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Original Article

Metabolism of Imiprothrin Isomers in Rats: Biotransformation and Excretion

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The biotransformation and excretion of imiprothrin ([2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl]methyl (1*R*)-*cis*, *trans*-chrysanthemate), a novel pyrethroid, was examined by dosing (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin orally to male and female rats at 1 (low dose) and 200 mg/kg (high dose). Elimination of the *trans*- and *cis*-isomers was rapid, and almost all of the dosed ^{14}C was excreted into urine, feces and expired air within 7 days, with the former route predominating (more than 83% of the dosed ^{14}C). ^{14}C-Tissue residues on the 7th day after administration were generally low in all dosed groups. There were no marked sex-related differences in the rate of ^{14}C-excretion and the ^{14}C-tissue residues within either treatment group. To elucidate metabolic pathways of imiprothrin, major urinary metabolites were isolated using (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin and chromatographic techniques, with identification by spectroanalyses (NMR and MS). Metabolites in urine or feces were also identified and quantified by HPLC analyses using these isolated metabolites as standards. No marked sex-related differences in the metabolite profiles were observed within dose groups. Based on the identified metabolites, the main metabolic reactions of imiprothrin in rats are 1) cleavage of the ester linkage, 2) cleavage of the imidomethylene linkage, 3) hydroxylation of the imidazolidine ring, 4) dealkylation of the 2-propynyl group and 5) oxidation at the ω-*trans*-methyl group in the isobutenyl side chain.

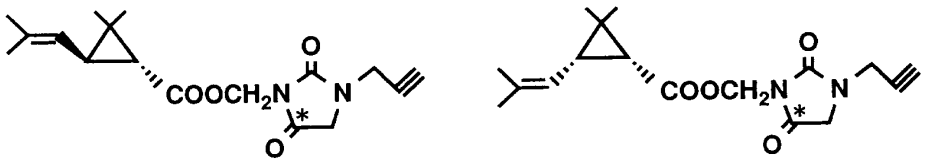
INTRODUCTION

The pyrethroid insecticide, imiprothrin [S-4056F, S-41311, [2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl]methyl (1*R*)-*cis*, *trans*-chrysanthemate], is a potent knockdown agent[1] which comprises two geometrical isomers [(1*R*)-*cis* and (1*R*)-*trans*] in the ratio of 1 : 4. The metabolism of chrysanthemic acid, which is the acid moiety of imiprothrin, has been well established from studies of various pyrethroids such as tetramethrin, phenothrin and cyphenothrin.[2-4] A common major metabolic reaction was found, being oxidation of the methyl group of the isobutenyl group to alcohol and acid derivatives. Therefore, the present study was conducted with the objectives of studying ^{14}C-excretion, ^{14}C-tissue residues and identification and quantification of metabolites in rats following a single oral administration of (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin.

MATERIALS AND METHODS

1. Chemicals

Unlabeled (1*R*)-*trans*-imiprothrin (96.9% purity) and (1*R*)-*cis*-imiprothrin (94.9% purity), (1*R*)-*trans*-[imidazolidinyl-5-^{14}C]imiprothrin (1.95 GBq/mmol) and (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin (1.95 GBq/mmol) (Fig. 1) were synthesized in our laboratory. The labeled preparations were purified by TLC developed in benzene/ethyl acetate, 4/1 (v/v) before use, the radiochemical purity being



(1*R*)-*trans*-isomer (1*R*)-*cis*-isomer

*: ^{14}C Labeled position

Fig. 1 Chemical structures of (1*R*)-*trans*- and (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin.

established to be >97%.

2. Chromatographic Procedures

TLC analysis was conducted essentially as described previously by Kaneko *et al.*[5] and Saito *et al.*[6] Pre-coated silica gel 60 F_{254} chromatoplates (Art. 5715, 20×20 cm, 0.25 mm layer thickness, E. Merck, Germany) were used for purification of ^{14}C-labeled compound and determination of its radiochemical purity. For preparative TLC, pre-coated silica gel 60 F_{254} chromatoplates (Art. 5744, 20×20 cm, 0.5 mm layer thickness, E. Merck) were applied. The following solvent systems were used in the present study: (A) *n*-butanol/acetic acid/water (6/1/1, v/v); (B) ethyl acetate/ethanol/water (7/2/1, v/v); and (C) benzene/diethyl ether (4/1, v/v).

Radioactive metabolites on TLC plates were detected by X-ray films (SB-5, Kodak, U.S.A.) exposed for about one week at 4°C and then developed with a Model M6B processor (Kodak).

HPLC was performed with a system consisting of an L6200 pump (Hitachi Ltd., Tokyo, Japan) fitted with a Cosmosil C18 column (ODS, 4.6×250 mm, Nacalai Tesque, Kyoto, Japan), an L4000 UV detector (Hitachi) and an LB 507A radioactivity monitor (Berthold, Germany). Chromatographic data were analysed using Chromatopac CR-7A (Shimazu, Kyoto, Japan).

3. Treatment of Animals and Sample Collection

SD male and female rats were purchased from Charles River Japan Inc. (Kanagawa, Japan) at 6 weeks of age and acclimatized for 1 week before use. Animals were given pelleted diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and water *ad libitum* throughout the study.

For the ^{14}C-excretion study, groups of five male and five female rats were given a single oral dose of (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin at 1 or 200 mg/kg. In the acute oral toxicity study of imiprothrin in rats,[7] LD_{50} values for male and female rats were more than 1800 and 900 mg/kg, respectively. Therefore, 1 mg/kg was selected for low dose as a no effect level, and 200 mg/kg was selected for high dose as the dose level which is supposed to exert some toxic or pharmacologic signs, but not to produce sever effects or high incidence of mortality to rats. Corn oil was used as a vehicle and given at 5 ml/kg. Rats dosed with the ^{14}C-labeled compounds were housed individually in glass metabolism cages (Metabolica CO_2®, Sugiyamagen Iriki Co., Ltd., Tokyo, Japan), and urine and feces were collected 1, 2, 3, 5 and 7 days after administration of the ^{14}C-labeled compounds. Expired air was collected 1, 2 and 3 days after the administration. On the 7th day after dosing, rats were killed by collection of blood from the abdominal aorta under anesthesia with diethyl ether and a total of 23–24 tissues removed.

