Original Article

Hydrolysis of *N*-Phenylimide Herbicide Flumioxazin and Its Anilic Acid Derivative in Aqueous Solutions

Toshiyuki KATAGI*

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,
4-2-1 Takatsukasa, Takarazuka, Hyogo 665-8555, Japan

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Hydrolysis of the *N*-phenylimide herbicide flumioxazin was studied at pH 2.5–9.0 and $25 \pm 1^\circ\text{C}$ by directly analyzing a buffered aqueous solution with column-switching high performance liquid chromatography. Flumioxazin was hydrolyzed *via* a base-catalyzed opening of the cyclic imide moiety with half-lives of 4.1 days, 16.1 hr and 17.5 min at pH 5.0, 7.0 and 9.0, respectively. The primary hydrolysis product was the anilic acid derivative which was subsequently degraded to the corresponding aniline and dicarboxylic acid *via* cleavage of the amide linkage under acidic and neutral conditions, along with a partial recyclization to flumioxazin. Kinetic analysis of the pH dependency of each reaction path showed that the acid-catalyzed cleavage of the amide bond in the anilic acid was an intramolecular reaction where the undissociated form of the carboxyl group participated.

Key words: hydrolysis of flumioxazin, reaction mechanism of hydrolysis, kinetic analysis, column-switching HPLC.

INTRODUCTION

Flumioxazin (**I**) (Sumisoya®, *N*-(7-fluoro-3,4-dihydro-3-oxo-4-prop-2-ynyl-2*H*-1,4-benzoxazin-6-yl)cyclohex-1-ene-1,2-dicarboximide) is one of the *N*-aryl-3,4,5,6-tetrahydrophthalimide herbicides developed by Sumitomo Chemical Co., Ltd. and selectively controls annual broad-leaved weeds.¹⁾ After being emitted into the environment, **I** is considered to be distributed on plants and soil and in water *via* spray drift and run-off events with concomitant transformations. Among the abiotic processes, hydrolysis is one of the most important transformations in water and soil and its profiles are indispensable to assess the impact of **I** and its degradates on the environment. In order to primarily assess the behavior of this class of herbicide in the aquatic environment, Katagi has previously examined the hydrolytic profiles of *N*-phenyl-3,4,5,6-tetrahydrophthalimide as a model compound that is common to them.²⁾ The hydrolysis proceeds *via* a base-catalyzed opening of the imide ring to form the corresponding anilic acid derivative which further degrades *via* intramolecular nucleophilic catalysis by the undissociated carboxylic acid adjacent to the amide linkage. However, a kinetic analysis on the re-cyclization of the

anilic acid failed to be conducted due to instability during extraction of the buffered aqueous solution under the acidic conditions. Similar hydrolytic processes have been spectrophotometrically studied for a series of cyclic imides^{3,4)} and the corresponding amic^{5–8)} and anilic acids.^{9–12)} Mechanisms of hydrolysis for these acids have also been studied kinetically^{13–15)} and theoretically,¹⁶⁾ supporting the intramolecular catalysis of the undissociated carboxyl group. These hydrolysis studies have been mostly conducted in aqueous organic solvents at higher concentrations than expected in the aquatic environment due to the low water solubility of these chemicals, but there may be some differences in the rates and routes of hydrolysis caused by the effects of solvent and salt, as compared with those in the aquatic environments.

The present paper first deals with hydrolysis of ¹⁴C-labeled **I** conducted at a realistic environmental concentration well below its water solubility at various pHs. A direct high-performance liquid chromatographic analysis using the column-switching method was applied to avoid the artificial degradation of the acid-labile hydrolysis product expected when the buffer solution was extracted under the acidic conditions with organic solvent. Hydrolysis of the corresponding ¹⁴C-labeled anilic acid derivative (**II**) (*N*-[7-fluoro-3-oxo-4-(2-propynyl)-2*H*-1,4-benzoxazin-6-yl]-3,4,5,6-tetrahydrophthalanilic acid), likely to be present in a dissociated

* To whom correspondence should be addressed.
E-mail: katagi@sc.sumitomo-chem.co.jp

Fig. 1. Proposed hydrolysis pathway of I and its anilic acid derivative II.

