

Distribution of Residues and Metabolism of Herbicide Buthidazole in Sugarcane from Root and Foliar Treatments

Norman S. Nomura and H. Wayne Hilton*

Radioactivity from the herbicide [^{14}C]buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2- ^{14}C -2-yl]-4-hydroxyl-1-methyl-2-imidazolidinone] exhibited typical xylem movement into the leaves of sugarcane. Over 12 weeks 85% was recovered in the plant from nutrient culture root absorption, while 42% remained in topically treated leaves. Normal senescent leaf abscission removed most of the root-absorbed and all of the foliar-applied radioactivity from the plants. The major metabolite was 3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-2-imidazolidinone, designated desmethylbuthidazole, constituting 54% and 70%, respectively, of the foliar residues at 12 weeks from root and foliar absorption. Buthidazole was next most abundant at 20% and 7%, respectively. The remaining ^{14}C was contained in a number of minor metabolites. The degree of conjugation and ethanol insolubility of buthidazole and desmethylbuthidazole increased with time; however, there was no evidence of increasing resistance to acid hydrolysis. Basic hydrolysis following acid treatment released only minor amounts of additional radioactivity, and bound residues in the fiber were negligible.

The herbicide buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone] has potential for control of many important weeds in Hawaiian sugarcane (*Saccharum* spp. hybrids). Tolerance of the vegetatively propagated crop to buthidazole is reasonably good, with some variation dependent on application dosage, crop cultivar, soil type, and rainfall. The herbicidal mode of action of buthidazole appears to be primarily the inhibition of photosynthesis and anthocyanin biosynthesis (Hatzios and Penner, 1980a; York et al., 1981). Buthidazole is active in soil against germinating weed seed and has foliar activity when applied to emerged weed vegetation (Weed Science Society of America, 1979). The pattern of distribution of buthidazole and its metabolites in most plant species from root or foliar treatment has been typical of xylem movement (Hatzios and Penner, 1980b,c; Haderlie, 1980).

Studies have been published on the metabolism of buthidazole in two animal species, lactating cows and laying hens (Atallah et al., 1980), in corn (maize) seedlings and alfalfa plants (Yu et al., 1980), and in alfalfa and quack grass (Hatzios and Penner, 1980c). Atallah (1977) reported earlier on a liquid chromatographic separation of several metabolites. In the animals, after a 14-day feeding period, numerous metabolites were identified as hydroxylated, oxidized, N-demethylated, and dehydrated derivatives and other products having a cleaved imidazolidinone ring. There were 5 major products among 12 identified by thin-layer chromatography and mass spectrometry.

Plant metabolism of buthidazole differed markedly between maize and alfalfa, each of which has a degree of tolerance to the herbicide. In short-term studies of 25 and 16 days for the respective species, maize altered 38% of the buthidazole to hydroxylated, N-demethylated, and N-methylol derivatives of the imidazolidinone ring. At 25 days, buthidazole remained the major product, present in the free form (43%) and as an acid-hydrolyzed conjugate (~20%).

Alfalfa degraded buthidazole more rapidly and completely than did the maize plants. While the metabolic pathway was considered similar, buthidazole constituted less than 4% of extracted radiocarbon from alfalfa at 16

days. The major metabolic products were formed by hydroxylation and subsequent cleavage of the imidazolidinone ring to urea and amine forms.

Quack grass, a susceptible weed species, metabolized less than 15% of the buthidazole in a 6-day treatment period compared to about 85% for alfalfa in the same length of time.

The herbicide tebuthiuron [1-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-1,3-dimethylurea] resembles buthidazole in its thiadiazole moiety. The reported metabolism of tebuthiuron in four animal species (Morton and Hoffman, 1976) and in barley and wheat (Lee and Ishizuka, 1976) principally involved the demethylation of the urea portion of the molecule at both nitrogen positions. However, evidence was presented for the animal species for the hydroxylation of the dimethylethyl group attached to the thiadiazole ring. Buthidazole has also shown such hydroxylation as a minor reaction in animals, but not in the plant species. If β -oxidation occurs in plants that have been examined for either herbicide, it is not a pathway of any apparent consequence.

In this paper we report the results of an investigation on the distribution of residues and the metabolic alteration of [^{14}C]buthidazole (I, Figure 1) applied by two separate routes: (a) to the roots of sugarcane plants growing in aqueous nutrient culture solution and (b) to the foliage of sugarcane growing under normal field conditions.

