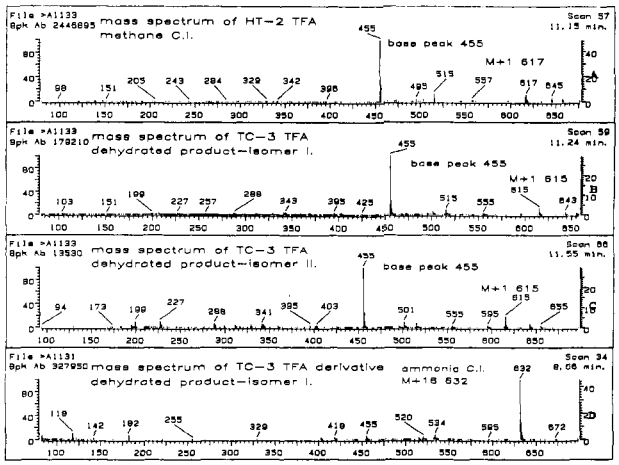

Figure 4. Mass spectra of di-TFA derivatives of (A) HT-2 toxin (+CI CH_4), (B) TC-3 isomer 1 (+CI CH_4), (C) TC-3 isomer 2 (+CI CH_4), and (D) TC-3 isomer 1 (+CI NH_3). Note the same base peak (M^+ 455) in all spectra (CH_4 CI) and a two proton difference in $M^+ + 1$ of T-2 and TC-3 isomers. The +CI in ammonia helps establish the molecular ion.

matography, one with a retention time of 11.2 min and the other 11.5 min (Table I). The mass spectrum of HT-2 at 70 eV shows a molecular ion of 616 in electron impact and 617 in positive chemical ionization (Figure 4) with a base peak of 455 in the latter. The TFA derivatives of TC-3 (both isomers) also show a base peak of 455 and a molecular ion at M^+ 615. Both isomers are dehydration products analogous to those found in TC-1 explained above. The identity of the molecular ion of TC-3 is further confirmed by the $M^+ + 18$ (632) shown in Figure 4 when detected by chemical ionization in NH_3.

Thus, the reactivity of TC-1 and TC-3 with trifluoroacetic acid anhydride forms isomeric dehydration products that simplifies procedures for the detection of C-3′ hydroxylated products of T-2 metabolism. This characteristic reaction has assisted us in detecting another T-2 derivative (TC-6) that produces isomers similar to the TFA reaction products of TC-1 and TC-3.

Registry No. III, 84474-35-1; VI, 78368-54-4; TC-1 (isomer I), 91860-58-1; TC-1 (isomer II), 91860-59-2; TC-3 (isomer I), 91860-60-5; TC-3 (isomer II), 91860-61-6.

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Metribuzin Metabolism in Soybeans. Characterization of the Intraspecific Differential Tolerance

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Differential tolerance of soybean [*Glycine max* (L.) Merr.] to [^{14}C]metribuzin was not due to absorption or translocation differences but due to the rate of metabolism. After 106-h treatment by subirrigation, the radioactivity in the susceptible cultivar "Semmes" was primarily in leaf interveinal tissue as unmetabolized metribuzin while in the tolerant cultivar "Coker 338" the majority of the radioactivity was in the more mature shoot tissue as polar metabolites and was restricted primarily to the vascular tissue. Differential tolerance was attributed to at least the following factors: (a) the restriction of metribuzin to the vascular tissue in tolerant "Coker 338" with movement mostly into the interveinal tissue in "Semmes", (b) the higher metribuzin concentration in "Semmes" leaves (9 μg/g of dry weight) than in "Coker 338" leaves (3 μg/g of dry weight), and (c) the higher rate of polar product (metribuzin conjugate) formation in "Coker 338".

Metribuzin, 4-amino-6-*tert* butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one, is an asymmetrical triazine herbicide used on soybeans. However, cultivar tolerance to metribuzin varies dramatically (Andersen, 1976). Intraspecific differential tolerance is apparently due to differential rates of metabolism (Mangeot et al., 1979; Smith and Wilkinson, 1974). Metribuzin is metabolized to polar and nonpolar metabolites and incorporated into the insoluble residue (Mangeot, et al., 1979; Smith and Wilkinson, 1974). Smith and Wilkinson (1974) reported that tolerance resulted from metribuzin detoxification through polar conjugate formation. Mangeot et al. (1979) indicated that formation of the 6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one (DA) nonpolar metabolite, numerous unidentified aqueous metabolites, and incorporation into the insoluble fraction all contributed to cultivar tolerance to metribuzin. In tomato, the polar metabolites have been identified as the β-D-(*N*-glucoside) and malonyl β-D-(*N*-glucoside) conjugates of metribuzin (Frear et al., 1983).