*, $\blacktriangle$: ^{14}C -labeled positions. K_a : dissociation constant of II. k_1 , k_2 and k_{-1} are the rate constants for opening of the imide ring of I, cleavage of the amide linkage of II and re-cyclization of II to I, respectively.

form (Fig. 1) under neutral and basic conditions, was also examined to clarify the degradation mechanism. Second, the pH dependence of the hydrolysis rates was numerically analyzed on the basis of the proposed pathways to explain the different product distribution at each pH.

MATERIALS AND METHODS

1. Chemicals

I and the corresponding aniline (III) (6-amino-7-fluoro-4-(2-propynyl)-2H-1,4-benzoxazin-3(4H)-one) were synthesized at Sumitomo Chemical Co., Ltd. II was prepared in our laboratory according to a reported method²⁾ and its chemical structure was confirmed by ^1H NMR (Hitachi R-90H FT-NMR at 90 MHz), IR (Nicolet ZDX FT-IR) and field desorption (FD) MS (Hitachi M-80) spectroscopies. ^1H NMR δ_{H} (DMSO- d_6) ppm: 1.50–1.72 (m, 4H, cyclohexene-4,5- CH_2), 2.31 (m, 1H, $\text{C}\equiv\text{CH}$), 2.40–2.56 (m, 4H, cyclohexene-3,6- CH_2), 4.57 (s, 2H, OCH_2CO), 4.68 (s, 2H, NCH_2), 7.02 (d, 1H, $J=9.7$ Hz, aromatic-H), 7.70 (d, 1H, $J=6.8$ Hz, aromatic-H), 9.67 (s, 1H, NH). IR ν_{max} (KBr disk) cm^{-1} : 3447 (O-H), 3276 (N-H); 1687, 1657 and 1643 (C=O). FD-MS m/z : 372 (M^+), 220, 152. The chemical purity of each compound was determined to be >99% by HPLC analysis. 3,4,5,6-Tetrahydrophthalic anhydride (IV) was purchased from Aldrich Chemical Co., Ltd. (Milwaukee) and treated with 1.05 molar equivalent of sodium hydroxide in acetonitrile/water (1/1, v/v) to prepare the corresponding di-acid (V). [$\text{Ph-}^{14}\text{C}$]-I uniformly labeled in the phenyl moiety (15.5 MBq mg^{-1}) and [$\text{THP-}^{14}\text{C}$]-I labeled at the 1- and 2-positions of the 3,4,5,6-tetrahydrophthalimido moiety (10.7 MBq mg^{-1}) were prepared in our

laboratory. [$\text{Ph-}^{14}\text{C}$]-II (14.7 MBq mg^{-1}) as a sodium salt was conveniently prepared by treating [$\text{Ph-}^{14}\text{C}$]-I with 1.05 molar equivalents of sodium hydroxide in acetonitrile/water (1/2, v/v), since the preparation of the undissociated II failed due to its rapid decomposition during neutralization. The radiochemical purity of each ^{14}C -preparation was determined to be >99% by HPLC analysis. The organic solvents and inorganic reagents of a special grade from Wako Pure Chemical, Ltd. (Osaka) were used without further purification. Pure water supplied from a Puric Model-R (Organo Co., Ltd.) equipped with a G-10 filter (Organo Co., Ltd.) with an electrical conductivity of $5.5 \times 10^{-8} \Omega^{-1} \text{ cm}^{-1}$ was used to prepare buffer solutions. PIC-A used in a HPLC mobile phase was purchased from Waters Co., Ltd. (Milford).

2. Measurement of pK_b

The pK_b value of III was determined by electrometric titration with a model Comtite-5 titrator (Hiranuma Institute Co., Ltd.) equipped with a pH meter, as previously reported.²⁾ A 1.0-ml aliquot of III in dimethylsulfoxide was added to 30 ml of pure water, resulting in a final concentration of 1 mM. This solution was titrated with 0.2 M HCl, and the pK_b value was obtained graphically from a titration curve.

3. Radioassay

The radioactivity in aqueous fractions was determined by liquid scintillation counting (LSC) with a Packard model 460CD Tri-carb scintillation spectrometer equipped with an automatic external standard in a low potassium glass vial, using 10 ml of a Packard Emulsifier-scintillator 299TM. The background level was about 30 dpm throughout the study.

