methylation and the other through hydroxylation of the *tert*-butyl group. The metabolism of isouron through N-demethylation is similar to the path reported for phenylurea herbicides (Goren, 1969; Onley ett al., 1968; Swanson and Swanson, 1968). This could occur through the hydroxylation of the *N*-methyl group in a microsome fraction (Frear, 1968; Frear et al., 1969). The formation of the β-glucoside of the hydroxymethyl methylurea derivative indicates that hydroxylation of the *N*-methyl group occurs before N-demethylation.

Further N-demethylation to form the urea derivative occurred at a slower rate. Many investigators have reported that aniline formation from phenylurea herbicides occurred in small quantities in plants (Geissbühler et al., 1975), but the amine derivative, 5-*tert*-butyl-3-aminoisoxazole, has not been detected in this study. As the substituted isoxazole amine is very volatile, it might have been lost during the isolation procedures and its formation in small amounts cannot be ruled out. Hydroxylation of the *tert*-butyl group of isouron also occurred to a limited extent. However, the hydroxylation of the group of the monomethylurea derivative proceeds much more rapidly. Hydroxylation of the *tert*-butyl group was observed in the metabolism of tebuthiuron (Morton and Hoffman, 1976) and buthidazole (Atllah et al., 1980) in animals. It is presumed from this study that the N-demethylation of isouron followed by the hydroxylation of the *tert*-butyl group is the major route of its metabolism in bean plants.

On the basis of the above interpretation, a scheme for the metabolism of isouron in bean plants is proposed in Figure 6.

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Plant Metabolism of Fluvalinate [α-Cyano-3-phenoxybenzyl 2-[2-Chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate]

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The metabolism of [*trifluoromethyl*-^{14}C]fluvalinate (1) was studied at the approximate field use rate (0.1 kg/ha) on cotton (leaves, squares, bolls), on tomatoes (leaves, fruit), and on leaves of tobacco, lettuce, and cabbage. In general, fluvalinate was metabolized predominantly by hydrolysis to the anilino acid 2 that was present in small amounts as the free acid (1–5% of applied dose) or in larger amounts (particularly with longer times posttreatment) as conjugated metabolites. 4′-Hydroxyfluvalinate (4) was detected in cotton and tomato leaves (1–2% of applied dose), and 2-chloro-4-(trifluoromethyl)aniline (3) was found in plant tissues (up to 4% of applied dose) and was also volatilized. Fluvalinate and its metabolites do not appear to translocate in plants. The approximate half-life of fluvalinate at 0.1 kg/ha, under greenhouse conditions, was greater than 6 weeks on lettuce and tomato leaves and fruit and about 4 weeks on cabbage, tobacco, and cotton. The asymmetric center of the alcohol moiety of fluvalinate is prone to partial epimerization on cotton leaves while the asymmetric center of the anilino acid portion seems relatively stable.

Fluvalinate [α-cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate, MAVRIK] is an insecticide with pyrethroid-like activity that has been selected from numerous synthetic analogues for commercialization by Zoecon Corporation (Henrick et al., 1980). As part of our efforts to determine the environmental fate of fluvalinate, we studied its degradation in several plant species and report our results herein.

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EXPERIMENTAL SECTION

Radiosynthesis. The anilino acid (2) was prepared from $Ba^{14}CO_3$ in six steps (Figure 1) under the direction of Dr. Ron Hale (Dynapol, Palo Alto, CA) using methods developed at Zoecon by Dr. R. J. Anderson. The crude

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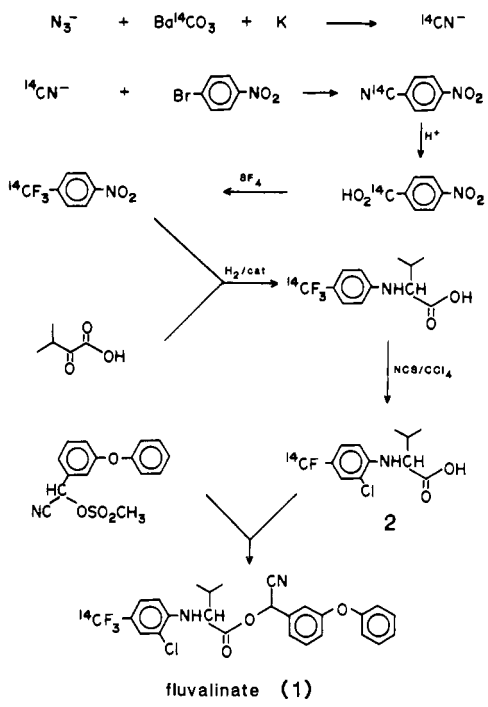

Figure 1. Radiosynthesis of [*trifluoromethyl*-^{14}C]fluvalinate.

sample of **2** was purified by liquid chromatography (LC) on a reversed-phase column (Lobar LiChroprep RP-8, 25 × 310 mm, E. Merck, Darmstadt) using acetonitrile–0.1 M phosphate buffer (45:55, pH 3) for elution. The progress of the chromatography was followed by a UV detector at 254 nm. The anilino acid (**2**, 5.5 mCi, 0.11 mmol, 33 mg) was reacted with *m*-phenoxybenzaldehyde cyanohydrin mesylate ester (46 mg, 0.15 mmol) in tetrahydrofuran–dimethylformamide (6:4, 1 mL) for 20 h at room temperature, giving a 96% conversion to [*trifluoromethyl*-^{14}C]-fluvalinate (**1**). The crude [^{14}C]fluvalinate was purified by LC on a silica gel column (Lobar, LiChroprep SI 60, 25 × 310 mm, E. Merck) using hexane–ether (5:1) for elution and monitoring the progress of separation with a hand-held Geiger counter (or alternatively UV detection at 254 nm). The resultant [*trifluoromethyl*-^{14}C]fluvalinate had a specific activity of 48.3 mCi/mmol (determined by mass spectrometry) and had a radiochemical purity of 99% (LiChrosorb RP-8 column, gradient of 60–90% methanol–0.1% acetic acid over 30 min). The ratio of diastereomers for **1** [i.e., [(αR,2*R*) + (αS,2*S*)]:[(αR,2*S*) + (αS,2*R*)]] was 48:52 based on normal-phase LC (LiChrosorb SI 100, 25 × 0.46 cm, E. Merck; elution with ether–pentane, 4:96).

