Original Article

Hydrolysis of *trans*-Tetramethrin in Buffered Aqueous Solutions

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Hydrolysis of a pyrethroid insecticide *trans*-tetramethrin (**I**) [3,4,5,6-tetrahydrophthalimidomethyl (1*RS*)-*trans*-chrysanthemate] was examined at $25 \pm 1^\circ\text{C}$, using the ^{14}C preparations labeled at 1-position of the cyclopropyl ring or at 1- and 2-positions of the 3,4,5,6-tetrahydrophthalimido moiety. HPLC analysis of the buffered aqueous solutions showed that **I** was degraded following pseudo-first-order kinetics at pH 5.0 with a rate of $4.08\text{--}4.99 \times 10^{-7} \text{ sec}^{-1}$, while it was rapidly hydrolyzed with initial rates of $7.82\text{--}8.75 \times 10^{-6} \text{ sec}^{-1}$ at pH 7 and $6.02\text{--}8.62 \times 10^{-4} \text{ sec}^{-1}$ at pH 9, followed by slower hydrolysis. HPLC and TLC cochromatographies with synthetic standards and LC-MS analysis showed that **I** was primarily hydrolyzed to the unstable tetrahydrophthalamic acid derivative of **I** *via* opening of the cyclic imido moiety. The ester linkage was subsequently cleaved to form *trans*-chrysanthemic acid and the unstable *N*-hydroxymethyl 3,4,5,6-tetrahydrophthalamic acid. The latter amic acid was stepwisely hydrolyzed by liberating formaldehyde to 3,4,5,6-tetrahydrophthalic acid *via* its amide derivative.

INTRODUCTION

Tetramethrin (Neo-Pyamin) is a synthetic pyrethroid characterized by its high knock-down and flushing-out activities¹⁾ and the *d-trans*-isomer is known to be most potent.²⁾ The metabolic studies of tetramethrin have revealed that ester cleavage and/or oxidation are the main degradation pathways in mammals^{3,4)} and a role of glutathione conjugation has been discussed in relation to its metabolism.⁵⁾ Since tetramethrin has a photo-labile chrysanthemic acid moiety, a variety of photo-reactions are considered to proceed similarly as in other synthetic pyrethroids.⁶⁾ The rapid photodegradation *via* isomerization, epoxidation and/or hydroxylation as well as ester cleavage has been previously reported in organic solvents.^{7,8)} For an environmental safety, hydrolysis of tetramethrin in aqueous phase should be first investigated but such information has not been available. Although

the gas chromatographic analysis after the alkaline hydrolysis has been reported for tetramethrin,⁹⁾ only chrysanthemic acid has been detected as a hydrolysis product.

This paper deals with hydrolysis of **I**, a major component of tetramethrin, in buffered aqueous solutions to determine its rates and routes in the aquatic environments. HPLC analysis of buffered aqueous solution was conducted instead of extraction to avoid an artificial degradation of hydrolysates. In order to confirm the chemical structures of **I** and its degradates, LC-MS spectra were measured by using the frit-FAB¹⁰⁾ and atmospheric pressure ionization (APCI)¹¹⁾ techniques.

MATERIALS AND METHODS

1. Chemicals

I, *trans*-chrysanthemic acid (**II**) and *N*-hydroxymethyl 3,4,5,6-tetrahydrophthalimide (**III**) were prepared in our laboratory. 3,4,5,6-Tetrahydrophthalic anhydride was purchased

