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Metabolism of the Herbicide Buthidazole in Corn Seedlings and Alfalfa Plants

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[^{14}C]Buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-^{14}C-yl]-4-hydroxy-1-methyl-2-imidazolidinone] was absorbed from nutrient solution and translocated by corn seedlings. Most of the radiocarbon in seedlings could be extracted by ethanol. Unextracted radiocarbon gradually increased to ~19% at day 25; however, acid hydrolysis released almost all of the conjugated metabolites in solids. Buthidazole was slowly but steadily metabolized. Unchanged buthidazole constituted 96% of the total radiocarbon at day 1, decreasing to 62% at day 25. The major degradation pathways were conjugation, hydroxylation, and N-demethylation. Alfalfa plants translocated and metabolized buthidazole more quickly and extensively than did corn seedlings. Unchanged buthidazole constituted 15% of total radiocarbon in alfalfa plants after 2 days but only 4% after 16 days. The major degradation pathway in alfalfa was hydroxylation of the imidazolidinone ring, followed by ring opening and subsequent N-demethylation and hydrolysis.

The herbicide buthidazole, code name VEL-5026, has shown promise for industrial vegetation control and for control of broadleaf and grassy weeds in tolerant crops. The mode of action, basis of selectivity, and metabolic fate of buthidazole have been reported for several crop and weed species (Hatzios, 1979; York, 1979; Hilton and Nomura, 1979). Although only gradually metabolized by tolerant crops, buthidazole was rapidly metabolized and eliminated by lactating cows and laying hens (Atallah et al., 1980) and small mammals (Yu and Atallah, 1976). The study reported here was undertaken to determine the uptake, translocation, and metabolism of buthidazole in corn seedlings (*Zea mays* L.) and alfalfa plants (*Medicago sativa* L.)—both of which have tolerance to this herbicide.

MATERIALS AND METHODS

Chemicals. [^{14}C]Buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-^{14}C-yl]-4-hydroxy-1-methyl-2-imidazolidinone] was synthesized by Velsicol Chemical Corp., Chicago, IL, and had a specific activity of 10.25 mCi/mmol and a radiochemical purity of greater than 98% by thin-layer chromatography. Buthidazole and seven model metabolites (Table I) were also synthesized by Velsicol; each was greater than 90% pure.

Treatment of Plants. Pioneer corn seeds (Minnesota 4201) were planted in vermiculite, nurtured with 50% Hoagland nutrient solution 1 (Hoagland and Arnon, 1938), and maintained under Gro-Lux lamps at 25 °C. Hoagland nutrient solution 1 consists of the following: potassium acid phosphate, 1 mM; potassium nitrate, 5 mM; calcium nitrate, 5 mM; magnesium sulfate, 2 mM; boron, 0.5 ppm; manganese, 0.5 ppm; zinc, 0.05 ppm; copper, 0.02 ppm; molybdenum, 0.05 ppm; iron, 1 ppm. After 8 days, corn seedlings (12–15 cm tall) were carefully separated from vermiculite under water and were individually placed in 24-mL glass scintillation vials each containing 1.1 ppm of [^{14}C]buthidazole in 10 mL of 50% Hoagland solution 1 (total of 9.3×10^5 dpm). Twenty-one corn seedlings were exposed for 24 h in this manner and then were transferred individually to fresh vials containing 20 mL of 50% Hoagland solution. The day of transfer to buthidazole-free solution was referred to as day 0; three plants were harvested for analysis at days 0, 1, 2, 4, 8, 14, and 25.

Additional corn seedlings were treated to generate sufficient quantitites of metabolites for mass spectrometric analysis. Routinely, 10 seedlings were placed individually in vials containing 20 mL of 50% Hoagland solution with 50 ppm of [^{14}C]buthidazole (7.6×10^5 dpm). In one case, five seedlings were placed individually in vials containing 20 mL of Hoagland solution 1 fortified with 200 ppm of buthidazole; seedlings thus treated were exposed for 3 days and then transferred to buthidazole-free 50% Hoagland solution for 20 days. Seedlings from each test were composited prior to analysis.

In another test designed to characterize unextractable radiocarbon in plant solids, six corn seedlings were placed individually in vials containing 20 mL of 50% Hoagland solution with 1.7 ppm of [^{14}C]buthidazole (3×10^6 dpm). The seedlings were held in the radiocarbon solution continuously for 21 days and then combined as a composite for analysis.