Radioassay and Chromatography. Thin-layer chromatography (TLC) was performed with silica gel GF plates (generally 1000 μm, Analtech) in the following solvent systems: SS 1 (hexane–ether, 1:1), SS 2 (hexane–ethyl acetate, 2:1), SS 3 (hexane–ethyl acetate–acetic acid, 12:9:0.1), SS 4 (hexane–ethyl acetate, 80:20), SS 5 (hexane–ethyl acetate, 5:2), SS 6 (hexane–ethyl acetate–acetic acid, 150:75:1), and SS 7 (hexane–ether, 10:1). Radiolabeled zones were located with a radiochromatogram scanner (Model 7201, Packard Instrument Co., Downers Grove, IL), and radiolabel on TLC plates was quantitated by liquid scintillation counting (LSC, Packard Model 2425 spectrometers with automatic external standardization). Unextractable ^{14}C-labeled residues were quantitated by combustion to $^{14}CO_2$ (Biological Material Oxidizer, Model OX-100LT, R. J. Harvey Instrument Co., Hillsdale, NJ) with collection in Oxifluor-CO_2 (NEN, Boston, MA) followed by LSC.

Both normal- and reversed-phase modes of liquid chromatography (LC) were utilized. Analysis by reversed-phase LC employed a Spectra-Physics (Sunnyvale, CA) instrument, Model 8000A, an ultraviolet (UV) detector at 254 nm, Model 8310, a LiChrosorb RP-8 column, 25 × 0.46 cm and 10 μm, and elution at 1.6 mL/min and 35 °C. Various mixtures of methanol–0.1% acetic acid were used: SS 8 (gradient 60–70% methanol over 15 min, 70–90% over 10 min, isocratic at 90% for 10 min), and SS 9 (isocratic at 65% methanol). The following conditions were used for normal-phase LC: Haskel Model 28030 pump; Spectra-Physics Model 8200 UV detector; LiChrosorb SI-100 column, 25 × 0.46 cm, 5 μm; elution at 1.6 mL/min with ether–pentane (2:98 for SS 10; 4:96 for SS 11), 50% water saturated. In general, LC analysis consisted of collection of timed eluate fractions for assay of ^{14}C by LSC.

Glucosides were analyzed by gas–liquid chromatography [GLC; Hewlett-Packard (Palo Alto, CA) Model 402 chromatograph using a 2 m × 2 mm i.d. glass column packed with 3% OV-17 on Chromosorb W, acid washed, DMCS treated]. Mass spectral (MS) analysis employed a Hewlett-Packard Model 5985 instrument in the electron impact mode at 70 eV. The metabolic glucosides were analyzed with ^{1}H NMR (Bruker WH-90 spectrometer, 90 MHz) by Dr. M. Maddox (Syntex Corp., Palo Alto, CA). The structures of synthetic standards were confirmed by ^{1}H NMR with a Varian (Palo Alto, CA) T-60 instrument.

Synthetic Standards. Authentic samples of fluvalinate (**1**), the anilino acid (**2**), and the haloaniline (**3**) were synthesized by the Zoecon Chemical Research Department.

4′-Hydroxyfluvalinate (**4**) was prepared by the following route. 3-(4-Methoxyphenoxy)benzaldehyde (497 mg, 2.18 mmol) in CH_2Cl_2 (10 mL) was treated with BBr_3 (1.15 g, 4.6 mmol) in CH_2Cl_2 (5 mL) at −10 °C for 1.5 h. The resultant phenol was isolated in 31% yield by preparative TLC (SS 1, R_f = 0.27). 3-(4-Hydroxyphenoxy)benzaldehyde (146 mg, 0.68 mmol) was converted to its cyanohydrin by reaction with NaCN (50 mg, 1.0 mmol) and $NaHSO_3$ (89 mg, 0.85 mmol) in a mixture of ether (2 mL) and water (1 mL) at room temperature for 1.5 h. This crude cyanohydrin (157 mg, 0.63 mmol) was esterified with **2** (221 mg, 0.75 mmol) in CH_2Cl_2 (2 mL) in the presence of dicyclohexylcarbodiimide (155 mg, 0.75 mmol) and 4-(dimethylamino)pyridine (9 mg, 0.08 mmol) for 1.5 h at room temperature. 4′-Hydroxyfluvalinate (**4**) was purified by preparative TLC (SS 2, R_f = 0.38). The overall yield for this two-step synthesis of **4** from 3-(4-hydroxyphenoxy)benzaldehyde was 40%: NMR ($CDCl_3$) δ 1.13 [m, 6, $CH(CH_3)_2$], 2.22 [m, 1, $CH(CH_3)_2$], 4.03 (m, 1, $CHCO_2$), 5.02 (d, 1, *J* = 8 Hz, NH), 5.83 (br s, 1, OH), 6.33 (s, 1, CHCN), 6.83 (s, 4, ar), ca. 7.1 (m, 7, ar); MS *m*/*z* (rel intensity) 520 (M^+, 0.8), 518 (M^+, 2), 252 (33), 250 (100), 248 (18), 206 (17).

An authentic sample of the peracetylated glucoside of **2** (i.e., **5**) was prepared in 55% yield from **2** (0.49 mmol) and 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (0.49 mmol) in the presence of dicyclohexylcarbodiimide (0.49 mmol) in pyridine (50 μL) and CH_2Cl_2 (4 mL) for 16 h at room temperature. From the NMR spectrum **5** consisted of a mixture of α and β anomers.

Metabolism Studies. When well established plants were treated with an emulsifiable concentrate of [*trifluoromethyl*-^{14}C]fluvalinate prepared as follows: [^{14}C]-fluvalinate (48.3 mCi/mmol, 99% radiochemical purity, 26.7%), emulsifiers (10.0%), and aromatic solvent (63.3%). The emulsifiable concentrate was diluted with water (0.5 mL) containing X-77 (0.045%, ADI Inc., Carmel, IN) as

a surfactant and applied to the plants via a sable hair brush with application rates ranging from 0.6 to 1 $\mu g/cm^2$ (ca. 0.1 kg/ha). After treatment, plants were maintained in greenhouses with nonfrosted glass windows that received natural lighting (February–August). Leaves were treated for all plants, and additional applications of fluvalinate were made to squares and bolls of cotton and to green tomato fruit.

Periodically various parts of the plants were harvested for analysis. After extraction of the ^{14}C-labeled residues ($CHCl_3$ and methanol), aliquots were quantitated by LSC and then analyzed by TLC (SS 3) and reversed-phase LC (SS 8). Control extractions at zero time indicate that [*trifluoromethyl*-^{14}C]fluvalinate is stable to the extraction procedure and in tissues at −18 °C prior to analysis. Unextractable ^{14}C-labeled residues were quantitated by LSC following combustion to $^{14}CO_2$.

