Metabolism of Halosulfuron-methyl by Corn and Wheat

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Halosulfuron-methyl is the active ingredient in a new class of herbicide products by Monsanto which are used for the control of broadleaf and sedge weeds in corn, turf, sugarcane, and other crops. The metabolic basis for the corn and wheat selectivity of halosulfuron-methyl was studied using cell suspension cultures and young seedlings. Both seedlings and cell suspensions were found to completely metabolize halosulfuron-methyl within hours of incubation, suggesting that corn and wheat tolerance to halosulfuron-methyl was due to rapid metabolism or detoxification. In marked contrast, in a halosulfuron-methyl sensitive species such as soybean very little metabolism occurred. The key metabolic route by which corn rapidly inactivates halosulfuron-methyl was found to be hydroxylation of the pyrimidine ring followed by rapid conjugation with glucose. The predominant pathway in wheat was O-demethylation of the methoxy group of the pyrimidine ring. Further oxidative biotransformation of the early primary metabolites led to the cleavage of the pyrimidine ring to form pyrazolesulfonamide as a terminal metabolite.

Keywords: *Sulfonylurea herbicide; halosulfuron-methyl; plant metabolism; wheat; corn; soybean; cell suspension cultures*

INTRODUCTION

Halosulfuron-methyl, methyl 3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1*H*-pyrazole-4-carboxylate, is the active ingredient in a new low use rate sulfonylurea herbicide which was recently registered by the U.S. Environmental Protection Agency (EPA) for postemergence broadleaf weed and sedge control in field corn, grain sorghum, turf, and sugarcane. It is formulated as a herbicide under the trade names of Permit for use on corn and grain sorghum, Manage for use on turf, and Sempra for use on sugarcane (Halosulfuron-methyl Technical Data Sheet, Monsanto Company). Halosulfuron-methyl has high unit herbicidal activity with use rates ranging from 18 to 70 g of active ingredient per hectare (g ai/ha). At this application rate, halosulfuron-methyl provides effective postemergence control of velvet leaf (*Abutilon theophrasti*), common cocklebur (*Xanthium strumarium*), and several other key broadleaf weeds (Bradshaw and Ricotta, 1993). Similar to other sulfonylureas, the site of action of halosulfuron-methyl is inhibition of acetolactate synthase (ALS), an enzyme involved in branched-chain amino acid biosynthesis (Ray, 1986; Schloss, 1990). Little is known about the metabolic behavior of halosulfuron-methyl and its degradation in plants. The metabolism of halosulfuron-methyl in corn, wheat, and soybean has now been studied using cell suspension cultures and young seedlings. The main objective of the present study was to elucidate the degradation pattern of halosulfuron-methyl in corn and wheat (tolerant crops) and to compare its metabolism in soybean (susceptible crop).

MATERIALS AND METHODS

Chemicals. Halosulfuron-methyl labeled with ^{14}C in the 4-position (Figure 1) of the pyrazole ring (25.7 mCi/mmol; radiochemical purity 98%) was synthesized at DuPont NEN Products, Boston, MA. Authentic standards of halosulfuron-methyl and metabolites **II**, **III**, **IV**, **VI**, and **VIII** were obtained from Nissan Chemical Industries, Ltd, Tokyo, Japan (see Yamamoto et al., 1992 and 1987, for relevant general synthetic methods). High-performance liquid chromatography solvents were obtained from Fisher Scientific (St. Louis, MO). All other reagents and solvents were obtained from commercial suppliers and used without further purification.

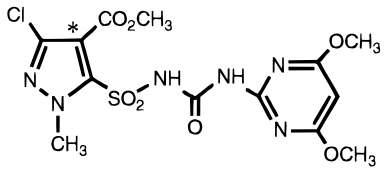

Figure 1. Chemical structure of halosulfuron-methyl. Asterisk shows position of ^{14}C radiolabel.

Plant Cell Cultures. Black Mexican sweet (BMS) corn suspension cells were cultured according to procedures described by Ludwig et al. (1985). Flasks containing about 50 mL of suspension media containing 5−10 mL of cells were shaken at 25 °C in the dark at 150 rpm overnight following the normal method of preparation. They were dosed in a sterile environment with stock solutions of [^{14}C]halosulfuron-methyl or its isolated ^{14}C-metabolites in acetone in order to give a final concentration in the range of 0.3−4.0 ppm. The cultures were incubated for 1, 3, 6, 24, 72, and 168 h after application. At the conclusion of the treatment period, the cells were immediately separated from the culture medium by vacuum filtration. The cells were weighed and extracted with aqueous organic solvent as described below. Flasks containing 50 mL of suspension media (minus cells) were dosed with the above dosing solutions as hydrolytic degradation controls. The same procedure was followed for experimentation with soybean and wheat cell suspension cultures. The soybean cells were derived from hypocotyls of aseptically germinated Peking variety soybean seeds (Gamborg et al., 1983), and the wheat cells (cv. Mustang) were obtained according to methods previously published (Wang and Nguyen, 1990).

Seedlings. Corn seeds (PN 3394) were germinated on moist filter paper inside plastic cups at room temperature in the dark. Seedlings (about 4 days old) were dosed with 7.6 μg (0.45 μCi) of [^{14}C]halosulfuron-methyl in 8 μL of acetone using a microsyringe to dot the stems with small droplets. Seedlings were returned to the darkness and eight treated seedlings were harvested 1, 3, 6, and 24 h after treatment. At harvest,

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seedlings were separated into shoots and roots, washed liberally with aqueous acetonitrile to remove the surface residues, and homogenized as described below. Soybean seeds (Williams 82) were germinated in finely-ground, wetted vermiculite. After 7 days of germination, seedlings were dosed with 7.6 μg (0.45 μCi) of [^{14}C]halosulfuron-methyl in 8 μL acetone as described above (stems were dosed below the cotyledon and above the surface of the vermiculite). After the application of halosulfuron-methyl, four sets of six treated seedlings were incubated for 1, 3, 6, and 24 h in the dark. At harvest, seedling shoot tissues were separated from the roots, washed extensively with aqueous acetonitrile to remove the surface residues, and homogenized as described below.

