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Kinetics and Mechanism of the Acid–Base Equilibrium and the Hydrolysis of Benzodiazepinooxazines[1]

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Ring-opening and ring-closing (acid–base equilibrium) reactions of the six-membered oxazine of benzodiazepinooxazines (BZINs) and the subsequent hydrolyses of the diazepine ring have been investigated kinetically and compared with the reactions of benzodiazepinooxazoles (BZOLs) which have the five-membered oxazolidine ring. The ring-closing reaction for BZINs is slower than that for BZOLs due to the increase in the degree of freedom for the moving moiety of BZINs. The rates of the ring-opening reactions are almost independent of the substituents at 12b-position (H-(1), CH_3-(2), and C_6H_5-(3)), indicating that an attacking proton may approach equally (non-sterically) to the lone pair of N_5 atoms. Possible conformational aspects of BZINs in solution are proposed. Cleavages (hydrolyses) of the diazepine ring occur at the C_{12b}-N_5 (iminium) bond for 2 and 3 and are 10—100 times faster than those for the corresponding BZOLs. For 1, in contrast to 2 and 3, hydrolysis of the amide bond (N_8-C_7) of the diazepine ring takes place instead of the iminium bond, similar to the case of 11b-hydrogen BZOL.

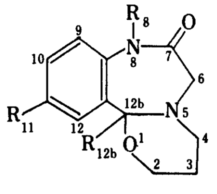
Keywords benzodiazepinooxazine; benzodiazepinooxazole; acid–base equilibrium; kinetics; oxazine ring-opening, -closing; diazepine ring; hydrolysis; reaction pathway; iminium bond; amide bond

Introduction

From the standpoint of drug behavior after oral administration, the acid–base equilibrium (oxazolidine ring-opening and ring-closing reactions) of benzodiazepinooxazoles (BZOLs) and the subsequent hydrolysis of the diazepine ring have been studied kinetically.[2-6] These reactivities of BZOLs which contain a five-membered oxazolidine ring fused to a seven-membered diazepine ring are found to be greatly influenced by the substituents at 11b-, 2-, 3-, and 7-positions.[2-6] It is thus of interest to investigate the reactivities of benzodiazepinooxazines (BZINs, six-membered oxazine rather than the oxazolidine of BZOLs) in order to compare the differences of kinetic behavior in the various ring systems. In this paper we described the reactivities of BZINs in comparison to those of BZOLs (Chart 1).

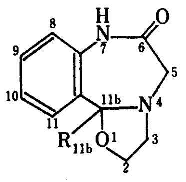
Experimental

Materials and Instruments Compounds **1**—**5** were synthesized by methods similar to those reported by Deriege *et al.*,[7] Miyadera *et al.*,[8] and Lemke and Hanze.[9] The chemical structures of these compounds were confirmed by elemental analyses as well as ^{1}H- and ^{13}C-nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) measurements. Melting points and the results of the elemental analyses of the compounds studied are listed in Table I. All other chemicals were purchased commercially and were of reagent grade, and used without further purification.



compound	R12b	R8	R11
1	H	H	H
2	CH3	H	H
3	C6H5	H	H
4	C6H5	CH3	H
5	C6H5	H	Cl

benzodiazepinooxazines



compound	R11b
6	H
7	CH3
8	C6H5

benzodiazepinooxazoles

Chart 1

Ultraviolet (UV) spectra were measured by Shimadzu UV-260 and UV-2200 spectrophotometers. A stopped-flow spectrophotometer (Otsuka Denshi RA-401) was used for the measurement of the reaction rates. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-FX 100 spectrometer at 100 and 25 MHz, respectively. An NEC microcomputer (PC-9801E) was used for the analyses of pH-rate profiles.

Kinetic Runs The buffer systems were the same as those empolyed in previous studies.[2-6] The rates of the oxazine ring-opening and ring-closing were measured by the pH-jump method using the stopped-flow instruments as reported previously.[2-4,6] The pseudo first-order rate constants (k_{obs}) for the reactions were determined by the ordinary first-order analysis and also by the Guggenheim method[10] in the case where the reaction end point is unknown. These analyses were performed directly using a Sord microcomputer (M223 mark III) linked to the stopped-flow instruments.

The hydrolysis of **1** was carried out at 70 °C. Aliquots of the sample solution were withdrawn at appropriate intervals and cooled to room temperature (25 °C). Their UV spectra were measured and compared with spectra of the model compounds *N*-acetyl-2-aminobenzaldehyde (**9**) and 2-aminobenzaldehyde-ethylenediimine (**10**).[6,11,12] All other reactions were carried out at 25 °C.