4. High Performance Liquid Chromatography (HPLC)

A Hitachi L-6000 liquid chromatograph equipped with a six-port automatic switching valve was first operated at a flow rate of 1.0 ml min^{-1} to flush the contents of the 2-ml loop onto a concentrator column by using a mobile phase of acetonitrile/distilled water (1/3, v/v) containing 5 mM PIC-A. At 3 min after injection, the mobile phase was switched (same as above except for the volumetric ratio, 2/3 (v/v)) to back flush the retained analytes onto an analytical column for separation at a flow rate of 1.0 ml min^{-1} with a Hitachi L-6200 liquid chromatograph. Sumipax ODS A-112 ($5 \mu\text{m}$, 6 mm i.d. $\times$ 15 cm; Sumika Analytical Service Ltd., Osaka) was used as both of the columns. The amounts of I and its hydrolysis products were quantified, using a Radiomatic flo-one/beta A-120 radiodetector (time constant, 6 sec). At the same time, UV traces at 250 nm were recorded with a Hitachi L-4000 variable wavelength UV monitor linked in series. At 45 min, the valve was switched again for the next analysis. The HPLC retention times of I–V are listed in Table 1. It was impossible in the HPLC analysis to distinguish between the undissociated and dissociated forms of II

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

Compound	TLC <i>R_f</i> value ¹⁾		HPLC retention time (min)
	A	B	
I	0.37	0.58	37.4
II ²⁾	0.00	0.47	9.2
III	0.31	0.47	10.6
IV	0.61	0.58	6.4
V	0.00	0.11	7.2

¹⁾ Solvent system A, hexane/ethyl acetate (1/1, v/v); Solvent system B, toluene/ethyl acetate/acetic acid (5/7/1, v/v/v).

²⁾ **II** was partly degraded to **III** and **V** when the acidic solvent system B was used.

and hence, the designation “**II**” was conveniently used in HPLC and TLC analyses and the subsequent kinetic analysis. The difference in retention time (about 0.3 min) between the two detectors was instrumentally corrected. The background level of the radiodetector was about 60 cpm sec⁻¹ and 10 cpm sec⁻¹ above background was detected. Since its counting efficiency was about 0.9, the detection limit was calculated to be about 0.1% of the applied ¹⁴C. Each peak was assigned on the basis of retention time comparisons.

5. Hydrolysis Study

The buffer solutions were prepared with the following compositions: pH 2.5–3.5, 10 mM HCl–10 mM sodium acetate; pH 4.0–5.0, 10 mM acetic acid–10 mM sodium acetate; pH 5.5–6.5, 10 mM disodium hydrogen phosphate–10 mM potassium dihydrogen phosphate; pH 7.0–9.0, 10 mM boric acid–10 mM sodium hydroxide. The ionic strength was adjusted to 0.1 by adding the appropriate amount of potassium chloride to each buffer. The pH of each buffer was checked with a model F-15 pH meter (Horiba Co., Ltd.) calibrated with standard buffer solutions at 25°C. The buffer solution was sterilized by autoclaving at 120°C and 1.5 psi for an hour with a model SD-30N (Tomy Seiko) before use.

The water solubility of **I** at 25°C is 1.79 ppm¹⁷⁾ and that of **II** is most likely to be much higher judging from its chemical structure. Therefore, the concentration of **I** and **II** was adjusted to 0.1 ppm, lower than their water solubilities. First, in order to examine the hydrolysis kinetics of **I** and **II** in the wide pH range of 2.5–9.0, a 0.1-ml aliquot of acetonitrile solution of [Ph-¹⁴C]-**I** (1.05 µg) or acetonitrile/distilled water (1/1, v/v) solution of [Ph-¹⁴C]-**II** (1.07 µg) was aseptically added in duplicate to each of the buffer solution (10 ml) in the heat-sterilized 20-ml vials with continuous stirring. The vials stoppered with Teflon-lined screw caps were kept in a Sanyo model SHR-200M incubator at 25 ± 1°C in darkness.

