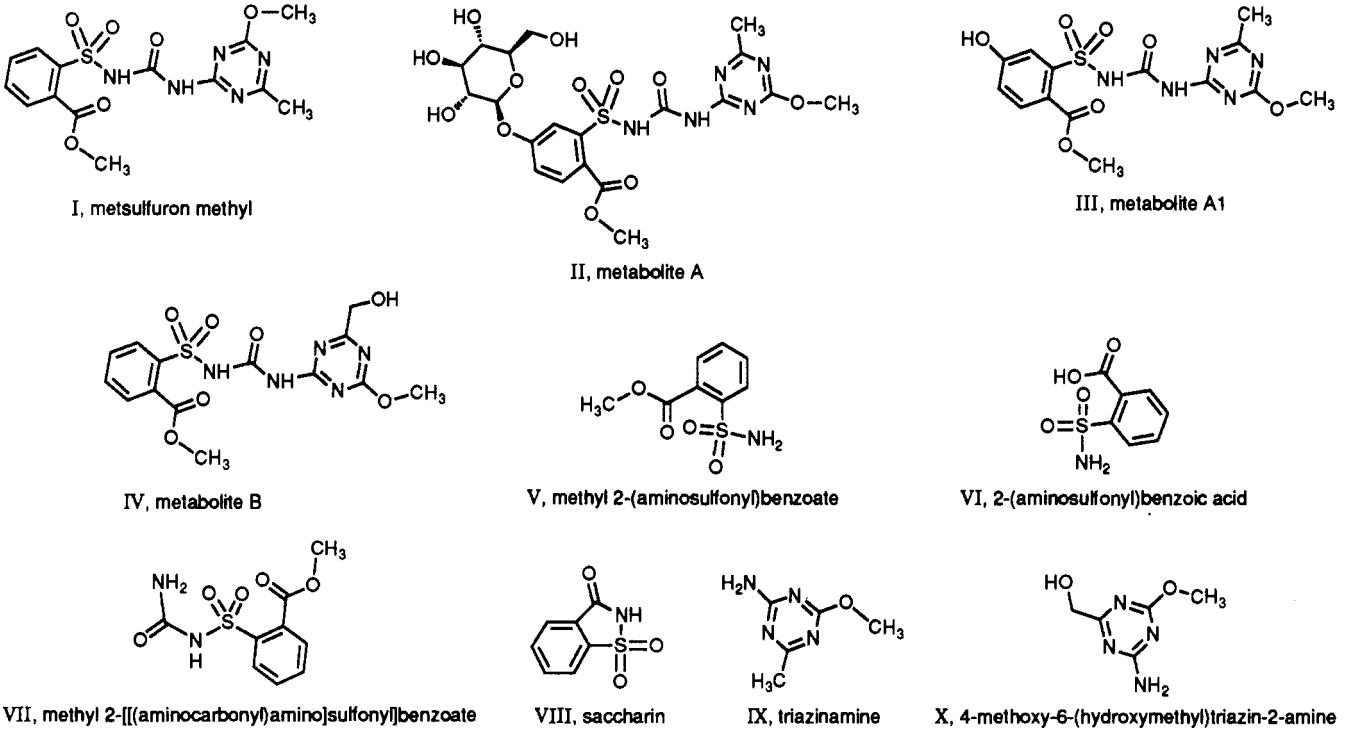

Figure 1. Structures and trivial names for metsulfuron methyl and suggested metabolites.

toradiography on Kodak AR-5 X-ray films. Visualized radioactive components were quantitated by LSC of the corresponding silica gel in a water/Formula-947 scintillation cocktail (1/3, v/v) gel.

Extracted straw (from mature barley only) was suspended in approximately 10 mL of 0.01 M potassium phosphate buffer (pH 6.0) and stirred overnight at ambient conditions. After stirring, the supernatant was separated by filtration. Radioactivity in the supernatant was quantitated by LSC in Formula-947 scintillation cocktail. Individual metabolites were separated and quantitated by HPLC system 3 (Table III). The extracted straw (or grain) was assayed for residual radioactivity by combustion analysis.