Extraction Procedure. The general extraction procedure for cell and seedling samples consisted of blending the entire sample in a high speed homogenizer for 5−10 min with 10−50 mL of ice-cold 40% aqueous acetonitrile followed by vacuum filtration through a Whatman glass fiber filter. The filter cake was re-extracted as necessary using the procedure described above until an insignificant amount of radioactivity was removed. Aliquots of the air-dried filter cakes were combusted and analyzed by liquid scintillation counting (LSC) in order to determine the amount of non-extracted radioactivity. The extracts were combined and concentrated by rotary evaporation at a bath temperature of <40 °C to a small volume for analysis by HPLC to determine the nature and the amount of each metabolite.

Radioassay. Total radioactivity present in samples was quantified by liquid scintillation counting (LSC) performed with Tracor Analytic Mark III model 6881 counters. The determination of the unextracted radioactive residue levels in samples was performed by combustion analysis using a Packard Tri-Carb automatic sample oxidizer (Packard Instrument Co., model 306). This instrument automatically oxidized the residues to $^{14}CO_2$ then trapped the $^{14}CO_2$ in vials of a solution of Carbo-Sorb and Permafluor V (Packard Instrument Co.). The vials were then counted by LSC. All samples were analyzed in at least three replicates.

High-Performance Liquid Chromatography (HPLC) with Radioactive Flow Detection. The HPLC system used for chromatographic analysis of sample extracts was comprised of the following components: A Rheodyne model 7125 syringe-loaded sample injector, a Waters model 680 gradient controller, Waters model 510 solvent pumps, a Waters model 440 or 484 variable wavelength UV detector (set at 254 nm), a Radiomatic Flo-One β radioactive flow detector with either a 2.5 mL liquid flow cell or a 250 or 500 μL XE solid scintillant cell, and an ISCO model 328 fraction collector. Atomflow liquid scintillation cocktail (NEN Co.; Boston, MA) was mixed with the column effluent at a flow rate of 9 mL/min yielding a ratio of 3:1 (cocktail:effluent). An Eldex model E-120-SRM pump (Radiomatic; San Carlos, CA) was used to pump the scintillation cocktail. The HPLC column was a Beckman Ultrasphere-ODS column (5 μm packing, 10 mm × 25 cm) in conjunction with a Brownlee Spheri-10 RP-18 precolumn (10 μM packing, 4 mm × 3 cm). The column was eluted (flow rate: 3 mL min^{-1}) with 90% water containing 0.1% trifluoroacetic acid and 10% acetonitrile for 5 min followed by a linear gradient to 100% acetonitrile over the next 30 min. The paired ion chromatography was performed under identical conditions except that the aqueous solvent contained 0.005 M tetrabutylammonium hydroxide in addition to 0.1% trifluoroacetic acid.

Isolation and Identification/Characterization of Metabolites. Isolation and purification of metabolites was achieved by repeated injection of ≤1.0 mL of the concentrated cell extracts or media onto a semipreparative HPLC column and manual collection of the metabolite fractions. The previously described HPLC instrumentation was also used for the preparative HPLC work under identical conditions except that the radioactive flow detector was fitted with a 500 μL XE solid scintillant cell which allowed the eluting radioactive components to be monitored during fraction collection. Radiochemically pure metabolites were prepared by pooling replicate metabolite fractions, removing the solvent by rotary evaporation, and dissolving the residue in aqueous acetonitrile. Where mass spectroscopy was used for metabolite identification, additional cleanup was provided by elution through SPE PrepSep silica gel or C18 columns. These purifications were performed using solvents which gradually changed from 100:0% hexane:ethyl acetate to 0:100% hexane:ethyl acetate when a silica column was used and 100:0% H_2O:acetonitrile to 0:100% H_2O:acetonitrile when a C18 PrepSep column was used. The eluents containing the metabolite fraction were concentrated under a stream of nitrogen gas and dissolved in methanol or acetone. Identification of metabolites was accomplished by HPLC cochromatography with authentic standards (when available) under one or more conditions and by mass spectral analysis.

A portion of the purified metabolite **V** (0.3 μg) was incubated with 0.2 mg of β-glucosidase (Sigma Chemical Co., St. Louis, MO) in 1 mL of 100 mM sodium acetate buffer, pH 4.8, at room temperature. Another portion of metabolite **V** was added to the above buffer solution in the absence of β-glucosidase in order to serve as a hydrolytic degradation control. After 15 min of incubation both samples were analyzed by HPLC to determine the extent of enzymatic digestion.