Second, a hydrolysis study using [Ph-¹⁴C]- and [THP-

¹⁴C]-**I** was similarly conducted at 0.1 ppm for up to 30 days to examine the product distribution at pH 5.0, 7.0 and 9.0 more in detail. A 1.0-ml aliquot of acetonitrile solution of each ¹⁴C-**I** (10.7 µg) was aseptically added to 100 ml of each buffer solution in a heat-sterilized 100-ml Erhlenmeyer flask with continuous stirring. The buffered aqueous solution at each pH was prepared in duplicate and kept at 25 ± 1°C in darkness. In order to avoid microbial contamination, all procedures up to the HPLC analysis were conducted on a clean bench (Hitachi, model CCV-811). The contamination of the buffer solution by fungi and bacteria was checked by a dilution plate method using Martin & Johnson and nutrient agar (mixtures of bacto-beaf extract, bacto-peptone and bacto-agar; DIFCO Laboratories) media.¹⁸⁾

In either experiment, the dissolved ¹⁴C in the 0.1-ml aliquot of each buffer solution was first quantified by LSC at each sampling to determine the concentration of ¹⁴C and the recovery at each sampling based on the applied ¹⁴C (*R₁*, %) was calculated. A 1.0-ml aliquot of each buffer solution was also analyzed by direct HPLC co-chromatography with a 0.2-ml aliquot of acetonitrile/distilled water (1/1, v/v) solution containing the unlabeled **I–V** co-injected. The ¹⁴C-recovery during each HPLC analysis (*R₂*, %) was also determined, by measuring the volume of an eluate and radioactivity in its 2.0-ml aliquot by LSC. The percentage of each degradate based on the applied ¹⁴C was obtained by calibrating the relative percent in the radio-HPLC analysis with both *R₁* and *R₂* values.

The HPLC fractions corresponding to **I** and its degradates were individually collected from the representative samples where formation of each degradate was maximal. **I** and **III** in the eluates were extracted three times with 20 ml of ethyl acetate followed by washing with distilled water. After treatment with anhydrous MgSO₄, the combined organic layer was concentrated under reduced pressure. In the case of **II** and **V**, the eluate was lyophilized by using a VirTis Freezemobile-3 and the resulting residue was dissolved in methanol. Precoated silica gel 60F₂₅₄ chromatoplates (20 × 20 cm, 0.25-mm layer thickness, E. Merck) were used for analytical purposes. The TLC *R_f* values of **I–V** in the two solvent systems are given in Table 1. The extracted or lyophilized samples were subjected to two-dimensional TLC co-chromatography with unlabeled authentic standards. The resolved radioactive spots and the unlabeled standards were detected by autoradiography and UV light fluorescence quenching, respectively, and their chemical identities were confirmed.

6. Kinetic Analysis

The apparent pseudo-first-order rates of hydrolysis (*k_{obs}*) for **I** and **II** were estimated by the least-square approximation method. By taking account of the previous hydrolysis studies on amic and anilic acids,^{13–15)} the hydrolysis pathway was assumed as shown in Fig. 1. When the concentrations of **I**, **II** and **III** (or **V**) are [**I**], [**II**] and [**III**] at time *t*,

respectively, the following differential Eq. (1)–(3) can be derived.

$$d[\text{I}]/dt = -k_1 [\text{I}] + k_{-1} [\text{II}] \quad (1)$$

$$d[\text{II}]/dt = k_1 [\text{I}] - (k_{-1} + k_2) [\text{II}] \quad (2)$$

$$d[\text{III}]/dt = k_2 [\text{II}] \quad (3)$$

Here, k_1 , k_{-1} and k_2 are the pseudo-first-order rate constants for hydrolysis of **I**, recyclization of **II** to form **I** and hydrolysis of **II** to **III** (or **V**), respectively. These differential equations were numerically solved by the Runge-Kutta method using the Model Maker program (version 2.0; Cherwell Scientific). Furthermore, the pH dependency of these rate constants was further analyzed using the Sigma-Plot program (version 5.0, SPSS Inc.) to understand the hydrolytic mechanism of **II** in more detail.