Determination of Equilibrium Constant The apparent equilibrium constants of BZINs were determined by a method similar to those reported previously.[2-4,6]

Results and Discussion

Oxazine Ring-Opening and -Closing Reactions Figure 1 shows the UV spectra of compound **1** in various pH buffer solutions. These spectra are attributed to equilibrium

TABLE I. Physical and Analytical Data for Benzodiazepinooxazines (BZINs)

Compd.	mp (°C) (lit.)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found) C	H	N
1	176—178 (175—176)[8]	Ethanol	$C_{12}H_{14}N_2O_2$	66.03 (66.24	6.46 6.35	12.83 12.82)
2	147—150	Ethanol	$C_{13}H_{16}N_2O_2$	67.22 (67.04	6.94 6.72	12.06 11.90)
3	220—222	Ether–ethanol	$C_{18}H_{18}N_2O_2$	73.45 (73.29	6.16 5.89	9.52 9.51)
4	150—152	Ethanol	$C_{19}H_{20}N_2O_2$	73.99 (74.28	6.55 6.64	9.09 9.11)
5	206—208 (220—222,[8] 213—216)[9]	Ethanol	$C_{18}H_{17}ClN_2O_2$	65.75 (65.49	5.21 5.18	8.52 8.58)

mixtures of the ring-opened iminium form (AF, acid free N_8 form) in acid solution and the ring-closed form (BF, basic free N_8 form) in weakly alkaline solution.[2–6] From the spectral data, similar to the case of BZOLs studied previously,[2–4,6] the apparent $\mathrm{p}k_{\mathrm{eq}}^{\mathrm{UV}}$ value ($-\log([\mathrm{BF}][\mathrm{H}^+]/[\mathrm{AF}])$) was determined to be 5.95.

Figure 2 illustrates the pH-rate profile for the oxazine ring-opening and ring-closing reactions of compounds **1** and **4**. The shapes of the profiles are similar to those for BZOLs reported previously,[2–4,6] suggesting that the reaction scheme shown in Chart 2 is applicable. In Chart 2, AC and AA represent acid cationic N_8 form and acid anionic N_8 form, respectively, and basic cationic and anionic forms are represented by BC and BA, respectively. The superscripts and subscripts of the rate constants have the following meanings: The superscripts H^+, 0, and OH^- represent the hydrogen ion-catalyzed, water-catalyzed or unimolecular (intramolecular), and hydroxide ion-catalyzed reactions, respectively. The first subscript indicates whether ring-opening (Op) or ring-closing (Cl) occurs, and the second one represents the cationic form (C), the free form (F) or the anionic form (A) at the 8 nitrogen (N_8) atom of the compound. $K_{\mathrm{a},1}$, $K'_{\mathrm{a},1}$, $K_{\mathrm{a},2}$, and $K'_{\mathrm{a},2}$ are the dissociation constants of the respective forms, and the dissociation processes are, in general, much faster than the processes of the ring-opening and the ring-closing.[2,4]

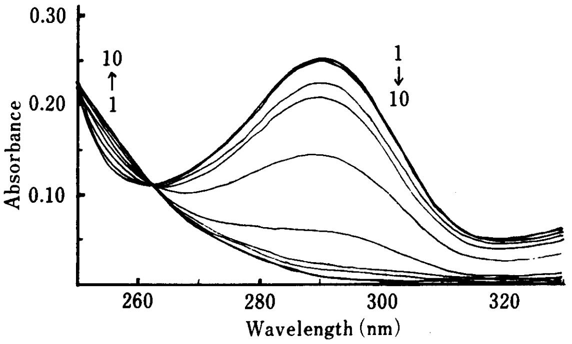


Fig. 1. UV Absorption Spectra of Compound **1** at Various pH Values

Concentration of **1** was 2.98×10^{-5} M. 1, pH 1.89; 2, 2.79; 3, 3.97; 4, 5.00; 5, 5.38; 6, 5.87; 7, 6,49; 8, 7.00; 9, 8.27; 10, 9.02.

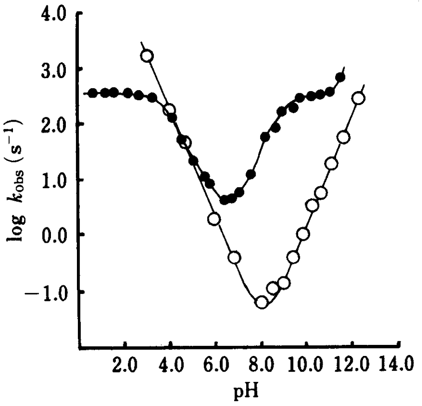


Fig. 2. The pH-Rate Profiles for Oxazine Ring-Opening and Ring-Closing Reactions of Compounds **1** and **4** at 25 °C

●, compound **1**; ○, compound **4**.