from Aldrich Chemical Co., Ltd. (Milwaukee) and treated with a 1.05 equivalent of sodium hydroxide in acetonitrile-distilled water (1/1, v/v) to prepare the corresponding acid (**IV**). 3,4,5,6-Tetrahydrophthalamic acid (**V**) was prepared by treating the anhydride with ammonium hydroxide.¹²⁾ The chemical structure of each compound was confirmed by ¹H NMR, IR and MS spectroscopies. The chemical purity of each compound was >99% as determined by HPLC analysis. [Acid-¹⁴C]-**I** labeled at 1-position of the cyclopropyl moiety (132 mCi/g) and [Alc-¹⁴C]-**I** labeled at 1- and 2-positions of the 3,4,5,6-tetrahydrophthalimide moiety (149 mCi/g) were prepared in our laboratory. The radiochemical purity of each preparation was >99% on the basis of the HPLC analysis. Chromotropic acid disodium salt from Tokyo Kasei (Tokyo) for quantification of formaldehyde, organic solvents and inorganic reagents in a special grade from Wako Pure Chemical Industries, Ltd. (Osaka) were used without further purification. Pure water with an electrical conductivity of $5.5 \times 10^{-8} \Omega^{-1} \text{cm}^{-1}$ was used for the preparation of buffer solutions. PIC-A (tetrabutylammonium phosphate) used in a mobile phase was purchased from Waters Co.,

Ltd. (Milford). The nutrient agar (mixtures of bacto-beef extract, bacto-peptone, and bacto-agar) used for examining sterility of buffered aqueous solutions was obtained from DIFCO Laboratories.

2. TLC

Precoated silica gel 60F₂₅₄ chromatoplates (20×20 cm, 0.25 mm layer thickness, E. Merck) were used for an analytical purpose. The TLC *R_f* values of **I–V** with the two solvent systems used in two-dimensional development are listed in Table 1. The resolved radioactive spots were detected by autoradiography with the developed TLC plates held under X-ray SB film (Eastman Kodak Company) at 4°C for a week. The unlabeled standard chemicals were detected by ultra-violet light fluorescence quenching.

3. HPLC

A Hitachi L-6200 liquid chromatograph was operated at a flow rate of 1.0 ml/min to flush the contents of the 2 ml loop onto a Sumipax ODS A-112 (5 μm, 6 mm i.d.×15 cm; Sumika Analytical Service Ltd., Osaka) column. The composition of mobile phase was changed stepwise as described in Table 2. The amount of **I** and its hydrolysis products were quantified, using a Radiomatic Flo-one/beta A-120 radiodetector (time constant, 6.0 sec) were a quantitation limit was about 500 cpm. At the same time, the UV absorbance at 240 nm was monitored by using a Hitachi L-4000 Variable Wavelength UV Monitor. The retention times of **I–V** are listed in Table 1.

Table 1 TLC *R_f* values and HPLC retention times of **I** and its degradates.

Compound	TLC <i>R_f</i> value ^{a)}		<i>R_t</i> (min) ^{b)}
	A	B	
I	0.40	0.67	32.0 ^{c)}
II	0.21	0.58	21.5 ^{c)}
III	0.12	0.47	16.6
IV	0.00	0.17	8.5
V	0.00	0.54, 0.17 ^{d)}	6.5
VI	— ^{e)}	—	24.0
VII	—	—	10.0

^{a)} A, hexane/ethyl acetate (1/1, v/v); B, toluene/ethyl acetate/acetic acid (5/7/1, v/v/v). ^{b)} Typical retention times in HPLC analysis (Table 2). ^{c)} Retention times of the *cis*-isomers of **I** and **II** were 31.5 and 21.8 min, respectively. ^{d)} **V** was unstable and partly degraded to **IV** on the TLC plate during development with the solvent system B. ^{e)} Not determined due to a rapid degradation during development.

Table 2 HPLC mobile phase conditions.

Time (min)	A ^{a)} (%)	B ^{a)} (%)	C ^{a)} (%)	Curve
0	20	0	80	
5	20	0	80	Isocratic
15	70	20	10	Linear
25	70	20	10	Isocratic
30	20	0	80	Linear
40	20	0	80	Linear

^{a)} Component of a mobile phase.

A, acetonitrile; B, methanol; C, distilled water containing $5 \times 10^{-3} \text{M}$ PIC-A. Flow rate was 1.0 ml/min throughout analysis.