RESULTS AND DISCUSSION

1. Hydrolysis of **I**

The total recovery of ^{14}C ($=R_1 \times R_2/100$) was 98.1–100.5% of the applied dose and therefore, neither adsorption of the radioactive compounds onto the glass surface and the HPLC columns nor their volatilization from solution was likely. The HPLC analysis immediately after an application of **I** showed that 95.1–101.1% (pH 2.5–7.0) and 89.1–92.3% (pH 7.5–9.0) of the applied ^{14}C were recovered as **I** unchanged, showing the susceptibility of **I** to alkaline hydrolysis. During the 30-day incubation, the buffer solutions were free from microbial contamination as evidenced by the dilution plate method in the two nutrient media used (data are not shown). The apparent hydrolysis rate constants ($k_{\text{obs}}^{\text{I}}$) in the pH range of 2.5–9.0 are summarized in Table 2. The corresponding half-life was calculated by the equation $0.693/(\text{rate constant})$ in the usual manner assuming the first-order kinetics. **I** was gradually hydrolyzed at pH 5.0 with a half-life of 4.1 days following the first-order kinetics,

while a more rapid dissipation with half-lives of 16.1 hr and 17.5 min was observed at pH 7 and 9.0, respectively. Constant $k_{\text{obs}}^{\text{I}}$ values around $(2.137 \pm 0.218) \times 10^{-6} \text{ sec}^{-1}$ were observed under the acidic conditions at and below pH 5.0. As with an increase of pH above 5.0, $k_{\text{obs}}^{\text{I}}$ greatly increased. The decline curve of **I** at about pH 7.0 showed a break at 2–4 days after an application and the two reactions were likely to be involved. In this case, the initial rate constant was conveniently calculated as $k_{\text{obs}}^{\text{I}}$. At and above pH 8.0, the reaction obeyed the first-order kinetics and the slope of $\log(k_{\text{obs}}^{\text{I}})$ vs. pH plot was approximately 1.0; $\log(k_{\text{obs}}^{\text{I}}) = 1.027 \text{ pH} - 12.49$ ($r^2 = 0.960$). These results indicate that the hydrolysis of **I** proceeds predominantly through neutral ($\text{pH} \leq 5$) or base-catalyzed ($\text{pH} \geq 8$) process, whereas both reactions are involved between pH 5.0 and 8. The second-order rate constant (k_{OH}^{I}) was estimated to be $32.4 \text{ M}^{-1} \text{ sec}^{-1}$ at and above pH 8.0 where the base-catalyzed process was dominant.

The alkaline hydrolysis of cyclic imides is known to follow the second-order kinetics via the $\text{B}_{\text{AC}}2$ mechanism, that is an initial attack by OH^- at the acyl carbon to form a tetrahedral intermediate, and the substitution of the *N*-hydrogen by phenyl causes an increase in the rate of ring opening by OH^- .⁴⁾ This substituent effect was detected for **I**, since its k_{OH} value was greater than that of 3,4,5,6-tetrahydrophthalimide ($1.3 \text{ M}^{-1} \text{ sec}^{-1}$) reported by Hückel and Müller.⁵⁾ In contrast, the k_{OH} value of **I** was much smaller than that of *N*-phenyl 3,4,5,6-tetrahydrophthalimide ($3.25 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$),²⁾ having a similar structure to **I**. Since the electron-withdrawing substituent in the phenyl ring is known to retard the alkaline hydrolysis of imides,⁴⁾ the fluorine atom at the 2-position of the phenyl moiety in **I** was considered to cause the decrease of the k_{OH} value. This electron-withdrawing effect was also ascertained by the potentiometric titration of **III**, whose pKb value (3.3) was smaller than that of aniline (4.6) as reported by Gutbezzall and Grundwald.¹⁹⁾

2. Hydrolysis of **II**

The loss of ^{14}C due to adsorption or volatilization was insignificant judging from the total recovery of ^{14}C ($>98\%$), and 80.5–92.5% (pH 2.5–6.5) and 97.8–102.0% (pH 7.0–8.0) of the applied ^{14}C were recovered as **II**. The apparent hydrolysis rate constants of **II** ($k_{\text{obs}}^{\text{II}}$) in the pH range of 2.5–8.0 are summarized in Table 2. In contrast to **I**, **II** was more susceptible to hydrolysis under the acid conditions and the half-lives at pH 5.0 and 7.0 were calculated to be 2.3 hr and 10.7 days assuming the first-order kinetics, respectively. A plateau was found at pH 2.5–4.0, while $k_{\text{obs}}^{\text{II}}$ was markedly reduced with an increase of pH at and above 4.5. Similar profiles of the apparent hydrolysis rate constant have been reported for *N*-phenyl 3,4,5,6-tetrahydrophthalanilic acid,²⁾ succinilic acids,¹⁰⁾ *N*-phenyl phthalanilic acids¹²⁾ and maleanilic acids²⁰⁾ and this is considered to be characteristic of intramolecular catalysis being participated in by the