The identification of the anilino acid (**2**) was based on coincidence of migration for radioactive bands with an authentic standard during TLC and reversed-phase LC. The anilino acid zone (from 5-week cotton leaves) was also methylated (CH_2N_2) for subsequent TLC analysis of the ester (R_f = 0.52, SS 4).

4′-Hydroxyfluvalinate (**4**) was isolated from cotton leaves by extraction, followed by TLC (R_f = 0.31, SS 5) and reversed-phase LC (SS 8, k' = 19.2). The trimethylsilyl ether of **4** was analyzed by GLC–MS and the mass spectrum of the isolated metabolite agreed with that of a synthetic standard of **4** with the significant displacement of certain ions by 2 mass units since the applied [^{14}C]fluvalinate had a specific activity of 48.3 mCi/mmol (hence, ca. 77 atom % ^{14}C for the CF_3 moiety): *m/z* (rel intensity) 594 (M^+, Cl = 37, C = 14, 1.1), 592 (M^+, 3.9), 254 (32), 252 (100), 250 (44), 206 (11). 4′-Hydroxyfluvalinate from tomato leaves was characterized by TLC and reversed-phase LC (SS 8).

The presumed polar conjugates from fluvalinate (i.e., origin zone from TLC in SS 3) were investigated to determine the identity of possible conjugates. Radiolabel in the origin zone was eluted from the silica with methanol. Aliquots were evaporated, then treated with 0.1 M KOH (5 mL) for cotton leaves (5 week), tomato leaves (6 week), and tobacco (8 week). After saponification at 38 °C for 16 h, the hydrolysates were acidified and then extracted with ether. An aliquot of the ether extract was analyzed by reversed-phase LC in SS 8. A separate portion of the TLC origin zone from cotton leaves (5 week) was treated with a mixture of β-glucosidase (3 mg, from almonds), sulfatase (4 mg, from *Helix pomatia*) and cellulase (5 mg, from *Aspergillus niger*) in citrate–phosphate buffer, pH 4.5 (all enzymes from Sigma Chemical Co., St. Louis, MO). Following a 16-h incubation at 37 °C, the hydrolysates were extracted with ether, and the concentrated ether extract was analyzed by TLC (SS 3) and reversed-phase LC (SS 8). A separate sample of the TLC origin zone from certain plant extracts was stirred overnight with pyridine (200 μL) and acetic anhydride (180 μL), and following partitioning between ether and water, the ether phase was analyzed by reversed-phase LC (SS 9).

So that the loss of volatile metabolites from [*trifluoromethyl*-^{14}C]fluvalinate could be monitored, a cotton plant with treated leaves was encased in an inverted Erlenmeyer flask with an opening at the top which permitted removal of air at a constant flow rate. This plant was maintained in the laboratory with only indirect sunlight (ca. 5000 lx). The evolved air stream was passed through a polyurethane foam plug (Gaymar Industries, Orchard Park, NY) to collect organic volatiles and then through a 5% KOH trap to collect $^{14}CO_2$. The foam plug was extracted periodically with ether, and an aliquot of the extract was analyzed by reversed-phase LC by coinjecting an authentic standard of the haloaniline (**3**, k' = 5.2 in SS 8).

The metabolism of the trifluoromethyl-^{14}C-labeled anilino acid (**2**, 48.3 mCi/mmol, 98.4% radiochemical purity) was studied on cotton leaves, cabbage leaves, and green tomato fruit (78–100 $\mu g/cm^2$, 7–9 kg/ha). Plants were treated and metabolites analyzed as described for [^{14}C]-fluvalinate. The origin zone from TLC of the 11-day cabbage extract (SS 6) was peracetylated (vide supra). This derivatized glucoside zone was purified by TLC (same solvent mixture) to give β-glucosides of **2** (R_f = 0.39) that were resolved further into a 1:1 mixture of (*R*)- and (*S*)-acid β-glucosides by reversed-phase LC (SS 9). The mixture of diastereomeric [i.e., (*R*)-acid and (*S*)-acid] β-glucosides gave the following spectral data: NMR (90 MHz, $CDCl_3$) δ 1.08 [d, 6, J = 6 Hz, $CH(CH_3)_2$], 1.81 (s, 1.5, $COCH_3$), 1.94 (s, 1.5, $COCH_3$), 2.06 (s, 12, $COCH_3$), ca. 2.0 [m, 1, $CH(CH_3)_2$], ca. 4.0 (m, 4, $CHCO_2$, pyranose at C-5 and C-6), ca. 5.1 (m, 4, NH, pyranose at C-2, C-3, and C-4), 5.75 (m, 1, pyranose at C-1 for β-glucoside), 6.6 (m, 1, ar), ca. 7.4 (m, 2, ar); MS *m/z* (rel intensity) 627 (0.3, M^+), 625 (0.5, M^+), 331 (13), 252 (57), 250 (97), 169 (100), 109 (86), 43 (98).

Translocation. Cotton, tomato, and cabbage plants were treated with [^{14}C]fluvalinate diluted with unlabeled fluvalinate (99% purity) to a dose rate of 1 $\mu g/cm^2$ (0.1 kg/ha). Plants were treated with emulsified fluvalinate as described previously and maintained in a greenhouse (10–32 °C).

Two cotton plants that had begun to blossom were treated over 90% of the upper leaf surfaces at 1 $\mu g/cm^2$. At 11 weeks posttreatment, two mature bolls were harvested from each plant. The seeds from each boll were removed for quantitation by LSC following combustion to $^{14}CO_2$. Cotton squares and bolls were treated on separate plants, and the mature seeds that developed therein were combusted likewise for determination of ^{14}C-labeled residues.

Two cabbage plants that showed signs of head formation were treated with [^{14}C]fluvalinate at 1 $\mu g/cm^2$. The upper surfaces of all leaves were treated except those touching the head itself. At 6.5 weeks posttreatment, both heads had matured and were harvested. The cabbage heads were minced for quantitation of an aliquot by combustion.

Possible translocation of ^{14}C into tomato fruit was examined for two plants (Ace variety), each with both treated leaves and fruit. Two green fruit (~30% of total fruit) and three small branches (~10% total leaf surface) were treated with [^{14}C]fluvalinate. After ripening of individual fruits, untreated fruits were harvested and extracted with methanol. Aliquots of the filtrate were quantiated by LSC. Aliquots of the dried residue were combusted to $^{14}CO_2$.

