drocarbons: these compounds may possibly play a role in selection of oviposition sites by the chalcid.

Comparison with Alfalfa Volatiles. Comparison of Table I with data obtained previously by the authors for alfalfa (Buttery et al., 1982a) shows many similarities and some marked differences. The nature of the volatiles in alfalfa flowers and clover flowers shows the major differences. Acetophenone, which is the major component of the clover flowers, does not occur in the alfalfa flowers. (*E*)-β-Ocimene, (*E*)-β-farnesene, and caryophyllene are common to both the alfalfa leaves (and pods) and the clover leaves (and pods). However, there are differences in the nature of the other terpene and sesquiterpene compounds between alfalfa and clover. With both alfalfa and clover the sesquiterpenes are at a higher relative concentration in the pods compared to the leaves (and flowers) of the same plant.

Preliminary Field Tests. Hexyl acetate, 2-hexanone, 2-heptanone, acetophenone, and acetoin appeared to be weak attractants for lygus bugs at the 1% concentration. None of the compounds tested was attractive to nitidulid beetles or seed chalcids. An unidentified humpbacked fly in the family Phoridae was strongly attracted to 1-phenylethanol at the 1% concentration. Unfortunately, this fly is not a pest and commonly feeds on decaying vegetation. We consider these data preliminary and not necessarily indicative of attractivity to the pest species. Some of the compounds tested are extremely volatile, and our baits may not have emitted an attractive concentration to the insect for more than several hours. Baits that control release of these compounds over a range of concentrations may give quite different results.

Registry No. 1-Penten-3-one, 1629-58-9; 2-hexanone, 591-78-6; 2-heptanone, 110-43-0; (*E*)-2-hexenal, 6728-26-3; 2-methylbutanol, 137-32-6; hexanol, 111-27-3; (*Z*)-3-hexenol, 928-96-1; (*E*)-2-hexenol, 928-95-0; acetoin, 513-86-0; 1-octen-3-ol, 3391-86-4; 2,3-dihydroxybutane, 513-85-9; (*Z*)-3-hexenyl acetate, 3681-71-8; acetic acid, 64-19-7; decyl acetate, 112-17-4; dodecyl acetate, 112-66-3; (*Z*)-β-ocimene, 3338-55-4; (*E*)-β-ocimene, 3779-61-1; longifolene, 475-20-7; caryophyllene, 87-44-5; (*E*)-β-farnesene, 18794-84-8; acetophenone, 98-86-2; methyl salicylate, 119-36-8; 1-phenylethanol, 98-85-1; 2-phenylethanol, 60-12-8; methyl cinnamate, 103-26-4.

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Fenvalerate Metabolism in Cotton Callus Tissue

Gayle H. Davidonis and Ralph O. Mumma*

The metabolism of fenvalerate in cotton callus tissue proceeded in a manner similar to that of other pyrethroids in intact plants. Using chlorophenyl-^{14}C-labeled fenvalerate 97.0% and 94.7% of the label was recovered from the tissue in 4 and 8 days, respectively. The [*chlorophenyl*-^{14}C]fenvalerate was metabolized to conjugates of 2-(4-chlorophenyl)-3-methylbutyric acid while benzyl-^{14}C-labeled fenvalerate was metabolized to numerous conjugates, some of which were conjugates of 3-phenoxybenzoic acid and 3-(4-hydroxyphenoxy)benzoic acid. No similar conjugates of either chlorophenyl-^{14}C- or benzyl-^{14}C-labeled fenvalerate were found, indicating conjugation of the parent nuclear chain does not seem to exist.

Fenvalerate [α-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)-3-methylbutyrate] is one of a number of synthetic pyrethroids that are used in controlling many insect pests of cotton and other crops. The metabolism of several pyrethroids has been examined in intact cotton plants under greenhouse and field conditions and in excised leaf disks (Ruzo and Casida, 1979). Under field conditions, about 30% of the ^{14}C-labeled permethrin was lost within 1 week (Gaughan and Casida, 1978). Permethrin, deltamethrin, and cypermethrin are degraded on or in cotton plants primarily by photoisomerization, ester cleavage, and conjugation reactions (Roberts, 1981; Ruzo and Casida, 1979; Wright et al., 1980). Plant tissue culture more efficiently utilizes the ^{14}C-labeled compounds and tends to optimize uptake and metabolism in pesticide studies compared to similar experiments under greenhouse or field conditions. In most tissue culture metabolism studies metabolites are qualitatively similar to intact plant studies. Plant tissue culture is advantageous in determining if the metabolites recovered are a result of true plant metabolism rather than that of microorganisms on the plant surface or photodegradation. This is important in evaluating short-term exposures of leaf disks to pesticides. Since fenvalerate is used on cotton to control lepidopterous pests (Ruscoe, 1980), we have examined the metabolic fate of

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fenvalerate in cotton callus tissue.