The objective of this study was to characterize, more definitively, the cause(s) of soybean intraspecific differ-

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ential tolerance to metribuzin.

EXPERIMENTAL SECTION

Chemicals. Technical- and analytical-grade metribuzin, analytical grades of DA, 4-amino-6-*tert*-butyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (DK), 6-*tert*-butyl-*as*-triazine-3,5-(2*H*,4*H*)-dione (DADK), and analytical-grade [5-^{14}C]metribuzin (sp act., 4.44 mCi/mmol) were obtained from Mobay Chemical Corp. Analytical-grade and labeled metribuzin purities were greater than 99%.

Plant Treatment and Extraction. "Coker 338" and "Semmes" soybeans were cultured in the greenhouse under supplemental light provided by metal halide lamps with a 15-h photoperiod. Noninoculated seeds were planted in coarse vermiculite and the surface was irrigated daily with tap water until 2 days after seedling emergence when 0.2 strength Hoagland's complete mineral nutrient solution (Hoagland and Arnon, 1950) was added by subirrigation. The Hoagland solution concentration was gradually increased to full strength a week later and thereafter. Plants were periodically sprayed with Dipel (Abbott Laboratories), a biological insecticide. Two days before treatment with metribuzin the plants were moved into a plexiglas chamber in the greenhouse for acclimation. Plants at the fourth trifoliate leaf stage were subirrigated with 1 μM [5-^{14}C]metribuzin (0.55 μCi/L solution) in 2 L of full strength Hoagland's solution for 106 h. At the midpoint of the experiment, 2 L of identical fresh solution was added to the treatment pans. The treatment pans contained four pots per pan and were covered with black polyethylene. The temperature varied from 22 to 34 °C. The sky was partly cloudy several days before and during treatment.

Following treatment, numerous shoots were autoradiographed by exposing to X-ray film for 3 weeks. The roots of the remaining plants were washed with water, rinsed with deionized water, and blotted dry. The plants were separated into roots, stems (including petioles and petiolules), and leaflets, and the plant tissues were lyophilized. Plant weights were determined before and after lyophilization. The lyophilized tissue was cut into small pieces and homogenized 5 min in 50 mL of 80% ethanol at maximum speed in a Sorvall omnimixer. During grinding the omnimixer cup was submersed in an ice–water bath. The extract was vacuum filtered (insoluble = "residue") on Whatman No. 1 filter paper with 20 mL of 80% ethanol/g of fresh weight at which point the eluant was colorless. The extraction efficiency for metribuzin was 87%. The filtrate was evaported to the water phase and filtered through a 0.65-μm Millipore filter (Millipore Filter Corp.) and rinsed with deionized water. The filtrate was partitioned twice with equal volumes of benzene (aqueous = "polar") (Smith and Wilkinson, 1974) and then filtered a second time on a 0.65-μm filter.

Analysis of Fractions. One milliliter of the aqueous extract was solubilized in 15 mL of Scintiverse (Fisher Scientific Co.) for scintillation counting while the remaining aqueous fraction was lyophilized and stored in a desiccator. The radioactivity was quantitated with a liquid scintillation spectrometer (Beckman, LS-100). The external standard ratios were used for quench correction, and all radioactivity was expressed as disintegrations per minute.

The plant residue from filtration was dried at room temperature and weighed. A sample of the residue (ca. one-tenth) was combusted with a Packard Tri-Carb Model B306 sample oxidizer. The CO_2 was absorbed in Carbosorb (Packard Co.) and solubilized in Permaflour V scintillation fluid (Packard Co.) and counted by liquid scintillation spectrometry. The benzene extract was quantitated by liquid scintillation in a toluene solution [5 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 2,2′-*p*-phenylene-bis[4-methyl-5-phenyloxazole] (dimethyl-POPOP) in 1 L of toluene]. The remaining benzene fraction was evaporated and then dissolved in 1 mL of benzene–acetonitrile (9:1 v/v). This fraction was cochromatographed with known standards on 20 × 20 cm Bakerflex (Baker Chemical Co.) thin-layer chromatography (TLC) sheets coated with silica gel G (250 μm) in a benzene–chloroform–*p*-dioxane (4:3:3 v/v/v) (Mangeot et al., 1979) solvent system. Chromatograms were developed to a height of 15 cm. The developed standards were sprayed with a fresh solution of 1% sodium nitrite in 1 N HCl followed by a 1% solution of *N*-naphthylethylenediamine dihydrochloride in 2 N HCl (Frear, 1968). The sheets were heated 3–5 min at 60 °C, and metribuzin and the three nonpolar metabolite standards were detected under long-wave UV light. The radioactivity on the developed samples was located by assaying on a Packard Model 7220/21 radiochromatogram scanner. The R_f values were 0.79, 0.67, 0.62, 0.55, and 0.03 for metribuzin, DA, DADK, DK, and unidentified metabolite, respectively.