MATERIALS AND METHODS

Root Absorption. Sugarcane plants, cultivar H59-3775, were established individually in 3 L of complete aerated culture solution. An amount of 0.10 mg (5 μCi ; sp act. 12.6 mCi/mM) of the labeled buthidazole was added as a single increment to each of the containers. Water and nutrients were added as needed to maintain volume. No phytotoxic symptoms were observed. Individual plants were removed 1, 4, 8, and 12 weeks after treatment and sectioned into component parts: apical spindle leaves, apical meristem, green leaves, senescent abscised leaves (present only at 8 and 12 weeks), stalk, vegetative seed piece, roots, and secondary basal shoots (present only at 4 weeks). Portions of each component were combusted in a Parr oxygen bomb (Hilton et al., 1972), and the radioactivity was determined by liquid scintillation spectrometry.

Portions of freshly chopped leaves were mixed with 50-mL portions of 95% ethanol and extracted with a Po-

*Experiment Station, Hawaiian Sugar Planters' Association, Aiea, Hawaii 96701.

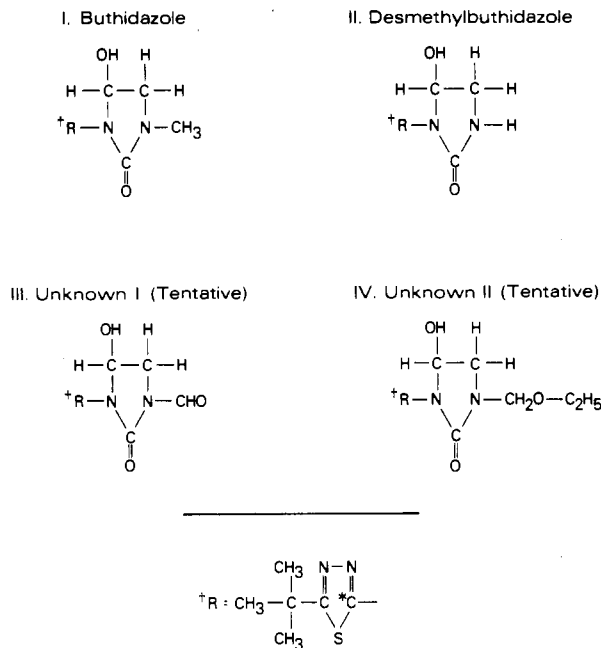


Figure 1. Representative structures of buthidazole and several metabolites.

lytron blender (Brinkmann Instruments, Inc.). The mixture was filtered with suction and the fibrous pulp reextracted twice with 50-mL portions of ethanol. The combined filtrates were placed in a 200-mL volumetric flask, and the pulp was freeze-dried for combustion and for further treatment.

Aliquots of 0.5 mL of the ethanol extracts were mixed with 10 mL of a commercial scintillation formulation (Aquasol, New England Nuclear, Inc.) and counted. Efficiency of an unquenched standard averaged 92%; quench corrections were applied by using an internal standard.

After removal of the ethanol in a rotary evaporator, 50 mL of water was added to the residue in the flask and mixed by placing the flask in an ultrasonic bath for 10 min. The mixture was then extracted 3 times with 50 mL of ethyl acetate. The organic phase was transferred to a 200-mL volumetric flask through a layer of anhydrous Na_2SO_4 ; 0.5-mL aliquots were assayed for radioactivity. The components of this solution were designated "free metabolites" (see Figure 2 for summary flow chart).

The aqueous fraction was acidified with 5 mL of 1 N HCl and refluxed for 30 min. After being cooled, the acid solution was extracted 3 times with 50-mL portions of ethyl acetate. Aliquots of the organic phase were assayed for ^{14}C and designated "acid-released metabolites".

The remaining aqueous fraction was mixed with 10 mL of 2.5 N NaOH and refluxed for 30 min. The "base-released metabolites" were extracted with ethyl acetate as before; the organic and remaining aqueous (water-soluble metabolites) phases were analyzed for radioactivity.

The residual fibrous pulp, after extraction of the ethanol-soluble metabolites, was refluxed for 30 min with 50 mL of 0.1 N HCl; this mixture was cooled and the pulp-acid-released metabolites were extracted with ethyl acetate. Aliquots of the organic phase were counted and the remainder was concentrated for thin-layer chromatography. The aqueous phase was counted. The pulp was again hydrolyzed for 30 min with 50 mL of 0.5 N NaOH, and the pulp-base-released metabolites were extracted with ethyl acetate, followed by counting of the organic and aqueous phases. No thin-layer analysis of the aqueous acid or of