Radioactivity in the methylene chloride, 1-butanol, or water fractions of the acetone/water extract of the grain was separated and quantitated by HPLC systems 4 and 5 (Table III). Radioactive compounds, separated by TLC or HPLC and detected by either autoradiography (TLC) or fraction collection (HPLC), were identified through cochromatography with authentic reference standards.

Preparation and Isolation of Metabolite A1 (III) for Mass Spectral Analysis. Field-grown Stevens winter wheat in the boot stage was treated with [^{14}C]metsulfuron methyl (specific activity 8.62 μCi/mg) in an aqueous spray solution containing 0.25% Surfactant WK at an application rate of 28 g/ha. After 3 weeks, this wheat was harvested and stored frozen at −20 °C until used. A 0.5-kg portion of this wheat, containing both straw and immature grain, was finely chopped and ultrasonically extracted with 80% acetone/20% water. This extract was concentrated and partitioned as previously described, and the 1-butanol fraction was applied to preparative preabsorbent 1000-μm silica gel TLC plates (Whatman PLKF) and developed in TLC solvent 1. Upon development, a broad radiolabeled band (R_f 0.4) was located on the TLC plates by plate scanning (Berthold linear analyzer). This band was removed by scraping and the radioactivity extracted with methanol. After evaporation of the solvent, the material was resuspended in 0.01 M potassium phosphate buffer (pH 6.0) containing 10 mg of β-glucosidase (Type II, Sigma Chemical Co.) and incubated overnight at room temperature. Then, the mixture was adjusted to pH 3 with phosphoric acid and extracted (3×) with methylene chloride. The methylene chloride extract was applied to an analytical silica gel thin-layer plate (500 m, E. M. Science) and developed in TLC solvent 1. A sharp radiolabeled band (R_f 0.30) was located, scraped, and extracted from the silica with methanol. After concentration to dryness and redissolving in acetonitrile, this material was chromatographed on HPLC system 1. Radiolabeled species were isolated by fraction collecting at 1-min intervals. Column effluent eluting between 6 and 8 min was extracted with methylene chloride, and the methylene chloride fraction reduced in volume before application to a 25 cm × 4.6 mm Zorbax SIL HPLC column (Du Pont) and chromatographed in a mobile phase of acetonitrile/CH_2Cl_2/acetic acid/H_2O (270/1500/25/3, v/v/v/v) at a flow rate of 2 mL/min, column temperature 30 °C (HPLC system 6). Material eluting between 7.4 and 8.0 min was collected, concentrated, and redissolved in methylene chloride. This chromatographically pure ^{14}C-labeled metabolite A1 (III, methyl 4-hydroxy-2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) was stored at 4 °C in this solvent. The final yield of metabolite A1 was 20 μg. Metabolite A1 isolated from ^{14}C-treated, field-grown wheat was analyzed on a Finnigan Model 45-21 mass spectrometer equipped with a Vespel probe.

Preparation of Metabolite A1 for Nuclear Magnetic Resonance Analysis. Stevens winter wheat seedlings (2 weeks after germination) were cut off at ground level and recut under water to minimize air bubble penetration into vascular tissue. The freshly cut seedlings were placed, cut-end down, into a 200 ppm solution of [^{14}C]metsulfuron methyl (specific activity 0.41 μCi/mg) in 0.01 M phosphate buffer, pH 7.0. After 1 day, plant tissue was removed from the [^{14}C]metsulfuron methyl solution. The wheat was extracted and metabolite A1 isolated from the β-glucosidase-treated wheat extract as described above. The chromatographically pure metabolite (1 mg) was dissolved in 100 atom % deuteriochloroform (Aldrich Chemical Co.). A Bruker 360-MHz nuclear magnetic resonance spectrometer was used to obtain the FT-NMR spectrum.

Treatment of Greenhouse-Grown Wheat. [*triazine*-2-^{14}C]-Metsulfuron methyl (2.7 mg, specific activity 35.4 μCi/mg) was foliarly applied (rate ~1 oz/acre) with a hand-held sprayer to a 1-ft^2 area of Waldron spring wheat at the 3- to 5-leaf stage. When the spray solution had dried on the leaf surfaces, representative plant samples were removed and immediately frozen for later analysis of the ^{14}C-labeled residues. Additional samples were collected at 1-, 2-, and 4-week intervals.