Table 2. Apparent hydrolysis rate constants of **I** and its anilic acid **II** at pH 2.5–9.0

pH	$k_{\text{obs}}^{\text{I}} \times 10^6$	$k_{\text{obs}}^{\text{II}} \times 10^6$	pH	$k_{\text{obs}}^{\text{I}} \times 10^6$	$k_{\text{obs}}^{\text{II}} \times 10^6$
2.5	2.401	522.2	6.0	3.742	15.03
3.0	1.866	485.8	6.5	7.962	3.825
3.5	2.029	438.7	7.0	11.97 ¹⁾	0.7490
4.0	2.202	369.2	7.5	41.42	0.3215
4.5	2.358	243.7	8.0	61.95	0.1119
5.0	1.964 ¹⁾	82.05	8.5	134.4	– ²⁾
5.5	2.513	32.95	9.0	659.9 ¹⁾	– ²⁾

$k_{\text{obs}}^{\text{I}}$ and $k_{\text{obs}}^{\text{II}}$: apparent hydrolysis rate constants of **I** and **II** in sec^{-1} .

¹⁾ Averaged values determined in hydrolysis of $[\text{Ph-}^{14}\text{C}]\text{-I}$ and $[\text{THP-}^{14}\text{C}]\text{-I}$. In other cases, $[\text{Ph-}^{14}\text{C}]\text{-I}$ and -II were used.

²⁾ Not determined.

undissociated carboxyl group. The $k_{\text{obs}}^{\text{II}}$ value at each pH was about one-tenth of that observed for *N*-phenyl 3,4,5,6-tetrahydrophthalic acid,²⁾ which was most likely to be mainly due to the electron-withdrawing effect by fluorine at the 2-position of the benzoxazin moiety. A similar retardation of rates has been reported for the malenic acids²⁰⁾ when chlorine was incorporated at the 2-position of the phenyl ring.

3. Product Distribution

During the 30-day incubation of [Ph-¹⁴C]- and [THP-¹⁴C]-I at pH 5.0, 7.0 and 9.0, I–V were identified by HPLC and two-dimensional TLC co-chromatographies with their unlabeled standards. I underwent an opening of the cyclic imide moiety to form II at pH values tested. At pH 5.0, II was a transient degradate common to both radiolabels and amounted to less than 4.7% of the applied ¹⁴C. In the case of [THP-¹⁴C]-I, IV was also detected transiently but its amount was less than 2.3%. III and V were the main degradates, amounting to 85.7% and 93.1% after 14 days, respectively, as summarized in Table 3. At pH 7.0, II was gradually produced with the degradation of I and its amount exhibited a maximum (60.2–70.3%) after 2–4 days (Fig. 2). However, III and V became the major degradates instead after 14 days with a concomitant decrease of II. At pH 9.0, II was the only degradate formed via rapid degradation of I after 0.5 hr with no other degradates detected by HPLC analysis, indicating that II was resistant to alkaline hydrolysis.

I was considered to be primarily hydrolyzed to II (faster reaction). Although II was resistant to hydrolysis under the alkaline conditions, it degraded to III and V (through IV) via cleavage of the amide linkage and partly reverted to I (slower reaction) at and below pH 7, as previously reported for *N*-methylphthalamic acid.⁷⁾ The break 2–4 days post-

application in the decline curve of I at about pH 7.0 was accounted for by these two reactions. Although a trace amount of IV was detected at and below pH 7.0 in this study, previous studies on the hydrolysis of amic acids have shown that one of the primary products is the corresponding acid anhydride.^{6,7)} This difference seemed to stem from the significant instability of IV in water, as previously reported by Eberson.²¹⁾

4. Kinetic Analysis

The rate constants (k_1 , k_{-1} and k_2) defined in the proposed hydrolysis pathway in Fig. 1 were estimated as listed in Table 4, by numerically solving the differential equations based on the product distribution observed in the hydrolysis of [Ph-¹⁴C]-II at pH 2.5–8.0. The product distribution in the 30-day hydrolysis study of [Ph-¹⁴C]-I at pH 7.0 could be

Fig. 2. Curve fitting to the product distribution in the hydrolysis of [Ph-¹⁴C]-I at pH 7.0. The solid lines were obtained by fitting the observed product distribution to the Eq. (1)–(3).