Table I. Injury Ratings of Soybean Cultivars to Metribuzin

cultivar	maturity class	injury rating,[a] % expt 1	expt 2	average[b]
"Coker 338"	VIII	98	94	96
"Coker 136"	V, VI	95	91	93
"Bossier"	VII	91	88	90
"Bienville"	VII	84	85	85
"Lee 68"	VI	77	80	79
"Hampton 266A"	VIII	69	68	69
"Bragg"	VII	61	66	64
"York"	V	57	65	61
"Arksoy"	VI	68	53	61
"Hardee"	VIII	56	64	60
"Coker 102"	VIII	22	29	26
"Tracy"	VI	26	24	25
"Semmes"	VII	18	15	17

[a] 0 = complete kill and 100 = no visible injury. [b] Average ratings are based on two experiments of five replications per experiment.

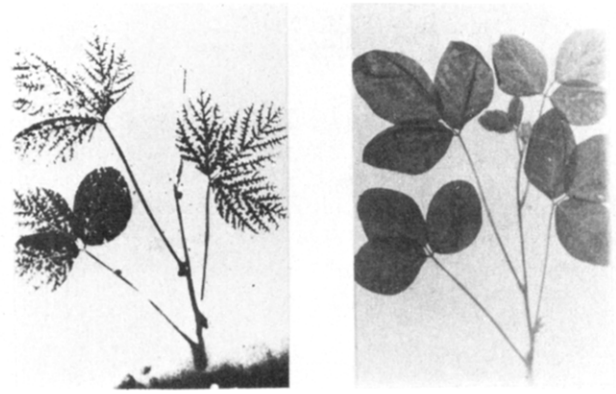

Figure 1. Autoradiograph (left) or tolerant "Coker 338" cultivar (right) subirrigated with 10^{-6} M [^{14}C]metribuzin (2.2 μCi) for 106 h.

RESULTS AND DISCUSSION

Absorption and Translocation. Thirteen soybean cultivars (maturity classes V–VIII) were visually rated for tolerance to 104-h exposure to subirrigated, unlabeled, technical-grade metribuzin (10^6 M) treatments in the greenhouse (Table I). The most tolerant, "Coker 338", and most susceptible, "Semmes", were used in all subsequent studies.

Metribuzin was readily absorbed by the roots and translocated to the shoots of both cultivars (Table II), but

Table II. Distribution of ^{14}C-Labeled Residues in Soybean Plants[a]

		fraction							
		μg/g of dry wt				%			
cultivar	organ	polar	nonpolar	residue	total	polar	nonpolar	residue	total[c]
"Coker 338"	leaf	9.19a	5.30b	3.89c	18.38b	50.0a	28.8b	21.2c	38.2b
	stem	9.36a	1.41c	6.89a	17.66b	53.0a	8.0d	39.0b	86.8b
	root	4.77b	1.77c	5.48b	12.02c	39.7b	14.7c	45.6a	25.0c
	av[b]	8.37	3.37	5.15	16.89	48.5	17.6	33.8	100.0
"Semmes"	leaf	3.71bd	14.31a	3.89c	21.91a	16.9d	65.3a	17.7c	59.3a
	stem	2.12cd	2.30c	3.89c	8.30d	25.5c	27.7b	46.8a	22.5c
	root	1.48c	2.12c	3.18c	6.71d	22.1c	31.6b	47.4a	18.2c
	av	2.77	8.14	3.74	14.64	19.8	50.7	29.7	100.0
"Coker 338" av vs. Semmes av		**[d]	**	**	**	**	**	**	

[a] Means within a column followed by a common letter are not significantly different ($P < 0.05$ by LSD). [b] av = weighted average of organs except % av total = leaf + stem + root or % av total = polar + nonpolar + residue. [c] % total = [total μg/(total leaf μg + total stem μg + total root μg)] × 100; % polar/nonpolar/residue = (μg/total μg) × 100. [d] (**) Significant at $P < 0.01$.

