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Metabolism of the Synthetic Pyrethroid Fenpropathrin in Plants

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The metabolic fate of fenpropathrin [(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate] in cabbages was studied by using ^{14}C preparations labeled separately at the cyano group and the benzyl and cyclopropyl rings. By foliar treatment in a greenhouse, the insecticide disappeared with the the initial half-life of approximately 11–12 days. The insecticide underwent ester cleavage, hydroxylation at either or both of the *gem*-dimethyl groups with subsequent oxidation to the carboxylic acid, hydroxylation at the 2- or 4-position of the phenoxy group, and hydrolysis of the CN group to the $CONH_2$ and COOH groups. Most of the carboxylic acids and alcohols thus produced occurred as glycoside conjugates. 2,2,3,3-Tetramethylcyclopropanecarboxylic acid, the acidic half of the molecule, was converted mainly to glucose conjugate in abscised leaves of apple and vine, to malonylglucoside in cabbage, orange, and bean plants, and to gentiobioside in tomato. $H^{14}CN$ in abscised leaves of cabbage was rapidly incorporated into β-cyanoalanine, asparagine, aspartic acid, and γ-glutamyl-β-cyanoalanine, with ultimate formation of $^{14}CO_2$ and bound ^{14}C residues. Little ^{14}C was detected in the shoot portions of bean plants grown to maturity in soils treated with ^{14}C-fenpropathrin at a rate of 1 ppm.

INTRODUCTION

Fenpropathrin [(*RS*)-α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate] (I) is a synthetic pyrethroid, which has been developed as a commercial name of Danitol or Meothrin (Matsuo et al., 1976). It possesses a great potential for the control of various insects and mites that infest the fruit plants, vegetables, and other crops (Fujita, 1981). Although the metabolic studies in rats (Crawford and Hutson, 1977) and soils (Roberts and Standen, 1977; Mikami et al., 1985a), and photodegradation (Takahashi et al., 1985) were already performed from the view point of environmental safety, little is known about the persistence and metabolism in plants. However, as estimated from the results of plant metabolism of other pyrethroid insecticides such as permethrin (Ohkawa et al., 1977; Gaughan and Casida, 1979), deltamethrin (Ruzo and Casida, 1979), and fenvalerate (Ohkawa et al., 1980; Mikami et al., 1985b), the ester hydrolysis is anticipated to be one of the major metabolic routes of I in plants. The ester hydrolysis of I results in the formation of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (VIII) from the acid moiety and α-cyano-3-phenoxybenzyl alcohol from the alcohol moiety. The latter compound is unstable and rapidly converted to 3-phenoxybenzaldehyde with a release of hydrogen cyanide. Although it has been clarified that 3-phenoxybenzaldehyde was rapidly metabolized to the corresponding acid and alcohol, with subsequent conjugation with various saccharides (More et al., 1978; Roberts and Wright, 1981; Mikami et al., 1984), metabolic fate of HCN or VIII in plants was not fully characterized.

In the present paper the metabolic fate of fenpropathrin in cabbage grown and treated in a greenhouse is reported, together with results of the subsequent work on the fate of hydrogen cyanide in abscised leaves of cabbage and of VIII in abscised leaves of apple, cabbage, kidney bean, orange, tomato, and vine. Uptake of the radiocarbon in soils treated with ^{14}C-labeled fenpropathrin by kidney bean plants was also examined.

MATERIALS AND METHODS

Chemicals. The following ^{14}C preparations were synthesized in Takarazuka Research Center of Sumitomo Chemical Co. Ltd. (Kanamaru et al., 1982): fenpropathrin labeled separately at the cyano group (^{14}CN), the C_1 position in the cyclopropyl ring (cyclopropyl-^{14}C), and the benzyl ring (benzyl-^{14}C) with the specific activity of 20.0, 25.6, and 15.7 mCi/mmol, respectively (radiolabeled positions are shown in Figure 6); 2,2,3,3-tetramethylcyclopropanecarboxylic acid (VIII) labeled at the C_1 position in the cyclopropyl ring (25.6 mCi/mmol). $K^{14}CN$ (60.2 mCi/mmol) was purchased from Radiochemical Center

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Table I. Fenpropathrin and Its Derivatives

no.	chemical structure
	Ester derivatives
I	α-cyano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
II	α-carbamoyl-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
III	α-carboxy-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
IV	α-cyano-3-(2-hydroxyphenoxy)benzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
V	α-cyano-3-(4-hydroxyphenoxy)benzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
VI	α-cyano-3-hydroxybenzyl 2,2,3,3-tetramethylcyclopropanecarboxylate
VII	α-cyano-3-phenoxybenzyl 2-hydroxymethyl-2,3,3-tetramethylcyclopropanecarboxylate
	Cyclopropane Derivatives
VIII	2,2,3,3-tetramethylcyclopropanecarboxylic acid
IX	2-(hydroxymethyl)-2,3,3-trimethylcyclopropanecarboxylic acid
X	5,6,6-trimethyl-3-oxabicyclo[3.1.0]hexan-2-one
XI	2,2-bis(hydroxymethyl)-3,3-dimethylcyclopropanecarboxylic acid
XII	5-(hydroxymethyl)-6,6-dimethyl-3-oxabicyclo[3.1.0]hexan-2-one
XIII	2-carboxy-2,3,3-trimethylcyclopropanecarboxylic acid
	Phenoxybenzyl Derivatives
XIV	3-phenoxybenzyl alcohol
XV	3-phenoxybenzoic acid
XVI	3-(2-hydroxyphenoxy)benzoic acid
XVII	3-(4-hydroxyphenoxy)benzoic acid
	Glucoside Conjugates
XVIII	1-*O*-(2,2,3,3-tetramethylcyclopropylcarbonyl)-β-D-glucopyranose
XIX	1-*O*-(2,2,3,3-tetramethylcyclopropylcarbonyl)-6-*O*-malonyl-β-D-glucopyranose
XX	1-*O*-(2,2,3,3-tetramethylcyclopropylcarbonyl)-β-D-gentiobiose heptaacetate

Ltd., Amersham, England. The radiochemical purity of all samples was more than 99%.