Established alfalfa sets (Funk's F-261, 34% California, 33% Washington, and 33% Idaho) in their second year of growth were obtained from Velsicol Agriculture Research Center, Woodstock, IL, in November. Alfalfa shoots were cut at the soil surface and were individually placed in vials containing 20 mL of full-strength Hoagland solution fortified with 0.8 ppm of [^{14}C]buthidazole (1.4×10^6 dpm). The plants were maintained continuously in the radiocarbon solution under Gro-Lux lamps at 25 °C until harvested in duplicate at days 2, 5, 6, 9, 12, and 16.

Thin-Layer Chromatography (TLC). Precoated silica gel G TLC chromatoplates (Macherey-Nagle and Co., distributed by Brinkmann Instruments, Inc.) were used for the separation of metabolites. Both one-dimensional

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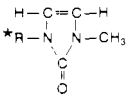
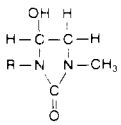
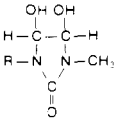
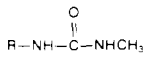
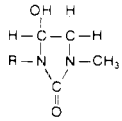
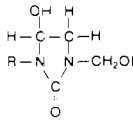
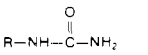
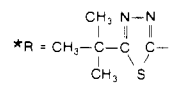
TABLE I. R_f Values on Silica Gel G TLC for Buthidazole and its Metabolites in Corn Seedlings and Alfalfa Plants.

Designation and Trivial Name	Structure	R_f Values in Solvent System[a] A	B	C	D	E	F	G
I. Dehydrate		0.62	0.40	0.34	0.40	0.37	—	—
II. Buthidazole		0.58	0.31	0.27	0.29	0.31	0.47	0.50
III. Dihydroxy		0.41	0.50	0.20	0.59	0.20	0.55	—
IV. Methyl Urea		0.40	0.42	0.31	0.49	0.20	0.49	0.40
V. Desmethyl		0.35	0.36	0.22	0.36	0.18	0.44	0.50
VI. Methylol		0.32	0.31	0.16	0.35	0.17	—	—
VII Amine	$R-NH_2$	0.20	0.30	0.29	0.49	0.16	0.35	—
VIII. Urea		0.18	0.28	0.22	0.47	0.14	0.30	0.32
IX. Unknown		0.71	0.42	0.41	0.46	—	—	—
X. Unknown		0.12	0.19	—	—	—	—	—
XI. Unknown		—	—	—	—	—	0.28	—
XII. Unknown		—	—	0.19	—	0.13	0.18	—
XIII. Unknown		—	—	0.16	—	0.08	0.13	0.19
XIV. Unknown		—	—	0.04	—	—	—	0.13
XV. Unknown		—	—	0.19	—	—	—	—
XVI. Unknown		—	—	0.16	—	0.11	—	0.17
XVII. Unknown		0.0	0.0	—	—	0.0	0.0	—

[a] Solvent System:
A — Methylene chloride-n-propanol (90:10)
B — Petroleum ether-diethyl ether-ethanol (50:35:15)
C — Benzene-dioxane-acetic acid (76:21:3)
D — Petroleum ether-diethyl ether-n-propanol (50:30:20)
E — Petroleum ether-chloroform-ethyl ether (7:2:1)
F — Acetonitrile-ethyl ether (3:7)
G — Acetonitrile-ethyl ether (1:1)



TLC and two-dimensional TLC were used to identify metabolites. Solvent systems used are listed in Table I. In the corn seedling study, TLC plates were developed first in solvent system A and then in B, C, D, or E. In the alfalfa study, TLC plates were developed first in solvent system E and then in C, F, or G.

Plant extracts were spotted alone or cochromatographed with seven reference standards (compounds I–V; VII, and VIII, Table I). Radioactive spots were located by radioautography using Kodak blue brand X-ray film; reference compounds were visualized under UV light. Quantitation of the radioactive spots was accomplished by scraping and counting the gel in Aquasol scintillation solution. For confirmation of ^{14}C metabolites, radioactive spots were scraped from TLC plates, extracted with either acetone or methanol, and then cochromatographed with the suspected reference standards in several other solvent systems.