4. Hydrolysis Study

The buffer solutions were prepared with the following compositions; pH 5.0, 0.01 M acetic acid/0.01 M sodium acetate (1/2, v/v) and pHs 7.0 and 9.0, 0.01 M H_3BO_3 +0.01 M KCl and 0.01 M NaOH mixed in an appropriate volumetric ratio. The buffer solution was sterilized by autoclaving at 120°C and 1.5 psi for an hour with a Model SS-325 autoclave (Tomy Seiko) before use. The concentration of **I** was adjusted to 0.3 ppm below the water solubility of tetramethrin (1.83 ppm at 25°C). The test solution at each pH was duplicately prepared by adding a 1.0 ml aliquot of acetonitrile solution of [Acid- ^{14}C]-**I** (4.16 μCi , 31.5 μg) or [Alc- ^{14}C]-**I** (4.83 μCi , 32.4 μg) to each buffer (100 ml) in the heat-sterilized Erlenmeyer flask under continuous stirring. The flasks were kept in a Sanyo Model SHR-200M incubator at $25 \pm 1^\circ\text{C}$ in darkness for 30 days. Since **I** was susceptible to hydrolysis at pHs 7 and 9, the hydrolysis study in a shorter period (<1 day) was separately conducted. At appropriate intervals, a 0.5 ml aliquot of each solution was taken in duplicate and the concentration of ^{14}C was determined by using a Packard model 460CD Tri-carb scintillation spectrometer. A 1.0 ml aliquot of the water sample was mixed with a 0.2 ml aliquot of the acetonitrile/distilled water (1/1, v/v) solution containing the unlabeled standards and the mixture was directly analyzed by HPLC. Microbial contamination was avoided by conducting all procedures up to HPLC analysis aseptically in a clean bench. The sterility of the buffered aqueous solutions was checked at the beginning and end of the study by the dilution plate method, using the medium developed by Martin & Johnson and the nutrient agar medium.¹⁸⁾ **I** and **II** were fractionated by HPLC and separately extracted with ethyl acetate. The fractions of **IV**, **V** and the two unstable degradates (**VI** and **VII**) were separately lyophilized. The extracted or lyophilized samples dissolved in methanol were subject to the two-dimensional TLC analysis together with the non-labeled synthetic standards in order to confirm a chemical structure of each degradate.

5. LC-MS

LC-MS spectra of **I** and its degradates in reaction mixtures were measured by using the following instruments: Hitachi M-1000 LC-MS equipped with an APCI interface¹¹⁾ (positive ion (PI) and negative ion (NI) modes; drift voltages were 130 V and -140 V, respectively) linked with a Hitachi L-6200 Liquid Chromatograph and an Intertsil ODS-2 (5 μm , 4.6 mm i.d. $\times$ 25 cm) column in series; JEOL JMS-LX1000 LC-MS equipped with a FAB interface (Xe gas, glycerol matrix; NI mode, -3 kV) linked with a Yokogawa LC-100 Liquid Chromatograph and a Sumipax ODS A-112 (5 μm , 6 mm i.d. $\times$ 15 cm) column in series. The mobile phase was similar to that used in the hydrolysis study, but PIC-A was replaced with 0.1 M ammonium acetate in the former LC-MS analysis. LC-MS analysis of **I** and its degradates was conducted at an exaggerated concentration. A 0.5 ml aliquot of acetonitrile solution containing non-labeled **I** (0.1 mg) was mixed well with 9.5 ml of 0.01 M borate buffer (pH 9), resulting in 10 ppm aqueous solution. After incubation at 25°C for 20 min, a 1.0 ml aliquot was subjected to LC-MS analysis.

6. Quantitation of Formaldehyde

Formaldehyde formed *via* hydrolysis of **I** was spectrophotometrically quantified according to the reported method by using chromotropic acid.¹⁴⁾ A standard response curve of formaldehyde was prepared for concentrations of between 0.5 and 5 μM . The buffered aqueous solution of **I** (0.3 ppm, 0.9 μM) at pH 9 was prepared similarly as in the hydrolysis study and a 1.0 ml aliquot was subjected to analysis after incubation for an hour at 25°C. Since acetonitrile was found to inhibit the complex formation, methanol was used as a cosolvent instead.