The unlabeled compounds, listed in Table I, were prepared for reference purposes in our laboratory. The compounds (XIV–XVII) were synthesized following the procedure of Unai and Casida (1977). XVIII was prepared by coupling of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with 2,2,3,3-tetramethylcyclopropanecarboxylic acid chloride in dichloromethane containing dry pyridine, with subsequent catalytic dehydrogenation by 5% Pd-C, as reported earlier (Mikami et al., 1984). XX was prepared, according to the methods of Nishikawa et al. (1975), from the silver salt of VIII and acetobromogentiobiose in dry, alcohol-free chloroform. The malonylglucoside conjugate (XIX) was prepared, according to the methods of Kasai et al. (1981), from malonic acid and XVIII in the presence of *N,N'*-dicyclohexylcarbodiimide in dry dioxane.

γ-Glutamyl-β-cyanoalanine was prepared according to the methods of Ressler and Patzkin (1961) and Ressler et al. (1963).

Asparagine, aspartic acid, malonic acid, gentiobiose, and KSCN were purchased from Wako Chemical Co., Ltd. (Japan). β-Glucosidase (almond) and cellulase (*Aspergillus niger*) were purchased from Sigma Chemical Co., St. Louis, MO.

Radioanalysis. Liquid scintillation counting (LSC), combustion analysis, and autoradiography were carried out as reported previously (Mikami et al., 1980).

Thin-Layer Chromatography (TLC). The extracts were applied as spots or bands on the precoated silica gel $60F_{254}$ chromatoplates (20 × 20 cm, 0.25 mm layer thickness, E. Merck Co., Darmstadt, Germany) and HPTLC cellulose chromatoplates (10 × 20 cm, 0.10 mm layer thickness, E. Merck) and were analyzed with various combinations of the solvent systems as follows: (A) hexane–toluene–acetic acid (3:15:2); (B) hexane–acetone (4:1); (C) toluene–ethyl formate–formic acid (5:7:1); (D) ethyl acetate–ethanol–water (4:2:1); (E) chloroform–methanol (1:1); (F) chloroform–ethyl acetate (1:1); (G) dichloromethane–ethyl formate–ethanol (50:30:1); (H) butan-1-ol–ethyl acetate–acetic acid–water (1:1:1:1); (I) butan-1-ol–ethanol–chloroform–17% NH_4OH (4:5:2:4); (J) butan-1-ol–ethanol–chloroform–17% NH_4OH (4:4:2:3). The solvent systems (A–H) were used for silica gel TLC plates, and (I–J) for cellulose TLC plates. The *Rf* values of I and the metabolites are summarized in Table II. The solvent systems for two-dimensional development are illustrated, for example, as follows: (A, B × 2) indicates development in the first direction with solvent system A and in the second direction twice with solvent system B. The radioactive spots were detected by autoradiography, and unlabeled chemicals were detected under UV light or by exposure to the following chromogenic reagents: (1) ferric nitrate in dilute nitric acid (thiocyanate) (Snell and Snell, 1949); (2) 5% $AgNO_3$ in NH_4OH or 2% triphenyltetrazolium chloride in 1 N NaOH–methanol solution (glucose); (3) 5% ninhydrin in butan-1-ol (amino acid).

Spectroscopy. Proton nuclear magnetic resonance (NMR) and ^{13}C NMR were determined, in either methanol-d_4 or D_2O, with Hitachi R-40 and R-900 spectrometers at 90 MHz, respectively. Field desorption mass spectra (FD-MS) were recorded with Hitachi M-80 spectrometer equipped with Model M-003 data system. GC/MS was carried out on Finnigan Model 4000 gas chromatograph mass spectrometer with Model 6111 data system. The glass column (50 cm × 2 mm i.d.) was packed with 5% PEG-20M Gas-Chrom Q (80–100 mesh) and maintained at a temperature of 110 °C. Helium was used as a carrier gas at a flow rate of 20 mL/min. The temperature of the injection port was 140 °C and the ion source was kept at 250 °C. The ionization energy and emission current were 20 eV and 150 μA, respectively.

Plant Treatment and Extraction. Treatment with [^{14}C]Fenpropathrin. Cabbage plants (*Brassica oleracea*, var *capitata* cv. Shikidori) at the 4–5th leaf stages were used in the experiment. The plants were allowed to grow in a greenhouse at 25 ± 2 °C before or after treatment. Each of the ^{14}CN-, cyclopropyl-, and benzyl-^{14}C labels in methanol (100 mL) was evenly applied to the upper surface of two 3rd–4th leaves at a rate of 22 μg per leaf (ca. 25 cm^2) by a microsyringe.

The cabbages were sampled at various time intervals by cutting off at ground level and separating them into the treated leaves and nontreated shoot portions. The treated leaves were rinsed with methanol (20 mL × 2) to remove the external radiocarbon (surface wash), cut into small

Table II. The *Rf* Values for Fenpropathrin and Its Metabolites

chemicals	*Rf* values in solvent systems[a]									
	A	B × 2	C	D	E	F	G × 2	H × 2	I × 2	J × 2
I	0.57	0.57	0.89							
II	0.31	0.13	0.67							
III	0.33	0.10	0.69							
IV	0.41	0.26	0.85							
V	0.27	0.19	0.65							
VI	0.16	0.19	0.71							
VII (cis)	0.23	0.22	0.62							
VII (trans)	0.25	0.22	0.62							
VIII	0.36	0.32	0.71							
IX (cis)	0.10	0.00	0.34							
IX (trans)	0.15	0.04	0.34							
X	0.26	0.49	0.62							
XI	0.01	0.00	0.31							
XII	0.03	0.07	0.28							
XIII (cis,trans)	0.30	0.00	0.55							
XIV	0.26	0.38	0.68							
XV	0.32	0.09	0.59							
XVI	0.25	0.00	0.52							
XVII	0.16	0.00	0.49							
XVIII				0.74	0.78					
XIX				0.35	0.35					
XX						0.48	0.55			
β-cyanoalanine								0.54	0.55	0.42
asparagine								0.39	0.37	0.26
aspartic acid								0.44	0.28	0.16
γ-glutamyl-β-cyanoalanine								0.48	0.32	0.20
KSCN								0.89	0.80	0.76

[a] Solvent systems A–H for silica gel TLC; solvent systems I–J for cellulose TLC.

pieces, and homogenized in a Waring blender for 7 min with 10 mL of methanol–chloroform–water (4:2:1) per gram of the plant material. The mixture was filtered and the residue was reextracted twice in the same way. The nontreated shoots were extracted as described above. Each of the combined filtrate was radiocounted, concentrated and analyzed by TLC in solvent systems A, B, and C. Through extraction, autoradiography, and LSC, 97.3% of fenpropathrin dosed was recovered from cabbage immediately after treatment with the ^{14}CN label at 22 μg per leaf.