For isolation of urinary metabolites, a total 2 g of (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin was dissolved in corn oil and dosed orally to 13 female rats for 5 consecutive days at 150 mg/kg/day to allow sufficient amounts of unknown metabolites in urine to be collected for spectroanalytical identification. The specific activity of (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin was adjusted to 3.17 MBq/mmol by isotopic dilution with unlabeled (1*R*)-*cis*-

imiprothrin. Rats dosed with the ^{14}C-labeled compound were housed in glass metabolism cages to achieve the separate collection of urine and feces as described above.

4. Radioanalysis

Radioanalysis was carried out essentially as described previously by Saito *et al.*[6] Radioactivity in urine and fecal extracts was quantified by liquid scintillation counting (LSC) with a Tri-Carb® 2500TR Liquid Scintillation Counter (Packard, U.S.A.) using Emulsifier Scintillator 299™ (Packard). Samples of fecal homogenates and unextractable fecal residues were combusted with a Tri-Carb® 307 Sample Oxidizer (Packard) or a Tri-Carb® 306 Sample Oxidizer (Packard) prior to LSC after being air-dried. Carbosorb 2® and Permafluor® E$^+$ (Packard) were used as a $^{14}CO_2$ absorbent and a scintillator, respectively. ^{14}C-Recovery was 96.0% or more for the combustion.

Radioactivity in 0–1 day, 1–2 day and 2–3 day urine samples was determined by LSC. Following the collection of urine and feces, each metabolism cage was washed with water to recover ^{14}C (cage-wash). The radioactivity in the cage-wash samples was assayed by LSC, being included in the urinary ^{14}C-excretion. The 3–5 day or 5–7 day urine was combined with the respective cage-wash samples and then radioassayed. Feces of individual rats on each collection day were homogenized in a 2-fold weight of water using an Excel Auto Homogenizer (Nihonseiki Co., Tokyo, Japan), the homogenate samples being combusted for radioassay. Expired air was collected 1, 2 and 3 days after administration using 10% NaOH solution for trapping the $^{14}CO_2$ expired. Aliquots of the solution were radioassayed by LSC using Hionic-Fluor™ (Packard) as a scintillator.

For the ^{14}C-tissue residue determinations, blood was divided into blood cell and plasma by centrifugation. Rat carcasses were minced with a meat chopper. Each tissue sample was combusted for radioanalysis.

5. Purification and Isolation of Metabolites

A flow diagram of the purification procedures is given in Fig. 2.

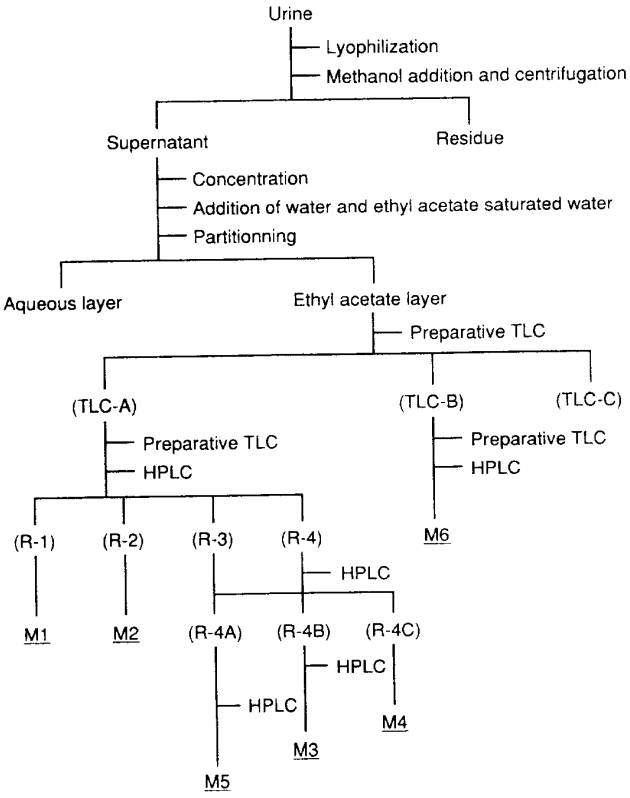


Fig. 2 Flow diagram of the purification procedures for metabolites.

5.1. Pretreatment of urine

The collected urine was lyophilized, and extracted with methanol three times. The extracts were concentrated, and dissolved in water. The solution was then extracted with 2 vols of ethyl acetate saturated with water three times. Approximately 70% of the total ^{14}C was recovered in the ethyl acetate layer. This layer was concentrated and the metabolites in the layer were separated by preparative TLC under solvent system (A) to give three radioactive fractions (TLC-A, *Rf*: 0.84–0.70; TLC-B, *Rf*: 0.61–0.52; TLC-C, *Rf*: 0.39–0.30), and the TLC-A and B fractions were used for purification of metabolites in the present study.

5.2 Metabolites 1 and 2 (M1 and M2)

The TLC-A fraction was re-separated by preparative TLC under solvent system (A). The TLC-A fraction was purified by HPLC. Flow rate and solvent characteristics were 1.0 ml/min for 10 min isocratic with 7% in water followed by a 15-min linear gradient to methanol. Four radioactive peaks (R-1, R-2,

R-3 and R-4; retention times: 6.3, 10.0, 26.5 and 27.4 min, respectively) were separated and their fractions were pooled separately. M1 and M2 were concentrated by lyophilization from the fractions R-1 and R-2, respectively.