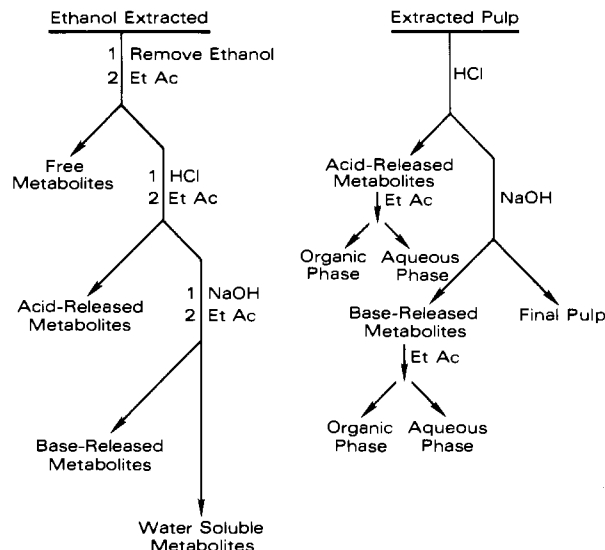


Figure 2. Flow chart of partitioned radiocarbon species in the analysis of leaves from sugarcane plants after [^{14}C]buthidazole treatment.

the base-hydrolyzed organic or aqueous phases was attempted because of insufficient radioactivity.

The extracted, hydrolyzed pulp was freeze-dried and analyzed by combustion for "bound" residue, defined as ^{14}C not removed by ethanol or by the acid/base hydrolysis procedure.

Foliar Absorption. Sugarcane plants were treated individually with a mixture of 0.10 mg (5 μCi) of labeled and 5.58 mg of unlabeled buthidazole to simulate foliar application of 0.5 kg/ha based on normal plant population. The chemical in 1 mL of 95% ethanol-water (1:3), containing 0.5% nonionic surfactant by volume (Tergitol 15-S-9; Union Carbide Co.), was applied equally with a microsyringe to the four youngest expanded leaves of each stalk. The treated area on each leaf approximated 80 cm^2 of the midsection of each leaf blade. There was no evident phytotoxicity. Duplicate plants were sampled at 1, 4, 8, and 12 weeks and sectioned as follows: spindle leaves; young leaves developed after treatment; older green leaves below the four treated leaves; senescent abscised leaf trash (if present); apical meristem; stalk; and the four treated leaves subsectioned into (a) treatment area, (b) distal area above treatment, and (c) proximal area below treatment. The extraction and analysis scheme paralleled that of the root treatment. In all the samples only treated leaves retained enough ^{14}C for subsequent isolation and identification of metabolites.

Thin-Layer Chromatography and Metabolite Identification. Only the free and acid-released extracts in ethyl acetate contained significant amounts of radioactivity for characterization. After evaporation of the ethyl acetate, the concentrates were first chromatographed in one dimension on precoated silica gel thin-layer plates of 1-mm gel thickness (PLQF 1000; Quantum Industries, Fairfield, NJ). One of two solvent systems was used: a mixture of dichloromethane-1-propanol (90:10) (system A) or a mixture of acetonitrile-diethyl ether- NH_4OH (50:50:3).

Metabolites isolated in sufficient quantities were co-chromatographed in two dimensions with reference standards on a 250- μm precoated silica gel thin-layer plate (F-254; Brinkmann Instruments, Inc.). Solvents used were system A followed by petroleum ether-diethyl ether-ethanol (50:35:15) (system B). The R_f values of two major products and ten unknowns are shown in Table I. For values of other known metabolites refer to Yu et al. (1980).

Table I. R_f Values on Silica Gel TLC for Buthidazole, Desmethylbuthidazole, and the Unknown Metabolites in Sugarcane^a

designation	R_f values in solvent system ^b	
	A	B
buthidazole	0.58	0.31
desmethylbuthidazole	0.35	0.36
unknown I	0.51	0.48
unknown II	0.57	0.52
unknown III	0.30	0.16
unknown IV	0.51	0.57
unknown V	0.67	0.63
unknown VI	0.62	0.48
unknown VII	0.28	0.48
unknown VIII	0.50	0.58
unknown IX	0.39	0.43
unknown X	0.53	0.45

^a For R_f values of other known metabolites, refer to Yu et al. (1980). ^b Solvent A: methylene chloride-1-propanol (90:10). Solvent B: petroleum ether-diethyl ether-ethanol (50:35:15).