Treatment with [^{14}C]2,2,3,3-Tetramethylcyclopropanecarboxylic Acid (VIII). In addition to cabbages, the following five species of plants were used in the experiment: apple (cv. Koogyoku), kidney bean (*Phaseolus vulgaris* L., cv. Nagauzura), mandarin orange (cv. Unshu), tomato (*Lycopersicon esculentum* Mill, cv. Fukuju No. 2), vine (cv. Neomasukatto). Matured leaves were removed as required. The petiole was immersed in water and cut an angle of 45° with a scalpel. Groups of two abscised leaves were immediately transferred to a 100-mL glass flask containing an aqueous solution (100 mL) of [^{14}C]VIII at a concentration of 1 ppm. After cultivation in a greenhouse for 5 days, they were shredded into small pieces and extracted with methanol–chloroform–water (4:2:1) in the precedures described above.

Another experiment was carried out with a total of 160 abscised leaves, supplied with 3 L of an aqueous solution of [^{14}C]VIII at 100 ppm for up to 5 days, to obtain large quantities of metabolites for characterization. The extracts were partitioned between diethyl ether (200 mL) and distilled water (300 mL) to separate the ^{14}C metabolites from the mass of colored plant materials. More than 95% of the radiocarbon remained in the aqueous fraction. After acidification to pH 3, the aqueous layer was partitioned with ethyl acetate to extract the starting material and malonylglucose conjugates. The ethyl acetate layer was concentrated, and the metabolites were purified by preparative TLC in solvent systems D and E. The remaining aqueous fraction was neutralized at 0–5 °C and lyophilized to give a yellow syrup. Some metabolites were purified by column chromatography on a Sephadex G-25 and Sephadex G-50 column (60 cm × 3 cm i.d.) and by repeated preparative TLC in solvent systems D and E.

Treatment with K^{14}CN. Groups of two abscised leaves of cabbage were cultivated in the filter-sterilized aqueous solution (100 mL) of K^{14}CN (50 μCi) for 2 days in a greenhouse. At specified intervals, the treatment leaves were extracted as described above. The extracts were analyzed by silica gel TLC in solvent system (H × 2) or by cellulose TLC in solvent systems (I × 2) and (J × 2).

Four hours later, some of the treated leaves were transferred to 100 mL of K^{14}CN-free distilled water in a glass flask and then cultivated for 2 days. The glass flask was placed in a 3-L glass jar, to which CO_2-free air was continuously supplied in order to lead $^{14}CO_2$ and/or H^{14}CN, volatilized from the treated leaves, to a 0.5 N NaOH solution (200 mL). For determination of $^{14}CO_2$, 1 mL of a 1 N $BaCl_2$ solution was added to 10 mL of the NaOH trap solution, and the precipitate ($Ba^{14}CO_3$) was separated by centrifugation. Both the supernatant phase and precipitate were radioassayed. In this method, more than 99% of $^{14}CO_2$ was precipitated, whereas less than 1% of $^{14}CN^-$ was precipitated.

Uptake of Radiocarbon in Soils by Bean Plants. Kodaira light clay and Azuchi sandy clay loam soils were utilized in the experiment. The detailed characteristics of these soils were reported previously (Mikami et al., 1980).

The cyclopropyl- or benzyl-^{14}C fenpropathrin (0.5 mg/mL of methanol) was applied to Kodaira (ca. 250 g) and Azuchi (ca. 400 g) soils at a rate of 1 ppm relative to dry soil, and the soils were mixed well and then placed into a plastic pot (11 cm i.d. × 7 cm in height). The treated soils were moistened to ca. 40% of the maximum water-holding capacity and incubated for 2 weeks at 25 ± 2 °C in the dark. Then, the 7-day old seedlings of kidney bean plants were transplanted into the pots and kept in a

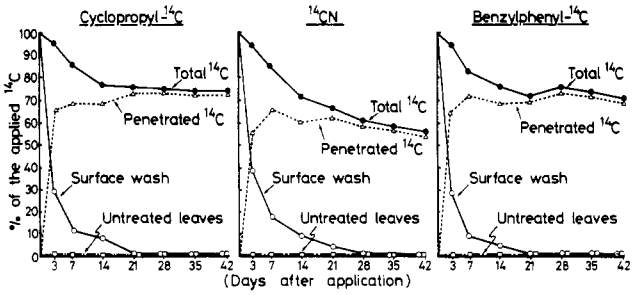

Figure 1. Distribution of radiocarbon in cabbages treated with [^{14}C]fenpropathrin.

greenhouse. About 30–50 mL of water was applied daily to each pot. At harvest 40 days after treatment, the plants were carefully pulled out from the soils, and the roots were washed with 200 mL of distilled water. The harvested plants were sectioned into the roots, shoots, and edible portions. Each part of the plants was extracted by homogenization with a Waring blender for 10 min by using 10 mL of chloroform–methanol (1:2) per gram of the plant material. The soils were extracted three times with methanol (3 mL/g) by shaking followed by centrifugation. The combined extracts of bean plants or soils were analyzed for ^{14}C by LSC and degradation products by TLC in solvent systems A, B, and C.

Characterization of Metabolites. Direct comparison by TLC of the metabolites from three ^{14}C-labeled preparations of I was used to distinguish products retaining the ester linkage from the hydrolysis products. The metabolites were identified by TLC cochromatography with authentic samples before or after hydrolysis, methylation, and/or acetylation.