5.3 Metabolites 3 and 4 (M3 and M4)

Fraction R-4 was repurified using the same HPLC system. Flow rate was 1.0 ml/min and 50% methanol in water was used as a mobile phase. Three radioactive peaks (R-4A, R-4B and R-4C; retention times: 9.1, 13.3 and 15.6 min, respectively) were separated and pooled. Fraction R-4A was mixed with fraction R-3, and fraction R-4B was purified again using the same HPLC system (flow rate 1.0 ml/min, mobile phase: 45% methanol in water). M3 in the purified R-4B fraction was concentrated by lyophilization. Fraction R-4C was also lyophilized and M4 was isolated.

5.4 Metabolite 5 (M5)

Fraction R-3 was repurified using the same HPLC system (flow rate was 1.0 ml/min, mobile phase: 40% methanol in water). One main peak was pooled. The metabolite in this fraction was lyophilized and named M5.

5.5 Metabolite 6 (M6)

The TLC-B fraction was re-separated by preparative TLC under solvent system (A) and then purified using the HPLC system (flow rate 1.0 ml/min, mobile phase: water). The metabolite in the fraction was lyophilized and named M6.

6. Analysis of Metabolites in Excreta

To analyze the metabolites in urine, mixed samples of one-twentieth of the 0–1 and 1–2 day urine from the five male or five female rats in the same dose groups were prepared. Similary, to separately quantify the fecal metabolites of male and female rats, 0–1 day fecal homogenates (about 1/20 of the total) were combined with 1–2 day homogenates (about 1/20 of the total) for each sex and dose group. The metabolites in the fecal homogenate mixtures were extracted with methanol (>5 vols) three times, the extracts being concentrated *in vacuo*. The urine and feces extracts were analyzed with or without mixing the isolated metabolite standards by HPLC to identify and quantify the contained metabolites. The gradient conditions used in the present study are shown in Table 1.

7. Spectrometry

The purified metabolites were subjected to spectroanalyses to determine their chemical structures. NMR spectra were obtained on a JEOL GSX-270 spectrometer (JEOL Ltd., Tokyo, Japan) operating at 270 MHz for ^{1}H and 67.5 MHz for ^{13}C. Chemical shifts were expressed in ppm units relative to deuterium solvents. Electron impact mass spectrometry (EI-MS) and secondary ion mass spectrometry (SI-MS) were performed with a Hitachi M-80B mass spectrometer (Hitachi Ltd.). In the EI-MS analysis on a positive ion mode, the ionization voltage was 70 eV and the accelerating voltage was 3.0 kV. For the SI-MS analysis, samples were introduced in a glycerol matrix and the spectra were recorded in a negative ion mode.

Table 1 Gradient conditions for HPLC analysis of metabolites in urine and feces.

Step No.	Time (min)	Concentration (%)		Event
		A[a]	B[b]	
1	0	100	0	Equilibration
2	15	100	0	Hold
3	10	80	20	Linear gradient
4	10	0	100	Linear gradient
5	10	0	100	Hold
6	5	100	0	Linear gradient
7	10	100	0	Hold
8	—	100	0	Column equilibration

[a] 0.1% trifluoroacetic acid (TFA) in water.

[b] Methanol.

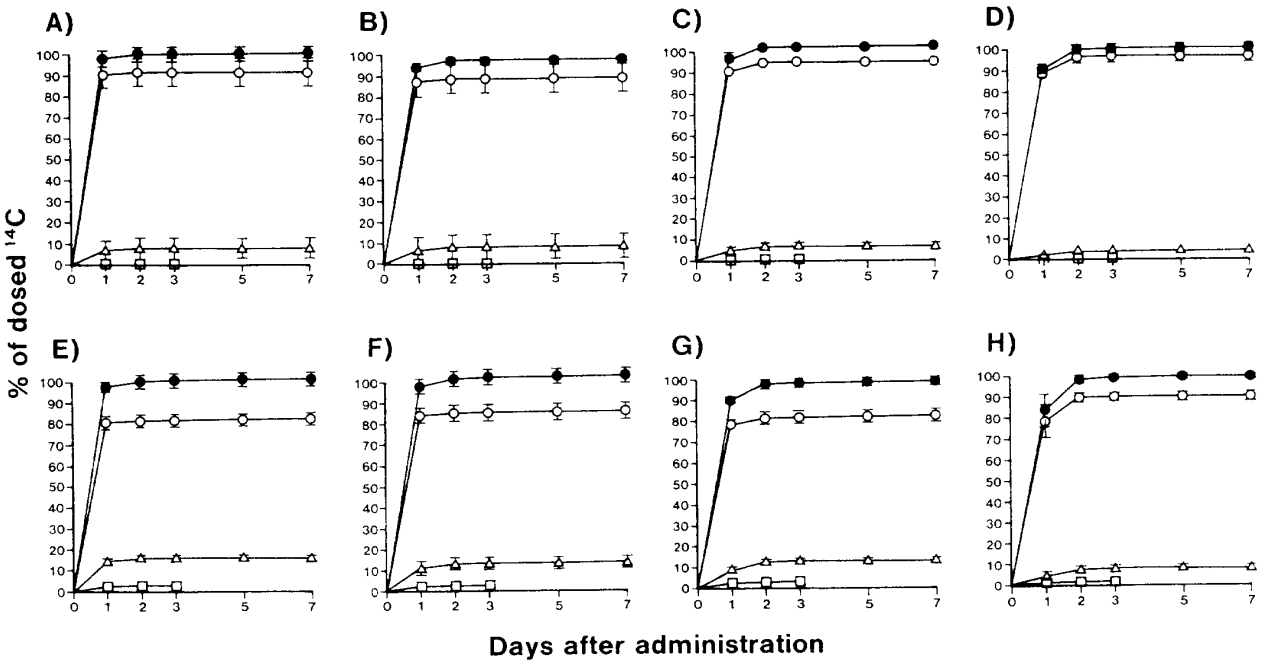


Fig. 3 Cumulative ^{14}C-excretion after a single oral administration of ^{14}C labeled (1*R*)-*trans*- (A–D) or (1*R*)-*cis*- (E–H) imiprothrin to male or female rats at 1 or 200 mg/kg.