Mass Spectrometry. High-pressure liquid chromatograph electrospray ionization mass spectra (HPLC/ESI/MS) were recorded with a Perkin Elmer-Sciex API 100 single-quadrupole mass spectrometer (Thorhill, Ontario, Canada) and processed with a Macintosh Power PC 8100/100 computer using the API 100/300 software v. 1.2. The sample was initially introduced into a Rheodyne (no. 8125) injector which was connected with an ABI140A Solvent Delivery System at a rate of 40 μL/min of mobile phases, linearly programmed from 10% to 95% acetonitrile in water containing 1% formic acid in 30 min. The HPLC column was an ABI C-18 microbore column (1 × 250 mm) in conjunction with an Upchurch Scientific guard column (Oak Harbor, WA). Ionization was achieved with high voltage application (−4000 to −4500 V) on the API unit. Nitrogen gas was used as the nebulizing gas. The spectrometer was generally scanned from 200 to 700 amu at a rate of 3.5 s. Negative ions were recorded. High-resolution fast atom bombardment mass spectra (HR/FAB/MS) were recorded with a JEOL SX-102A double-sector high-resolution mass spectrometer (Tokyo, Japan) and processed with a Hewlett Packard Apollo Series 425 computer using the JEOL Complement X software. The samples were deposited on a thin layer of glycerol and then introduced into the mass spectrometer by direct probe. Ionization was achieved with a fast atom gun producing 3 kV xenon atoms at 10 mA emission current. The mass spectrometer was adjusted at 5000 resolution and was voltage-scanned from 365 to 461 amu for 6.25 s with 0.20 s inter-scan time. Two glycerol peaks at *m/z* 367 and 459 were used as the reference standards for high-resolution analysis. Negative ions were recorded.

RESULTS AND DISCUSSION

Metabolism of Halosulfuron-methyl in Cell Cultures. The distribution of ^{14}C-radioactivity in the cell suspension cultures of corn, wheat, and soybean during a seven day incubation with halosulfuron-methyl is shown in Table 1. For corn and wheat most of the applied ^{14}C-radioactivity was found in the tissue culture medium, whereas a relatively small amount of applied activity was extractable from the cells. In the soybean cell suspension, practically all of the ^{14}C-radioactivity was found in the culture medium. Despite the low partition of radioactivity between media and cells, HPLC analysis of the culture media revealed that halosulfuron-methyl was rapidly degraded in BMS corn and wheat cell cultures whereas in soybean very little breakdown of halosulfuron-methyl was detected. Table 1 demonstrates that while 86% of the applied radioactivity was recovered from the soybean cell culture as unchanged halosulfuron-methyl at the end of a seven-day incubation period, no parent halosulfuron-methyl

Table 1. Time Course Study with Halosulfuron-methyl[a]

species	incubation time (h)	applied radioactivity (% of dose)[b] culture media	applied radioactivity (% of dose)[b] cell extracts[c]	total radioactivity as unchanged parent (%)
corn	1	99	0.3	70
	3	95	1	13
	6	102	1	4
	24	91	6	0
	72	91	9	0
	168	85	14	0
wheat	1	100	0	86
	3	85	15	10
	6	95	5	0
	24	98	2	0
	72	95	4	0
	168	98	2	0
soybean	1	100	0	96
	3	100	0	95
	6	97	0	96
	24	98	0	94
	48	101	0	92
	120	98	0	89
	168	100	0	86

[a] Distribution of ^{14}C-radioactivity in cells and culture media of corn, wheat, and soybean after application of [^{14}C]halosulfuron-methyl [b] Results represent a single experiment and are expressed as percentages of applied dose; replicate experiments gave similar results. [c] In all samples, generally less than 1% of applied activity was found transformed to non-extractable residues.

was detected in both wheat and corn cell cultures after only 6 h of incubation. Figure 2 shows HPLC radiochromatograms comparing the extent of the metabolism of ^{14}C-halosulfuron-methyl in corn, wheat, and soybean tissue culture media after 3 h of incubation. In the case of corn and wheat suspension cultures, the radioactivity consisted of mostly polar metabolites and small amounts of unchanged parent compound. Since no metabolism of halosulfuron-methyl occurred in the control media in the absence of cells, the extensive breakdown of halosulfuron-methyl in corn and wheat cell cultures is assumed to be due to uptake of halosulfuron-methyl by the cells and subsequent excretion of polar metabolites into the culture fluid. HPLC radiochromatograms (Figure 2) show that in addition to halosulfuron-methyl (**I**), there are seven major radiolabeled metabolites. To allow identification/characterization of the halosulfuron-methyl metabolites, the seven radioactive metabolites produced by corn and wheat were separated by HPLC and purified as described under Materials and Methods. The structures and HPLC retention times for these metabolites are shown in Table 2. Metabolites **II**, **III**, **IV**, **VI**, and **VIII** were identified by co-chromatography with authentic reference standards followed by mass spectroscopic confirmation.

Identification of Metabolite V. Metabolite **V** was the major component in both corn and wheat cell extracts but also was present in the media at significant levels. Since glucose conjugation is a common metabolic transformation in most sulfonylurea herbicides metabolism by plants, the purified metabolite **V** was subjected to β-glucosidase digestion. Metabolite **V** was quantitatively converted by β-glucosidase to yield a less polar product. The product of β-glucosidase digestion was found to co-chromatograph with metabolite **II**, 5-hydroxyhalosulfuron. Additionally, a portion of the radiochemically pure metabolite **V** was treated with 1 M hydrochloric acid at room temperature. Mild acid hydrolysis of metabolite **V** also produced 5-hydroxyhalosulfuron (**II**) as an intermediary product which under