Table II. Distribution of Radiocarbon in Sugarcane Plants Treated with [¹⁴C]Buthidazole: Root Uptake from Nutrient Solution

plant part	% of total radiocarbon in plant			
	1 week	4 weeks	8 weeks	12 weeks
spindle	0.51	0.08	n.s. ^a	n.s.
apical meristem	0.36	0.04	0.01	n.s.
green leaves	80.40	86.85	50.39	19.63
detached leaves	n.a. ^a	n.a.	38.53	66.83
stalk	4.40	2.60	2.46	3.82
seed piece	1.45	1.01	0.62	0.45
roots	12.88	9.09	8.00	9.28
secondary shoots	n.a.	0.32	n.a.	n.a.
total ¹⁴ C in plant, % of applied	32.99	76.81	77.86	85.47

^a n.a., none available. n.s., no significant radioactivity above background.

Detection consisted of radioautography on X-ray film (Kodak No-screen medical film, NS 5T), requiring from 3 to 30 days, depending on the intensity of the radioactivity. The spots were scraped from the plate and extracted with ultrasonic mixing into 10 mL of ethyl acetate. Small portions (0.5 mL) were counted. Material was collected from duplicate runs for identification.

Isolated metabolites were analyzed at Velsicol Chemical Co. laboratories in Chicago, IL, with a Hewlett-Packard Model 5982A mass spectrometer coupled with a Hewlett-Packard Model 5934A dual-disk data system. The samples were analyzed by direct inlet probe by using two modes: electron ionization (EI) at 70 eV and chemical ionization (CI) with methane as the ionizing gas at a pressure of 0.1 torr.

RESULTS

Root Absorption. Radioactivity from [¹⁴C]buthidazole applied to the roots of sugarcane plants growing in nutrient culture solution was absorbed and translocated, apparently in the xylem, to the green leaves. The quantity absorbed during the experiment increased from 33% at 1 week to 85% at 12 weeks, with 4% remaining in the nutrient medium (Table II). Within 1 week, 80% of the absorbed activity could be located in the leaves and 13% in the roots. At 12 weeks 86% had been stored in the leaves: 19.6% in the green leaves on the plant and 66.8% in leaves that had absorbed the activity, become naturally senescent, and

Table III. Extractability of Radiocarbon in Sugarcane Leaves after Root Treatment with [¹⁴C]Buthidazole

	% of total radiocarbon in green and detached, dry leaves					
	1 week	4 weeks	8 weeks	12 weeks		
ethanol extracted						
free metabolites	82.4	56.1	31.2	(49.4)	25.5	(8.6)
acid released	6.4	24.1	31.6	(16.1)	36.6	(5.0)
base released	n.s. ^a	0.8	1.2	(0.6)	n.s.	(n.s.)
water soluble	n.s.	0.7	1.6	(0.9)	3.9	(0.1)
unextracted						
acid released						
organic phase	6.2	11.2	24.5	(27.6)	19.5	(71.3)
aqueous phase	0.6	1.1	1.5	(2.9)	5.3	(8.4)
base released						
organic phase	n.s.	1.7	2.5	(1.8)	n.s.	(3.7)
aqueous phase	0.9	1.3	1.5	(1.1)	4.1	(2.9)
residual pulp	n.s.	n.s.	0.3	(0.2)	n.s.	(1.9)
total	96.5	97.0	95.9	(100.6)	94.9	(101.9)

^a n.s., no significant radioactivity above background.

detached from the plant. The roots appeared to retain about 9% and the stalk 2.5–4% near the end of the experiment. No other section, spindle, meristem, seed piece, or secondary shoots, contained significant amounts of radioactivity.

The summary in Table III of the free and the acid/base-hydrolyzed metabolites indicates that acid hydrolysis released most of the radioactive metabolites in a form soluble in ethyl acetate. Ethanol-soluble residues, both free and acid released, decreased with time and the ethanol-insoluble, acid-hydrolyzable metabolites in the fibrous pulp increased. In particular, the senescent detached leaves (dry trash) at 12 weeks contained most of their radioactivity in a conjugated form that was only released by acid hydrolysis.

Thin-layer chromatography, confirmed with a mass spectrometer, revealed the primary root-absorbed metabolite of buthidazole in sugarcane to be the desmethyl derivative (II, Figure 1), present partly in a free and partly in an acid-released form (Table IV). The parent buthidazole decreased throughout the time period. Desmethylbuthidazole was the major constituent of the acid-released substances that were ethanol soluble as well as ethanol insoluble. The soluble free and acid-released metabolites decreased and the acid-released metabolites from the pulp increased with time. Buthidazole and the desmethyl derivative were partially conjugated in an acid-hydrolyzable form. Acid hydrolysis of the parent compound did not yield desmethylbuthidazole.