Some of the ester metabolites of I were hydrolyzed in 0.1 N NaOH solution (1 mL) at 25 °C for 24 h. The hydrolysis products were extracted with ethyl acetate (2 mL × 3) at pH 2.

The glucoside conjugates of VIII were subjected to hydrolysis by β-glucosidase or cellulase (2 mg) in 0.04 M acetate buffer (pH 4.5) at 37 °C for 12 h. The enzymatic cleavage was complete under the conditions because no further cleavage occurred on either longer incubation or reincubation with enzymes. The released aglycon was extracted with ethyl acetate (1 mL × 3) for radioassay by LSC and analysis by TLC. The aqueous fraction, containing the released sugars, was analyzed directly by TLC in butan-1-ol–propan-2-ol–water (130:47:23) or in butan-1-ol–acetone–water (4:5:1). In parallel, a certain volume of the aqueous layer was subjected to the colorimetric analysis at 620 nm by the anthrone–H_2SO_4 method (Trevelyan and Harrison, 1952) to determine the content of glucose.

Some components of the glucoside conjugates were acetylated by treatment with acetic anhydride (0.5 mL) and pyridine (1 mL) at 80 °C for 1 h.

RESULTS

Distribution of Radiocarbon in Cabbages Treated with [^{14}C]Fenpropathrin (I). After foliar application of [^{14}C]I to cabbages, the radiocarbon remaining on the treated leaves (surface wash fraction) promptly decreased, with concomitant increase of the ^{14}C penetrated into the plant tissues (Figure 1). Most of the recovered ^{14}C resided in the treated leaves, with less than 1.2% of the dose in nontreated shoots of the plants. These findings indicate that I and its metabolites hardly translocate from the application site to other parts of the plants. After 42 days, 73–75% of the applied ^{14}C was recovered from cabbages treated with the cyclopropyl- and benzyl-^{14}C labels, compared with 56% from cabbages treated with the ^{14}CN label.

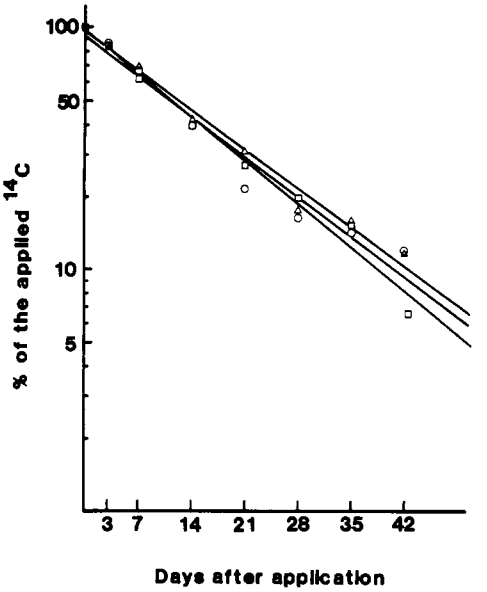

Figure 2. The rate of disappearance of fenpropathrin from the treated leaves of cabbages: (○) cyclopropyl label; (□) cyano label; (△) benzyl label. Regression analysis equations [cyclopropyl label, $Y = 90.8 \exp(-0.054X)$, $r^2 = 0.956$; cyano label, $Y = 97.2 \exp(-0.059X)$, $r^2 = 0.982$; benzyl label, $Y = 94.8 \exp(-0.053X)$, $r^2 = 0.984$].

As shown in Figure 2, I disappeared from the treated leaves according to the first-order kinetics and decreased to 6–12% of the initial dose after 42 days. The half-life of the disappearance of I, calculated by the least square approximation method, was approximately 11–12 days.

Identification of Metabolites in Cabbages. The analysis by TLC showed that only unchanged I was present in the surface wash fraction of the treated leaves. In contrast, a number of metabolites were present in the plant extracts, in free and conjugated forms. More than six free products were detected by TLC on silica gel plates in solvent system A. Each product, separated by preparative TLC, was identified by two dimensional cochromatography with synthesized compounds, in the following solvent systems: I, II, and III (A, B × 2); VII, X, and XII (A, C). VII hydroxylated at the methyl group cis to the carboxyl group was partially converted to X and 3-phenoxybenzaldehyde via spontaneous ester bond cleavage on silica gel TLC plates.

The conjugated metabolites, located at the origin of the TLC plates in solvent system A, were scraped off, eluted with methanol–water (1:1), and then developed again by TLC in solvent system D. The individual conjugates were subjected to β-glucosidase and/or cellulase hydrolysis, and the released aglycons were identified by two dimensional cochromatography, in the following solvent systems: IV, V, and XIV (A, B × 2); VIII, IX, XIII–XVII (A, C). As with VII, IX hydroxylated at the methyl group cis to the carboxyl group and XI hydroxylated at both of the *gem*-dimethyl groups underwent the cyclization reaction to form X and XII, respectively, on silica gel TLC plates. The aglycons with the *Rf* values of 0.16 and 0.11 by TLC in solvent system A were tentatively identified by cochromatography of the acid and alcohol moieties, released on the alkaline hydrolysis. On treatment with 0.1 N NaOH solution, the former ester product gave a mixture of XVI and XVII from the alcohol moiety, and a mixture of IX and X from the acid moiety. Therefore, the aglycon was considered to be a mixture of α-cyano-3-(2-hydroxyphenoxy)benzyl 2-(hydroxymethyl)-2,3,3-trimethylcyclo-

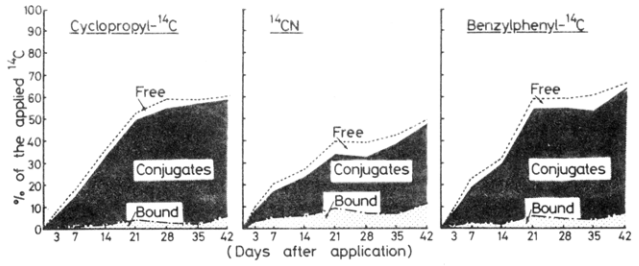

Figure 3. The amounts of free and conjugated metabolites and bound ^{14}C residues in cabbages treated with [^{14}C]fenpropathrin.

propanecarboxylate (XXI) and α-cyano-3-(4-hydroxyphenoxy)benzyl 2-(hydroxymethyl)-2,3,3-trimethylcyclopropanecarboxylate (XXII). Meanwhile, the latter ester product released a mixture of XVI and XVII from the alcohol moiety and XII from the acid moiety. Therefore, the aglycon was considered to be a mixture of α-cyano-3-(2-hydroxyphenoxy)benzyl 2,2-bis(hydroxymethyl)-3,3-dimethylcyclopropanecarboxylate (XXIII) and α-cyano-3-(4-hydroxyphenoxy)benzyl 2,2-bis(hydroxymethyl)-3,3-dimethylcyclopropanecarboxylate (XXIV). In addition, two minor aglycons with the *Rf* values of 0.20 and 0.26 by TLC in solvent system A were present. On treatment with 0.1 N NaOH solution, they afforded XVI and XVII from the alcohol moieties, but the hydrolysis products from the acid moieties have yet to be characterized.