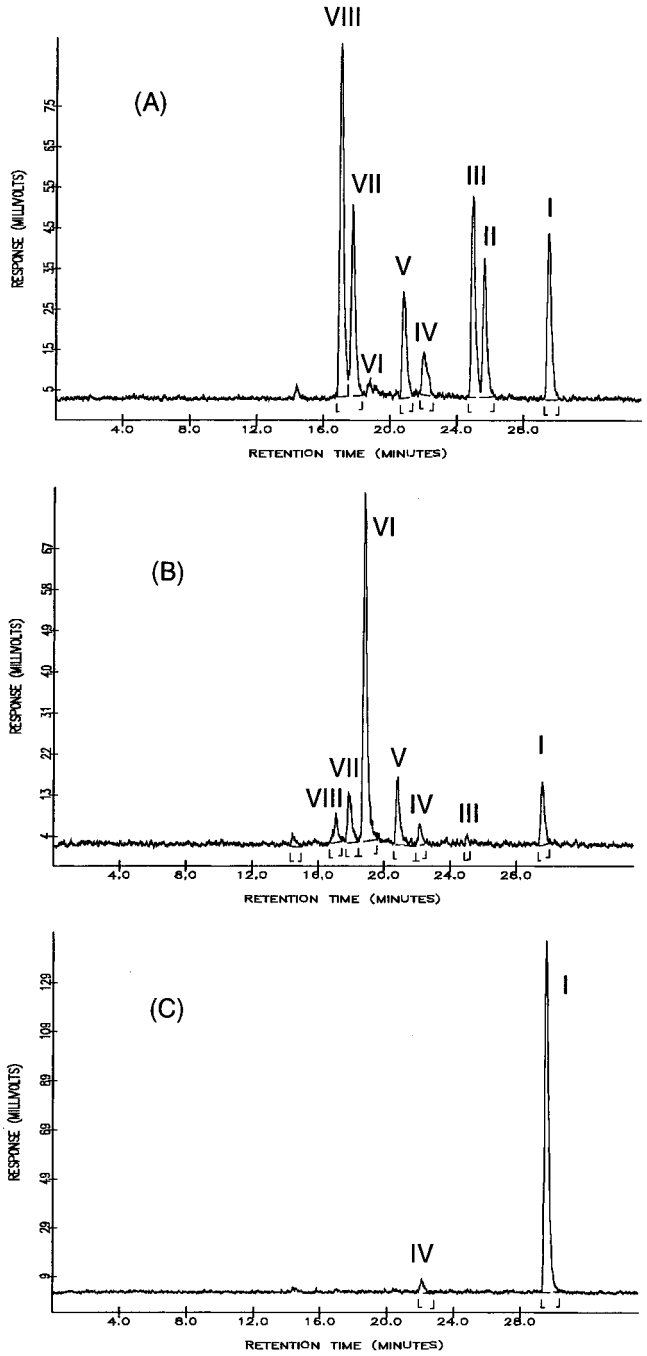
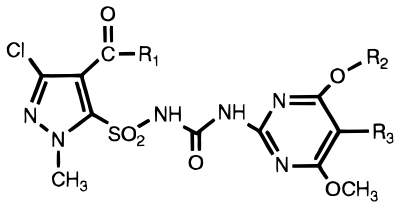


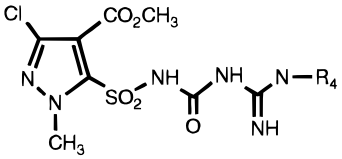
Figure 2. HPLC radiochromatograms of [^{14}C]halosulfuron-methyl and metabolites in cell suspension medium (A) corn, (B) wheat, and (C) soybean. See Materials and Methods for chromatography conditions. The numbered metabolites correspond to the metabolites described in Table 2.

the reaction conditions slowly converted quantitatively to metabolite **IV** (pyrazolesulfonamide) by hydrolysis of the sulfonylurea bridge. The result of enzymatic digestion and mild acid hydrolysis clearly indicated that the site of metabolic transformation (possibly glycosylation) in metabolite **V** was carbon-5 of the pyrimidine ring with the pyrazole portion of the molecule unaltered. Final structural characterization of metabolite **V** was obtained by mass spectroscopy. Mass spectrum of metabolite **V** showed a prominent deprotonated molecular ion at *m/z* 612 which was 178 mass units (equivalent to one hexose molecule) heavier than parent halosulfuron-methyl ion, suggesting the identity of metabolite **V** as a hexose conjugate of 5-hydroxyhalosulfuron. Although from the MS data it was not

Table 2. Chemical Structures of Identified and Proposed Metabolites of Halosulfuron-methyl



(A)



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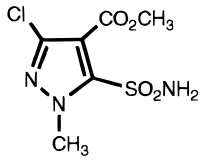

(C)

compd	common name	HPLC (RT,[a] min)	(M − H)$^-$ [b]	structure	substrate R_1	R_2	R_3	R_4
I	halosulfuron-methyl	29.2	433,[c] 435,[d], 437[e]	A	CH_3	CH_3	H	
II	5-hydroxyhalosulfuron	25.4	449, 451, 453	A	CH_3	CH_3	OH	
III	halosulfuron acid	24.7	419, 421, 423	A	H	CH_3	H	
IV	pyrazolesulfonamide	22.1	242, 244, 246	C				
V	5-hexosehalosulfuron	21.0	611, 613, 615	A	CH_3	CH_3	$O-C_6H_6O_5$	
VI	halosufuron desmethyl	18.1	419, 421, 423	A	CH_3	H	H	
VII	dihydroxyhalosulfuron	17.8	451, 453, 455	B				$=C(OCH_3)COCO_2H$
VIII	halosulfuron guanidine	17.1	337, 339, 341	B				H_2

[a] RT, retention time. [b] Negative-ion ESI/MS data. [c] Based on ^{12}C and ^{35}Cl atoms. [d] Based on ^{12}C and ^{37}Cl and/or ^{12}C and ^{14}C atoms. [e] Based on ^{12}C and ^{37}Cl and ^{14}C and/or ^{12}C and two ^{37}Cl and/or ^{12}C and two ^{14}C atoms.