With ¹⁴C recoveries approximating 85%, plus 4% remaining in the nutrient solution, it is unlikely that extensive degradation occurred in sugarcane beyond desmethylbuthidazole. Since most of the radiocarbon—except that contained in the roots—deposits in the leaves that subsequently senesce and detach from the stalk, the desmethyl derivative, at least in its conjugated forms, has considerable stability. The relative stability of the desmethyl metabolite is unimportant as a final plant residue, as the detached leaves do not constitute a part of the harvested crop.

There were small but measurable quantities of three known and eight unknown intermediate metabolites with no consistent patterns of increase with time. The known metabolites have appeared in other crops (Yu et al., 1980) or in animals (Atallah et al., 1980) and have varying alterations in the imidazolidinone portion of the molecule. The three were tentatively identified as a dihydroxy-

Table IV. Identity^a and Percent Distribution of Radiocarbon in Sugarcane Leaves after Treatment of Roots with [¹⁴C]Buthidazole^b
 % of total radiocarbon recovered in the leaves

	buthidazole	desmethyl- buthidazole	urea	dehydrate	dihydroxy	I	II	III	IV	V	VI	VII	VIII	IX	X	total
1 week																
free	72.76	9.07				0.13	0.33	0.57	0.05							82.40
acid released	0.23	5.66				0.09			0.11							6.40
pulp (acid)	3.57	2.14							0.29							6.20
total	76.56	16.87				0.22	0.33	0.57	0.16		0.29					95.00
4 weeks																
free	47.50	8.49				2.89	1.05	0.11	0.14	0.78						56.10
acid released	0.53	18.46				0.36		0.24								24.09
pulp (acid)	2.06	8.78														11.20
total	50.09	35.73				3.25	1.05	0.35	0.14	0.78						91.39
8 weeks (green leaves)																
free	19.87	7.49				1.24	0.80		0.36	3.84					0.42	31.20
acid released		27.75				0.80	0.64		0.26	0.36	0.68					31.61
pulp (acid)	1.08	21.19				2.04	1.44		0.62	0.34	0.20					24.51
total	20.95	56.43				2.04	1.44		0.62	4.54	0.88				0.42	87.32
8 weeks (detached leaves)																
free	21.06	18.93	6.82			2.59	0.70		1.24	0.18						49.40
acid released	0.02	11.61	1.14			0.75		0.26	0.29					0.18		16.08
pulp (acid)	1.30	23.95			0.70			0.29	1.37							27.61
total	22.38	54.49	7.96		0.70	3.34	0.70	0.55	2.61	0.18				0.18		93.09
12 weeks (green leaves)																
free	19.65	5.85				1.94	2.09		0.97							25.50
acid released		30.83			0.77				2.44							36.60
pulp (acid)		17.06				1.94	2.09		3.41							19.50
total	19.65	53.74			0.77	1.94	2.09		3.41							81.60
12 weeks (detached leaves)																
free	5.16	3.08	0.36			0.13	0.10									8.60
acid released		4.77						2.78	2.71							5.00
pulp (acid)	11.56	54.25				0.13	0.10	2.78	2.71							71.30
total	16.72	62.10	0.36			0.13	0.10	2.78	2.71							84.90

^a Identity by two-dimensional thin-layer cochromatography of authentic standards and mass spectrometry verification. ^b Organosoluble radiocarbons of the free and acid-released metabolites only. Substances released by base hydrolysis are not included.

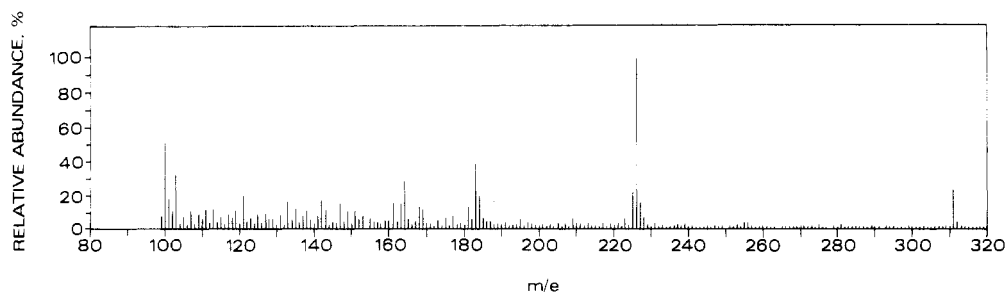


Figure 3. Mass spectrum of buthidazole metabolite unknown I (EI mode) isolated from sugarcane leaves.