Analysis of ^{14}C-Labeled Residues from Greenhouse-Grown Wheat. Surface residues were removed from plant tissue with use of a methanol wash (1 min) followed by a chloroform wash (1 min). Plant tissue was then extracted in 80% acetone/20% H_2O on a Waring blender. Cellular debris was separated from the extract by filtration and washed with additional extraction solvent. Residual radioactivity in the cellular debris was determined by combustion analysis.

Table I. Concentration of Total Radiolabeled Residues in Field Wheat and Barley Treated with Metsulfuron Methyl, as a Function of Time

time, weeks	ppm as [*phenyl*-U-^{14}C]-metsulfuron methyl	
	wheat	barley
0	1.87	2.29
1	0.76	0.86
2	1.08	0.71
4	0.12	0.32
9 (straw)	0.07	0.22
9 (grain)	<0.01	0.03

The acetone/water extract was reduced in volume by rotary evaporation, and the residue was resuspended in distilled water. The mixture was adjusted to pH 10 with 28% ammonium hydroxide and extracted with *n*-hexane. The aqueous phase was then lowered to pH 3.2 by addition of phosphoric acid and sequentially extracted with methylene chloride and 1-butanol. The radioactivity in each fraction was determined by LSC in Atomlight scintillation cocktail (New England Nuclear).

The three fractions derived from the original 80% acetone/20% water extract (i.e., methylene chloride, 1-butanol, and water) and the two surface washes (methanol and chloroform) were each evaporated to dryness by rotary evaporation. The residues from the methanol wash, the 1-butanol fraction, and the water fraction were redissolved in methanol, while the chloroform wash was redissolved in chloroform and the methylene chloride fraction was redissolved in methylene chloride. These fractions, along with appropriate reference standards, were analyzed by TLC system 1 (Table II) at 4 °C. After development, radioactivity was located by autoradiography on Kodak AR-5 X-ray film and/or with a Berthold LB-2852 automatic scanning TLC linear analyzer. Identification of the separated compounds was based on cochromatography with authentic standards.

RESULTS

Field-Grown Wheat Treated with [*phenyl*-U-^{14}C]-Metsulfuron Methyl: Concentration of Total Radiolabeled Residues in Wheat Tissue. The concentration of radioactive residues in tissues from wheat foliarly treated with [^{14}C]metsulfuron methyl decreased from 1.87 ppm immediately after application to 0.07 ppm in the straw at harvest. The grain contained less than 0.01 ppm at harvest. (See Table I.)

Extraction of Radiolabeled Residues from the Wheat Tissue. Radioactive residues in the wheat tissues were, with the exception of mature straw samples, readily extractable with 80% acetone/20% H_2O. On an average, 90% of the radioactivity in the 0-, 1-, 2-, and 4-week wheat samples was extracted with use of this solvent mixture. An additional 7% (on an average, of the total radioactivity) was extractable with the pH 7 phosphate buffer. Most of the residues in the mature straw could not be extracted by the 80% acetone/20% H_2O extraction solvent. However, the remaining phosphate buffer extractable and unextractable (bound) residues were present at concentrations of 0.02 and 0.03 ppm, respectively. Mature grain samples were not analyzed because the total residues were less than 0.01 ppm.

Identification of Radiolabeled Residues. Radiolabeled residues present in the methylene chloride, 1-butanol, and aqueous fractions were analyzed in either HPLC system 1 (methylene chloride soluble) or system 2 (1-butanol and aqueous soluble). Identification was made by comparison of the retention times of the radiolabeled compounds with those of authentic reference standards [see Table II (TLC) and Table III (HPLC) for the chromatographic behavior of metsulfuron methyl and its wheat metabolites]. Methylene chloride extracts contained metsulfuron methyl and an unknown with a retention time of 5.0 min in HPLC system 1 (metabolite A1; Figure 1); methyl 2-(aminosulfonyl)benzoate (V), a suggested hydrolysis product of metsulfuron methyl similar to that seen with a similar sulfonylurea herbicide, sulfometuron methyl (Harvey et al., 1985), was also present in the methylene chloride extracts. The major radiolabeled compound in the 1-butanol extracts was an unknown with a retention time of 5.6 min in HPLC system 2 [metabolite A (II, methyl 4-(β-D-glucopyranosyloxy)-2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]-sulfonyl]benzoate) Figure 1]. Suggested hydrolysis products of metsulfuron methyl were also present in the 1-butanol fraction and in the aqueous fraction. An additional compound, methyl 2-[[(aminocarbonyl)amino]-sulfonyl]benzoate (VII), was also detected. This compound could conceivably occur as a result of hydrolysis of metsulfuron methyl on the triazinamine side of the urea bridge. The small amount of radioactivity in the pH 7 phosphate buffer extracts was not characterized because of the low level (average of 7% of the total radioactivity in the 0-, 1-, 2-, and 4-week samples) of radioactive species in the presence of the solubilized plant debris. All fractions contained some radioactivity that was either spread diffusely throughout the HPLC chromatograms or was unretained in HPLC system 2. These residues correspond to a concentration of only 0.01 ppm in mature straw.