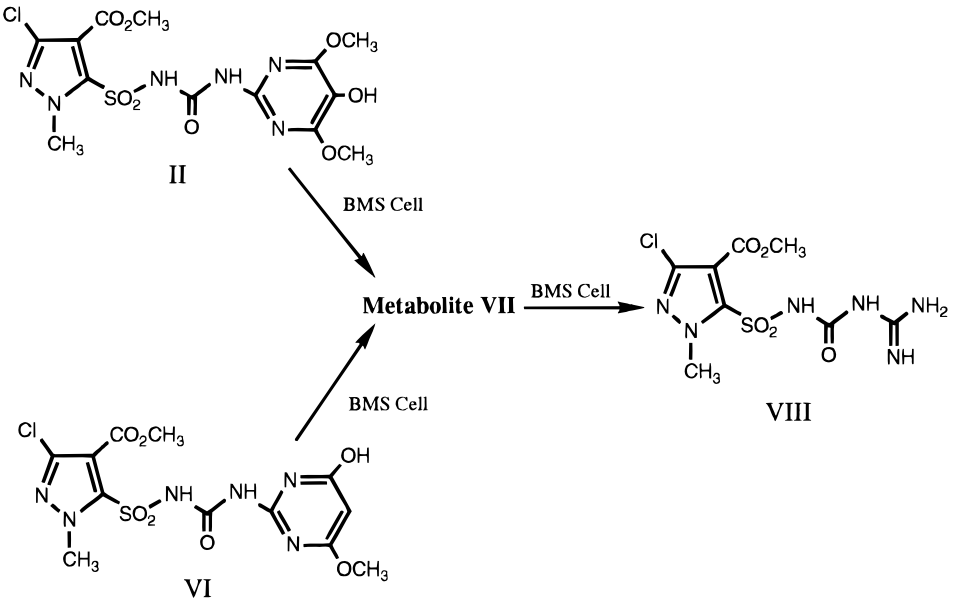

Figure 3. Biotransformation of halosulfuron-methyl metabolites in BMS corn cell suspension.

possible to determine which hexose was conjugated to halosulfuron-methyl, on the basis of the rapid cleavage of metabolite **V** with β-glucosidase we believe the conjugate would most probably be glucose. Plant monosaccharide conjugation of xenobiotics involving hexoses other than glucose is rare (Lamoureux et al., 1986).

Identification of Metabolite VII. Metabolite **VII** was present in both corn and wheat cell cultures. The proportion of **VII** generally increased with incubation time. When radiochemically pure 5-hydroxyhalosulfuron **II** or halosulfuron desmethyl **VI** were incubated in BMS cell, metabolite **VII** was found to be the major product formed. Furthermore, metabolite **VII** upon further incubation in the BMS cell was partially converted to metabolite **VIII** as depicted in Figure 3.

Mass spectrum of the radiochemically pure portion of metabolite **VII** showed a prominent deprotonated molecular ion of 451 which was 18 mass units heavier than halosulfuron-methyl ion. High-resolution mass spectroscopy confirmed the molecular formula of the metabolite as $C_{12}H_{13}ClN_6O_9S$ which indicates a gain of two oxygen atoms and loss of a CH_2 moiety from halosulfuron-methyl parent ($C_{13}H_{15}ClN_6O_7S$). On the basis of these data and known metabolic reactions, two possible structures for metabolite **VII** can be postulated as shown in Figure 4.

Both structures **VIIA** and **VIIB** are consistent with the above data. However, the HPLC retention time of metabolite **VII** under paired ion chromatography conditions is about 5 min longer than that observed for this compound under reverse phase conditions (data not shown). This result seems to be consistent with the structure **VIIA** which contains the ionizable carboxylic acid group. All attempts at derivatization (methylation and acetylation) of metabolite **VII** resulted in the hydrolysis of the sulfonylurea bridge to yield substituted pyrazolesulfonamide (data not shown). In the absence of an authentic synthetic standard of metabolite **VII**, the structural assignment is considered equivocal. However, examination of the mass spectral data and the fact that metabolite **VII** is the product of further metabolism of both halosulfuron desmethyl (**VI**) and 5-hydroxyhalosulfuron (**II**) in addition to being a