Metabolism of Fluvalinate Stereoisomers. A racemate of (α*R*,2*S*)- and (α*S*,2*R*)-[^{14}C]fluvalinate was isolated from the radiosynthetic preparation (a mixture of four stereoisomers) by normal-phase LC (SS 11). Cotton leaves were treated at 0.1 kg/ha as described above, and the plants were maintained in a greenhouse for 3 weeks (Jan 1981). Periodically leaves were harvested and extracted ($CHCl_3$–methanol). A portion of the extract was analyzed by TLC (SS 3) to determine the amount of unmetabolized fluvalinate. The fluvalinate TLC zone was scraped, eluted, and analyzed by normal-phase LC (SS 10).

Other cotton leaves were treated at 0.1 kg/ha with nonradiolabeled (α*S*,2*R*)- or (α*RS*,2*R*)-fluvalinate (obtained from Dr. T. E. Baer, Zoecon) and maintained in a green-

Table I. Distribution of Metabolites after Treatment at 0.1 kg/ha with [*trifluoromethyl*-^{14}C]Fluvalinate

commodity and treatment	% of applied dose: fluvalinate (1)	anilino acid (2)	TLC origin zone[a]	unextractable ^{14}C	total recovery
cotton leaves (cv. Acala SJ-4)					
2 weeks	71	2	8	0.5	84
9 weeks	15	2	22	3	45
cotton squares					
10 weeks	27	3	10	9	53
cotton bolls					
9 weeks	40	3	10	9	66
tobacco leaves (cv. NC 95)					
2 weeks	65	2	16	0.6	85
8 weeks	43	3	11	1.6	64
lettuce (Green Ice)					
12 days	86	0.7	5	0.01	93
lettuce (Romaine)					
5 weeks	77	3	16	2	100
tomato leaves (Pearson)					
6 weeks	61	2	37	2	106
tomato fruit (Pearson)					
3 weeks	72	0.4	3	12	96

[a] TLC development with hexane–ethyl acetate–acetic acid, 12:9:0.1.

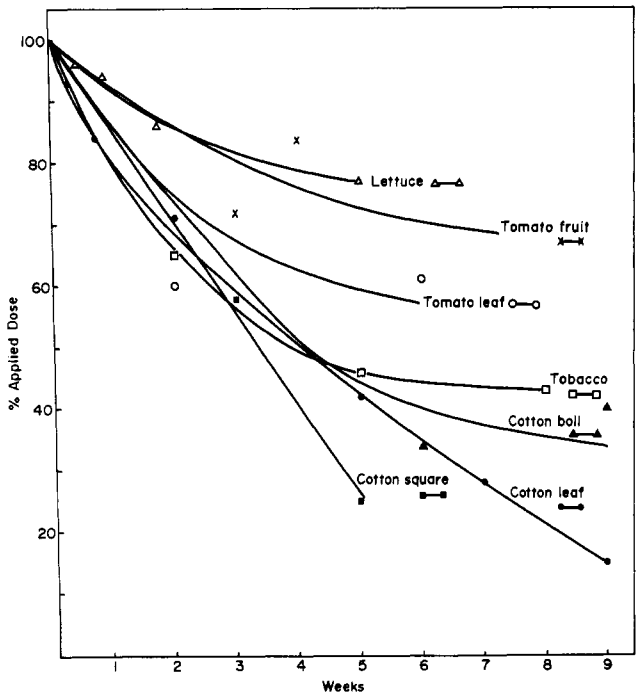

Figure 2. Recovery of fluvalinate from plants after treatment at 0.1 kg/ha.

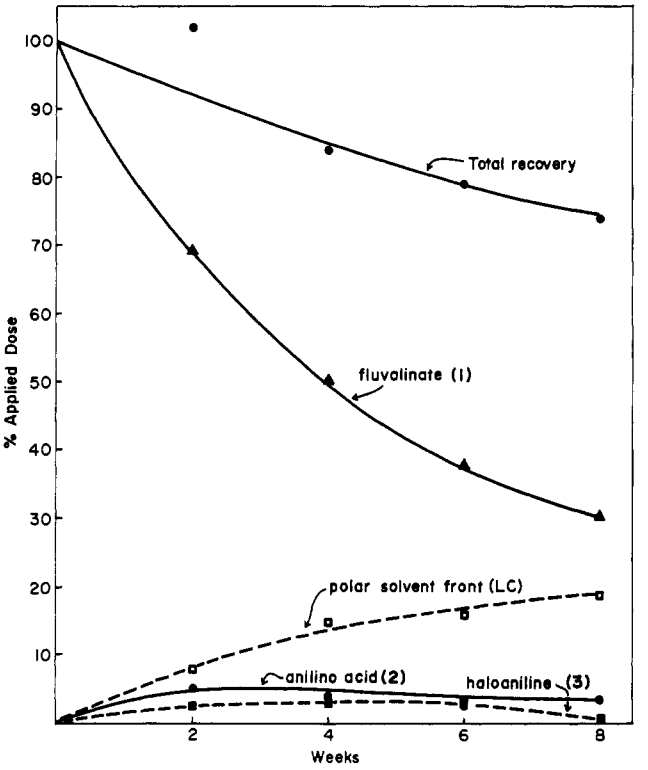

Figure 3. Metabolism of [^{14}C]fluvalinate by cabbage (Ferry Morse Round Dutch) after treatment at 0.1 kg/ha (LC analysis in SS 8).

house with exposure to sunlight (Feb 1981). Periodically leaves were extracted with acetonitrile to isolate fluvalinate that was purified by TLC (SS 3). After spiking samples with [^{14}C]fluvalinate (~30 000 dpm, 0.1 μg) to monitor recoveries, the fluvalinate isomers were derivatized to their respective menthyl esters under the following conditions: 1-mL conical vial; fluvalinate (10–50 μg); *l*-menthol (10 mg); concentrated HCl (300 μL); magnetic stirring at 92 °C for 2 h with the cap off and 14 h with the cap on. The menthyl esters were purified by TLC (SS 7) and then analyzed by normal-phase LC (SS 11).

Separate plots of cotton plants in Guaira, San Paulo State, Brazil, were treated by D. Ragsdale with (α*RS*,2*RS*)- and (α*RS*,2*R*)-fluvalinate at 0.2 and 0.1 kg/ha, respectively. The plants received three successive spray treatments at 7–10-day intervals (Jan–Feb 1981) and then were harvested 7 days after the last application. Samples of leaves were frozen in dry ice for delivery to Zoecon and subsequent analysis of fluvalinate stereoisomers by the menthylation procedure detailed above.