Extracts of corn seedlings that were treated with a high concentration of buthidazole were spotted as a band on 0.5-mm preparative TLC plates and developed in solvent system A. The ^{14}C bands were scraped, extracted, and further purified on TLC using other solvent systems.

Mass Spectrometry (MS). Mass spectra were obtained by using a Hewlett-Packard Model 5982A quadrupole mass spectrometer by direct inlet probe in either the electronic impact (EI, 70 eV) or chemical ionization (CI, methane) mode. Output from the MS was monitored with a Hewlett-Packard Model 5934A dual-disk data system.

Sample Analysis. *Corn Seedlings.* Entire seedlings were taken on days 0, 1, 2, or 4 and analyzed as single samples—i.e., shoot and root were taken together. For remaining samples, the shoot and root were analyzed separately. Plants were cut into small pieces and blended in 50 mL of ethanol. After suction filtration, the plant solids were reextracted with 50 mL of ethanol and the filtrates were combined. Solids, after extraction, were air-dried overnight before combustion analysis. The volume of the combined ethanol filtrates was measured and 0.5-mL duplicate samples were radioassayed. The remaining filtrate was rotary evaporated to complete dryness. Fifty milliliters of distilled water was added to the residue which was then extracted 3 times with 50 mL of ethyl acetate. Aliquots of the combined ethyl acetate extract (free metabolites) was radioassayed. The aqueous fraction was acidified with 5 mL of 1.0 N HCl, refluxed for 30 min, allowed to cool, and extracted 3 times with 50 mL of ethyl acetate. Aliquots of the organic layer (acid-released metabolites) were radioassayed. Ten milliliters of 2.5 N NaOH was added to the aqueous layer, and the mixture was refluxed for 30 min, allowed to cool, and extracted 3 times with 50 mL of ethyl acetate. Aliquots of the organic layer (base-released metabolites) and aqueous layer were radioassayed. The ethyl acetate layer from free as well as acid and base treatments was separately dried over anhydrous sodium sulfate, filtered, and rotary evaporated to dryness. The flask was rinsed with acetone which was subsequently transferred to a centrifuge tube for evaporation to a suitable volume for TLC analysis.

Alfalfa Plants. Shoots were chopped and blended in a Virtis blender with 50 mL of ethanol for 10 min. The macerate was suction-filtered and the solids were reex-

Table II. Average Radiocarbon Content of Corn Seedlings Treated with [*thiadiazolyl-2-*^{14}C]Buthidazole for 24 Hours and Then Transferred to 50% Hoagland Solution for Designated Periods[a]

day after transfer to ^{14}C-free soln	0	1	2	4	8	14	25
% radiocarbon[b]	14.60	12.28	12.52	13.76	15.97	12.91	12.79

[a] Each corn seedling was treated in a 10-mL Hoagland solution (50%) containing 10.5 μg of [^{14}C]buthidazole (9.3×10^5 dpm) for 24 h. Radiocarbon in the solution was decreased by an average of 17.2% for the 24-h period. [b] Radiocarbon content in corn seedlings expressed as percent of total activity in the treatment solution.

tracted with 50 mL of ethanol and filtered, and the extracts were combined. A portion of the dried solids was combusted and radioassayed. The combined ethanol extract was rotary evaporated to 1 mL, quantitatively transferred to a separatory funnel with 20 mL of distilled water and 50 mL of ethyl acetate, and shaken. The aqueous layer was reextracted twice with 50 mL of ethyl acetate. An aliquot of the combined ethyl acetate extract (free metabolites) was radioassayed, and the remaining extract was prepared for TLC analysis. The remaining aqueous fraction was adjusted to pH 1.0 by using 2.0 N HCl, shaken, permitted to stand for 20 min, and then extracted 3 times with ethyl acetate. An aliquot of the combined ethyl acetate layer (acid-released metabolites) was radioassayed and the remaining layer was prepared for TLC analysis. Aliquots of the aqueous layer (water-soluble metabolites) were also radioassayed.