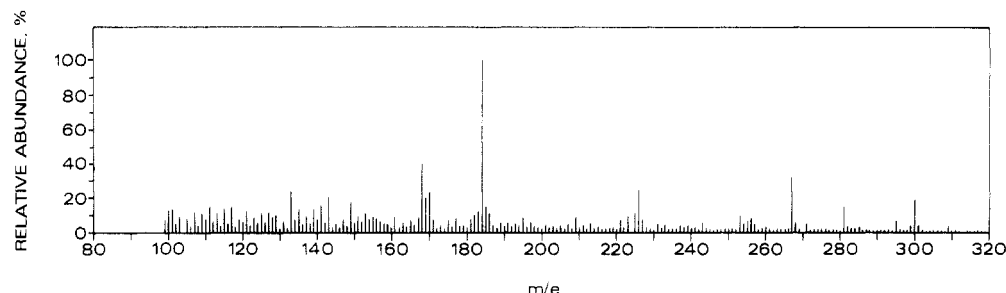


Figure 4. Mass spectrum of buthidazole metabolite unknown II (EI mode) isolated from sugarcane leaves.

buthidazole [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4,5-dihydroxy-1-methyl-2-imidazolidinone], a dehydratebuthidazole with water removal to form a double bond at the 4,5 position of the imidazolidinone ring, and a urea derivative [3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]urea]. Unknown I was tentatively identified by mass spectrometry (Figure 3) as buthidazole aldehyde (III, Figure 1) and unknown II (Figure 4), again tentatively, as the ethyl ether of *N*-methylolbuthidazole (IV, Figure 1), probably formed as an artifact. Quantities for identification were very small. These metabolic products seem to indicate relatively little cleavage of the imidazolidinone ring, with no apparent increase with time. At no time did the total metabolites other than desmethylbuthidazole exceed 11% of the total radiocarbon in the plant; at 12 weeks the quantity was less than 6%. No single minor metabolite accounted for more than 4% and was usually less than 2%. Only unknowns III and IV could be said to have increased as conjugated products insoluble in ethanol and released by acid.

Foliar Absorption. Radioactivity from foliar treatment with [^{14}C]buthidazole did not transport from the treated leaves to any extent (Table V). Minor amounts appeared early in untreated leaves but were considered to be wind-abraded contamination from upper treated leaves. Substantial distal movement occurred away from the treatment zone on each leaf, but the minor radioactivity found in the proximal untreated area can probably be considered to be contamination.

Total radioactivity in or on the leaves from the surface treatment decreased with time: most of the loss (43%) occurred in the first week, with subsequent loss totaling 58% after 12 weeks. No activity was found in any other portion sampled, such as the meristem and new leaves appearing after treatment. The losses from the leaf surface could have resulted from volatility, wind and water abrasion, biological or chemical degradation, or perhaps other forces. Buthidazole in solution is known to be relatively stable to photodegradation (Diaz, 1974).

Free and soluble metabolites decreased with time (Table VI); most radiocarbon not extracted with ethanol could be released from the pulp with acid. Chromatography and mass spectrometry again showed desmethylbuthidazole to

Table V. Distribution of Radiocarbon in Sugarcane Plants Treated with [^{14}C]Buthidazole: Foliar Intake

plant part	% of total radiocarbon in plant			
	1 week	4 weeks	8 weeks	12 weeks
spindle	n.s. ^a	0.09	n.s.	n.s.
meristem	n.s.	n.s.	n.s.	n.s.
treated leaves				
treatment area	64.99	65.79	54.20	63.26
distal area	31.31	25.30	43.93	34.39
proximal area	2.68	5.38	1.87	2.35
untreated green leaves	1.01	3.44	n.s.	n.s.
untreated detached dry leaves	n.a. ^a	n.a.	n.s.	n.s.
stalk	n.s.	n.s.	n.s.	n.s.
^{14}C recovery, whole plant, % of applied	56.55	54.94	36.80	41.62

^a n.a., none available. n.s., no significant radioactivity above background.

Table VI. Extractability of Radiocarbon in Sugarcane Leaves after Foliar Treatment with [^{14}C]Buthidazole

	% of total radiocarbon in leaves			
	1 week	4 weeks	8 weeks	12 weeks
ethanol extractable				
free metabolites	27.6	19.4	12.8	8.9
acid released				
organic phase	37.4	33.9	28.1	16.0
base released				
organic phase	0.9	1.0	0.6	n.s. ^a
aqueous phase	1.2	1.2	1.0	0.7
unextractable				
acid released				
organic phase	23.8	34.9	43.4	58.7
aqueous phase	2.7	3.3	4.0	6.3
base released				
organic phase	2.1	1.7	3.1	1.7
aqueous phase	1.1	1.4	2.3	2.5
residual pulp	0.3	0.3	0.3	0.3
total	96.8	97.1	95.6	93.3

^a n.s., no significant radioactivity above background.