Amounts of Metabolites. As shown in Figure 3, a large proportion of the metabolites occurred in conjugated forms, with the unconjugated free products amounting to a total of less than 5% of the applied ^{14}C. After 42 days, the unextracted bound ^{14}C residues amounted to 5.1–7.5% of the dose in plants treated with the cyclopropyl and benzyl labels, and 11.3% with the CN label.

The amounts of I and its metabolites in cabbages 28 and 42 days after treatment are shown in Table III. The major metabolites were glycoside conjugates of XXIII and XXIV and XII, which amounted to 20.7–22.0% and 11.1% of the dose, respectively. In addition, VII, XIII, XVI, XVII, XXI, and XXII, which occurred mainly in conjugated forms, were detected in relatively higher amounts. Each of the other identified metabolites amounted to less than 2% of the dose.

Metabolism of [^{14}C]2,2,3,3-Tetramethylcyclopropanecarboxylic Acid (VIII) in Abscised Leaves. In order to characterize the chemical structures of conjugated metabolites of fenpropathrin in cabbages, further metabolic studies were performed by using VIII as a starting material. The abscised leaves of cabbages were treated with an aqueous solution of [^{14}C]VIII at 1 ppm for up to 5 days. Uptake of the radiocarbon occurred readily and 79% of the dose was recovered from the plant leaves (Table IV). The analysis by TLC showed that one major product (designated as T-6) and four minor products (T-1, T-2, T-3, and T-7) were present in the plant extracts. The parallel experiments were also carried out with the abscised leaves of apple, kidney beans, orange, tomato, and vine. Although there was a considerable variation between species in the amounts taken up, VIII was rapidly converted in plants to more polar products (T-1, T-2, T-3, and T-6). Further, one major metabolite (T-5) was present in extracts of the apple and tomato leaves.

Some components (2–15 mg) were isolated from the abscised leaves and treated with [^{14}C]VIII at 100 ppm for 5 days. Each of the purified samples was subjected to cellulose hydrolysis, and the released aglycon and sugar moiety were identified by TLC cochromatography. Furthermore, the ^{14}C aglycon and unlabeled glucose were estimated by LSC and colorimetric analyses, respectively, and the molar ratios of glucose to the aglycon were determined. The results are summarized in Table V.

The component T-1 was identified as 1-*O*-((2,2,3,3-tetramethylcyclopropyl)carbonyl)-β-D-glucopyranose, based on the following results. On treatment with cellulose, T-1 released an equimolar mixture of VIII and glucose. T-1 showed a molecular ion of *m/z* 304 by FD-MS, and the *Rf* values by TLC in solvent systems D and E were consistent with those of the synthesized XVIII.

Each component of T-2, T-3, and T-4 released an equimolar mixture of IX and glucose. The acetylated

Table III. Amounts of Fenpropathrin and Its Metabolites in Cabbages 28 and 42 Days after Foliar Application of ^{14}C Labeled Preparations[a]

	% of the applied ^{14}C					
	cyclopropyl-^{14}C		^{14}CN		benzyl-^{14}C	
	28	42	28	42	28	42
treated leaves	74.5	73.2	59.9	55.8	74.2	75.3
surface wash	0.9	0.4	1.4	0.9	2.0	0.5
I	0.6	0.3	1.0	0.6	1.7	0.4
others	0.3	0.1	0.4	0.3	0.3	0.1
extracts	71.0	67.7	51.8	43.6	68.2	67.3
I	15.8	11.7	16.9	6.0	12.9	11.3
II	0.7	<0.1	0.3	<0.1	0.9	<0.1
III (free)	0.4	0.4	0.6	0.3	0.7	<0.1
(conj)	0.4	0.2	0.6	0.4	0.6	0.7
IV (free)	0.1	<0.1	0.4	<0.1	0.4	<0.1
(conj)	0.2	0.1	0.1	0.1	0.1	0.2
V (conj)	1.3	0.7	1.6	1.0	0.6	0.8
VII (free)	0.4	0.3	0.5	0.5	0.6	0.2
(conj)	3.5	4.0	3.4	4.3	4.2	4.0
VIII (conj)	0.9	0.8				
IX (conj)	1.1	1.0				
X	0.1	<0.1				
XII (free)	0.8	0.9				
(conj)	11.3	11.1				
XIII (conj)	3.7	4.2				
XIV (conj)					0.1	0.1
XV (conj)					0.8	1.1
XVI (conj)					6.9	7.4
XVII (conj)					4.5	4.6
XXI (conj)	4.8	4.5	4.6	4.5	5.9	6.2
XXII (conj)						
XXIII (conj)	20.3	22.0	18.6	20.7	19.4	21.6
XXIV (conj)						
others	5.2	5.8	4.2	5.8	9.6	9.1
bound ^{14}C	2.6	5.1	6.7	11.3	4.0	7.5
untreated shoots	0.9	1.2	0.6	0.7	0.4	0.4
total	75.4	74.4	60.5	56.5	74.6	75.7

[a] conj = conjugate.