Results are compared to metabolism studies of fenvalerate in bean (Ohkawa, et al., 1980) and cotton plants and in cotton leaf disk studies of deltamethrin (Ruzo and Casida, 1979).

MATERIALS AND METHODS

Chemicals. Chlorophenyl-^{14}C-labeled fenvalerate was supplied by the Shell Development Co. (radiochemical purity 97%; specific activity 14.9 mCi/mmol). Benzyl-^{14}C-labeled fenvalerate (radiochemical purity 97%; specific activity 59.7 mCi/mmol) was supplied by J. O. Nelson of the University of Maryland. The unlabeled standards 2-(4-chlorophenyl)-3-methylbutyric acid and 3-phenoxybenzoic acid were supplied by Shell, and 3-(4-hydroxyphenoxy)benzoic acid was a gift from D. M. Soderlund.

Chromatography. Three solvent systems were used for thin-layer chromatography (TLC) on silica gel 60 F-254 plastic plates (0.25-mm thickness): toluene–diethyl ether–acetic acid (75:25:1), hexane–acetone–acetic acid (25:25:1, three developments), and butanol–acetic acid–water (6:1:1). Solvent migration was 16 cm.

Radioanalysis. Methanol-insoluble residues were combusted in a Packard Tri-Carb sample oxidizer. The radioactivity of the fractions was measured by liquid scintillation counting (Beckmann, Model LS 8000) in ACS (Amersham) solvent. Metabolites were located by autoradiography and cochromatography with nonlabled standards.

Cotton Callus Tissue Cultures. Cotton (*Gossypium hirsutum* var. Coker 310) seeds were delinted and surface sterilized in 2% sodium hypochlorite, rinsed in sterile distilled water, and allowed to germinate on moistened sterile filter paper. Cotyledon sections were removed and transferred to Linsmaier and Skoog's agar medium (1965) with the following modifications: 30 g/L glucose instead of sucrose, 2 mg/L naphthaleneacetic acid, and 1 mg/L kinetin. After callus had been initiated the tissue was transferred every 2 months and grown under continuous low-intensity fluorescent light. Callus was grown for 6 or 7 weeks prior to use of test compounds. Tissue in each of two flasks (4.4 and 4.8 g) was injected with 15 μL of benzyl-^{14}C-labeled fenvalerate (1.5 μCi) in methanol, and the tissue was harvested after 4 days. Callus tissue was also injected with chlorophenyl-^{14}C-labeled fenvalerate (2.2 μCi) and two flasks were harvested after 4 days (7.4 and 9.2 g) and the remaining two after 8 days (8.8 and 8.1 g).

Metabolite Extraction. Callus tissue from each flask was ground in a VirTis "45" tissue homogenizer with 50 mL of methanol. The homogenate was filtered and washed with methanol. The filtrate was concentrated by rotary evaporation. The methanol insoluble residues that remained after extraction were dried at ambient temperature and combusted. The culture media were frozen and extracted with methanol. The cotton extracts were examined with the previously mentioned TLC solvent systems. Some components from TLC bands were eluted with methanol and subjected to a 24-h treatment with 5 mg of β-glucosidase (pH 5.0) at ambient temperature (26 °C). Some components were also treated with 0.1 N HCl for 1, 5, or 20 h at 100 °C, and the products were TLC chromatographed. Acid-hydrolyzed material was eluted from thin-layer plates and analyzed by mass spectrometry (AEI Model MS-950).