A and E: males at 1 mg/kg, B and F: females at 1 mg/kg, C and G: males at 200 mg/kg, D and H: females at 200 mg/kg. ●: total, ○: urine, △: feces, □: expired air.

RESULTS

1. ^{14}C-Excretion

Data for cumulative ^{14}C-excretion into urine, feces and expired air during the 7 days after a single oral administration of (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin are shown in Fig. 3. Radiocarbon was almost completely excreted into urine, feces and expired air within 7 days in all dosed groups (98–103%). The urinary ^{14}C-excretion was 83–97%, while the fecal excretion was 16% or less. However, the urinary excretion of ^{14}C for the *trans*-isomer was slightly larger (89–97%) than that for the *cis*-isomer (83–91%). ^{14}C-Excretion into the expired air was less than 3% of the dosed ^{14}C. Most of the dosed ^{14}C was rapidly eliminated within 2 days (98–102%) in all treatment groups. No marked sex differences in the ^{14}C-elimination were observed within groups.

2. ^{14}C-Tissue Residues

Data for ^{14}C-tissue residues on the 7th day after a single oral dose of *trans*- and *cis*-isomers are shown in Table 2. For the low dose groups, the level was the highest in liver, being 8.1–8.4 and 20.9–27.2 ppb in rats treated with *trans*- and *cis*-isomers, respectively. For the high dose groups, the levels were relatively high in liver, blood cell and skin and hair, being 1.2–2.2 and 2.4–5.9 ppm in rats treated with *trans*- and *cis*-isomers, respectively. There were no marked differences in the ^{14}C-tissue residues between males and females within either treatment group. The levels were lower in rats treated with the *trans*-isomer than in those treated with the *cis*-isomer within dose groups.

3. Identification of Isolated Metabolites

The chemical structures of (1*R*)-*cis*-imiprothrin and isolated metabolites were confirmed by NMR and MS (Table 3).

In the ^{1}H NMR spectrum of M2, there were no proton signals of the chrysanthemic acid moiety, indicating that the ester linkage was cleaved. Moreover, the signals corresponding to the imido-methylene group of (1*R*)-*cis*-imiprothrin disappeared. The other proton signals were very similar to those of (1*R*)-*cis*-imiprothrin. The ^{13}C NMR spectrum of M2

Table 2 ^{14}C-Tissue residues in male and female rats on the 7th day after single oral administration of (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin at 1 (low dose) and 200 mg/kg (high dose).[a)]

Tissue	Low dose (ng imiprothrin equivalents/g tissue)				High dose (μg imiprothrin equivalents/g tissue)			
	Trans		*Cis*		*Trans*		*Cis*	
	Male	Female	Male	Female	Male	Female	Male	Female
Adrenal	<3.0	<2.4	13.4±2.63	9.0±1.06	1.0±0.07	<0.5	2.8±0.74	1.1±0.60
Blood	3.2±0.96	2.5±0.20	17.0±2.05	10.7±2.34	1.0±0.07	1.0±0.14	3.2±0.44	1.5±0.23
Blood cell	5.4±0.54	4.6±0.39	21.3±3.28	13.5±0.99	1.9±0.12	1.8±0.24	4.9±0.55	2.6±0.17
Plasma	1.2±0.20	<0.6	11.6±2.65	6.6±1.05	0.2±0.03	<0.1	1.8±0.21	0.8±0.21
Bone	4.4±1.40	3.3±0.96	19.0±2.40	13.9±1.70	0.8±0.06	0.4±0.07	3.9±0.68	1.5±0.33
Bone marrow	1.6±0.74	<1.2	7.9±1.09	5.6±1.62	0.3±0.02	0.3±0.15	1.6±0.42	0.8±0.20
Brain	<0.6	<0.6	1.9±0.24	1.5±0.24	<0.1	<0.1	0.4±0.06	0.2±0.03
Caecum	1.9±0.44	1.6±0.17	10.1±1.42	8.0±2.34	0.3±0.03	0.3±0.06	2.0±0.51	0.8±0.16
Carcass	3.5±0.56	3.0±0.84	12.4±2.12	8.8±1.97	0.7±0.10	0.5±0.10	2.6±0.33	1.4±0.39
Fat	<1.8	<1.6	5.1±0.84	3.4±1.22	<0.2	<0.5	1.3±0.22	0.6±0.28
Heart	2.1±0.32	1.9±0.33	9.4±1.47	6.7±0.85	0.4±0.02	0.3±0.03	1.8±0.31	0.8±0.11
Kidney	4.8±0.79	4.5±0.52	15.9±2.39	12.7±1.19	0.7±0.03	0.6±0.08	3.1±0.44	1.5±0.21
Large intestine	1.9±0.26	1.6±0.39	8.9±1.14	8.6±3.79	0.3±0.02	0.3±0.07	1.6±0.39	0.8±0.16
Liver	8.4±2.48	8.1±2.28	27.2±4.25	20.9±0.31	1.7±0.18	1.2±0.34	5.9±1.11	2.4±0.23
Lung	2.8±0.28	1.9±0.20	12.3±1.73	8.5±1.15	0.5±0.05	0.4±0.03	2.3±0.38	1.0±0.15
Mandibular gland	2.0±0.23	1.7±0.32	8.6±1.47	6.1±1.59	0.4±0.03	0.2±0.01	1.7±0.34	0.7±0.11
Muscle	1.9±0.35	1.7±0.30	9.2±1.38	6.5±2.60	0.4±0.04	0.2±0.05	1.8±0.41	0.6±0.10
Pancreas	1.4±0.24	0.9±0.38	6.9±1.16	5.1±0.89	0.3±0.03	0.2±0.03	1.6±0.35	0.6±0.13
Skin and hair	7.0±2.21	5.2±2.43	24.9±5.45	11.1±2.35	2.1±0.77	2.2±1.53	4.6±0.95	2.5±0.82
Small intestine	1.4±0.39	1.3±0.10	7.3±0.94	6.4±1.77	0.3±0.03	0.2±0.07	1.4±0.23	0.7±0.16
Spinal cord	1.7±3.27	<1.0	2.6±0.33	1.9±0.53	<0.2	<0.3	0.6±0.18	0.3±0.04
Spleen	2.4±0.20	1.7±0.37	9.8±1.58	8.4±1.93	0.5±0.05	0.3±0.03	1.9±0.33	0.9±0.16
Stomach	2.0±0.39	1.7±0.17	10.4±1.39	8.2±1.90	0.4±0.03	0.2±0.05	2.1±0.26	0.9±0.17
Thymus	1.7±0.35	1.4±0.21	7.4±1.88	6.2±0.88	0.3±0.04	0.2±0.04	1.6±0.36	0.8±0.27
Thyroid	<7.0	<8.9	12.1±2.97	9.5±0.93	<1.2	<1.8	2.5±0.39	1.1±0.24
Testis	1.2±0.21	N.A.[b)]	5.6±0.73	N.A.	0.2±0.03	N.A.[b)]	1.1±0.18	N.A.
Ovary	N.A.	<1.6	N.A.	8.0±1.72[c)]	N.A.	<0.3	N.A.	0.9±0.15[c)]
Uterus	N.A.	2.8±1.14	N.A.	12.8±3.36	N.A.	0.3±0.14	N.A.	1.1±0.20