The solid remaining after the original ethanol extraction was subjected to enzyme hydrolysis (β-glucuronidase, from *Helix pomatia* with sulfatase activity, 97 600 units/mL, Sigma Chemical Co., St. Louis, MO). After transfer of the solids to a 250-mL Erlenmeyer flask, 50 mL of pH 4.5 sodium acetate buffer and 1 mL of the enzyme solution were added and the solution was incubated on a shaking water bath at 37 °C. After 1 h, another 1.0 mL of enzyme was added and the incubation continued for an additional hour. The mixture was then suction-filtered and the aqueous layer extracted twice with ethyl acetate. The combined ethyl acetate extracts (enzyme-released metabolites) were radioassayed. This fraction contained insufficient radioactivity for TLC analysis. The aqueous layer was added to the solids, adjusted to 1.0 N with HCl, and refluxed for 30 min. After being cooled, the mixture was suction-filtered, and both the aqueous solution (water-soluble metabolites after enzyme and acid hydrolysis) and the solids (unextracted metabolites) were radioassayed.

Radioassay. Liquid samples were assayed directly in either Econofluoror or Aquasol scintillation solution (New England Nuclear). Plant solids were combusted by using a Packard Model 305 sample oxidizer. $^{14}CO_2$ was trapped in ethanolamine and counted in Oxyprep counting solution (New England Nuclear). The efficiency of $^{14}CO_2$ recovery was determined by combusting known amounts of [^{14}C]-hexadecane. Recoveries ranged from 80 to 95% and were used to determine the actual radiocarbon content in combusted samples.

All samples were counted in a Mark III liquid scintillation system, Model 6880 (Searle Analytical, Inc.), for 10 or 20 min. Counting efficiency was automatically determined by the external standard pulse method via an efficiency vs. external standard pulse curve stored in the microprocessor unit of the counter. Counting efficiency ranged between 70 and 85%. Data were corrected for the background value of control samples.

RESULTS AND DISCUSSION

Absorption and Translocation. When corn seedling roots were immersed in the [^{14}C]buthidazole solution for 24 h, the radiocarbon content in the solution decreased by an average of 17.2%. Between 12 and 16% of the radiocarbon in the solution was accounted for in the corn seedlings (Table II). The radiocarbon content in the corn seedlings remained fairly constant over the 25-day period in the ^{14}C-free solution, indicating that the corn plants did not significantly degrade the [^{14}C]thiadiazolyl ring to $^{14}CO_2$ or other volatile products. The ratio of radiocarbon in roots to shoots remained fairly constant for plants harvested at days 8, 14, and 25. This indicates that after the initial translocation of buthidazole and its metabolites between root and shoot, no further translocation occurred after 8 days or, less likely, that translcation in both directions was equal. From day 8 on, ~86% of the radiocarbon was in the shoots and 12% in the roots (Table III).

About 24% of the [^{14}C]buthidazole in the nutrient solution was absorbed and translocated by alfalfa shoots in 2 days (Table IV). The amount of absorption reached a plateau at about day 6 (52%) and remained near that level through day 16.

Metabolites in Corn Seedlings. Ethanol-extracted radiocarbon constituted 98% of the total in corn seedling at day 0 (Table III). However, the extractability gradually decreased to 81% at day 25. At the same time, radio-

Table III. Characteristics of Radiocarbon in Corn Seedlings Treated with [*thiadiazolyl-2-*^{14}C]Buthidazole

	% total ^{14}C in seedlings					
	extracted in ethanol					
day after transfer to ^{14}C-free soln	free	acid released	base released	water soluble	solids[a]	^{14}C recovery
day 0 shoot and root	78.57	21.35	0.60	2.44	1.64	104.60
day 1 shoot and root	59.87	26.09	0.53	2.78	2.09	91.37
day 2 shoot and root	61.93	21.81	0.94	4.37	3.06	92.12
day 4 shoot and root	55.94	26.51	1.72	6.97	4.22	95.38
day 8 shoot	48.44	20.02	0.31	1.67	13.04	83.48
root	1.67	6.38	0.12	0.82	1.80	10.79
day 8 shoot and root total	50.11	26.40	0.43	2.49	14.84	94.27
day 14 shoot	48.28	22.53	0.75	2.68	7.20	81.44
root	1.59	5.49	0.62	4.43	1.33	13.34
day 14 shoot and root total	49.87	28.02	1.37	7.11	8.53	94.79
day 25 shoot	48.56	17.97	1.10	6.24	17.77	91.64
root	1.43	5.93	0.38	1.88	1.31	10.93
day 25 shoot and root total	49.99	23.90	1.48	8.12	19.08	102.57

[a] Before acid hydrolysis.