Characterization of Metabolite A. Studies of the metabolism of chlorsulfuron in wheat (Sweetser, et al., 1982) have shown that the major metabolite of this sulfonylurea herbicide was the glucose conjugate of a hydroxylated chlorsulfuron. These results suggested that metabolism by wheat of the sulfonylurea herbicide under investigation in this study, metsulfuron methyl, may follow

Table II. Thin-Layer Chromatographic[a] Behavior of Metsulfuron Methyl and Suggested Metabolites

no.	compound	R_f
I	metsulfuron methyl	0.58
II	metabolite A	0.09
III	metabolite Al	0.16
IV	metabolite B	0.42
V	methyl 2-(aminosulfonyl)benzoate	0.80
VI	2-(aminosulfonyl)benzoic acid	0.17
VIII	saccharin	0.38
IX	triazinamine	0.83
X	(hydroxymethyl)triazinamine	0.63

[a] Silica gel thin-layer chromatography plates were developed with methylene chloride/methanol/28% NH_4OH (144/50/6, TLC system 1). Plates were developed at either ambient temperature or 4 °C.

Table III. High-Performance Liquid Chromatography (Retention Times in Minutes)

no.	system[a]				
	1	2	3	4	5
I	6.9			20.9	6.2
II	5.6	5.6		13.9	
III	5.0				
V	2.8	3.8	12.0		2.8
VI		1.9			
VII		3.1			
VIII		1.2	3.5	3.8	

[a] All separations were performed on a 25 cm × 4.6 mm Zorbax C8 chromatographic column at a flow rate of 2 mL/min and a column temperature of 45 °C. The mobile phase in all HPLC systems was a mixture of acetonitrile (A) and pH 2.2 H_2O (formic or phosphoric acid; B). Chromatographic conditions: (1) 30% A/70% B; (2) 20% A/80% B; (3) sequentially, 2% A for 5 min, a linear gradient up to 10% A over 10 min, 100% A for 5 min; (4) sequentially, linear gradient from 10% A to 40% A over 20 min, 40% A for 5 min, 100% A for 5 min; (5) 35% A/65% B.