Table VII. Identity^a and Percent Distribution of Total Radiocarbon in Sugarcane Leaves after Treatment of Leaves with [¹⁴C]Buthidazole^b

	% of total radiocarbon in leaves													total
	buthidazole	desmethyl- buthidazole	urea	I	II	III	IV	V	VI	VII	VIII	IX	X	
1 week														
free	8.89	18.71												
acid released		30.29	0.68	1.50	3.86	0.26	0.19	0.49			0.15			
pulp (acid)	2.24	18.99	0.41	0.48	0.31	0.31	0.26	0.33	0.26	0.21				
total	11.13	67.99	1.09	1.98	4.17	0.57	0.45	0.82	0.26	0.21	0.15			88.82
4 weeks														
free	12.22	7.18												19.40
acid released	0.79	26.17		3.27	1.23	0.44	0.48	1.43		0.10				33.91
pulp (acid)	2.72	29.21	0.54	0.98		0.40			0.83	0.22				34.90
total	15.73	62.56	0.54	4.25	1.23	0.84	0.48	1.43	0.83	0.32				88.21
8 weeks														
free	5.81	6.99												12.80
acid released		25.64		0.92	0.93		0.31	0.18	0.11					28.09
pulp (acid)	2.29	37.63		0.85	0.56		0.72	0.57	0.39	0.09			0.30	43.40
total	8.10	70.26		1.77	1.49		1.03	0.75	0.50	0.09			0.30	84.29
12 weeks														
free	2.24	6.66												8.90
acid released	0.05	14.11		0.70	0.72	0.15	0.05	0.12	0.05	0.02	0.03			16.00
pulp (acid)	4.46	49.06	0.64	1.35	0.46	0.48		0.60	0.40	0.41	0.72	0.12		58.70
total	6.75	69.83	0.64	2.05	1.18	0.63	0.05	0.72	0.45	0.43	0.75	0.12		83.60

^a Identity by two-dimensional thin-layer cochromatography of authentic standards and mass spectrometry verification.

^b Organosoluble radiocarbons of free and acid-released metabolites only. Substances released by base hydrolysis are not included.

be the primary metabolic product: 65% of the radiocarbon remaining in or on the plant after the first week was present in this form, with little subsequent change (Table VII). The parent buthidazole declined slightly from 11 to 7% between 1 and 12 weeks, but it was readily apparent that most metabolic alteration took place in the first week.

At the 12-week harvest, all of the treated leaves had senesced and abscised from the plants, as was also true of the same leaves on the control plants, removing all the radiocarbon from the growing plant.

Minor known and unknown metabolites appeared in the chromatograms as in the root-absorption experiment, again with no particular pattern of increase with time. Any one unknown metabolite accounted for a maximum of less than 4% of the extracted radiocarbon. There was a tendency for the minor unknown products to become less ethanol soluble, but they were acid hydrolyzed from the pulp.

DISCUSSION

Cultivars of sugarcane are grown in Hawaii as a 2-3-year crop. Weed control is necessary during the first 4-6 months of growth of each crop until the leaf canopy shades out weed seed germination and growth of perennial rootstocks. Herbicide residues absorbed by crop roots or leaves during the early growth have an unusually long period—generally 18 months or more—for dilution and depletion. Residues from herbicides that translocate in the transpiration system, as buthidazole and its metabolites do, concentrate in the leaves as the transpired water evaporates (Hilton et al., 1976). The top five green leaves are most active photosynthetically and in concentrating mobile substances, especially from a nutrient culture source. As a new leaf expands and becomes active over a 10-12-day period, the lowest green leaf gradually loses metabolic function, becomes senescent, and detaches from the stalk. With a more-or-less constant 10-15 green leaves per stalk, the total active life of each leaf may be 65-75 days, dependent somewhat on cultivar and environmental growth condition. Leaf abscission provides an excretory mechanism for soluble salts and any other mobile substances such

as pesticides and their metabolites that are carried and deposited by the transpired water but which do not cross over into the phloem. Since herbicides are used only during early crop growth, and since residues in the leaves are lost during senescence and detachment, significant or even measurable amounts of herbicide residues are unlikely to appear in the harvested crop.

The accumulated dry leaf trash may be burned in the field prior to harvest or separated by washing and de-trashing mechanisms at the raw sugar factory before the sugar juice is extracted from the stalks. The fibrous pulp (bagasse) and any portion of the recoverable leaf trash is burned as fuel for the sugar mills. Public power generation from sugarcane fiber is increasing rapidly in Hawaii, although the sugar mills and their surrounding communities have long benefited from this indigenous fuel.