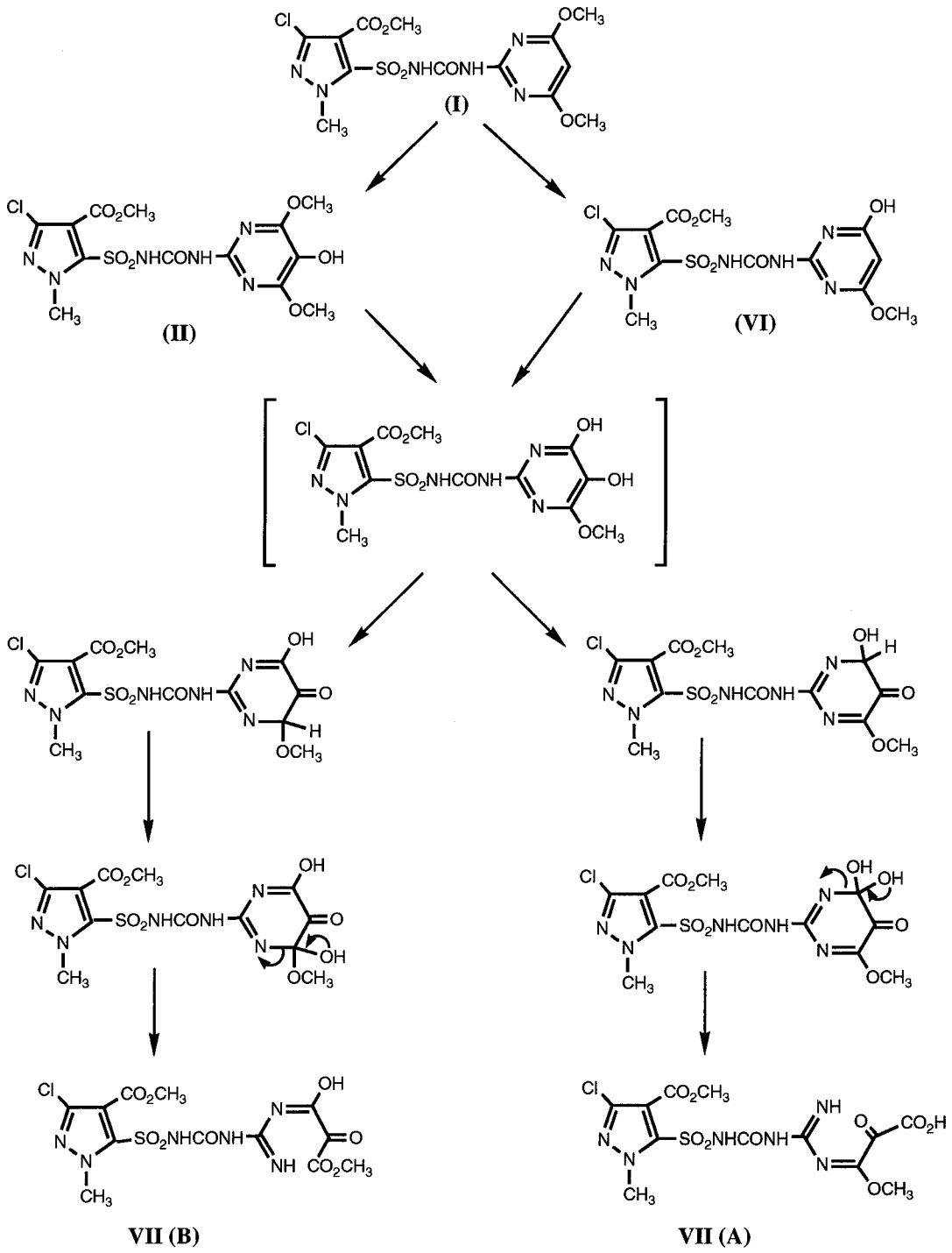

Figure 4. Proposed mechanism of formation of dihydroxyhalosulfuron, metabolite **VII**.

metabolic precursor to halosulfuron guanidine (**VIII**), support our structural assignments.

Halosulfuron-methyl metabolism was found to be qualitatively similar in the corn and wheat systems (Table 3). However, the major metabolite produced by wheat (halosulfuron desmethyl) was only a minor metabolite in corn cell suspension culture. The time course of in-vitro metabolism of halosulfuron-methyl in wheat cell suspension revealed that the major early metabolite produced by wheat is halosulfuron desmethyl (VI) resulting from oxidative demethylation of the methoxy substituent of the pyrimidine ring. In the corn cell suspension, however, metabolites arising from oxidation of carbon-5 of the pyrimidine ring to yield 5-hydroxyhalosulfuron followed by glucose conjugation, and deesterification of the carbomethoxy group were observed to be the predominant early metabolites. Metabolic detoxification by different pathways for the same herbicide by different plant species has been reported before for other sulfonylurea herbicides, chlorsulfuron (Hutchison et al., 1984) and thifensulfuron methyl (Brown et al., 1993, 1990; Cotterman and Saari, 1989). The dissimilarity of the early metabolic profile of halosulfuron-methyl in corn and wheat is less obvious at later incubation periods. The reason for this observation is the fact that additional oxidative biotransformation of the pyrimidine moiety of both halosulfuron desmethyl and 5-hydroxyhalosulfuron results in the formation of the same metabolite (metabolite **VII**). Although halosulfuron acid (**III**) was formed early in the corn cell suspension, its concentration never reached greater than 18% of the dose in corn and 1% of the dose

Table 3. Percent Composition of [^{14}C]Halosulfuron-methyl and Metabolites in Cells and Culture Media of Corn, Wheat, and Soybean during the Time Course Study with Halosulfuron-methyl

species	incubation time (h)	% applied radioactivity (% of dose)[a] I	II	III	IV	V	VI	VII	VIII
corn	1	70	3	8	3	1	0	5	9
	3	13	10	16	4	9	1	14	26
	6	4	0	18	5	27	1	16	29
	24	0	0	16	26	10	0	13	29
	72	0	0	16	19	6	0	24	34
	168	0	0	17	8	11	0	24	27
wheat	1	86	0	0	3	1	9	0.5	0.5
	3	10	0	1	3	14	58	8	4
	6	0	0	0	4	16	60	13	3
	24	0	0	0	3	15	44	28	0
	72	0	0	0	2	12	38	37	6
	168	0	0	0	2	5	35	46	8
soybean	1	96	0	0	3	0	0	0	0
	3	95	0	0	3	0	0	0	0
	6	96	0	0	4	0	0	0	0
	24	94	0	0	5	0	0	0	0
	48	92	0	0	7	0	0	0	0
	120	89	0	0	11	0	0	0	0
	168	86	0	0	14[b]	0	0	0	0

[a] Total concentration of halosulfuron and metabolites detected in the cell extracts and suspension media. For structures see Table 2. [b] This metabolite was also formed (about 6% of dose) in the hydrolytic degradation control flask (suspension media in the absence of soybean cells) after 168 h of incubation.

Table 4. Uptake of [^{14}C]Halosulfuron-methyl by Corn and Soybean Seedlings with Time[a]

time (h)	corn seedlings surface residue	corn seedlings shoots uptake	corn seedlings roots uptake	soybean seedlings surface residue	soybean seedlings shoots uptake	soybean seedlings roots uptake
1	70	21	2	70	16	0
3	56	31	4	52	46	0.2
6	48	46	2	57	48	0.4
24	29	57	1	42	39	5

[a] Results represent a single experiment and are expressed as percentages of applied dose.

in wheat (see Table 3). Additionally, no further metabolism of halosulfuron acid was observed since in all other metabolites isolated in corn and wheat cell cultures the carbomethoxy moiety was intact.