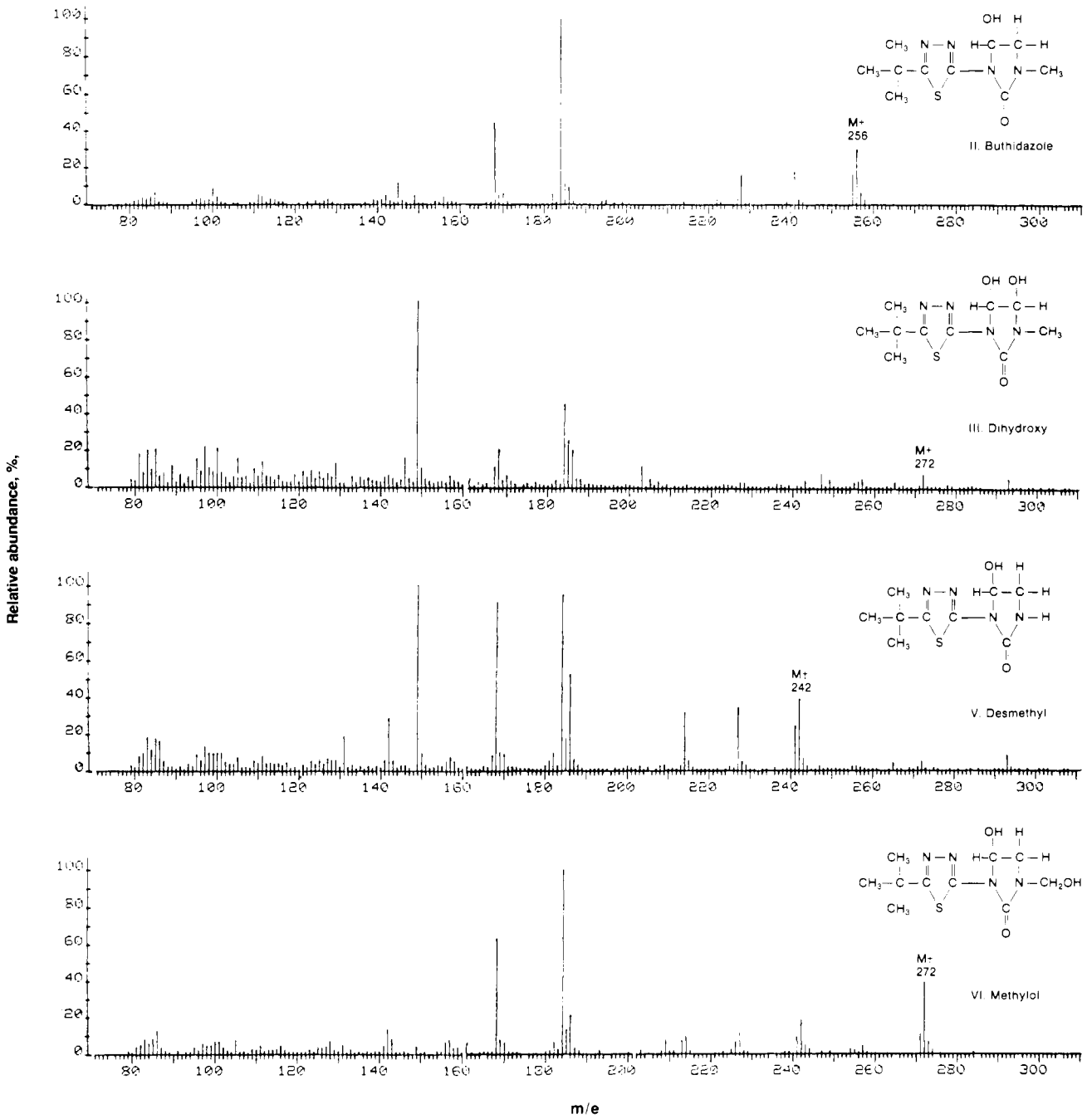


Figure 1. Mass spectra (EI) of buthidazole and its metabolites isolated from corn seedlings.