RESULTS AND DISCUSSION

1. Hydrolysis Rate

Hydrolysis of **I** at pH 5 followed pseudo-first-order kinetics and the apparent rate constants (k) in an average were calculated to be 4.99×10^{-7} and $4.08 \times 10^{-7} \text{ sec}^{-1}$ for [Acid- ^{14}C]- and [Alc- ^{14}C]-labeled compounds, respectively, by the least square approximation method (correlation coefficient (r)= -0.99) on the

basis of the amounts of **I** at 0–30 days. In contrast, the decline curves of **I** at pHs 7 and 9 showed breaks at 2–4 days and 45–60 min, respectively. Almost the similar hydrolytic profiles have been previously reported for *N*-phenyl 3,4,5,6-tetrahydrophthalimide¹⁵⁾ and two reactions were likely to be involved. The apparent hydrolysis rates of **I** due to the slower reaction were first estimated on the basis of the data at 3–14 days (pH 7) and 1–24 hr (pH 9). The average initial k values at each pH were then calculated by subtracting its contribution from the data at 0–1 day (pH 7) and 0–30 min (pH 9) in the reported manner¹⁵⁾ as follows: $7.82 \times 10^{-6} \text{ sec}^{-1}$ (pH 7), $6.02 \times 10^{-4} \text{ sec}^{-1}$ (pH 9) for [Acid-¹⁴C]-**I**; $8.75 \times 10^{-6} \text{ sec}^{-1}$ (pH 7), $8.62 \times 10^{-4} \text{ sec}^{-1}$ (pH 9) for [Alc-¹⁴C]-**I**. The slope in the log k vs. pH plot¹⁵⁾ was 0.8 ($r=0.99$), showing that the second-order base-catalyzed process was dominant in hydrolysis of **I**. The second-order rate constant was calculated to be $3.41 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$ which was larger than that of tetrahydrophthalimide ($43.3 \text{ M}^{-1} \text{ sec}^{-1}$).¹⁶⁾ The k value reported for hydrolysis of *N*-phenyl 3,4,5,6-tetrahydrophthalimide¹⁵⁾ was about ten times as large as that of **I** at each pH. These differences in the k value would be, at least in part, due to a substituent effect at nitrogen of the imide moiety, as previously discussed by Hargreaves *et al.*¹⁷⁾

2. Product Distribution

The total recovery of ¹⁴C was 96.2–101.7% during the experiment, indicating that neither the chemical and physical adsorption of radioactive compounds onto the flask and the HPLC column nor their volatilization loss was of significance. The HPLC analysis immediately after an application of ¹⁴C-**I** showed that 100.0% (pH 5), 100.0% (pH 7) and 81.7–96.2% (pH 9) of the applied ¹⁴C were recovered as **I**, indicating a rapid hydrolysis of **I** under the alkaline conditions. Dilution plate method using the two different nutrient media showed that the buffer solutions were free from microbial contamination during the experiment and therefore the degradation observed was only due to chemical hydrolysis.

The product distribution at each pH is summarized in Table 3. The main degradates at pH 5 were **II** (from [Acid-¹⁴C]-**I**) and **IV** (from [Alc-¹⁴C]-**I**), whose amounts gradually increased to 67.1–70.6% (**II**) and 65.6–66.6% (**IV**) of the applied ¹⁴C at 30 days, respectively. The unidentified degradates amounted to less than 3.8% of the applied ¹⁴C throughout the experiment. At pH 7, **I** was almost completely hydrolyzed to **II** and **IV** during 30-days incubation. The two degradates were additionally observed except **II** and **IV**. One of them was 3,4,5,6-tetrahydrophthalamic acid (**V**) characteristic to [Alc-¹⁴C]-**I**. It showed a maximum amount (15.2–15.7%) at Day-1 but

Table 3 Amounts of **I** and its degradation products.

Compound	% of the applied ¹⁴ C ^{a)}					
	pH 5		pH 7		pH 9	
	14 days		1 day		20 min	
	Ac ^{b)}	Alc ^{c)}	Ac	Alc	Ac	Alc ^{d)}
I	50.9	58.0	49.3	47.0	48.9	34.3
II	47.7	— ^{e)}	46.5	—	36.2	—
IV	—	42.0	—	29.9	—	1.4
V	—	—	—	15.4	—	20.5
VI	—	—	4.2	6.3	13.7	12.2
VII	—	—	—	—	—	28.2
Others	1.4	< 0.1	< 0.1	1.4	1.2	3.4

^{a)} Averaged values between the duplicate experiments. ^{b)} [Acid-¹⁴C]-**I**. ^{c)} [Alc-¹⁴C]-**I**. ^{d)} After 30-days incubation, **IV** (68.1%) and **V** (31.9%) were only detected on the HPLC chromatogram. ^{e)} Not detected.