Metabolism of Halosulfuron-methyl in Corn and Soybean Seedlings. Metabolism of halosulfuron-methyl in corn and wheat suspension cultures in vitro as described in the previous section was rapid and extensive. The in vivo metabolism of halosulfuron-methyl was also examined using young seedlings of corn and soybeans in order to compare the rate and extent of uptake in these species. After treatment of the seedlings with a solution of [^{14}C]halosulfuron-methyl in acetone, the seedlings were placed in the dark at room temperature. At harvest, seedling shoot tissues were thoroughly washed in order to remove surface residue. Table 4 summarizes the endogenous ^{14}C-activity in the seedling shoot and root tissues with respect to time in terms of percentage of uptake. In a 24-h period, 58% of the applied activity was taken up by corn seedlings which was comparable to that obtained from soybean seedlings (44% of applied radioactivity). These results demonstrated that under our experimental conditions halosulfuron-methyl was taken up by both soybean and corn seedlings to a similar extent.

Despite similarity in uptake levels, the extent of metabolism of halosulfuron-methyl was dramatically different in corn and soybean. Figure 5 compares the

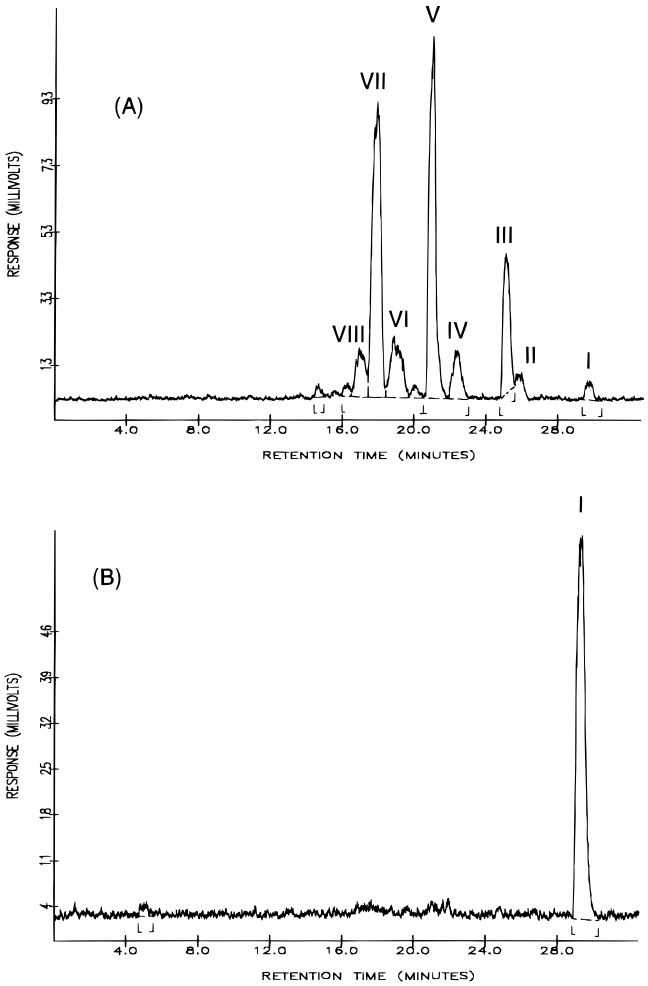

Figure 5. HPLC radiochromatograms of [^{14}C]halosulfuron-methyl metabolism by (A) corn seedlings and by (B) soybean seedlings. See Materials and Methods for chromatography conditions. The numbered metabolites correspond to the metabolites described in Table 2.

HPLC analysis of shoot tissue extracts of soybean and corn. Approximately 98% of halosulfuron-methyl taken up by corn seedlings was metabolized in a 24-h period. However, soybean demonstrated very little metabolism of halosulfuron-methyl in the same time period. Despite the fact that soybean plants took up more than 48% of applied halosulfuron-methyl, all of the activity remained unchanged.

Crop tolerance to herbicides may result from several factors. In most cases, however, the most important factors are reduced herbicide uptake and translocation, or rapid metabolic inactivation of the herbicide by tolerant crops (Koeppe and Brown, 1995). We have shown that halosulfuron-methyl was readily taken up by both corn and soybean, indicating that limited uptake is not responsible for corn's tolerance to halosulfuron-methyl. Our results suggest that corn and wheat tolerance to halosulfuron-methyl is due to their ability to rapidly detoxify halosulfuron-methyl to nonherbicidal metabolites. Halosulfuron-methyl metabolites **III**, **VI**, and **IV** showed no herbicidal activity in whole plant testing (unpublished, Monsanto Co.). Metabolites **II** and **V**, due to the polar substituents on the 5-position of the pyrimidine ring, are expected to be herbicidally-inactive based on work with other sulfonylureas (Brown and Cotterman, 1994). In contrast, soybean, a halosulfuron-methyl susceptible crop, is incapable of significant metabolic inactivation of halosulfuron-methyl. Our