Table IV. Uptake and Metabolism of 2,2,3,3-Tetramethylcyclopropanecarboxylic Acid in Abscised Leaves of Various Plants over a 5-Day Period

	% of the applied ^{14}C					
	apple	bean	cabbage	orange	tomato	vine
abscised leaves	35.9	80.1	79.0	7.6	20.6	8.4
extract	35.6	79.9	78.8	7.2	20.4	8.1
VIII	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
T-1	21.5	14.7	3.2	3.0	2.0	6.6
T-2,3	5.2	3.2	3.4	0.2	0.2	0.4
T-4	1.3					0.1
T-5	5.4	2.8			12.8	0.7
T-6	0.7	56.2	70.5	3.1	4.3	0.1
T-7			1.5	0.4		
others	1.5	3.0	0.2	0.5	1.1	0.2
bound	0.3	0.2	0.2	0.4	0.2	0.3
aqueous solution	58.3	25.3	14.1	64.5	77.7	83.5
VIII	57.1	24.5	13.2	63.8	76.1	83.0
others	1.2	0.8	0.9	0.7	1.6	0.5
total	94.2	105.4	93.1	72.1	98.3	91.9

Table V. Analytical Data for Conjugated Metabolites of VIII and Their Acetyl Derivatives

metabolites	TLC, *Rf*[a]	FD-MS, *m/z*	cellulase treatment			molar ratios, sugars/aglycon
			aglycon	sugars	fatty acid	
T-1	0.80	304	VIII	glucose	[c]	1.0
T-2	0.70	530[b]	IX	glucose	[c]	1.1
T-3	0.66	530[b]	IX	glucose	[c]	1.2
T-4	0.63	488[b]	IX	glucose	[c]	1.1
T-5	0.55	760[b]	VIII	glucose	[c]	2.1
T-6	0.44		VIII	glucose	malonic acid	1.2
T-7	0.30		IX	glucose	malonic acid	

[a] Silica gel TLC in ethyl acetate–ethanol–water (4:2:1). [b] Data for the acetylated derivative. [c] Not detected.

derivative of T-2 and T-3 showed the same molecular ion of *m/z* 530 by FD-MS. Therefore, these metabolites were tentatively identified as 1-*O*-((2-(hydroxymethyl)-2,3,3-trimethylcyclopropyl)carbonyl)-β-D-glucopyranose, in which the glucose moiety was linked to the carboxy group of cis and trans isomers of IX through β-glycoside linkage. In contrast, the acetylated derivative of T-4 showed a molecular ion of *m/z* 488 by FD-MS, and therefore T-4 was identified as 1-*O*-(1,2,2-trimethyl-3-carboxycyclopropyl)methyl-β-D-glucopyranoside.

The component T-5 released two molar equivalents of glucose to one of VIII, and upon acetylation gave one less polar product with a molecular ion of *m/z* 760 by FD-MS. The *Rf* values of the acetylated derivative in solvent systems F and G were consistent with those of the synthesized XX. Thus, T-5 was identified as 1-*O*-((2,2,3,3-tetramethylcyclopropyl)carbonyl)-6-*O*-β-D-glucopyranosyl-β-D-glucopyranose.

Component T-6 released IX, glucose, and malonic acid in molar ratios of 1.0:1.2:0.8. Malonic acid was identified and estimated as follows. An aliquot of the reaction mixture with cellulase was diluted by 100-fold with methanol and an ethereal solution of diazomethane to give malonic acid dimethyl ester. The dimethyl ester was directly analyzed by GC/MS and identified by comparison of the retention time (*Rt* 4.6 min) and the mass spectra (molecular ion, *m/z* 132) with those of an authentic sample. Quantitation was carried out by selected ion monitoring/mass spectrometry at *m/z* 101 and 132. Because a limited hydrolysis to T-1 occurred on silica gel TLC plates in solvent system D, it is likely that component T-6 is a malonate hemiester derivative of T-1. The substitution position of the glucose moiety with malonic acid was determined by comparison of ^{13}C NMR spectra (D_2O) of T-1 and T-6. The spectrum of T-6 differed from that of T-1 primarily by the additional signals derived from the methylene (51.8 ppm) and carbonyl carbons (173.8 and 174.0 ppm), and the lower shift of the signal (from 63.1 to 67.3 ppm) derived from the C-6 position of the glucose moiety. Further, T-6 showed the identical *Rf* values by TLC in solvent systems D and E with those of the synthesized XIX. Based on the above evidences, T-6 was identified as the malonylglucose conjugate of VIII, in which malonic acid was linked to the hydroxymethyl group in the glucose moiety.

The component T-7 released IX, glucose, and malonic acid. Because a limited hydrolysis to T-4 occurred on silica gel TLC plates in solvent system D, it is likely that T-7 is a malonyl glucoside conjugate of IX. Further characterization has yet to be carried out.

All of the conjugated metabolites were cleaved by β-glucosidase. The exception was malonyl glucoside conjugates (T-6 and T-7), which were resistant to β-glucosidase hydrolysis but were completely cleaved by cellulase.

Metabolism of $K^{14}CN$ in Abscised Leaves. In order to clarify the metabolic fate of HCN released on ester bond cleavage of fenpropathrinin in cabbages, further studies were performed with $K^{14}CN$ as a starting material. When the abscised leaves of cabbage were supplied with an aqueous solution of $K^{14}CN$ for 48 h, at least six ^{14}C metabolites were detected in the plant extracts. Among them, β-cyanoalanine, asparagine, aspartic acid, and γ-glutamyl-β-cyanoalanine were identified by silica gel or cellulose TLC cochromatography with authentic samples in solvent system (H × 2), (I × 2), or (J × 2). In addition, three minor products with the *Rf* values of 0.93, 0.65, and 0.21 on cellulose TLC plates in solvent system (I × 2) were detected. They were not identical with SCN^- and glutamic acid. The amounts of these metabolites are shown in Table VI. At 2 h after treatment, β-cyanoalanine, asparagine, aspartic acid, and γ-glutamyl-β-cyanoalanine constituted 8, 23, 9, and 50% of the radiocarbon in the plant extracts, respectively. These metabolites reached maximum amounts at 4 or 8 h after treatment, and gradually decreased thereafter. In contrast, the bound ^{14}C increased with time.