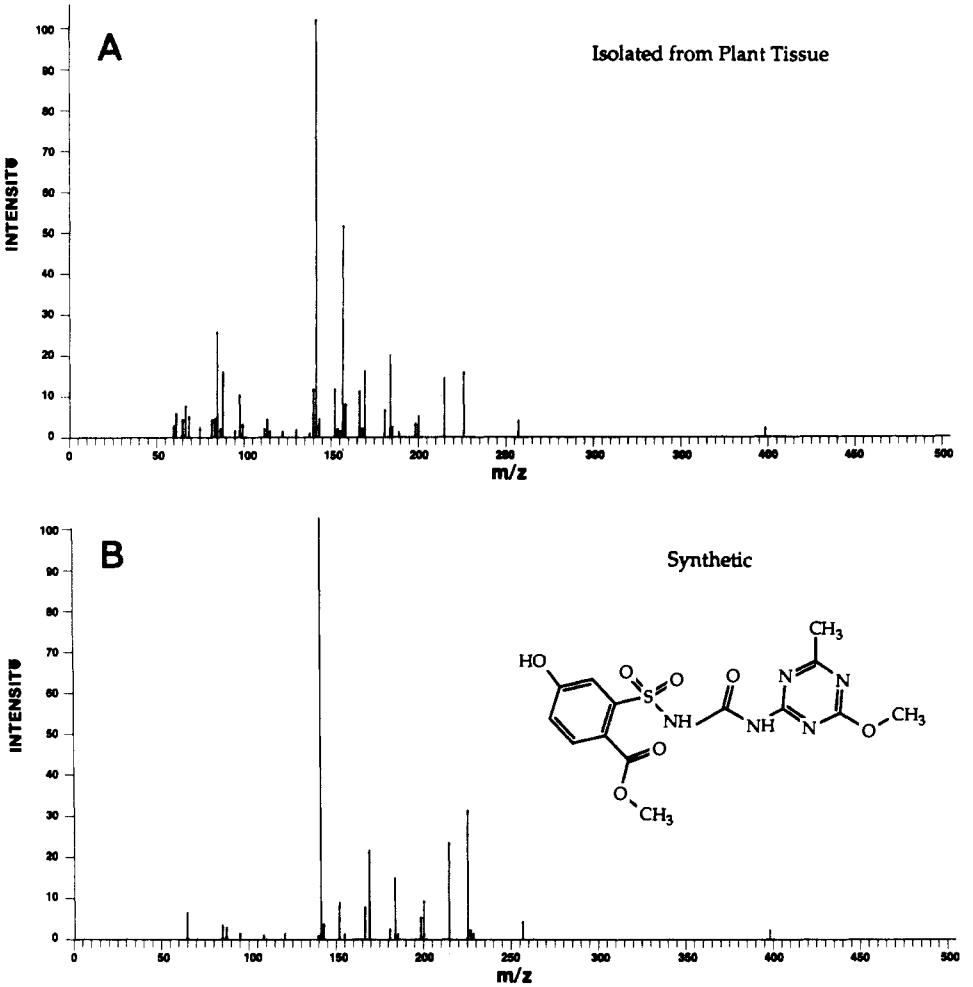

Figure 2. Mass spectrum of metabolite A1 isolated from wheat (A) and synthetic metabolite A1 (B). The following experimental conditions were used: solvent, methylene chloride; instrument, Finnigan Model 45-21 mass spectrometer; probe, Vespel; source temperature, 120 °C.

a similar pattern. The purified [^{14}C]metabolite A1 generated through enzymatic cleavage of metabolite A (isolated from field-grown wheat) comigrated with synthetic metabolite A1 in both HPLC system 1 (retention time 7.0 min) and in HPLC system 2 (retention time 7.4 min). When it was analyzed by mass spectrometric techniques, metabolite A1 had a weak parent ion (Figure 2) of mass 398 (M + 1) consistent with the structure proposed of a hydroxylated metsulfuron methyl. The mass spectrum of metabolite A1 (III) purified from wheat exhibited a close resemblance to the mass spectrum of the synthetic 4-hydroxy metabolite (Figure 2). Examination of the NMR spectrum of metabolite A1 (III) (Figure 3) confirmed the location of the hydroxyl groups (position 4 of the phenyl ring). The splitting pattern in the aromatic region of the spectrum was identical with that obtained from the synthetic standard (spectrum not shown). The sharp peak at 7.1 ppm was due to a chloroform impurity in the sample.

Metabolite A was prepared from synthetic metabolite A1 by the method of Yamaha and Cardini (1960). Metabolite A prepared in this manner was used as a chromatographic standard for the analysis of extracts of field wheat and barley treated with metsulfuron methyl.

Concentration of Radiolabeled Residues in Wheat Tissue. Metabolite A is the primary extractable metabolite of metsulfuron methyl in wheat. Metabolite A1, the exocon of metabolite A, is presumably the primary metabolite, which is conjugated with glucose (or another carbohydrate) to yield metabolite A. The concentration of metsulfuron methyl decreases rapidly with a first half-life of 1 week (Table IV). The concentration as a percent of the total residue of metabolite A increases rapidly during the first 2 weeks and then decreases as it presumably degrades to other metabolites (Figure 4). The concentration of any of the previously mentioned hydrolysis products of metsulfuron methyl did not exceed 0.15 ppm at any time. In mature straw, these metabolites had decreased to a concentration of 0.01 ppm or less.