RESULTS AND DISCUSSION

With these incubations 94.7–97.0% of the applied [^{14}C]fenvalerate label was recovered in the tissue. On the average, 1% of the applied label was recovered from the agar media. A small amount of methanol-insoluble ^{14}C residue was recovered (0.3–0.6%). Radiolabel distribution of fenvalerate and its metabolites from cotton tissue are given in Table I and the R_f values of these metabolites are presented in Table II. About 70% of the applied [^{14}C]-fenvalerate remained unmetabolized in 4 days, while 60% was left in 8 days. All metabolites were recovered as conjugates. Acid hydrolysis of all the metabolites from chlorophenyl-^{14}C-labeled fenvalerate yielded only Cl-Vacid [2-(4-chlorophenyl)-3-methylbutyric acid], which was confirmed by mass spectrometry. One conjugate was tentatively identified as the glucoside of Cl-Vacid and it could be hydrolyzed with β-glucosidase (conj-2). The benzyl-^{14}C-labeled fenvalerate was also metabolized to conjugates that appeared in three TLC regions (Table II). Phenoxybenzyl (PB) conjugate complex 3 can be cleaved with β-glucosidase to yield three products. One of these products cochromatographs with 3-phenoxybenzoic acid (PB-acid). PB conjugate complexes 1 and 2 yielded several products when cleaved with β-glucosides, and both also yielded products that were identified as PB-acid and 3-(4-hydroxyphenoxy)benzoic acid (4'-OH-PB-acid) by cochromatography. Hydrolysis products composed 16.8% of the material in PB conjugate complex 1 and of that 2.1% was PB-acid and 5.2% was 4'-OH-PB-acid. In PB conjugate complex 2 36.5% of the complex was hydrolyzed

Table I. Distribution of ^{14}C-Labeled Fenvalerate and Its Metabolites in Cotton Tissue after Injection of 1.5 μCi of Benzylic Labeled and 2.2 μCi of Chlorophenyl-Labeled Fenvalerate into the Tissue

	% of applied ^{14}C[a]		
	benzylic labeled,	chlorophenyl labeled	
	4 days[b]	4 days[b]	8 days[b]
callus tissue	97.0	96.1	94.7
extractable ^{14}C	96.7	95.8	94.1
fenvalerate	71.4	70.1	59.7
Cl-Vacid conj complex 1		18.1	24.2
Cl-Vacid conj 2		3.4	3.4
Cl-Vacid conj 3		2.0	4.4
PB-conj complex 1	7.5		
PB-conj complex 2	13.3		
PB-conj complex 3	2.2		
other metabolites	2.3	2.2	2.4
extract residue	0.3	0.3	0.6

[a] Each value is an average of two replicates. [b] Days after injection.

Table II. TLC R_f Values for Fenvalerate and Its Metabolites from Cotton Callus Tissue after Thin-Layer Chromatography[a]

	R_f			
compound	A	B	C	D
fenvalerate	0.82	0.97	0.68	0.74
Cl-Vacid	0.74	0.97	0.40	0.72
PB-acid	0.66	0.97	0.32	0.71
4'-OH-PB acid	0.59	0.00	0.13	0.71
Cl-Vacid conj complex 1	0.00	0.06	0.00	0.30
Cl-Vacid conj 2	0.00	0.13	0.00	0.41
Cl-Vacid conj 3	0.00	0.12	0.00	0.45
PB-conj complex 1	0.00	0.00	0.00	0.00, 0.07, 0.13, 0.25, 0.30
PB-conj complex 2	0.04	0.11	0.00	0.37, 0.42, 0.52
PB-conj complex 3	0.11	0.22	0.00	0.56

[a] A = hexane-acetone-acetic acid (25:25:1); B = hexane-acetone-acetic acid (25:25:1), three developments; C = toluene-diethyl ether-acetic acid (75:25:1); D = butanol-acetic acid-water (6:1:1).

and of that 1.6% was PB-acid and 19.3% was 4'-OH-PB-acid. All three PB complexes readily cleaved with HCl to yield PB-acid while complexes 2 and 3 also yielded 4'-OH-PB acid along with two or three additional products.

Ohkawa et al. (1980) found that in greenhouse-grown bean plants, fenvalerate metabolism included ester cleavage, hydrolyses of the CN group to $-CONH_2$ and -COOH groups, hydroxylation at the 2'- and 4'-phenoxy positions, oxidation of the 3-phenoxybenzyl alcohol (PB-alc), derived by hydrolysis, to PB-acid, and conjugation of the resulting carboxylic acids and alcohols with sugars. They detected only one Cl-Vacid conjugate in beans while we have found several in cotton callus tissue. Part of the diversity of the cotton Cl-Vacid conjugates may be due to species differences, and it is interesting to note that some of the [*chlorophenyl*-^{14}C-]fenvalerate metabolites recovered in preliminary work with soybean callus tissue incubated with fenvalerate also do not cochromatograph with those recovered from cotton. Treatment of the soybean metabolites with β-glucosidase and acid hydrolysis yielded Cl-Vacid.