[a)] Data represent mean values±S.D. of five rats.
[b)] Not analyzed.
[c)] Data represent mean value of four rats.

exhibited the presence of six carbons and EI-MS showed a molecular ion peak at *m/z*: 138. Consequently, M2 was identified as 2,4-dioxo-1-(2-propynyl)-imidazolidine (PGH).

Similarly, the proton signals of the chrysanthemic acid moiety and the imido-methylene group were not detected in the ^{1}H NMR spectrum of M1. The signals of the methylene protons in 2-propynyl group were clearly separated [4.40 ppm (dd) and 3.94 ppm (dd)] implying the effects of a nearby proton with a chiral carbon. Furthermore, the signal corresponding to the two methylene protons in the imidazolidine ring changed into one for a proton, and the proton was shifted to the lower field (5.31 ppm), compared with the signal of

Table 3 Spectrometric data for isolated metabolites and (1*R*)-*cis*-imiprothrin.

Compounds	NMR (ppm)		MS (*m/z*)
M2	^{1}H NMR (CD_3OD)	2.82 (1H, t, J=2.6 Hz),	EI-MS (positive)
		4.07 (2H, s), 4.22 (2H, d, J=2.6 Hz)	138 (M^+)
	^{13}C NMR (CD_3OD)	32.5, 51.2, 74.5, 78.0, 158.0, 173.3	
M1	^{1}H NMR ($(CD_3)_2CO$)	2.78 (1H, t, J=2.6 Hz, 2.0 Hz)	EI-MS (positive)
		3.94 (1H, dd, J=17.8 Hz, 2.6 Hz)	154 (M^+)
		4.40 (1H, dd, J=17.8 Hz, 2.6 Hz)	
		5.31 (1H, s)	
	^{13}C NMR ($(CD_3)_2CO$)	29.4, 73.6, 78.6, 79.4, 155.3, 172.2	
M6	^{1}H NMR (CD_3OD)	4.0 (2H, s)	EI-MS (positive)
	^{13}C NMR (CD_3OD)	48.8, 161.0, 176.0	100 (M^+)
M3	^{1}H NMR (CD_3OD)	1.31 (3H, s), 1.34 (3H, s)	SI-MS (negative)
		1.93 (3H, s),	363 ($M-H)^-$
		1.99 (1H, d, J=8.6 Hz)	
		2.17 (1H, brt, J=6.6 Hz, 9.2 Hz)	
		2.79 (1H, t, J=2.6 Hz, 2.0 Hz)	
		4.04 (1H, dd, J=17.8 Hz, 2.6 Hz)	
		4.49 (1H, dd, J=17.8 Hz, 2.6 Hz)	
		5.36 (1H, s)	
		5.52 (2H, m),	
		7.08 (1H, brd, J=9.2 Hz)	
M4	^{1}H NMR (CD_3OD)	1.31 (3H, s), 1.34 (3H, s)	SI-MS (negative)
		1.92 (3H, s),	347 ($M-H)^-$
		1.99 (1H, d, J=8.6 Hz)	
		2.17 (1H, brt, J=7.9 Hz, 8.6 Hz)	
		2.85 (1H, t, J=2.6 Hz)	
		4.13 (2H, s), 4.29 (2H, d, J=2.6 Hz)	
		5.51 (2H, m),	
		7.08 (1H, brd)	
M5	^{1}H NMR (CD_3OD)	1.31 (3H, s), 1.34 (3H, s)	SI-MS (negative)
		1.93 (3H, s),	309 ($M-H)^-$
		1.99 (1H, d, J=8.6 Hz)	
		2.16 (1H, brt, J=8.6 Hz, 9.2 Hz)	
		4.05 (2H, s),	
		5.50 (2H, m),	
		7.08 (1H, brd, J=9.2 Hz)	
(1*R*)-*cis*-imiprothrin	^{1}H NMR ($CDCl_3$)	1.18 (3H, s), 1.23 (3H, s),	EI-MS (positive)
		1.63 (1H, d, J=8.6 Hz)	318 (M^+)
		1.68 (3H, s), 1.75 (3H, s)	
		1.92 (1H, t, J=8.6 Hz, 8.6 Hz)	
		2.37 (1H, t, J=2.6 Hz, 2.0 Hz)	
		4.04 (2H, s), 4.27 (2H, d, J=2.6 Hz)	
		5.34 (1H, d, J=8.6 Hz), 5.49 (2H, m)	

the two protons of M2 (PGH) at 4.07 ppm. The ^{13}C NMR spectrum of M1 exhibited the presence of six carbons, similar to those of PGH except that the imidazolidine ring signal was shifted to the lower field at 79.4 ppm as compared with 51.2 ppm. These observations suggested that one methylene proton in the imidazolidine ring was replaced with a functional group. The EI-MS showed a molecular ion peak at *m/z*: 154. Therefore, the replacement group was considered to be a hydroxy group. Consequently, M1 was identified as 5-hydroxy-2,4-dioxo-1-(2-propynyl)-imidazolidine (PGH-OH)

In the ^1H NMR spectrum of M6, proton signals of the chrysanthemic acid moiety were not detected, indicating that the ester linkage was cleaved. The signals corresponding to the imido-methylene and 2-propynyl groups were also not detected. The ^{13}C NMR spectrum of M6 exhibited the presence of three carbons and EI-MS showed a molecular ion peak at *m/z*: 100. Therefore, M6 was identified as 2,4-dioxo-imidazolidine (hydantoin, HYD).