Fig. 1 Amounts of degradates in the hydrolysis of [Alc- ^{14}C]-I at pH 9.

Amounts of II were from [Acid- ^{14}C]-I.

disappeared after a week. The other degradate (VI) was transiently detected at a retention time of *ca.* 24 min in HPLC with its amount of less than 7% within 4 days. Since VI was formed from both of the ^{14}C preparations of I, it was considered that VI retained both ester and amide linkage in the molecule. The other unknown degradates amounted to less than 3.5% of the applied ^{14}C . The formation and decline of hydrolysis products at pH 9 are shown in Fig. 1. II was the main degradate from [Acid- ^{14}C]-I. VI was transiently detected within 2 hr and showed a maximum yield (15.9–17.2%) at Min-10. In the case of [Alc- ^{14}C]-I, the product distribution similar to that at pH 7 was observed except that another degradate (VII) was detected at a retention time of 10.0 min. Since VII was detected only with the [Alc- ^{14}C] preparation, it seemed to be

formed *via* cleavage of the ester linkage or successive hydrolysis of the imide ring. The amount of VII showed a maximum value at 20 min (27.7–28.6%) but rapidly decreased thereafter. After 1 day, the main degradates were IV and V, amounting to 67.3–68.8% and 31.2–32.7% at 30 days, respectively. At any pH tested, III formed *via* cleavage of the ester linkage of I was not detected. Neither I nor II underwent geometrical isomerization to the corresponding *cis*-isomer at any pH tested, as clarified by HPLC analysis.

3. Product Identification

I, II, IV and V detected by radio-HPLC were tentatively identified by cochromatography with the corresponding non-labeled authentic standards, by comparing their retention times. The chemical identity of each compound was further confirmed by two-dimensional TLC cochromatography. During the development on TLC plate, V was found to be partly degraded to IV. V seemed to be unstable under the acidic conditions, judging from the structural similarity to phthalamic acid which easily degrades to phthalic acid.¹⁸⁾ The chemical structures of I, II and V were also identified by LC-MS as follows: I, APCI (PI mode), m/z 349 ($\text{M}+\text{NH}_4^+$), 332 ($\text{M}+\text{H}^+$); II, frit-FAB (NI mode), m/z 259 ($\text{M}-\text{H}^- + \text{glycerol}$), 167 ($\text{M}-\text{H}^-$); V APCI (PI mode), m/z 187 ($\text{M}+\text{NH}_4^+$), 170 ($\text{M}+\text{H}^+$), 153 (acid anhydride of $\text{IV}+\text{H}^+$), API (NI mode), m/z 168 ($\text{M}-\text{H}^-$). The similar MS spectrum has been previously reported for I by using a thermospray technique.¹⁹⁾

VI and VII isolated by HPLC from the reaction mixture at pH 9 were first subjected to the TLC analysis. However, they were found to be degraded during the TLC development. VI afforded the several unresolved spots including IV and V on TLC. In the case of VII, the two spots due to IV and V were detected. In order to identify the chemical structures of VI and VII, more amounts of these degradates were prepared in a separate experiment. The borate buffer (pH 9) containing ^{14}C -I at 1.5 ppm was prepared in the presence of 5% acetonitrile and incubated for 20 min at 25°C. A 2.0 ml aliquot was analyzed by HPLC and the fractions corresponding to VI and VII were

Fig. 2 LC-MS spectrum of **VI** by using a frit-FAB interface (negative ion mode).

Fig. 3 LC-MS spectra of **VII** by using an API interface.