RESULTS AND DISCUSSION

The topical application of fluvalinate (1) to several plant species resulted in a half-life of ca. 4 weeks for cotton (leaves, squares, bolls), tobacco, and cabbage (Figures 2 and 3). For applications to tomatoes (leaves, fruit) and lettuce the half-life was somewhat longer (6 weeks). However, we noticed that the rate of fluvalinate degradation by plants varied with the greenhouse environmental conditions.

Primary Metabolites. The distribution of fluvalinate and its metabolites shown for cabbage in Figure 3 was typical for all plants with only minor differences (Table

I). Hydrolysis was the main, initital metabolic pathway for fluvalinate in plants, an observation consistent with the plant degradation of other pyrethroids [i.e., deltamethrin (Ruzo and Casida, 1979), permethrin (Gaughan and Casida, 1978; Ohkawa et al., 1977), cypermethrin (Wright et al., 1980), fenvalerate (Ohkawa et al., 1980), and phenothrin (Nambu et al., 1981)]. The major free metabolite of fluvalinate in all plants was the anilino acid (2), which resulted from hydrolysis of 1. In most plant samples 2 was present at a steady-state concentration of ca. 1–5% of the applied dose, but on lettuce and tomatoes, fluvalinate was more refractory to degradation, thus producing lesser amounts of 2.

4′-Hydroxyfluvalinate (4) was identified in the leaves of cotton and tomatoes (1.1 and 1.6% of applied dose, respectively), but this metabolite was less abundant in other plant extracts. The presence of low levels of 4′-hydroxylated, intact esters is consistent with metabolic data for other pyrethroids. However, we found no evidence for conjugated hydroxy esters as reported for 2′- and 4′-hydroxypermethrin in beans (Gaughan and Casida, 1978).

While in general the recovery of the applied radiolabel was quite satisfactory, we explored a trend toward reduced ^{14}C recovery as a function of time posttreatment. By trapping volatile organic degradation products on a polyurethane plug, we identified the haloaniline (3) as the major metabolite that was responsible for a loss of recoverable ^{14}C at extended times after treatment. About 90% of the volatilized ^{14}C was contributed by 3. Although the haloaniline was continuously evolved (reaching 6% of the applied dose after 4 weeks for cotton leaves maintained under reduced light in the laboratory), it was not observed at greater than 4% of the radiolabel in plant tissues. The loss of ^{14}C from plants as $^{14}CO_2$ was negligible.

The following plant-derived products have been reported for other pyrethroids, but the analogous compounds from fluvalinate were not found: (1) 3-(2,2-dichlorovinyl)-2-(hydroxymethyl)-2-methylcyclopropanecarboxylic acid from permethrin on beans and cotton (Gaughan and Casida, 1978); (2) decarboxylated fenvalerate, a photoproduct on beans (Ohkawa et al., 1980); (3) conversion of CN to $CONH_2$ and COOH for fenvalerate (Ohkawa et al., 1980) and cypermethrin (Roberts, 1981).

Conjugates. The anilino acid (2) appeared to be converted readily to polar conjugates that were manifest as an increase in radioactivity remaining at the origin after TLC. Saponification of the polar products from the TLC origin zone produced 2 in at least 60–70% yield from the leaves of cotton (5 week), tobacco (8 week), and tomatoes (6 week). Acetylation of the TLC origin conjugates demonstrated that the glucoside of 2 (i.e., deacetylated 5) represented <0.5, <0.5, and 0.6% of the applied dose for leaves of cotton, tobacco, and lettuce, respectively, but the glucoside of 2 represented 9% of the applied ^{14}C for tomato leaves, according to results from reversed-phase LC analysis. An LC analysis of the underivatized TLC origin zone conjugates revealed a complex mixture of products from all plants. Enzymatic cleavage of the polar, origin-zone conjugates with β-glucosidase and sulfatase was ineffective at hydrolyzing conjugates from 5-week cotton leaves.

In order to further probe the identity of possible anilino acid conjugates, the metabolism of 2 was examined on cotton, cabbage, and tomatoes (Table II). Reversed-phase LC analysis of the acetylated, TLC origin conjugates from cabbage revealed that the glucoside of 2 (i.e., deacetylated 5) represented about 50% of the polar conjugates (4% of applied dose). A 1:1 mixture of (*R*)-2 and (*S*)-2 β-glucoside diastereomers was isolated for identification by 90-MHz proton NMR and mass spectrometry. GLC and NMR data indicated that only β-glucosides of 2 were present (<1% α-glucosides) as judged by the resonance of the hydrogen at C-1 of the pyranose (δ = 5.75 for β-glucoside vs. ca. 6.3 for an α-glucoside).

Table II. Metabolism of Trifluoromethyl-^{14}C-Labeled Anilino Acid (2) at 7–9 kg/ha by Plants

	% of applied dose
cotton leaves (11 days)	
extract	77.7
anilino acid (2)	67.3
haloaniline (3)	1.2
polar conjugates	4.4
residual solids	0.4
total recovery	78.1
cabbage leaves (11 days)	
extract	76.4
2	55.8
3	7.4
polar conjugates	8.1
β-glucoside of 2	4.0
unknown conjugate of 2 (possible malonyl glucoside of 2)	2.0
residual solids	1.5
total recovery	77.9
tomato fruit (12 days)	
extract	88.1
2	78.8
3	1.6
polar conjugates	3.5
residual solids	0.9
total recovery	89.0

An unknown glycoside of 2 was methylated and peracetylated and then purified by TLC (R_f = 0.26, hexane–ethyl acetate–acetic acid, 150:75:1) and LC (k' = 15.8, SS 9). Mass spectral analysis of this unknown suggested a conjugate of 2 with glucose plus malonic acid [cf. Dutton et al. (1976)]; however, we were unable to isolate enough of this conjugate for structural confirmation by ^1H NMR.

It is evident from the data in Table II that 2 applied at 7–9 kg/ha is neither readily degraded nor conjugated, in marked contrast to the apparent facile conjugation of residues of 2 generated metabolically from fluvalinate applied at 0.1 kg/ha. In agreement with plant metabolism data for cypermethrin (Wright et al., 1980), fenvalerate (Ohkawa et al., 1980), deltamethrin (Ruzo and Casida, 1979), and permethrin (Ohkawa et al., 1977; Gaughan and Casida, 1978) the acid portion of fluvalinate conjugates with glucose. However, we found no direct evidence for disaccharide conjugates of 2 [cf. Wright et al. (1980)], but considering the apparent complexity of the polar metabolite fraction, the presence of such conjugates is a distinct possibility.