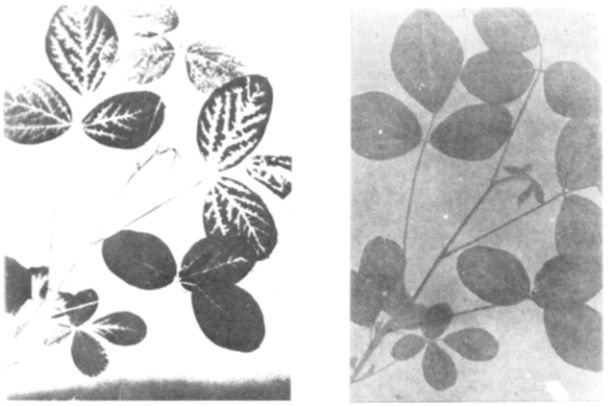

Figure 2. Autoradiograph (left) of susceptible "Semmes" cultivar (right) subirrigated with 10^{-6} M [^{14}C]metribuzin (2.2 μCi) for 106 h.

"Coker 338" absorbed 30% more [^{14}C]metribuzin than "Semmes" (48.06:36.92). The radioactivity in "Coker 338" shoots was restricted mostly to the older, more mature plant parts with little radioactivity moving into the growing points and was restricted largely to the veins (Figure 1). Comparing all the autoradiographs of "Coker 338" shoots indicated that the leaves with the greatest veinal restriction of ^{14}C occurred at the uppermost leaves, was second most prevalent among the lower leaves (including unifoliate), and was least prevalent (though common) among leaves at the intermediate height on the plant.

The radioactivity in "Semmes" shoots was mostly in the interveinal leaf tissue (Figure 2). A greater accumulation of radioactivity was noted in the growing points of "Semmes" shoots when compared to "Coker 338" plants. Results from all the autoradiographs of "Semmes" shoots indicated that the youngest leaves often had the radioactivity somewhat restricted to the veins, although less so than in "Coker 338". The remaining "Semmes" leaves had no ^{14}C restriction.

Whereas the autoradiographs of both cultivars showed a striped appearance in the stem (^{14}C concentration in strands), the radioactivity appeared more concentrated in "Coker 338" stem than in "Semmes" stem. The restriction of ^{14}C in the older, more mature plant tissue (veins) of tolerant "Coker 338" and increased ^{14}C movement into the younger tissue (interveinal) of susceptible "Semmes" is consistent with whole plant studies showing increased tolerance to metribuzin with age (da Silva and Warren, 1976). This analysis of the ^{14}C movement expands our knowledge on the distribution of metribuzin and metabolites within plant tissue.

The data in Table II confirm that the tolerant "Coker 338" plants translocated more metribuzin and metabolites to the shoots (36.04 μg/g of dry weight) than did "Semmes" (30.21 μg/g of dry weight), eliminating translocation as the cause of differential tolerance. The translocated metribuzin and metabolites were uniformly distributed in "Coker 338" between the leaves (38%) and stems (37%) (Table II). However, in "Semmes", the metribuzin and metabolites were primarily in the leaves (59%).

Metabolism. The nonpolar metribuzin was metabolized to polar and nonpolar metabolites and was incorporated into the insoluble fraction (Table II). In the nonpolar fraction there was a low percentage of radioactivity in "Coker 338" stems and a high percentage in "Semmes" leaves, which are, presumably, a reflection of their differential metabolism. The high percent of nonpolar in "Semmes" leaves was, apparently, due to the low rate of metabolism resulting in high accumulation of metribuzin in leaves followed by symptom development. Both cultivars formed the DA, and an unidentified nonpolar metabolite as assayed by TLC scanning. The cultivars had the same trend in percent nonpolar radioactivity remaining in the organs, stems < roots < leaves, which reflects metabolic rates of metribuzin conversion into the polar and residue fractions in the order of stems > roots > leaves.

The data from the TLC scans of the nonpolar fractions indicated that ca. 60, 20, and 20% were unaltered metribuzin, DA metabolite, and unidentified product, respectively, in "Coker 338" leaves. The values in "Semmes" leaves were ca. 62, 23, and 16%, respectively. Therefore, "Coker 338" contained ca. 3 μg of metribuzin/g of dry weight and "Semmes" ca. 9 μg/g of dry weight in the leaves after 106-h metribuzin treatment. Thus, phytotoxicity must be caused by 9 or less μg of metribuzin/g of dry weight in photosynthetic tissue. Assuming metribuzin's primary mode of action to be photosynthesis inhibition (Eue, 1972; Trebst and Wietoska, 1975), the restriction of metribuzin in "Coker 338", but not "Semmes", to the veinal region with lower photosynthetic capacity may help explain the differential tolerance.