In the ^1H NMR spectra of M3, M4 and M5, the signals corresponding to three protons of the methyl group in the isobutenyl side chain disappeared. However, in the M3 spectrum, other signals were very close to those of PGH-OH. SI-MS showed a quasi-molecular ion peak at *m/z*: 363 $(M-H)^-$. Therefore, M3 was concluded to be [4-hydroxy-2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl]methyl (1*R*)-*cis*-2,2-dimethyl-3-((*E*)-2-carboxyl-1-propenyl) cyclopropanecarboxylate (*ωt*-acid-*cis*-PGH-OH). In the case of the ^1H NMR spectrum of M4, other signals were very similar to those of PGH. SI-MS showed a quasi-molecular ion peak at *m/z*: 347 $(M-H)^-$, and therefore, M4 was identified as [2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl]-methyl (1*R*)-*cis*-2,2-dimethyl-3-((*E*)-2-carboxyl-1-propenyl)cyclopropanecarboxylate (*ωt*-acid-*cis*-PGH). In the ^1H NMR spectrum of M5, the signals corresponding to the 2-propynyl group of imiprothrin were not detected. SI-MS showed a quasi-molecular ion peak at *m/z*: 309 $(M-H)^-$. Therefore, M5 was concluded to be [2,5-dioxo-1-imidazolidinyl]methyl (1*R*)-*cis*-2,2-dimethyl-3-((*E*)-2-carboxyl-1-propenyl)cyclopropanecarboxylate (*ωt*-acid-*cis*-HYD).

TLC *Rf* values and HPLC retention times for the isolated metabolites and parent compounds are shown in Table 4.

4. *Metabolites in Urine and Feces*

The metabolites in pooled (0–2 day) urine and fecal extracts for males or females in all dosed groups were analyzed by HPLC using the isolated metabolites as standards (Table 5).

For the *trans*-isomer, the amounts of PGH-OH were the greatest among the urinary metabolites (32.9–51.6% of the dosed ^{14}C),

Table 4 Summary of TLC *Rf* values and retention times for metabolite standards and the parent compounds.

Compounds	*Rf*			Retention time[d] (min)
	Solvent system			
	A[a]	B[b]	C[c]	
HYD	0.46	0.61	0.00	4.7
PGH-OH	0.64	0.85	0.03	12.9
PGH	0.58	0.83	0.05	21.4
(1*R*)-*trans*-imiprothrin	0.67	0.98	0.32	40.1
(1*R*)-*cis*-imiprothrin	0.68	0.96	0.34	40.0
ωt-acid-*cis*-HYD	0.57	0.78	0.00	35.4
ωt-acid-*cis*-PGH-OH	0.70	0.94	0.00	37.6
ωt-acid-*cis*-PGH	0.65	0.90	0.01	38.0

[a] A: *n*-butanol/acetic acid/water=6/1/1.
[b] B: ethyl acetate/ethanol/water=7/2/1.
[c] C: benzene/diethyl ether=4/1.
[d] Operating conditions are shown in Table 1.

Table 5 Amounts of metabolites in urine and feces after a single oral administration of ^{14}C-labeled (1*R*)-*trans*- or (1*R*)-*cis*-imiprothrin to male or female rats at 1 (low dose) or 200 mg/kg (high dose).

Metabolite	% of dosed ^{14}C							
	Trans				*Cis*			
	Low dose		High dose		Low dose		High dose	
	Male	Female	Male	Female	Male	Female	Male	Female
Urine								
PGH-OH	51.6	50.0	35.5	32.9	28.3	27.4	32.4	28.3
HYD (Hydantoin)	9.7	12.6	13.2	14.1	12.8	15.5	9.3	13.0
PGH	5.8	3.1	27.4	29.9	5.9	5.6	18.3	27.4
UK-1	9.1	5.9	5.7	3.6	5.3	3.5	5.9	2.6
PMG	11.8	9.7	10.8	10.7	18.2	17.2	11.2	10.9
ωt-acid-*cis*-PGH-OH +*ωt*-acid-*cis*-PGH	—	—	—	—	N.D.	3.8	1.2	2.9
ωt-acid-*cis*-HYD	—	—	—	—	1.9	8.0	1.9	3.2
Others	3.3	7.4	2.3	5.1	7.7	4.7	1.5	1.1
Not recovered	0.4	0.0	0.0	0.0	1.7	0.0	0.0	0.3
Total	91.8	88.8	95.0	96.3	81.7	85.6	81.7	89.5
Feces								
Parent compound	1.4	0.8	2.3	N.D.	1.9	0.8	0.9	0.4
PGH-OH	2.7	2.6	0.3	0.2	N.A.	N.A.	N.A.	N.A.
ωt-acid-*cis*-PGH-OH +*ωt*-acid-*cis*-PGH	—	—	—	—	1.1	1.3	1.8	1.4
ωt-acid-*cis*-HYD	—	—	—	—	2.3	4.8	2.2	2.2
PMG	0.6	0.4	N.D.	N.D.	0.7	0.5	0.5	0.2
Others	2.6	3.4	3.4	3.0	6.3	3.0	3.9	1.6
Not recovered	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Unextracted	0.7	1.3	0.7	0.5	3.7	3.1	3.6	1.5
Total	8.0	8.5	6.7	3.7	16.0	13.5	12.9	7.4

N.A.: not analyzed, N.D.: not detected, PMG: HPLC void peak containing polar metabolites (polar metabolite group).

followed by hydantoin (HYD) (9.7–14.1%). Another identified metabolite was PGH. No parent compound was observed. Although the parent compound was observed in the fecal extracts, the amounts were 2.3% or less. In the high dose group, marked increases in the proportions of PGH were observed in the urinary metabolites (male 27.4% and female 29.9%) in comparison with the low dose group (male 5.8% and female 3.1%). However, there were no sex differences in the metabolite profiles of (1*R*)-*trans*-imiprothrin within either dose group.