Translocation. Fluvalinate and its degradation products are not significantly translocated in plants. The stems of treated cotton leaves and squares were analyzed and never contained more than 0.05% of the applied dose. Treatment of cotton leaves, squares, or bolls with 1 resulted in negligible ^{14}C-labeled residues in cotton seeds (<0.2% of applied dose, <16 ppb) although trace levels of ^{14}C in cotton seeds increased slightly in the treatment order leaves < squares < bolls. Fluvalinate and metabolites are translocated neither from treated cabbage leaves into the head nor into untreated tomato fruit. The minimal translocation of fluvalinate and its metabolites within plants agrees with the limited internal mobility of permethrin (Ohkawa et al., 1977) and fenvalerate (Ohkawa et al., 1980) in beans.

Table III. Treatment of Cotton Leaves (cv. Acala SJ-4) with a Mixture of ($\alpha R,2S$)- and ($\alpha S,2R$)-[^{14}C] Fluvalinate at 0.08 kg/ha. Analysis is by Normal-Phase LC without Conversion to Menthyl Esters

exposure time, days	% of applied dose recovered		% fluvalinate isomers	
	CH_3OH-$CHCl_3$ extract (1)	unextractable residue	($\alpha R,2R$) + ($\alpha S,2S$)	($\alpha R,2S$) + ($\alpha S,2R$)
0	92 (92)	0.5	7	93
0[b]	emulsion		3	97[a]
0[a,c]	stock radiochemical		3	97
1	86 (72)	0.4	38	62
3	74 (74)	0.2	21	79
7	82 (70)	1.4	23	77
14	65 (42)	1.2	26	74
21	61 (36)	1.5	42	58

[a] Purity was >99% for ($\alpha R,2S$)- plus ($\alpha S,2R$)-fluvalinate immediately after LC purification. After several days in acetone (<1 μg/mL), isomerization occurred. Racemization also occurs for a 1 mg/mL acetone solution of ($\alpha S,2R$)-fluvalinate (~6% over ~1 month at −20 °C). [b] Emulsion allowed to stand 0.5 h without application to leaves. [c] Isomeric purity of radiochemical before making up emulsion.

Table IV. Degradation of Fluvalinate Stereoisomers on Cotton Foliage

	days post-treatment	% fluvalinate recovered	% yield of menthyl esters	% total menthyl esters			
				$\alpha R,2R$	$\alpha S,2S$	$\alpha R,2S$	$\alpha S,2R$
greenhouse							
$\alpha RS,2R$	0	82	48	48	<1	<1	50
	3	46	63	47	0.9	1.1	51
	7	39	45	48	1	2	50
$\alpha S,2R$	0	80	33	3	<1	8	89
	3	43	75	19	3	6	72
	7	59	50	11	1	5	83
Brazil							
$\alpha RS,2RS$	0[a]	100	43	23	19	32	26
	7	94	15	24	20	30	26
$\alpha RS,2R$	0[a]	100	43	43	5	7	45
	7	92	13	43	2	6	49

[a] Active ingredient only. Not applied to cotton plants.

Fluvalinate Stereoisomers. Several experiments were designed to explore the possible preferential degradation and epimerization of fluvalinate isomers. Most of the plant metabolism studies utilized ($\alpha RS,2RS$)-[^{14}C]fluvalinate, which was a mixture of four isomers. Using normal-phase LC it was possible to separate enantiomeric pairs of fluvalinate isomers that were present in a 48:52 ratio initially for [($\alpha R,2R$) + ($\alpha S,2S$)]:[($\alpha R,2S$) + ($\alpha S,2R$)]. Two weeks after treatment of cotton leaves and tobacco, the remaining fluvalinate was reisolated from plants maintained in a greenhouse, and LC analysis showed ratios of 51:49 and 50:50 for [($\alpha R,2R$) + ($\alpha S,2S$)]:[($\alpha R,2S$) + ($\alpha S,2R$)] in cotton and tobacco, respectively. Hence, these data suggest equal dissipation rates for the enantiomeric pairs.

The environmental epimerization of the two asymmetric centers in fluvalinate was monitored by treating cotton foliage in a greenhouse with a racemate of $\alpha R,2S$ and $\alpha S,2R$ isomers. Epimerization at either asymmetric center would result in fluvalinate isomers (i.e., $\alpha R,2R$ or $\alpha S,2S$) that could be resolved from the $\alpha S,2R/\alpha R,2S$ pair by normal-phase LC. Using this methodology it was determined that one or both asymmetric centers epimerize although the isomerization appeared to be relatively slow since fluvalinate reisolated from cotton after 3 weeks was still not fully racemized to a mixture of $\alpha R,2R$, $\alpha S,2S$, $\alpha R,2S$ and $\alpha S,2R$ isomers (Table III).

The final, most unambiguous method for epimerization analysis involved isolation of fluvalinate from plants and derivatization of each isomer to its corresponding menthyl ester adduct. The method of Horiba et al. (1980) was modified to allow analysis of individual fluvalinate stereoisomers on a 10-μg scale without the necessity of using radiolabeled compound. Under the optimized conditions the racemization caused by the derivatization method amounted to only 1–2% as determined by analysis of pure ($\alpha S,2R$)- and ($\alpha RS,2R$)-fluvalinate. Kinetic resolution of the stereoisomers during menthyl ester formation resulted in deviations of −10, −4, +1, and +11% from the expected values for the $\alpha R,2R$, $\alpha S,2S$, $\alpha R,2S$, and $\alpha S,2R$ isomers, respectively.

Cotton leaves were treated at 0.1 kg/ha with ($\alpha S,2R$)- and ($\alpha RS,2R$)-fluvalinate and maintained in a greenhouse with exposure to sunlight. Periodically leaves were extracted to isolate fluvalinate that was derivatized to menthyl esters. From the data in Table IV we conclude that ($\alpha RS,2R$)-fluvalinate is stable to racemization under greenhouse conditions. ($\alpha S,2R$)-Fluvalinate is partially isomerized to the $\alpha R,2R$ isomer, but after 1 week most of the $\alpha S,2R$ isomer is still intact stereochemically. Hence, the asymmetric center of the alcohol portion of fluvalinate is prone to partial racemization on cotton leaves while the asymmetric center of the anilino acid seems relatively stable. This conclusion was corroborated by analysis of foliage from cotton plants in a Brazilian field. The plants were treated 3 times at 7–10-day intervals with ($\alpha RS,2RS$)- or ($\alpha RS,2R$)-fluvalinate at 0.2 and 0.1 kg/ha, respectively. Seven days after the last treatment, cotton leaves were hand harvested for transport back to Zoecon for analysis by the menthyl ester derivatization method. From the data in Table IV and Figure 4 we conclude that there is no preferential loss of individual fluvalinate isomers and ($\alpha RS,2R$)-fluvalinate is relatively stable to environmental isomerization on cotton leaves in a Brazilian field.