"Coker 338" plants metabolized ca. 50% and "Semmes" 20% of the total metribuzin absorbed to the polar fraction (Table II). "Coker 338" leaves and stems formed a significantly more polar product than the roots. In "Semmes" the percent polar product was higher in stems and roots than in leaves. The percent polar product was higher in all "Coker" organs than in "Semmes" organs. Thus, polar product formation is an important factor in soybean differential tolerance. Soybean differential tolerance can thus be attributed to the following factors: (a) the restriction of metribuzin to the vascular tissue in tolerant "Coker 338" but not in susceptible "Semmes", (b) the higher metribuzin

concentration in "Semmes" leaves, and (c) the higher rate of polar product formation in "Coker 338".

The amount of radioactivity in the residue fraction was equal in the leaves of the two cultivars (Table II). In stems, the level of residue radioactivity was significantly higher in "Coker". However, the restriction of radioactivity in stems to the vascular strands, which have low levels of chlorophyll and thus photosynthesis, argues against residue incorporation as a cause of "Coker" tolerance. Furthermore, the toxiphoric group on metribuzin, the primary amine (Draber and Buchel, 1969), is probably conjugated or sterically hindered before incorporation into the residue. Residue incorporation was higher in roots of "Coker". However, roots do not carry out photosynthesis, thus eliminating this as a cause of differential tolerance. For these reasons incorporation into the residue is not thought to explain differential tolerance.

The restriction of radioactivity to the vascular tissue is interpreted as being caused by metribuzin metabolism to a product that will not penetrate through a membrane. Thus "Coker" traps the products in the veins. "Semmes" metabolizes metribuzin at a much lower rate; thus metribuzin penetrates through the membranes and inhibits photosynthesis.

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Maillard Polymers Derived from D-Glucose, D-Fructose, 5-(Hydroxymethyl)-2-furaldehyde, and Glycine and Methionine

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Water soluble, nondialyzable Maillard polymers having molecular weights in excess of 16 000 were prepared from 5-(hydroxymethyl)-2-furaldehyde (HMF), D-glucose, or D-fructose and glycine. Similar polymers were prepared from the latter sugars and methionine. In all cases, the polymers showed no absorption maxima in the 220–320-nm range. For the preparations derived from the latter two sugars, elemental analyses were similar and indicated a nitrogen content of over 6%. Elemental analyses suggest that the polymer is composed of 1 mol of sugar and 1 mol of glycine minus about 3 mol of water. Studies using 90 atom % enriched D-glucose-*1*-^{13}C, glycine-*1*-^{13}C, and glycine-2-^{13}C as precursors in the reactions and ^{13}C NMR as a probe show that both carbon atoms of glycine are incorported into the polymer and that C-1 of D-glucose appears as a substituted methyl group. The NMR data further suggest that the main monomeric (dialyzable) products are unreacted sugar or amino acid and 1-deoxy-1-(*N*-glycino)-D-fructose derivatives (Amadori compounds).

The reaction of reducing sugars with amino acids or protein to produce brown polymers was orginally described by Maillard. Since that time, this reaction has been the subject of numerous studies, and more recently several symposia have been held on the subject (Eriksson, 1982; Waller and Feather, 1983). Although monomeric food flavor and aroma compounds, reductones, and ultraviolet-absorbing compounds are known to be produced in the reaction, the origin and constitution of the polymers are not, at present, well understood. Barbetti and Chiappini (1976a,b) have studied some model systems recently, as have Ledl and co-workers (Ledl, 1982a,b; Ledl and Severin, 1982) and Velisek and Davidek (1976a,b). More recently (Imasato et al., 1981; Bobbio et al., 1981), reports have appeared that describe the preparation and fractionation of melanordins from D-glucose, D-fructose, D-xylose, and glycine. Analytcal data, including elemental analyses and IR and acetylation data were reported.

A knowledge of the chemical constitution of Maillard polymers is desirable, since they are known to contribute to the discoloration of many foods and may have an effect on the digestibility and mineral binding properties of processed foods.

The purpose of this report is to describe the isolation of Maillard polymers derived from D-glucose, D-fructose, or 5-(hydroxymethyl)-2-furaldehyde and glycine and to report analytical data (UV, NMR, and elemental analyses)

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