(i), positive ion mode; (ii), negative ion mode.

individually collected. Since these degradates were not detected at pH 5, they were considered to be unstable under the acidic conditions. Therefore, a 0.5 ml aliquot of each fraction was mixed well with 0.5 ml of 0.01 M acetate buffer (pH 5.0) and the reaction mixture was re-analyzed by HPLC after an incubation for 30 min at 25°C in order to get more structural information. HPLC re-analysis demonstrated that **IV** (22.1%), **V** (14.5%) and **VII** (35.7%) were formed from the [Alc-¹⁴C]-**VI** and **II** (70.1%) from the [Acid-¹⁴C]-**VI**. **I** (7.1–9.8%) was also detected from both ¹⁴C-preparations of **VI**. The deviation from first-order kinetic in the hydrolysis at pHs 7 and 9 was likely to be due to the presence of the partial formation of **I**. **IV** (62.1%) and **V** (37.9%) were similarly formed from **VII** under the acidic conditions. Since **V** was partly degraded to **IV** and considered to be acid-labile by analogy of *N*-alkyl derivatives²⁰⁾ and phthalamic acid,¹⁸⁾ it was suggested that both **VI** and **VII** had a structure of 3,4,5,6-tetrahydrophthalamic acid (**V**) and that **VI** also had **II** as a substructure in its molecule.

LC-MS spectrum (frit-FAB) of **VI** exhibited the *m/z* value be 440 (*M*–H[–]+glycerol), 348 (*M*–H[–]), 180 (*M*–H[–] of **III**), and 167 (*M*–H[–] of **II**) in the NI mode (Fig. 2). In the case of **VII**, the *m/z* values (APCI) detected were 217 (*M*+NH₄⁺), 200 (*M*+H[–]), 187 (*M*+NH₄⁺ of **V**) and 170 (*M*+H⁺ of **V**) in the PI mode and 198 (*M*–H[–]) and 168 (*M*–H[–] of **V**) in the NI mode (Fig. 3). LC-MS and HPLC analysis of the degradates strongly suggested that **VI** and **VII** were *N*-(1*RS*)-*trans*-chrysanthemoyloxymethyl 3,4,5,6-tetrahydrophthalamic acid formed *via* opening of the imide ring of **I** and *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalamic acid formed *via* cleavage of the ester linkage of **VI**, respectively.

4. Hydrolysis Pathways

I possessed both an ester linkage and a cyclic imide moiety in its molecule and the two reaction sites feasible to hydrolysis was considered. It is unlikely that the ester linkage was primarily cleaved, since **III** was not detected at any pH tested and **VI** was first detected at pH 9 followed by formation of **VII**, as demonstrated in Fig. 1. Although hydrolytic profiles of

pyrethroids possessing the *trans*-chrysanthemoyloxymethyl moiety have not been studied previously, the apparent hydrolysis rates of (1*R*)-*trans*-cypermethrin and fenpropathrin at 25°C have been reported to be $5.6 \times 10^{-9} \text{ sec}^{-1}$ and $9.1 \times 10^{-9} \text{ sec}^{-1}$ (pH 5), $3.7 \times 10^{-8} \text{ sec}^{-1}$ and $5.9 \times 10^{-8} \text{ sec}^{-1}$ (pH 7), and $2.7 \times 10^{-8} \text{ sec}^{-1}$ and $5.1 \times 10^{-8} \text{ sec}^{-1}$ (pH 9), respectively.^{21,22)} These values showed that the change of a substituent at 3-position of the cyclopropyl ring caused an insignificant effect on the rate of hydrolysis at the ester linkage. Since the apparent hydrolysis rate of **I** at each pH was about 100 times as large as these values, the faster hydrolysis seemed to be mainly due to the opening of the imide ring. The second-order rate constant of tetrahydrophthalimide in alkaline hydrolysis has been reported to be $43.3 \text{ M}^{-1} \text{ sec}^{-1}$ ¹⁶⁾ and the substitution of *N*-hydrogen with the electron-withdrawing COOCH₂ moiety is expected to accelerate the opening of the imide ring. Such an alkaline reaction was known to be kinetically effective at extremely low hydroxide concentration (pH $\geq$ 3),¹⁷⁾ which accounted for the large hydrolysis rate constant under the acidic conditions.