3-Phenoxybenzyl Moiety. Although in this work we did not follow the fate of the alcohol portion of fluvalinate, hydrolysis of several related pyrethroids (i.e., deltamethrin, cypermethrin, and fenvalerate) produces α-cyano-3-phenoxybenzyl alcohol, which is also the initial product of fluvalinate hydrolysis. The metabolic disposition of the alcohol-radiolabeled portion from the intact pyrethroid has

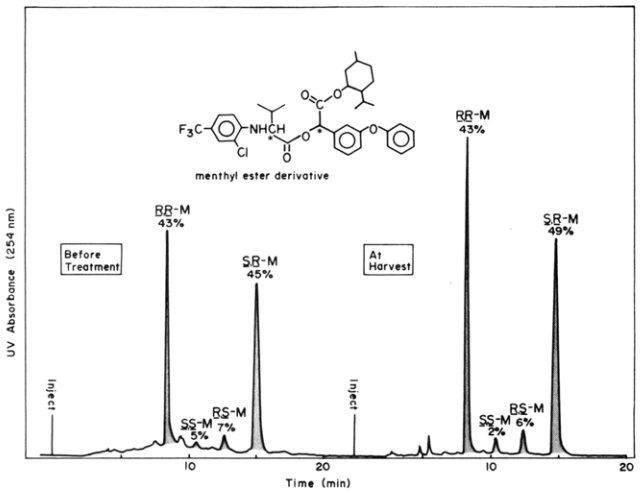

Figure 4. Normal-phase LC analysis of stereoisomers from Brazilian cotton foliage 1 week after three successive applications of (αRS,2R)-fluvalinate at 7–10-day intervals at 0.1 kg/ha. The following were used for LC analysis: 5-μm LiChrosorb SI 100 column; UV detection at 254 nm; elution with ether–pentane (2:98) at 1.6 mL/min; 3 μg of (αRS,2R)-fluvalinate as menthyl ester (R,R-M and S,R-M) derivatives.

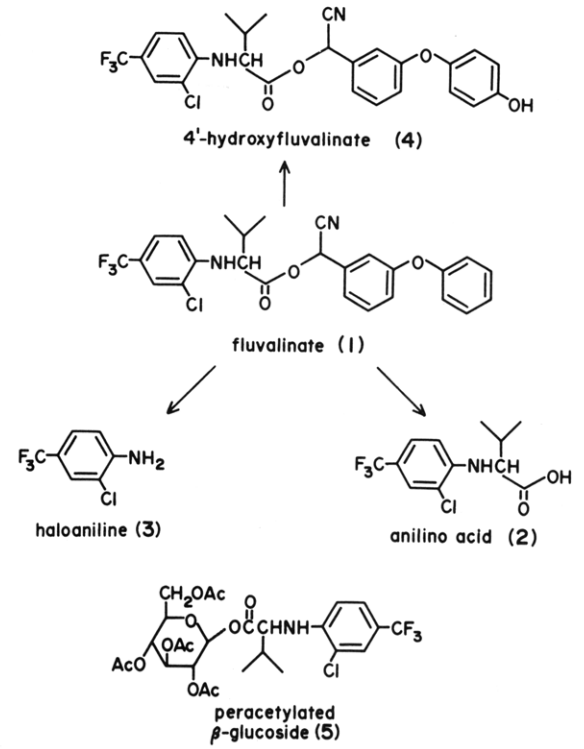

Figure 5. Plant degradation products from [*trifluoromethyl*-^{14}C]fluvalinate.

been reported on plants for deltamethrin [cotton (Ruzo and Casida, 1979)], cypermethrin [apples (Roberts, 1981)], and fenvalerate [beans (Ohkawa et al., 1980)]. The initial hydrolytic product of these pyrethroids is α-cyano-3-phenoxybenzyl alcohol, which not surprisingly has not been detected as a free metabolite since this cyanohydrin is inherently unstable under ambient conditions, losing HCN to form 3-phenoxybenzaldehyde. 3-Phenoxybenzaldehyde is either oxidized or reduced to the corresponding acid or alcohol. However, the *free* 3-phenoxy-substituted benzaldehyde, benzoic aicd, and benzyl alcohol metabolites are generally minor residues [with the exception of cotton leaf disks in vitro (Ruzo and Casida, 1979)].

Glycoside conjugates represent the major portion of the ^{14}C-labeled residue in plants from metabolism of the 3-phenoxybenzyl moiety. The initial α-cyano-3-phenoxybenzyl alcohol itself is alleged to form sugar conjugates when plants are treated with fenvalerate (Ohkawa et al., 1980) and deltamethrin (Ruzo and Casida, 1979) although chemical structures for the metabolites have not been assigned. The presence of these cyanogenic glycosides is particularly interesting since there are related natural products that release HCN upon hydrolysis. However, HCN from plants in the presence of sunlight is expected to be efficiently converted to CO_2 (Mikami et al., 1980). Unknown conjugates similar to these cyanogenic glycosides contained α-carboxy-3-phenoxybenzyl alcohol from beans treated with fenvalerate (Ohkawa et al., 1980). However, these α-carboxy-3-phenoxybenzyl conjugates are alleged to arise from α-carboxyfenvalerate (i.e., fenvalerate with an α-carboxyl moiety vs. an α-cyano moiety) so such glycosides would not be expected from fluvalinate.

3-Phenoxybenzoic acid and 3-phenoxybenzyl alcohol are the predominant aglycons found in plants. In the cases of fenvalerate (Ohkawa et al., 1980) and deltamethrin (Ruzo and Casida, 1979), preliminary evidence suggests that these residues exist in part as glucosides, as well as other unknown glycosides. An elegant study by More et al. (1978) has shown conclusively that 3-phenoxybenzoic acid itself is converted in several plant species to ester conjugates of glucose, glucosylarabinose, and glycosylxylose. Whereas the glucose ester of 3-phenoxybenzoic acid was the major conjugate in cotton, the disaccharide esters were most abundant in other crops (Wright et al., 1980). Similarly, 3-phenoxybenzyl alcohol is metabolized by cotton leaves to glucose and glucosylpentose derivatives (Roberts and Wright, 1981). Unknown glycosides of 2′- and 4′-hydroxy-3-phenoxybenzoic acid have been reported also (Ohkawa et al., 1980; Roberts, 1981).