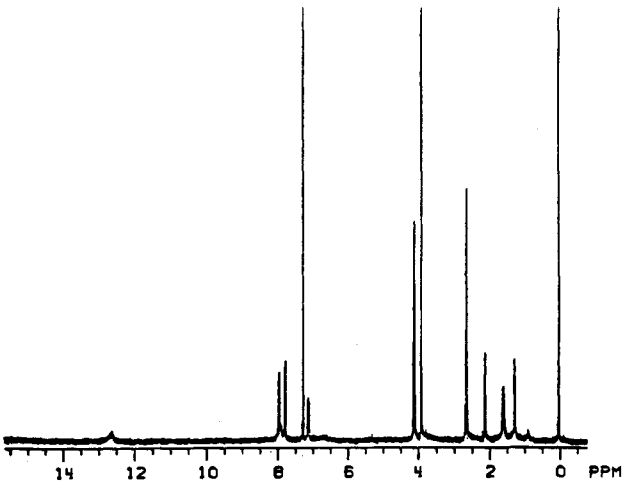

Figure 3. NMR spectrum of metabolite A1 purified from wheat. The following experimental conditions were used: solvent, deuteriochloroform; instrument, Bruker 360-MHz nuclear magnetic spectrometer; spectrum type, FT-NMR.

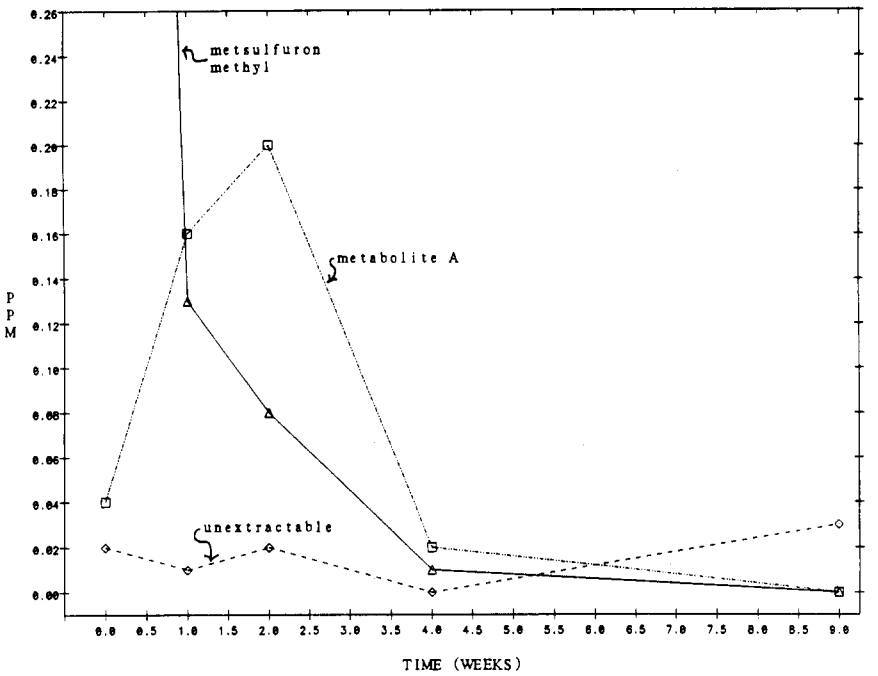

Figure 4. Residues of metsulfuron methyl, metabolite A, and unextractable residues in wheat tissue as a function of time (expressed as ppm metsulfuron methyl equivalents).

Table IV. Concentration of Metsulfuron Methyl, Metabolite A, Metabolite A1, and Unextractable Residues in Wheat as a Function of Time

time, weeks	ppm as [*phenyl*-U-^{14}C]metsulfuron methyl			
	I	II	III	unextr
0	1.40	0.04	0.04	0.02
1	0.13	0.16	0.03	0.01
2	0.08	0.20	0.03	0.02
4	0.01	0.02	0.01	<0.01
9 (straw)	<0.01	<0.01	<0.01	0.03
9 (grain)	<0.01	<0.01	<0.01	<0.01

Although metabolite A is the primary extractable metabolite of metsulfuron methyl in wheat, the concentration of unextractable ("bound", Table IV) radioactivity is actually the major residue in mature wheat. The kinetics of formation of the bound residues (Figure 4) suggest that bound residues arise from further metabolism of metabolite A, since the increase in the level of bound residues accompanies a decrease in the concentration of metabolite A.