Table VI. Uptake and Metabolism of $H^{14}CN$ in Abscised Leaves of Cabbages over a 2-Day Period

	% of the applied ^{14}C after treatment			
	2h	4h	8h	48h
treated leaves	10.5	14.6	15.5	15.0
extract	7.6	10.9	11.0	7.3
β-cyanoalanine	0.6	0.9	0.9	0.6
asparagine	1.8	2.4	2.8	1.1
aspartic acid	0.7	1.0	1.1	1.3
γ-glutamyl-β-cyanoalanine	3.8	5.4	5.1	2.4
others	0.7	1.2	1.1	1.9
bound	2.9	3.7	4.5	7.7
aqueous solution	86.9	79.6	83.1	63.2
total	97.4	94.2	98.6	78.2

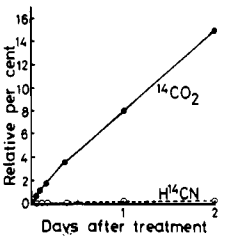

Figure 4. The volatile radiocarbon from the abscised leaves of cabbage treated with $K^{14}CN$. (The abscised leaves were treated with an aqueous solution of $K^{14}CN$ for 4 h and then transferred to $K^{14}CN$-free distilled water. The vertical line shows the relative percent to the total radiocarbon taken up 4 h after treatment.)

Some of the treated leaves were transferred to $K^{14}CN$-free distilled water in order to trap the radiocarbon volatilized from the leaves. The results are shown in Figure 4. Most of the radiocarbon trapped in the NaOH solution was considered to be $^{14}CO_2$, because 98% of the radioactivity was precipitated as $Ba^{14}CO_3$ by addition of 1 N $BaCl_2$ solution.

Uptake of Radiocarbon in Soils by Bean Plants. After 40-day cultivation of bean plants in soils treated with

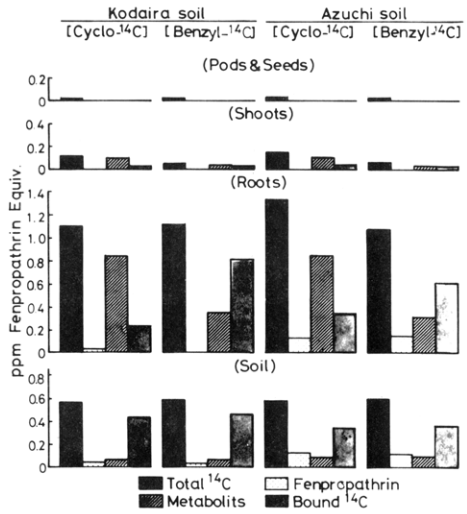

Figure 5. Uptake of radiocarbon in soils treated with [^{14}C]fenpropathrin by bean plants.

the cyclopropyl or benzyl label of I at 1 ppm, the concentrations of ^{14}C in pods and seeds, shoots, and roots were determined (Figure 5). The detection limit of ^{14}C was 0.01–0.02 ppm, based on a minimum determination of 2 × 10^3 dpm/g plant material following sample combustion. The concentrations in the edible portions were 0.02 ppm, compared with 0.08–0.20 ppm, 1.1–1.4 ppm, and 0.56–0.60 ppm in shoots, roots, and soils, respectively. The root portions contained unchanged I along with the degradation products in soils such as II, III, V, and VI. The radiocarbon in roots appears to be due at least in part to direct contamination of the soil ^{14}C residues, because small particles of soil are apparently attached to the roots. In addition, III, V, and VI were extracted from the roots mainly in the free forms but not in conjugated forms.

In contrast, I was not detected in the shoots. The pods and seeds were not analyzed for the parent compound, because of a quite low content of ^{14}C. These evidences suggest that although fenpropathrin residues in soils are taken up by bean plants to some extents, there was no essential translocation of the residues to other portions of the plants.

DISCUSSION

After foliar treatment, fenpropathrin disappeared from the treated leaves of cabbages with an initial half-life of approximately 11–12 days under greenhouse conditions. The rate of disappearance of some other pyrethroids with the 3-phenoxybenzyl moiety has also been determined under greenhouse conditions. The time required for half of the insecticides to disappear was less than one day for phenothrin in bean and rice plants (Nambu et al., 1980), 8 days for deltamethrin in cottons (Ruzo and Casida, 1979), 7–9 days (Ohkawa et al., 1977) and 1–2 weeks (Gaughan and Casida, 1978) for permethrin in bean plants, and 12–14 days for fenvalerate in bean and cabbage plants (Ohkawa et al., 1980; Mikami et al., 1985b). Although it is difficult to make valid comparison of the degradation rate, due to variation of the plant species and environmental conditions tested, it appears that the rate of disappearance of I in cabbages is within the ranges of those of other pyrethroids in plants.

Figure 6 shows the proposed metabolic pathways for fenpropathrin in cabbages. The insecticide underwent ester bond cleavage, hydrolysis of the CN group to the $CONH_2$ and COOH groups, hydroxylation at either or both of the *gem*-dimethyl groups with subsequent oxidation to the carboxylic acid, and hydroxylation at the 2- and 4-

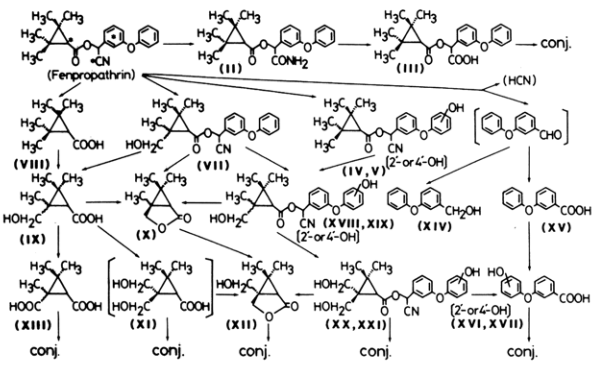

Figure 6. The proposed metabolic pathways for fenpropathrin in cabbages (*, radiolabeled positions).