Conclusions. The degradation of fluvalinate by plants is similar to that reported for other pyrethroids (Figure 5). The major ^{14}C-labeled residues in plants consist of the anilino acid **2** and its glycoside conjugates, in addition to unmetabolized fluvalinate. In agreement with Ohkawa et al. (1980), who found no difference in the degradation of (2S)-fenvalerate compared to that of the 2RS mixture on beans, we found that individual fluvalinate stereoisomers dissipate at apparently equal rates. However, in contrast to the reported stability of the benzylic center of deltamethrin on cotton foliage (Ruzo and Casida, 1979), the benzylic asymmetric center of fluvalinate is susceptible to slow epimerization.

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Metabolism of Fluvalinate by a Lactating Dairy Cow

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When a lactating cow was given a single oral dose (1 mg/kg) of [*trifluoromethyl*-^{14}C]fluvalinate, 53, 42, and 0.9% of the applied dose were excreted in urine, feces, and milk, respectively, after 8 days. The major urinary metabolites consisted of the anilino acid (**2**), which arose from hydrolysis of fluvalinate (**1**), and β-glucuronide conjugates of **2**, representing 6–19 and 63–76% of the urinary ^{14}C, respectively. Fecal ^{14}C-labeled residues consisted of **1**, **2**, and the bile acid conjugates of **2**, which were present as 47, ca. 11, and ca. 13% of the fecal ^{14}C. Although tissues, in general, contained only traces of radiolabel, fluvalinate contributed at least 70% of the ^{14}C-labeled residue in milk and fat.

As part of our continued study of the metabolic fate of fluvalinate [α-cyano-3-phenoxybenzyl 2-[2-chloro-4-(trifluoromethyl)anilino]-3-methylbutanoate, ZR-3210, MAVRIK], we now report the degradation of this pyrethroid by a lactating cow. For the preceding paper in this series see Quistad et al. (1982a).

EXPERIMENTAL SECTION

Radioassay and Chromatography. Radioactivity was quantitated by liquid scintillation counting (LSC) alone or in conjunction with sample combustion to $^{14}CO_2$ (Quistad et al., 1982a). Thin-layer chromatography (TLC) on silica gel utilized the following solvent systems: SS 1 (hexane–ethyl acetate–acetic acid, 12:9:0.1), SS 2 (hexane–ethyl acetate, 5:1), SS 3 (hexane–ethyl acetate, 1:1), SS 4 (ether–hexane, 2:1), SS 5 (hexane–ethyl acetate–acetic acid, 150:75:1), SS 6 (hexane–ethyl acetate, 2:1), SS 7 (ether–hexane, 1:1), SS 8 (hexane–ethyl acetate, 1:2), and SS 9 (hexane–ethyl acetate, 3:1).

The analysis of ^{14}C-labeled residues by reversed-phase and normal-phase liquid chromatography (LC) has been described (Quistad et al., 1982a). The following mixtures of methanol–0.1% acetic acid were used for reversed-phase LC: SS 10 (gradient 60–70% methanol over 15 min, 70–90% over 10 min, isocratic at 90% for 10 min); SS 11 (gradient 55–75% methanol over 20 min, isocratic at 75% for 5 min, gradient 75–90% over 10 min); SS 12 (65% methanol); SS 13 (80% methanol); SS 14 (isocratic at 75% methanol for 15 min, gradient 75–90% over 5 min, isocratic at 90%); SS 15 (85% methanol); SS 16 (isocratic at 80% methanol for 15 min, 80–90% over 5 min); SS 17 (gradient 40–90% methanol over 30 min). Normal-phase LC employed mixtures of ether–pentane, half-saturated with water: SS 18 (3:97), SS 19 (30:70), SS 20 (15:85), SS 21 (75:25), and SS 22 (90:10).

Synthetic Metabolite Standards. The fluvalinate and synthetic metabolite standards used in this study were racemic unless otherwise noted. The preparations of [*trifluoromethyl*-^{14}C]fluvalinate (**1**), anilino acid (**2**), the amide of **2**, the glycine conjugate of **2** (i.e., **6**), the taurine conjugate of **2**, cis and trans lactones of hydroxy acid **3**, and 4′-hydroxyfluvalinate (**5**) are reported elsewhere (Quistad et al., 1980, 1982a; Staiger et al., 1982). The glutamic acid conjugate of **2** (as its dimethyl ester) was synthesized from dimethyl glutamate and the acid chloride of **2**. 4′-Methoxyfluvalinate was prepared by reacting the cyanohydrin of 3-(4-methoxyphenoxy)benzaldehyde with **2**. The haloaniline **4** was prepared by T. Mastre (Zoecon) and the (*R*)-anilino acid [(*R*)-**2**] was provided by Dr. Ted Baer (Zoecon).

The synthesis of the methylated, peracetylated glucuronides of **2** is summarized in Figure 1. Glucurone (D-glucurono-3,6-lactone, 40 g, 0.23 mol) was stirred 1 h with methanol (300 mL) containing sodium hydroxide (0.11 g, 3 mmol) according to Bowering and Timell (1960). Evaporation of the methanol gave methyl D-glucopyranuronate, which was peracetylated according to Bowering and Timell (1960) to give a 54% yield of methyl 1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate (mp 111–115 °C). Methyl 1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate (5.0 g, 13 mmol) was reacted with 31% HBr in acetic acid (25 mL) for 16 h at room temperature to give methyl 2,3,4-tri-*O*-acetyl-1-bromo-1-deoxy-D-glucopyranuronate [cf. Bowering and Timell (1960)], which was not purified but was treated immediately with water and Ag_2CO_3–Celite [from $AgNO_3$ (4.5 g), Celite (4.0 g), and Na_2CO_3 (1.5 g) according to Fieser and Fieser (1969)] to give methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate in 88% yield [cf. Pravdic and Keglevic (1964)]. The anilino acid (**2**, 188 mg, 0.64 mmol) reacted with methyl 2,3,4-tri-*O*-acetyl-D-glucopyranuronate (213 mg, 0.64 mmol) in the presence of dicyclohexylcarbodiimide (131 mg, 0.64 mmol) and 4-(dimethylamino)pyridine (8 mg, 0.06 mmol) in CH_2Cl_2 (5 mL) to give **7** in 39% yield. The structure of **7** was confirmed by ^{1}H nuclear magnetic resonance (NMR) and mass spectroscopy. Together with chromatographic data, this evidence showed the synthetic material to consist of a 1:1 mixture of derivatized α- and β-glucuronides. The four diastereomeric glucuronide derivatives were separable by LC (SS 12) and the methylated, peracetylated α-glucuronides resolved as a single peak from the corresponding β-gluc-

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