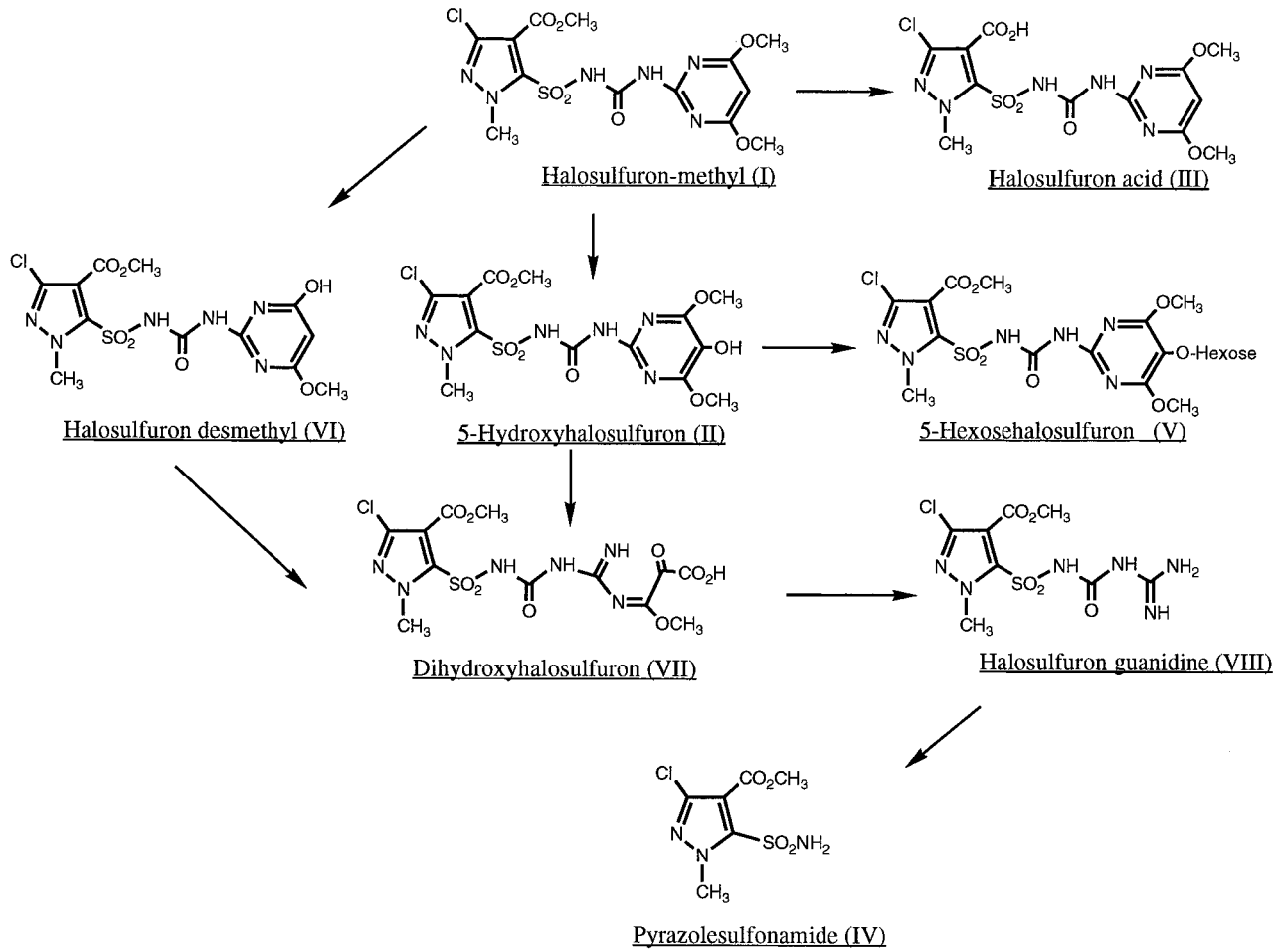

Figure 6. Proposed pathways for halosulfuron-methyl metabolism in corn and wheat.

results are in agreement with previous research on other sulfonylurea herbicides which attribute the rate of metabolic inactivation as the basis for the crop tolerance (Brown and Cotterman, 1994; Brown, 1990; Harms et al., 1990).

The predominant metabolites identified in corn seedlings were the same as those observed in BMS corn cell suspension cultures. Differences in halosulfuron-methyl metabolism in the corn cell cultures and seedlings were only quantitative, not qualitative. This observation is in agreement with several published studies for other agrochemicals in which the metabolism in cell cultures was comparable to that observed in whole plants (Harms, 1992; Swisher, 1987; Sandermann, 1984).

Metabolic Pathways. From the structures of metabolites identified, the pathways shown in Figure 6 are proposed for the degradation of halosulfuron-methyl in corn and wheat. Halosulfuron-methyl is rapidly metabolized in corn and wheat by three concurrent pathways: de-esterification, oxidative O-demethylation, and hydroxylation of the pyrimidine ring. The most significant early metabolites in corn are 5-hydroxyhalosulfuron and its glycosyl conjugate. Formation of glycosyl conjugates is a common fate for sulfonylurea herbicides in plants (Beyer et al., 1987). It is widely accepted that these conjugates are formed in two distinct steps, oxidation followed by glycosylation. The oxidation step is thought to be catalyzed by the action of a mixed function oxidase, cytochrome P450 monooxygenase (Diehl, 1995; Fonne-Pfister et al., 1990). The conjugation with glucose generally results from the action of a different enzyme, most likely a UDP-glucose glucosyltransferase (Lamoureux and Rusness, 1986). The other two most significant early metabolites, halosulfuron acid and halosulfuron desmethyl, are formed as a result of deesterification and O-demethylation of halosulfuron-methyl, respectively. O-demethylation is likely derived from hydroxylation of the methoxy substituent, catalyzed by mixed function oxygenases to form the chemically unstable formyl adduct which decomposes to the corresponding hydroxy analog (Beyer et al., 1987). De-esterification is typically thought to involve plant hydrolytic enzymes. However, it has been reported that deesterification may also be catalyzed by mixed function oxidases (O'Keefe et al., 1987; Feng et al., 1995). The importance of plant cytochrome P450 in the detoxification mechanism of halosulfuron-methyl is currently under investigation.

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