Table IV. Translocation of Radiocarbon by Alfalfa Plants Treated with [^{14}C]Buthidazole in Hoagland Solution[a]

sampling intervals (days)	2	5	6	9	12	16
% ^{14}C translocated[b]	23.9	31.9	52.3	55.1	75.4	54.9

[a] Each alfalfa plant was treated in 20 mL of Hoagland solution containing 0.8 ppm of [^{14}C]buthidazole (1.4×10^6 dpm). [b] Radiocarbon content in alfalfa plants expressed as percent of radioactivity in the treatment solution.

carbon in the solids steadily increased from 1.6% at day 0 to 19% at day 25. Ethanol extractables were partitioned between ethyl acetate and water. Radiocarbon partitioned to the ethyl acetate fraction (free metabolites) decreased from 79% at day 0 to ~50% at day 25. The acid-released metabolites remained at ~25%. The base-released metabolites also remained fairly constant at ~1%. The water-soluble metabolites increased from 2.4% at day 0 to 8.1% at day 25.

Shoot and root were analyzed separately for samples taken at days 8, 14, and 25. Radiocarbon retained in shoots was mostly in the free form (~48.5%). The acid-released metabolites in shoots constituted ~20% of the ^{14}C in corn seedlings. In contrast, most of the radiocarbon in roots were acid-released metabolites (6%). The free metabolites in roots constituted ~1.5% of the radiocarbon in corn seedlings. Thus, most of the radiocarbon in corn roots was conjugated and was rendered extractable only after acid hydrolysis.

Buthidazole was the major component in both free and acid-released radiocarbon. Detailed TLC analysis of radiocarbon in shoots and roots is shown in Table V. About 96% of radiocarbon in day 0 seedlings was buthidazole. The amount steadily decreased to 62% at day 25. The free buthidazole decreased from 76% at day 0 to 43% at day

Table V. Identity of Radiocarbon in the Shoot and Root of Corn Seedlings Treated with [^{14}C]Buthidazole

samples		metabolite identity and % distribution									
		I[b]	II	III	V	VI	VII	VIII	IX	X	XVII
day 0											
free	S and R[a]	0	75.51	0	0.40	0.55	0	0	0.05	0.29	1.76
acid	S and R	0	20.16	0.08	0.10	0.10	0	0	0.49	0.38	0.04
day 1											
free	S and R	0	55.17	0.57	0	0.41	0.37	0	0.08	0.31	2.96
acid	S and R	0	23.87	0.21	0.31	0.21	0.27	0.08	0.55	0.03	0.55
day 2											
free	S and R	0	58.33	0.28	0	0.74	0	0.15	0.17	0.16	2.07
acid	S and R	0	18.69	0.41	1.08	0.48	0	0	0.53	0.54	0.09
base	S and R	0	0.06	0	0	0	0	0	0	0.21	0.67
day 4											
free	S and R	0	52.34	0	0	0.67	0	0.17	0	0.09	2.66
acid	S and R	0	22.51	0.27	1.80	0.53	0	0	0.52	0.80	0.08
day 8											
free	S	0	42.31	0.48	0	1.11	0	0	0	2.28	0.26
free	R	0	1.08	0.06	0	0.02	0	0	0	0.44	0.08
acid	S	0.13	16.00	0.43	2.31	0.54	0	0	0.31	0.13	0.11
acid	R	0	6.01	0.03	0.14	0	0	0	0.13	0.04	0.04
day 14											
free	S	0	44.49	0	0	1.67	0	0	0	0	2.12
free	R	0	0.62	0.03	0	0.08	0	0	0	0.05	0.81
acid	S	0.57	16.51	0.73	2.06	0	0	0	1.32	0.88	0.47
acid	R	0	4.70	0.05	0.13	0	0	0	0	0.53	0.09
base	S	0	0.15	0	0.09	0	0.06	0.05	0	0.12	0.28
base	R	0	0.09	0	0	0	0	0	0	0.13	0.40
day 25											
free	S	0	42.48	0.72	0.67	2.58	0	0	0.64	0	1.45
free	R	0	0.50	0	0	0.03	0	0	0	0	0.90
acid	S	0.94	13.13	0.18	1.60	0.55	0	0	0.66	0	0.90
acid	R	0	5.49	0	0	0	0	0	0	0.16	0.28
base	S	0.0	0.17	0	0.06	0	0.06	0.06	0	0.22	0.55
base	R	0									

[a] S stands for shoot and R for root. [b] I, dehydrate; II, buthidazole; III, dihydroxy; V, desmethyl; VI, methylol; VII, amine; VIII, urea; IX, unknown; X, unknown; XVII, TLC origin.