Field-Grown Barley Treated with [*phenyl*-U-^{14}C]-Metsulfuron Methyl: Concentration of Total Residues in the Barley Tissue. The concentration of radioactive residues in tissues from barley foliarly treated with [^{14}C]metsulfuron methyl decreased from 2.29 ppm immediately after application to 0.22 ppm in the straw and 0.03 ppm in the grain of mature barley (Table V).

Extraction of Radiolabeled Residues from the Barley Tissue. Radioactive residues in the barley tissues were, with the exception of mature straw samples, readily extractable with the 80% acetone/20% H_2O extraction solvent. On an average, 94% of the radioactivity in the 0-, 1-, 2-, and 4-week barley samples was extracted with use of this solvent mixture. In mature straw, only 52% of the radioactivity (0.11 ppm) was extracted (into the 80% acetone/20% H_2O solvent). An additional 15% of the total radioactivity (0.03 ppm) was removed by the pH 6 phosphate buffer, leaving 33% of the total (0.07 ppm) as unextracted (bound) residue. Mature grain samples contained 0.02 ppm acetone/H_2O extractable radioactivity and 0.01 ppm unextractable radiolabeled residues.

Table V. Concentration of Metsulfuron Methyl, Metabolite A, Metabolite A1, and Unextractable Residues in Barley as a Function of Time

time, weeks	ppm as [*phenyl*]-U-^{14}C]metsulfuron methyl			
	I	II	III	unextr
0	1.91	0.04	0.06	0.11
1	0.40	0.22	0.05	0.05
2	0.18	0.22	0.05	0.03
4	0.03	0.17	0.02	0.03
9 (straw)	0.01	0.02	0.02	0.07
9 (grain)	<0.01	<0.01	<0.01	0.01

Identification of Radiolabeled Residues in Barley Tissue. Radiolabeled residues present in the methylene chloride, 1-butanol, aqueous fractions from barley sampled at 0–4 weeks, and mature straw were analyzed by TLC. Radiolabeled compounds in the phosphate buffer (from mature straw) and the methylene chloride, 1-butanol, and aqueous fractions of the mature grain were analyzed by HPLC. Identification was made by comparison of the retention times of the radiolabeled compounds with those of authentic reference standards (see Tables II and III). As was seen in the analysis of wheat extracts, methylene chloride extracts contained metsulfuron methyl, metabolite A1, and methyl 2-(aminosulfonyl)benzoate. The major radioactive compound in the 1-butanol extracts was metabolite A. The metsulfuron methyl hydrolysis products were also present in the 1-butanol and aqueous fractions. The grain contained less than 0.01 ppm of any of the identified metabolites. All fractions contained some unidentified radioactivity that did not cochromatograph with standards in TLC system 1 or HPLC systems 3–5. These residues correspond to 0.03 ppm in mature straw and 0.02 ppm in grain.

Concentration of Radiolabeled Residues in Barley Tissue. Metabolite A is the primary extractable metabolite of metsulfuron methyl in barley. As was observed in wheat, the concentration of metabolite A, expressed as a percent of the total radioactive residues in the barley, as a function of time (Table V), follows the behavior expected for a metabolite of metsulfuron methyl. The decrease in concentration of metsulfuron methyl with time

coincided with an increase in the concentration of metabolite A, followed by its subsequent decrease. Suggested metsulfuron methyl hydrolysis products did not exceed a concentration of 0.04 ppm at any of the sampling intervals. In mature straw, the hydrolysis products were present at a concentration of 0.01 ppm or less.

As was observed in wheat, the major radiolabeled residue in mature barley was bound material.

Metabolism of [*triazine-2*-^{14}C]Metsulfuron Methyl in Greenhouse-Grown Wheat. Radioactivity in the methanol, $CHCl_3$, and CH_2Cl_2 extracts was comprised mainly of parent compound, while radioactivity in the 1-butanol and water extracts was primarily metabolites. The extremely low levels of radioactivity in the 1-hexane fractions were not characterized.