Similar metabolite profiles were observed for the urine and feces from rats treated with the *cis*-isomer. The amounts of PGH-OH were also the greatest among the urinary metabolites (27.4–32.4% of the dosed ^{14}C), followed by hydantoin (HYD) (9.3–15.5%). Other identified metabolites were PGH, *ωt*-acid-*cis*-HYD, *ωt*-acid-*cis*-PGH-OH and *ωt*-acid-*cis*-PGH. No parent compound was observed. As fecal metabolites, *ωt*-acid-*cis*-HYD, *ωt*-acid-*cis*-PGH-OH and *ωt*-acid-*cis*-PGH were observed along with the parent compound. However, the proportion of each metabolite was less than 4.8%. Although marked increases in the amounts of PGH were also observed in the urinary metabolites of the high dose group (male 18.3% and female 27.4%) in comparison with the low dose (male 5.9% and female 5.6%), no sex differences in the metabolite profiles appeared within either dose group.

DISCUSSION

In a previous preliminary metabolism study of rats treated orally with (1*R*)-*trans*-[imidazolidinyl-5-^{14}C]imiprothrin or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin, urinary excretion of both compounds was more than 75% of the dosed ^{14}C within 7 days, a greater range of metabolites being observed in the urine of rats given the *cis*-isomer as compared with the *trans*-isomer (data not shown). Since metabolite profiles for males and females were very similar, those isolated from female urine using (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin were investigated in the present study.

In the ^{1}H NMR spectra of M1, M2 and M6 (PGH-OH, PGH and HYD, respectively) (Table 3), the signals corresponding to the imido-methylene group of imiprothrin disappeared, which has been reported in metabolism studies of tetramethrin in rats by Kaneko *et al.*[4] and Tomigahara *et al.*[8] In addition, the signals corresponding to the 2-propynyl group was not detected in the ^{1}H NMR spectrum of M6 (HYD). In the metabolism of a pyrethroid insecticide, Etoc® (S-4068SF),[9] having a C-linked 2-propynyl group in an alcohol moiety, the major metabolic reaction concerning the 2-propynyl group was found to be oxidation of the C-1 or C-2 positions. However, no metabolites oxidized at the 2-propynyl group were detected in the present case, and the major metabolic reaction was dealkylation of the N-linked 2-propynyl group of imiprothrin, suggesting a difference in stability between C–C and N–C bonds of the 2-propynyl group. In the ^{1}H NMR spectra of M3, M4 and M5 (*ωt*-acid-*cis*-PGH-OH, *ωt*-acid-*cis*-PGH and *ωt*-acid-*cis*-HYD, respectively), the signals corresponding to three protons of the methyl group in the isobutenyl side chain were not observed, and the common spectrum was a typical oxidation at the *ω-trans*-methyl group in the isobutenyl side chain (*ωt-trans*-carboxylic acid), which is well-known from metabolism studies of many pyrethroid insecticides.[2-4,8-10] Detailed analysis of other unidentified radioactive peaks by HPLC showed PMG to be a void peak containing polar metabolites while UK-1 was considered to be an oxidized metabolite of PGH-OH (unpublished data). Therefore, almost all the urinary metabolites were identified in the present study.

Radiocarbon from (1*R*)-*trans*- or (1*R*)-*cis*-[imidazolidinyl-5-^{14}C]imiprothrin was almost completely eliminated from the treated rats within 7 days after administration and ^{14}C-tissue residues on the 7th day after administration were generally low in all dosed groups. Radiocarbon excreted into urine from the *trans*-isomer amounted to 89–97% of the dose, while that from the *cis*-isomer was 83–91%. The slight changes in the excretion profiles between *trans*- and *cis*-isomers are considered to be mainly due to the difference in hydrolysis by esterase(s) between *trans*- and *cis*-isomers, which is well-known to occur with many pyrethroids.[2-4,9] Therefore, the *trans*-isomer is cleaved more easily and excreted into urine to a larger extent than the *cis*-isomer, which is consistent with the larger amounts of urinary ester cleaved metabolites (PGH-OH, HYD and PGH) for the *trans*-isomer (66–77%) as compared with those for the *cis*-isomer (47–69%) in the present study. However, urinary ^{14}C-excretion of the both imiprothrin isomers was high (more than 83% of the applied dose) and the differences in the ^{14}C-excretion profiles between *trans*- and *cis*-isomers were slight in comparison with the phenothrin,[2] tetramethrin,[3] cyphenothrin[4] and Etoc®[9] cases. These results imply that both imiprothrin isomers are better substrates for the esterase(s) of rats *in vivo* as compared with other pyrethroid insecticides. In addition, the noted excretion of *ω-trans*-carboxylic acid-type metabolites (*ωt*-acid-*cis*-PGH-OH, *ωt*-acid-*cis*-PGH and *ωt*-acid-*cis*-HYD) into urine clearly contributed to the increase in urinary ^{14}C-excretion from the *cis*-isomer.