25. The acid-released buthidazole remained constant at ~20%. A small amount of buthidazole (less than 0.3%) was released after base hydrolysis. As shown in Table I, metabolites of buthidazole detected in corn seedlings were designated as dehydrate (I), dihydroxy (III), desmethyl (V), methylol (VI), amine (VII), and urea (VIII). These metabolites existed in both free and conjugated forms. The respective amount of different metabolites at day 0 and day 25 were 0 and 0.9% for dehydrate, 0.5 and 2.3% for desmethyl, and 0.7 and 3.2% for methylol. Dihydroxy remained constant at ~0.8%. Trace amounts of amine (0.1%) and urea (0.07%) were detected in some of the samples. Small amounts of three unidentified metabolites were also found but together accounted for only 4.7%. Metabolites were identified by TLC cochromatography. In addition, buthidazole, dihydroxy, desmethyl, and methylol metabolites were also isolated from corn seedlings and confirmed by mass spectrometry (Figure 1). The spectra of the metabolites isolated from the corn seedlings were identical with that of the corresponding authentic reference standard except that two background ion fragments at m/e 293 and m/e 149 were present in dihydroxy and desmethyl spectra. The molecular ions of each sample were unequivocally established by MS–CI spectra.

Solids from the corn seedlings that were exposed to [^{14}C]buthidazole continuously for 21 days were refluxed with 0.1 N HCl for 30 min and then extracted with ethyl acetate. The distribution of radiocarbon in various fractions was as follows: ethyl acetate layer, 81.3%; water layer, 8.6% solid, 5.4%. TLC analysis of the organic layer showed that buthidazole was the major component (87.2%). The following metabolites were also detected: dehydrate (1.3%), dihydroxy (3.6%), desmethyl (3.7%), methylol (1.4%), urea (1%), IX (0.4%), X (0.8%), and XI (0.6%). It is apparent that most of the radiocarbon in the solids after ethanol extraction was strongly bound but could be released by acid hydrolysis.

Metabolites in Alfalfa Plants. Over 75% of the radiocarbon in alfalfa plants was extracted by ethanol (Table VI); however, the proportion of free metabolites decreased with time. For example, ~50% of the radiocarbon was free metabolites at day 2, but the amount decreased to ~35% at day 16. About 16% of the radiocarbon in alfalfa plants was released upon acid hydrolysis and 21% was water soluble. β-Glucuronidase released only a small amount (2%) of radiocarbon from the solids. Upon acid hydrolysis, most of the remaining radiocarbon in solids was rendered water soluble. Unextracted radiocarbon in the solids after enzyme and acid treatment was only ~0.8%.

In contrast to buthidazole stability in corn seedlings, the herbicide was rapidly metabolized in alfalfa plants. Buthidazole constituted 14.5% of the radiocarbon in the ethyl acetate extract in the day 2 sample and then decreased sharply to ~3% at day 6 and remained at this level for the duration of the experiment (Table VI). The major metabolite was the amine, which was primarily in the free form. There was a trend for the amine metabolite to increase as the experiment progressed—amine increased from 10% at day 2 to 19% at day 16. Two other major metabolites were methylurea and dihydroxy, both were largely in the free form. The urea and desmethyl were present in free and conjugated forms. Several minor metabolites, which were relatively polar, could not be identified because of the low levels of radiocarbon present.

Metabolic Pathway. Buthidazole was translocated and steadily metabolized by both corn seedlings and alfalfa plants. The rate of degradation by alfalfa plants was greater than that by corn seedlings. Because both species