Inspection of the autoradiograms and linear scans obtained from thin-layer chromatography of the plant extracts detected radiolabeled compounds comigrating with metsulfuron methyl, metabolite A1, metabolite B [IV, methyl 2-[[[[4-(hydroxymethyl)-6-methoxy-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]benzoate] (Figure 1), the triazinamine [IX, 4-methoxy-6-methyl-1,3,5-triazin-2-amine] (Figure 1), and its hydroxy analogue (X) (Figure 1). Metabolite B was not detected in extracts of wheat treated with [*phenyl*-U-^{14}C]metsulfuron methyl. Except for metsulfuron methyl, none of these compounds comprised more than 9% of the total radioactivity at any sampling time. As was seen with field wheat or barley treated with [*phenyl*-U-^{14}C]metsulfuron methyl, the major radiolabeled metabolite in [*triazine-2*-^{14}C]metsulfuron methyl treated wheat was metabolite A (identified solely on the basis of its chromatographic behavior). This compound (present mainly in the 1-butanol fraction) increased steadily with time (when expressed as a percent of the total radioactivity) and accounted for 13% of the total radioactivity in the 4-week sample.

DISCUSSION AND CONCLUSION

The recommended use rate of Ally herbicide is $^1/_{10}$ oz/acre of a formulation containing 60% metsulfuron methyl. Thus, the level of metsulfuron methyl applied to wheat and barley in the present study was eight times the expected use rate of metsulfuron methyl. The tolerance for metsulfuron methyl and metabolite A1 in green forage is 5 ppm (*40 CFR*, Section 180). In spite of the exaggerated levels of metsulfuron methyl applied in the present work, the levels of *total* radioactive residues expressed as ppm metsulfuron methyl in the straw and grain of mature wheat were below the tolerances in straw of 0.1 and 0.05 ppm in grain for combined metsulfuron methyl and metabolite A1. Similar results were observed in mature barley where the concentration of metsulfuron methyl in straw was 0.01 ppm and the concentration in grain was <0.01 ppm. Residues of metsulfuron methyl in green forage immediately after application of this exaggerated level of metsulfuron methyl never exceeded 1.91 ppm. The metabolites of metsulfuron methyl observed in wheat and barley extracts are consistent with the presence of dual metabolic pathways. Metabolism occurs by hydroxylation of the parent compound followed by conjugation with glucose or by hydroxylation of the methyl substituent on the heterocycline ring. Hydrolysis of the sulfonylurea bridge resulting in the formation of methyl 2-(aminosulfonyl)benzoate, 2-(aminosulfonyl)benzoic acid, saccharin [VIII, 1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide], methyl 2-[[(aminocarbonyl)amino]sulfonyl]benzoate (VII), the triazinamine (IX), and its hydroxy analogue (X) can also occur. A schematic diagram of this metabolic pathway as described can be found in the reference by Beyer et al. (1987). Bound residues were the apparent terminal residue of metsulfuron methyl in wheat and barley.

Bound residues customarily arise when herbicides, especially aromatic herbicides, are applied to plants (Hatzios and Penner, 1982) and can arise from complexation or incorporation of herbicide metabolites into plant natural products. The kinetics of disappearance of metsulfuron methyl and appearance of metabolite A in wheat and barley support a mechanism of this sort in the metabolism of metsulfuron methyl. Transient formation of metabolite A and bound residues accompanied the disappearance of metsulfuron methyl. The appearance of bound residue was retarded compared to the formation of metabolite A, suggesting that after metabolite A is formed it, or further degradation products of it, is further complexed to natural plant constituents, rendering them unextractable with the method employed.

We have referred to metabolite A as the glucose conjugate of metabolite A1, based on release of this exocon upon β-glucosidase treatment. However, release of metabolite A1 from metabolite A upon β-glucosidase treatment is not proof of a glucose conjugate; β-glucosidase (EC 3.2.1.21) can hydrolyze other glycosidic linkages (Heyworth and Walker, 1962). Nevertheless, synthetic metabolite A, prepared from synthetic metabolite A1 and UDP–glucose with wheat germ UDP–glucosyl transferase, did cochromatograph with metabolite A from wheat and barley.

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