The rate constants and dissociation constants in Chart 2 were determined by procedures similar to those empolyed previously.[2–4,6] The values obtained along with the $\mathrm{p}K_{\mathrm{eq}}^{\mathrm{UV}}$ values are summarized in Table II. For the sake of comparison, Table II also shows the values for compounds **6**—**8** obtained previosuly.[4,6] The solid curves in Fig. 2 were

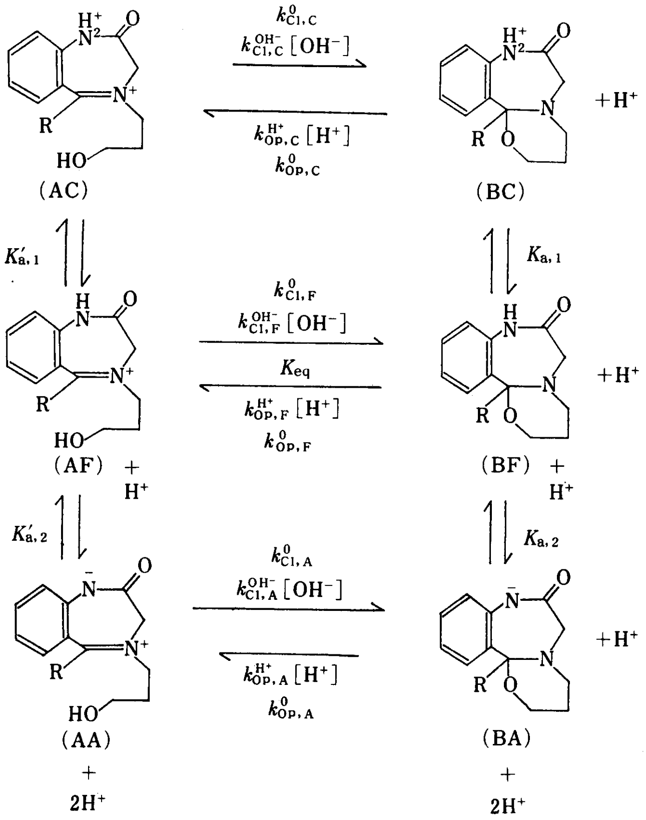


Chart 2

TABLE II. Estimated Rate Constants and Equilibrium Constants[a]

Compound	$k^{\mathrm{H}^+}_{\mathrm{Op,F}}\ \mathrm{s}^{-1}\mathrm{M}^{-1}$	$k^0_{\mathrm{Op,F}}+k^0_{\mathrm{Cl,F}}\ \mathrm{s}^{-1}$	$k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}\ \mathrm{s}^{-1}\mathrm{M}^{-1}$	$k^0_{\mathrm{Cl,A}}\ \mathrm{s}^{-1}$	$k^{\mathrm{OH}^-}_{\mathrm{Cl,A}}\ \mathrm{s}^{-1}\mathrm{M}^{-1}$	$\mathrm{p}K'_{\mathrm{a},2}$	$\mathrm{p}K_{\mathrm{a},2}$	$\mathrm{p}K^{\mathrm{UV}}_{\mathrm{eq}}$
1	2.23×10^6	1.87	3.72×10^7	0	1.43×10^5	8.79	13.33	5.95
2	c.n.d.[b]	c.n.d.[b]	2.04×10^5	0	2.80×10^1	9.58	c.n.d.[b]	8.37
3	2.10×10^6	0	1.40×10^4	0	1.20×10^1	9.60	c.n.d.[b]	8.59
4	2.14×10^6	0	1.39×10^4	—[c]	—[c]	—[c]	—[c]	8.51
5	7.33×10^5	0	1.85×10^4	0	2.93×10^1	8.95	c.n.d.[b]	8.00
6	1.45×10^6	7.16	5.87×10^9	c.n.m.[d]	c.n.m.[d]	c.n.m.[d]	c.n.m.[d]	6.23
7	c.n.d.[b]	c.n.d.[b]	1.81×10^7	0	6.34×10^5	9.76	c.n.d.[b]	7.5
8	7.90×10^4	5.83×10^1	5.42×10^7	0	1.20×10^6	9.38	>12[e]	7.2

[a] At 25 °C; in the presence of 4% (v/v) ethanol. [b] c.n.d. means "could not determine" because of relatively fast subsequent hydrolysis. [c] The 8-nitrogen atom has no proton to be deprotonated. [d] c.n.m. means "could not measure" because of fast reaction. [e] The absorbance change due to the deprotonation of the 7-nitrogen atom was not observed.