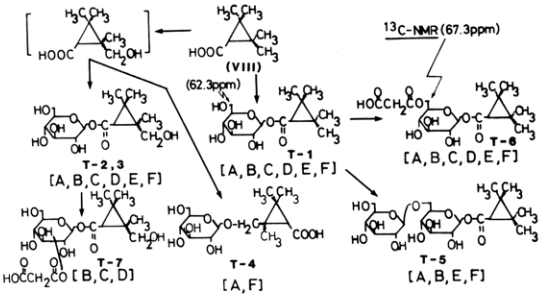

Figure 7. The proposed metabolic pathways of 2,2,3,3-tetramethylcyclopropanecarboxylic acid (VIII) in abscised leaves of plants: [A] apple, [B] bean, [C] cabbage, [D] orange, [E] tomato, [F] vine.

position of the phenoxy group. The resultant carboxylic acids and alcohols were conjugated with sugars. There were two different types of conjugates, the minor one a glucoside readily cleaved by β-glucosidase and the major one a glycoside poorly cleaved by this enzyme but completely cleaved by cellulase.

As shown in Figure 7, 2,2,3,3-tetramethylcyclopropanecarboxylic acid (VIII) was converted mainly to malonylglucoside, along with small amounts of the glucoside, and glucose and malonylglucose conjugates of IX in abscised leaves of cabbage. The malonylglucosides were resistant to β-glucosidase hydrolysis but were completely cleaved by cellulase, whereas the glucose conjugates were readily hydrolyzed by β-glucosidase. As with the conjugates of VIII, other metabolites of fenpropathrin might occur as glucose and malonylglucose conjugates in plants. The metabolic pathways for VIII were somewhat plant specific. The glucose ester was a main product in apple and vine plants, while the malonylglucoside was predominant in orange, cabbage, and bean plants. In tomato, the gentiobiocide was predominant. The structures of the components were closely analogous to those found for the metabolites of XIV (Roberts and Wright, 1981), XV (More et al., 1978; Mikami et al., 1984), 2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylic acid (Wright et al., 1980), and 2-(4-chlorophenyl)-3-methylbutyric acid (Mikami et al., 1985b) in plants.

The cyano group was released as HCN on hydrolysis of the ester linkage. Although metabolic fate of HCN derived from pyrethroids possessing the α-cyano moiety, namely cypermethrin, deltamethrin and fenvalerate, was not persued yet, it has been clarified that HCN was rapidly incorporated into various components of natural products (Resler et al., 1963; Tschiersh, 1964, Blumenthal et al., 1968; Nartey, 1968, 1969 and 1970; Miller and Conn, 1980). β-Cyanolanine, asparagine, aspartic acid, γ-glutamyl-β-cyanoalanine, formamide, and thiocyanate have been

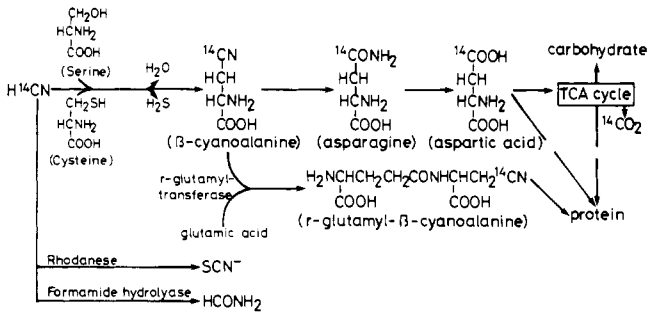

Figure 8. Metabolic fates of hydrogen cyanide in higher plants.

shown to be some of the major products of cyanide assimilation in higher plants (Figure 8). When the abscised leaves of cabbages were treated with K^{14}CN, most of the radiocarbon in plants was converted to β-cyanoalanine, asparagine, aspartic acid, and γ-glutamyl-β-cyanoalanine at an early stage after treatment. The amino acids and dipeptide could be subsequently assimilated by general biosynthetic pathways into the soluble metabolites connected with respiration and protein and carbohydrate syntheses. On prolonged cultivation, $^{14}CO_2$ was evolved in some degrees from the treated leaves, probably via decarboxylation of the α-ketoglutaric acid in a citric acid cycle and/or photosynthetic mineralization of the ^{14}C labeled glucose. Meanwhile, incorporation of the radiocarbon into the plant constituents like starch and cellulose resulted in formation of the unextractable bound ^{14}C residues. Thus, it seems that the released H^{14}CN from the α-cyano esters is ultimately converted at least in part to $^{14}CO_2$ and bound ^{14}C residues. The conclusion was in good accord with the observations of lower recovery of ^{14}C and of higher amounts of bound ^{14}C residues in cabbages treated with [^{14}CN]fenpropathrin, as compared with those treated with the cyclopropyl and benzyl labels (Figures 1 and 3).

Thiocyanate was not detected in extracts of the cabbages treated with K^{14}CN. Thus, metabolic fate of HCN in higher plants was different from that in mammals (Ruzo et al., 1978; Ohkawa et al., 1979).

The identified metabolites of fenpropathrin in cabbages were analogous to those in mammals except for the metabolites derived from HCN and the nature of the conjugating moieties. It has been clarified that upon oral administration to rats the glucoside conjugates of XIV and XV were absorbd mainly as their corresponding aglycons, after cleavage of the glycoside linkage by gut microflora, and completely eliminated as a mixture of metabolites very similar to those derived from XIV and XV, respectively (Crayford and Hutson, 1980; Mikami et al., 1985c). Further, conjugation with glucose significantly lowered the acute oral toxicity of XIV and XV in mice (Miyamoto and Mikami, 1983).

Registry No. I, 39515-41-8; II, 97335-24-5; III, 97335-25-6; IV, 97280-53-0; V, 97280-54-1; VI, 97280-55-2; VII, 66403-98-3; VIII, 15641-58-4; *trans*-IX, 66280-09-9; *cis*-IX, 97280-66-5; X, 97280-56-3; XI, 97280-57-4; XII, 96475-60-4; XIII, 17219-45-3; XIV, 13826-35-2; XV, 3739-38-6; XVI, 35101-26-9; XVII, 35065-12-4; XVIII, 97280-58-5; XIX, 97280-59-6; XX, 97280-60-9; HCH, 74-90-8; β-cyanoalanine, 923-01-3; asparagine, 70-47-3; aspartic acid, 56-84-8; γ-glutamyl-β-cyanoalanine, 16051-95-9.

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