Since **VI** was the tetrahydrophthalamic acid derivative, an intramolecular acid-catalyzed cleavage of the amide linkage by the adjacent carboxyl group could be considered as a hydrolysis mechanism besides the cleavage of the ester linkage. This type of reaction has been extensively studied for various amic acids.^{20,23)} The anhydride of **IV** is unstable in the aqueous media²⁴⁾ and therefore aminomethyl *trans*-chrysanthemate and **IV** should be formed as products *via* this catalytic reaction mechanism, while **II** and **VII**, and/or **V** due to an instability of **VII**, were the products *via* the cleavage of the ester linkage. At pH 5, any kind of degradate characteristic to [Acid-¹⁴C]-preparation was not detected except **II** and **IV** was only detected in the hydrolysis of [Alc-¹⁴C]-**I**. Since both aminomethyl *trans*-chrysanthemate and **VII** were considered to be very unstable judging from their chemical structures (hemiaminal derivatives) and **V** was also unstable under the acidic conditions, it was difficult to determine which mechanism was favored. The acid-catalyzed ester cleavage was considered to be much slower than

the base-catalyzed process as reported for pyrethroids^{21,22} and the intramolecular catalytic cleavage of the amide linkage was an extremely rapid process. Supposing that the hydrolytic profile of the ester linkage in **I** is almost the same as those of *trans*-cypermethrin and fenpropathrin, the acid-catalyzed intramolecular reaction might be most probable in the degradation of **VI**.

In contrast with the amide cleavage, the ester cleavage of **VI** was considered to become dominant at pH 7 and 9. The product distribution was in good agreement with that predicted from the ester cleavage mechanism. Although **VII** was not detected at pH 7 at any sampling time, it might be due to its instability even at pH 7. Furthermore, it is known that the intramolecular catalysis becomes insignificant under the neutral and alkaline conditions,¹⁵ which also suggests the ester cleavage pathway. The partial recyclization of **VI** to **I** was considered to proceed *via* nucleophilic attack of the amide nitrogen at the adjacent carboxyl carbon similarly as reported for *N*-methylphthalamic acid.²³ Meanwhile, the spectrophotometric method using a chromotropic acid showed the quantitative formation of formaldehyde (0.9–1.0 μM) from **I** (0.3 ppm, 0.9 μM) at pH 9. Since **VII** was a transient species showing a maximum amount at 20 min and pH 9 and decreased afterwards with a concomitant increase of **V** (Fig. 1), it was con-

sidered that formaldehyde was liberated from **VII**.

On the basis of these results, the hydrolysis pathways are proposed in Fig. 4. **I** was hydrolyzed to **VI**, which was subsequently degraded to **VII** and **II** and partly reverted to **I**. By liberating formaldehyde, **VII** was further degraded to **V** followed by its degradation to **IV**. Although the hydrolysis rate and route were dependent on pH tested, **I** was found to finally degrade to **II** and **IV**.

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Fig. 4 Hydrolysis pathways of **I**.

*, •, ¹⁴C-labeled positions.

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要 約

trans-Tetramethrin の加水分解

片木敏行

[¹⁴C]*trans*-tetramethrin (**I**) の 25±1°C での加水分

解速度は HPLC 分析により, $4.08\sim4.99\times10^{-7}\text{sec}^{-1}$ (pH 5), $7.82\sim8.75\times10^{-6}\text{sec}^{-1}$ (pH 7), $6.02\sim8.62\times10^{-4}\text{sec}^{-1}$ (pH 9) と求められ, 塩基触媒的に分解することがわかった. HPLC, 2D-TLC, LC-MS (APCI & Frit-FAB, 正負イオンモード) 分析により **I** は, イミド環の開裂により水中で不安定な **I** のアミド酸を生成し, さらにエステル結合の開裂により *trans*-菊酸とアルコール部分由来の水中で不安定なアミド酸に分解することがわかった. アルコール部分はクロモトロピック酸を用いた吸光法により, ホルムアルデヒドと 3,4,5,6-テトラヒドロフタルアミド酸に分解し, 最終的には対応するジカルボン酸にまで分解することが明らかとなった.