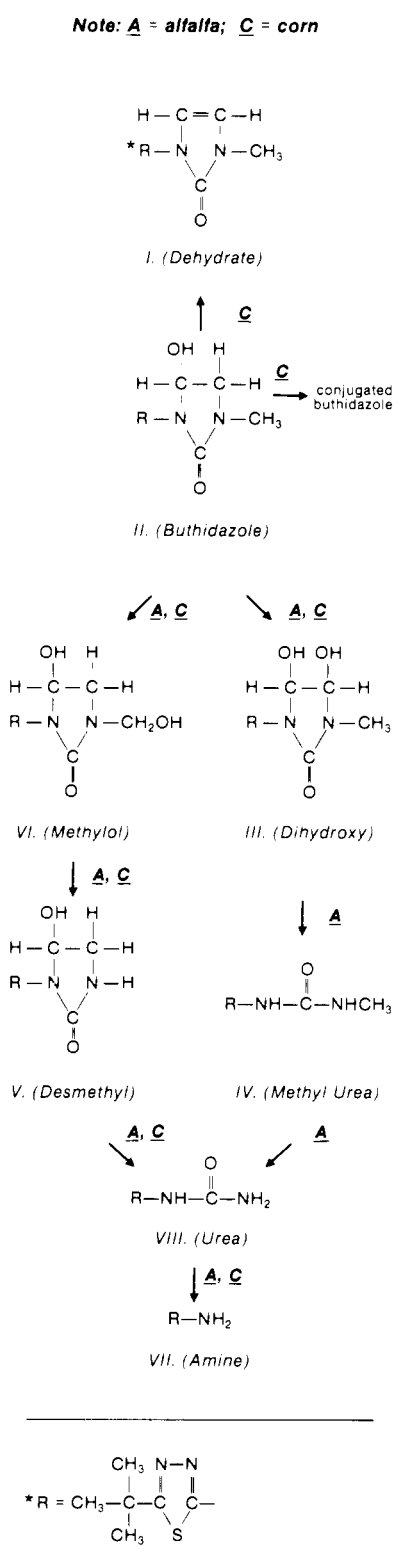


Figure 2. Proposed metabolic pathways of buthidazole in alfalfa plants and corn seedlings.

show tolerance toward the herbicide under field conditions, it appears that the selectivity of buthidazole does not depend on the metabolic capacity of these plant species.

The metabolism of buthidazole in corn seedlings proceeds primarily by the conjugation reaction, although ring and *N*-methyl hydroxylation accompanying the N-demethylation also occurred. In alfalfa plants, the major pathways were ring hydroxylation and ring opening accompanying N-demethylation and hydrolysis. The proposed metabolic pathway for buthidazole in corn seedlings and alfalfa plants is shown in Figure 2.

Table VI. Identity of Radiocarbon in Alfalfa Plants Treated with [*thiadiazolyl-2-*^{14}*C*]Buthidazole for Various Time Periods

metabolites	treatment period and % of total ^{14}C in plants: day 2	days 5-6	day 9	day 12	day 16
ethanol extractables					
free					
II, buthidazole	14.5	3.4	3.3	2.6	3.8
III, dihydroxy	13.4	3.8	0	0	0
IV, methylurea	3.3	19.8	0	6.4	1.7
V, desmethyl	4.6	0	0	0	0
VII, amine	9.6	3.1	13.6	17.1	18.8
VIII, urea	1.4	5.1	0	0	2.3
XI, unknown	1.6	0	0	0	0
XIII, unknown	0	0	4.3	5.3	8.1
XIV, unknown	0	1.9	0	0	0
XV, unknown	0	0	4.3	0	0
XVI, unknown	0	0	4.3	0	0
XVII, unknown	1.8	1.0	11.7	1.7	0.6
total:	50.2	38.1	41.5	33.1	35.3
acid released					
II, buthidazole	0	0	0	2.2	0
IV, methylurea	0	3.6	0	0	0
V, desmethyl	2.6	3.0	0	0	7.7
VII, amine	6.1	0	0	0	0
VIII, urea	0	0	0	7.8	3.0
XI, unknown	0	0	0	1.3	0
XII, unknown	2.2	2.6	4.7	0	0
XIII, unknown	0	4.0	4.7	0	11.1
XVII, unknown	2.1	6.0	1.7	1.9	2.1
total:	13.0	19.2	11.1	13.2	23.9
water solubles	12.4	23.3	21.3	32.1	17.4
total ethanol extractables	75.6	80.6	73.9	78.4	76.6
solids					
enzyme released	1.3	1.2	2.0	2.8	1.9
water solubles	5.0	4.6	9.3	8.6	5.6
unextracted after enzyme and acid release	0.4	0.6	1.2	1.1	0.5
total:	6.7	6.5	12.5	12.5	8.0
total ^{14}C recovery	82.3	87.1	86.4	90.9	84.6

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