Both bean and cotton plants metabolize the benzyl part of the molecule in a similar manner. In beans, [*benzyl*-^{14}C]fenvalerate was metabolized to free PB-alc, PB-acid, 2'-HO-PB acid, 4'-HO-PB acid, and α-cyanophenoxybenzyl alcohol along with their conjugates (Ohkawa et al., 1980). The alcohol moiety of deltamethrin was metabolized by cotton leaves to 3-phenoxybenzaldehyde, the corresponding alcohol and acid, 4'-HO-PB acid, in addition to conjugates of PB-alc, PB-acid, and α-cyanophenoxybenzyl alcohol (Ruzo and Casida, 1979). In this short-term treatment (5 h) of cotton leaf disks with deltamethrin, good uptake was achieved but little metabolism took place (Ruzo and Casida, 1979).

The metabolism of cypermethrin in cotton plants produced conjugates of the acid moiety that have been tentatively identified as glycosylxylose and glucosylarabinose esters (Wright et al., 1980). In excised cotton leaves [^{14}C]-3-phenoxybenzoic acid was converted to the glucose ester and disaccharide conjugates (More et al., 1978).

In greenhouse-grown bean plants, about 45% of the applied label was recovered as fenvalerate 60 days after treatment with labeled fenvalerate (Ohkawa et al., 1980). Fenvalerate had a half-life of approximately 2 weeks under green-house conditions (Ohkawa et al., 1980), while under field conditions it had a half-life of 2 days in cotton (Holmstead et al., (1978). Decarboxylfenvalerate, $CONH_2$-fenvalerate, and COOH-fenvalerate recovered from bean plants were considered to be produced in whole or part via photochemical and/or physiochemical reaction (Ohkawa et al., 1980). In a study of photodecomposition on cotton under field conditions, decarboxylfenvalerate, Cl-Vacid, PB-acid, PB-alc, PB-aldehyde, and 3-phenoxybenzyl cyanide were detected (Holmstead et al., 1978). In 4 days axenic cotton callus tissue metabolized 30% of the fenvalerate to numerous conjugates, and after 8 days 40% of the fenvalerate was metabolized. No attempt was made to optimize the metabolism, and direct addition to liquid media may increase the rate of degradation.

The fate of fenvalerate in cotton callus tissue is similar to the metabolism of fenvalerate in bean plants as well as the metabolism of the alcohol moiety of deltamethrin and cypermethrin in cotton plants. Therefore, cotton callus tissue is an excellent source of fenvalerate plant metabolites since sterile conditions can be maintained over a longer period than for leaf disks.

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Registry No. Fenvalerate, 51630-58-1.

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Metabolism of Deltamethrin by Cow and Chicken Liver Enzyme Preparations

M. Humayoun Akhtar

Deltamethrin is metabolized by an enzyme or enzymes in various fractions of liver homogenates from cow and chicken. Studies that employed acid- and benzyl-labeled insecticide showed the main metabolic pathway to be due to cleavage of the ester bond. The enzyme(s) responsible for ester bond cleavage was (were) located equally in both the soluble and microsomal fractions of a chicken liver homogenate. In cow liver homogenate, enzymatic activity was higher in the microsomal fraction. The metabolites identified were 3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylic acid, 3-phenoxybenzaldehyde, 3-phenoxybenzyl alcohol, 3-phenoxybenzoic acid, 3-(4-hydroxyphenoxy)benzyl alcohol, and 3-(4-hydroxyphenoxy)benzoic acid. GC and HPLC methods for analyses of various compounds are detailed.

Synthetic pyrethroids are the new generation of pesticides that are being developed as good substitutes for unwarranted organochlorine and toxic organophosphorus insecticides. One of the important members of this family is deltamethrin, which is also known by other names, e.g., RU-22974, NRDC-161, OMS-198, Decamethrin, Decis, and K-Orthin.

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