Compared with other xylem-translocated herbicides whose metabolism in sugarcane has been studied, buthidazole differs mainly in that its transformation to desmethylbuthidazole leads to a product that is apparently quite stable to further alteration. Conjugation of buthidazole and desmethylbuthidazole takes place to acid-labile ethanol-soluble and ethanol-insoluble substances; the 4-hydroxyl position common to each compound seems the most likely point of attachment although the conjugating moieties are not known. The ethanol-insoluble desmethylbuthidazole product(s) increases (increase) considerably with time at the expense of soluble derivatives, which may imply—without further verification—that glucosides may first be formed and become less soluble due to polysaccharide attachment in older tissue. The conversion of buthidazole to desmethylbuthidazole is particularly rapid from the leaf surface application. Data from Velsicol Chemical Co. (Diaz, 1974) indicate that desmethylbuthidazole is not a photolytic product from buthidazole applied to a glass surface or in water, nor is it a hydrolysis product. The conversion could be enzymatic, microbial, or both. Enzymatic action seems most likely since the same product appears from root absorption and since other plants, notably maize, may follow the same general met-

abolic alteration pathway but do not accumulate the desmethyl derivative.

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Fate of Fluridone in Sediment and Water in Laboratory and Field Experiments

Derek C. G. Muir* and Norbert P. Grift

The fate of the aquatic herbicide fluridone [1-methyl-3-phenyl-5-[(3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone] was studied in sediment-water systems in culture flasks, in pond water exposed to sunlight, and in small ponds by using either a carbonyl-¹⁴C- or *N*-methyl-¹⁴C-labeled compound. The half-life of fluridone in sediment was 12 months under laboratory conditions (25 °C) and about 17 weeks under field conditions. In the laboratory study, the major degradation product of fluridone in sediment was fluridone-acid [1,4-dihydro-1-methyl-4-oxo-5-[3-(trifluoromethyl)phenyl]-3-pyridinecarboxylic acid; II]. II accounted for 48-54% of the radioactivity that was extracted from sediments incubated for a 26-month period. Two phenolic compounds, 4-hydroxyfluridone [1-methyl-3-(4-hydroxyphenyl)-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone; III] and the 2-hydroxy derivative (V), were identified as minor breakdown products of fluridone in sediment (0.5-2.5% of radioactivity). Desphenylfluridone [1-methyl-3-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone; I], II, III, and V were identified in aqueous solutions of fluridone held in Pyrex flasks in sunlight. The half-life of fluridone in ponds treated at 100 µg/L was 2-3.5 days, and III and V were identified at low levels (0.05-0.5 µg/L) in water sample extracts. No major degradation products of fluridone were identified under field conditions due to the apparent extensive photodegradation of the compound.

Fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone] is an experimental aquatic herbicide for the control of vascular aquatic plants (Arnold, 1979; Dechoretz and Frank, 1978). It has also been tested for weed control in cotton (Webster et al., 1977). Studies on the disappearance of fluridone in aquatic systems have indicated that it has an average half-life of about 5 days in pond and lake waters (West et al., 1979; Muir et al., 1980). Dissipation of the herbicide in sediments and terrestrial soils under field conditions has been reported to be much slower than in water. Half-lives of fluridone in clay and fine sandy loam soils ranged from about 50-100 days (Banks et al., 1979). In sediments half-lives ranging from about 3 months to greater than 12 months have been observed (West et al., 1979; Muir et al., 1980).

No major degradation products of fluridone were found in water or sediment sample extracts from ponds treated

at recommended rates of application (West et al., 1979; Muir et al., 1980) despite the overall rapid disappearance of the herbicide. It seemed likely that the degradation products were either extremely labile in water or that they could not be determined by the analytical techniques used in the field studies. Laboratory studies have shown that fluridone degrades rapidly in deionized water (half-life of 23 h) under artificial sunlight (West et al., 1979). Thus, photolysis may be a major pathway for disappearance of fluridone in water.

The purpose of the present work was to identify the major degradation products of fluridone in sediment and water by using laboratory studies and to compare the results with those of field studies on the disappearance of fluridone in small artificial ponds.

MATERIALS AND METHODS

Analytical Standards. Fluridone, 4-hydroxyfluridone [1-methyl-3-(4-hydroxyphenyl)-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone], fluridone-acid [1,4-dihydro-1-methyl-4-oxo-5-[3-(trifluoromethyl)phenyl]-3-

*Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Manitoba, Canada R3T 2N6.