Table 3. Amounts of I and its degradation products in the hydrolysis of ¹⁴C-I at pH 5.0, 7.0 and 9.0

Compound	% of the applied ¹⁴ C											
	pH 5.0				pH 7.0				pH 9.0			
	Ph- ¹⁴ C		THP- ¹⁴ C		Ph- ¹⁴ C		THP- ¹⁴ C		Ph- ¹⁴ C		THP- ¹⁴ C	
	Incubation period ¹⁾											
	1 d	14 d	1 d	14 d	1 d	14 d	1 d	14 d	0.5 h	14 d	0.5 h	14 d
I	81.2	13.7	75.5	5.7	40.7	13.5	32.1	9.9	34.3	<0.1	29.3	<0.1
II	4.7	<0.1	4.2	<0.1	53.2	27.6	63.0	28.1	63.6	99.3	73.0	98.9
III	13.2	85.7	-	-	3.8	57.5	-	-	<0.1	<0.1	-	-
IV	- ²⁾	-	<0.1	2.3	-	-	<0.1	6.2	-	-	<0.1	<0.1
V	-	-	18.2	93.1	-	-	3.3	55.5	-	-	<0.1	<0.1
Others	<0.1	<0.1	<0.1	<0.1	1.8	<0.1	<0.1	1.2	<0.1	<0.1	<0.1	1.4
Total	99.1	99.4	97.9	101.1	99.5	98.6	98.4	100.9	97.9	99.3	102.3	100.3

¹⁾ "d" and "h" mean day and hour, respectively. ²⁾ Not applicable.

Table 4. Results of kinetic analysis on hydrolysis of [Ph-¹⁴C]-II

pH	Rate constants (sec ⁻¹)		
	$k_1 \times 10^4$	$k_{-1} \times 10^5$	$k_2 \times 10^6$
2.5	3.92 ± 0.76	43.2 ± 4.2	441.3 ± 14.5
3.0	2.31 ± 1.34	38.4 ± 6.1	281.2 ± 20.2
3.5	1.79 ± 0.48	41.0 ± 3.2	241.5 ± 10.8
4.0	2.42 ± 1.30	30.0 ± 6.2	180.9 ± 19.4
4.5	0.57 ± 0.32	18.6 ± 1.6	112.5 ± 5.1
5.0	0.12 ± 0.03	6.9 ± 0.3	38.4 ± 1.10
5.5	0.73 ± 0.12	9.9 ± 1.0	16.2 ± 1.64
6.0	0.77 ± 0.06	1.3 ± 0.6	8.4 ± 0.25
6.5	0.11 ± 0.01	0.68 ± 0.07	2.8 ± 0.07
7.0	0.27 ± 0.12	0.57 ± 0.26	0.98 ± 0.019
7.5	0.54 ± 0.04	0.53 ± 0.05	0.41 ± 0.025
8.0	1.70 ± 0.82	0.70 ± 0.05	0.16 ± 0.017

k_1 , k_2 and k_{-1} : rate constants for opening of the imide ring of **I**, cleavage of the amide linkage of **II** and re-cyclization of **II** to **I**, respectively.

simulated fairly well by using equations obtained through the kinetic analysis, as demonstrated in Fig. 2, indicating that the hydrolysis pathway proposed is rational. Although the k_1 values showed larger deviations at and below pH 4.0, the pH dependence above pH 4.5 was likely to show the base-catalyzed hydrolysis of **I**. Both k_{-1} and k_2 values also show pH dependencies and their contribution to k_{obs} was significant at and below pH 4.5. In order to examine the hydrolysis mechanisms of **II** in more detail, these pH dependencies were further analyzed. The pH profiles of the hydrolysis rate of **II** strongly suggested that the undissociated carboxylic acid group in **II** participates in the acid-catalyzed cleavage of the amide linkage. Thus, k_2 can be expressed by the following equation, as previously reported by Bender *et al.*⁶⁾