Remarkable increases in the proportions of PGH were detected in the urine of animals treated with the high dose of both isomers. In contrast, the proportions of the oxidized form, PGH-OH, which is a main metabolite of imiprothrin, was decreased. From the metabolite profiles, the major metabolic reactions of imiprothrin were concluded to be the oxidation by mixed function oxygenase or ester hydrolysis by the esterase(s). Therefore, the metabolite profile of imiprothrin depends on these

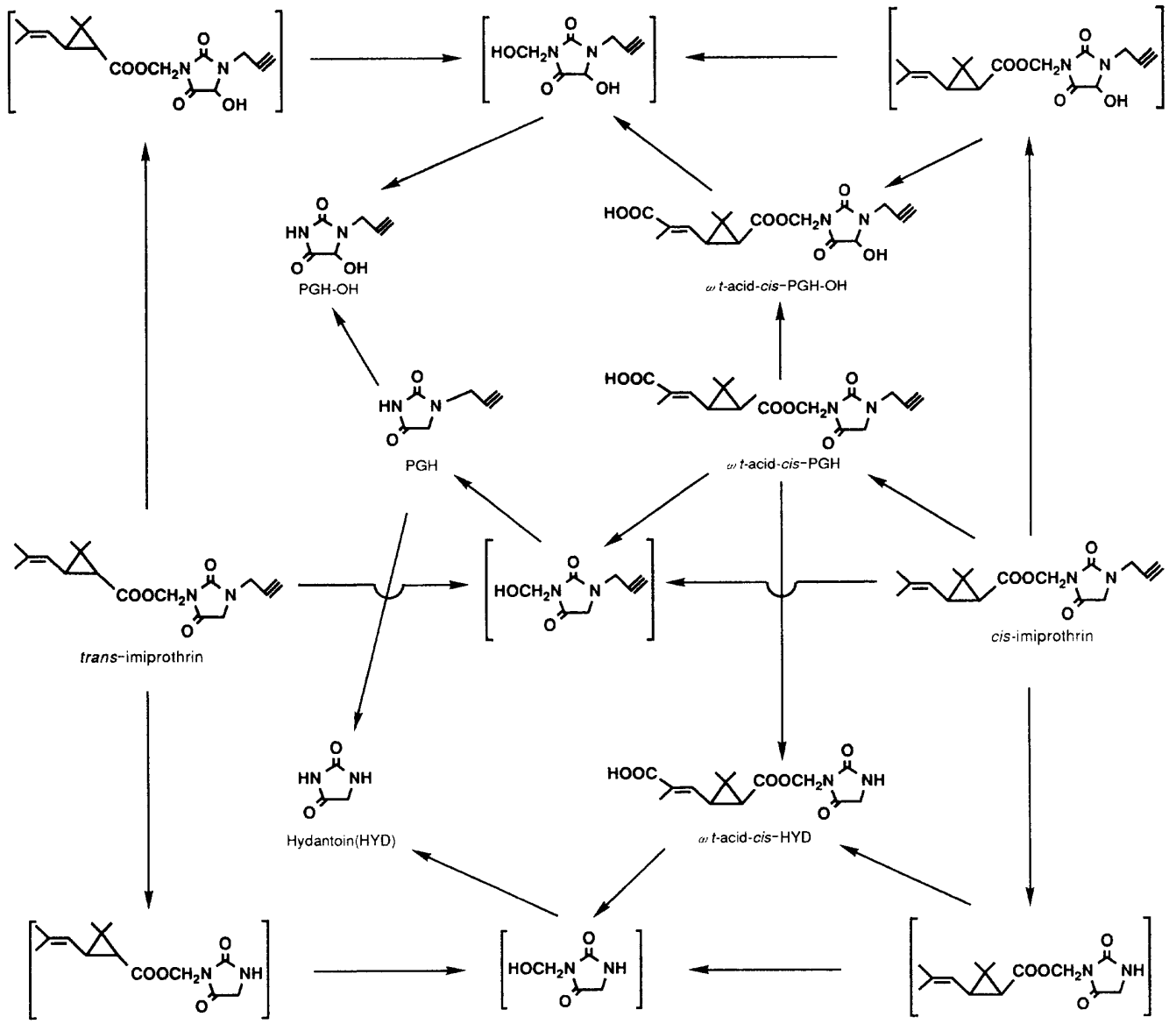


Fig. 4 Proposed metabolic pathways of imiprothrin in rats.

enzyme activities and the dose of imiprothrin applied. These results imply that saturation of the oxidation activity toward imiprothrin occurs in the high dose group rats while this is not the case for ester hydrolysis.

The findings in the present study suggest that both imiprothrin isomers are readily absorbed, metabolized and rapidly eliminated into urine and feces. On the basis of the results, major biotransformation reactions of imiprothrin in rats are 1) cleavage of the ester linkage, 2) cleavage of the imido-methylene linkage, 3) hydroxylation of the imidazolidine ring, 4) dealkylation of the 2-propynyl group and 5) oxidation at the *ω-trans*-methyl group in the isobutenyl side chain. The proposed metabolic pathways of imiprothrin are shown in Fig. 4.

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要　　約

イミプロスリン異性体のラットにおける代謝：生体内変換

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新規ピレスロイド系殺虫剤イミプロスリン[2,5-dioxo-3-(2-propynyl)-1-imidazolidinyl]methyl (1*R*)-*cis*, *trans*-chrysanthemate の *trans* および *cis* 体のアルコール側 ^{14}C 標識体を雌雄ラットに1および200 mg/kg の割合で一回経口投与した．その結果，すべての投与群で ^{14}C は，速やかに，投与後7日目までにほぼ完全に尿，糞および呼気中に排泄され，組織残留量も全般的に低値を示した．^{14}C 排泄率および ^{14}C 組織残留に顕著な性差は認められなかった．*cis* 体のアルコール側 ^{14}C 標識体を用いて尿中から単離，構造決定した代謝物を用いて同定された排泄物中の代謝物より，代謝反応として 1) エステル結合の開裂，2) イミドメチレン結合の開裂，3) イミダゾリジン環の水酸化，4) 2-プロピニル基の脱離，5) 酸側イソブテニルのメチル基の酸化を認めた．イミプロスリンの両異性体の代謝物に顕著な性差は認められなかった．