$$k_2 = (k_w + k_H[H^+]) / (1 + K_a/[H^+]) \quad (4)$$

where k_w , k_H and K_a are the first-order neutral and second-order acid-catalyzed rate constants and the dissociation constant of the carboxylic acid of **II**, respectively. Based on the pH-dependency of the k_2 values, a non-linear curve fitting was conducted using the Sigma Plot program and the best fit for Eq. (4) ($r^2=0.998$) was achieved, as shown in Fig. 3, with the following values; $k_w = (2.410 \pm 0.081) \times 10^{-4} \text{ sec}^{-1}$, $k_H = (6.416 \pm 0.356) \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$ and $K_a = (3.885 \pm 0.445) \times 10^{-5}$ ($\text{p}K_a=4.41$). The corresponding values for *N*-phenyl 3,4,5,6-tetrahydrophthalanilic acid have been reported by Katagi²⁾ as follows; $k_w = 5.171 \times 10^{-3} \text{ sec}^{-1}$, $k_H = 0.362 \text{ M}^{-1} \text{ sec}^{-1}$ and $\text{p}K_a=3.94$. The differences in the k_w and k_H values are considered to arise from the electron-withdrawing effect by fluorine at the 2-position. The measurement of $\text{p}K_a$ of **II** failed due to the rapid hydrolysis of **II** under the acidic conditions, but the calculated value was close to that of the corresponding *N*-phenyl derivative. These results strongly suggest that the undissociated

Fig. 3. Kinetic analysis for acid-catalyzed cleavage of the amide linkage of **II**.

The solid line was obtained by fitting the estimated k_2 values to the equation (4) where $k_w = 2.410 \times 10^{-4} \text{ sec}^{-1}$, $k_H = 6.416 \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$ and $K_a = 3.885 \times 10^{-5}$.

carboxyl group participates in the intramolecular catalytic cleavage of the amide linkage of **II**.

Since the re-cyclization of **II** was considered to proceed under acidic and neutral conditions, water and proton are likely to participate in this reaction. However, the equation describing the pH dependency of k_{-1} failed to well reproduce the pH-profiles of k_{-1} . Therefore, the following equation including the contribution of the $1/[H^+]$ term was used instead.

$$k_{-1} = (k'_w + k'_H[H^+] + k'_o/[H^+]) / (1 + K_a/[H^+]) \quad (5)$$

The $1/[H^+]$ term may represent contributions from the base-catalyzed cyclization of uncharged intermediates or from the water-catalyzed cyclization of intermediates having a unit negative charge. A non-linear curve fitting was conducted and the best fit for Eq. (5) ($r^2=0.982$) was achieved as shown in Fig. 4, with the following values; $k'_w = (4.278 \pm 0.337) \times 10^{-4} \text{ sec}^{-1}$, $k'_H = (1.160 \pm 0.150) \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$, $k'_o = (4.868 \pm 0.568) \times 10^{-10} \text{ M sec}^{-1}$ and $K_a = (4.191 \pm 1.254) \times 10^{-5}$ ($\text{p}K_a=4.38$). The $\text{p}K_a$ value estimated was in good agreement with that from the pH profiles of k_2 . The comparisons of k'_w , k'_H and k'_o values showed that the mechanisms represented by the first and third terms are predominant in the cyclization of **II** at the pH tested. By analogy with the cyclization of *N*-methylphthalamate to *N*-methylphthalimide,⁷⁾ the tetrahedral intermediate formed *via* addition of hydroxide ion at one of the carbonyl carbons in the cyclic imide moiety of **I** and its protonated form were most likely to be involved in the cyclization reaction.

The product distribution in the hydrolysis of **I** and its anilic acid **II** was successfully explained by the reaction pathways shown in Fig. 1, as demonstrated by the kinetic analyses. The pH dependency of the product distribution and each reaction path was found to be the same as that reported for cyclic imides,^{3,4)} amic^{5,8)} and anilic acids⁹⁻¹²⁾

Fig. 4. Kinetic analysis for re-cyclization of **II** to **I**. The solid line was obtained by fitting the estimated k_{-1} values to the equation (5) where $k'_w = 4.278 \times 10^{-4} \text{ sec}^{-1}$, $k'_H = 1.160 \times 10^{-3} \text{ M}^{-1} \text{ sec}^{-1}$, $k_o = 4.868 \times 10^{-10} \text{ M sec}^{-1}$ and $K_a = 4.191 \times 10^{-5}$.

examined spectrophotometrically at much higher concentrations in an aqueous organic solvent. Therefore, it is considered that the hydrolytic profiles of *N*-phenylimides including **I** are not very sensitive to the effects of either solvent or salt.

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