calculated by applying the values in Table II to equations which were derived in a manner similar to that used for BZOLs.[2,4,6)] The following equations were used:

a) pH < *ca.* 4.0

$$k_{\mathrm{obs}}=\frac{[\mathrm{H}^+]}{K_{\mathrm{a},1}+[\mathrm{H}^+]}(k^{0}_{\mathrm{Op,C}}+k^{\mathrm{H}^+}_{\mathrm{Op,C}}[\mathrm{H}^+])\fallingdotseq\frac{k^{0}_{\mathrm{Op,C}}[\mathrm{H}^+]}{K_{\mathrm{a},1}+[\mathrm{H}^+]} \tag{1}$$

b) *ca.* 4.0 < pH < *ca.* 8.0

$$k_{\mathrm{obs}}=k^{\mathrm{H}^+}_{\mathrm{Op,F}}[\mathrm{H}^+]+k^{0}_{\mathrm{Op,F}}+k^{0}_{\mathrm{Cl,F}}+k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}[\mathrm{OH}^-] \tag{2}$$

c) pH > *ca.* 8.0

$$\begin{aligned}k_{\mathrm{obs}}&=(k^{0}_{\mathrm{Cl,F}}+k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}[\mathrm{OH}^-])f_{\mathrm{AF}}+(k^{0}_{\mathrm{Cl,A}}+k^{\mathrm{OH}^-}_{\mathrm{Cl,A}}[\mathrm{OH}^-])f_{\mathrm{AA}}\\&=\frac{1}{[\mathrm{H}^+]+K'_{\mathrm{a},2}}\{k^{0}_{\mathrm{Cl,F}}[\mathrm{H}^+]+k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}K_{\mathrm{W}}+k^{0}_{\mathrm{Cl,A}}K'_{\mathrm{a},2}\\&\quad+k^{\mathrm{OH}^-}_{\mathrm{Cl,A}}(K_{\mathrm{W}}/[\mathrm{H}^+])K'_{\mathrm{a},2}\}\end{aligned} \tag{3}$$

where f_{AF} and f_{AA} are the molar fractions of AF and AA, respectively. K_{W} is the ionic product of water and is assumed to be $1\times10^{-14}\mathrm{M}^2$. The observed data points fit well to the calculated lines. The plateau portion found in the acid region for compound **1** suggests the involvement of 8 nitrogen atom protonation in the ring-opening rate. From the analysis of this region (from Eq. 1) the values of $k^{0}_{\mathrm{Op,C}}$ and $\mathrm{p}K_{\mathrm{a},1}$ were estimated to be 1.50×10 ($\mathrm{s}^{-1}\mathrm{M}^{-1}$) and 3.83, respectively. The following characteristics can be read from the determined kinetic constants in Table II: (1) The $k^{\mathrm{H}^+}_{\mathrm{Op,F}}$ value (ring-opening reaction) for **1** is similar to that for **6** and the $k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}$ value (ring-closing reaction) for **1** is about 1/200 of that for **6**. (2) Although the $k^{\mathrm{H}^+}_{\mathrm{Op,F}}$ values for **1**—**4** are almost the same, the values of $k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}$ decrease with the increase in the bulkiness of 12b-substituents. (3) The substituens at 8- and 11-positions do not influence the $k^{\mathrm{H}^+}_{\mathrm{Op,F}}$ and $k^{\mathrm{OH}^-}_{\mathrm{Cl,F}}$ values (compare the results among compounds **3**—**5**).

These observations may be interpreted as follows. The ring-closure to oxazine is considered to be more difficult than that to oxazolidine because of the increased freedom of the moiety (2 or 3 carbon chain containing terminal oxygen atom). Also the bulkiness of 12b-groups may prevent the ring-closure.

The ring-opening reaction ($k^{\mathrm{H}^+}_{\mathrm{Op,F}}$) occurs through an approach of a proton to the lone pair of N atoms at 5-position. The reaction is almost independent of the substituents at 12b-positions, indicating that the proton may approach equally to the lone pair of N_5 atoms among compounds **1**—**5**. Under the conditions that the diazepine ring is in a fixed conformation, that is, that the C_6 atom is not inverted, the oxazine ring of BZINs adopts 4 possible conformations ($\mathrm{R}_{\mathrm{I-IV}}$) as shown in Chart 3. Since the carbon atom at 12b-position is chiral, *i.e.*, BZIN is asymmetric, only R-enantiomer is considered here. Construction of the Dreiden molecular model for BZINs lead the following characteristics in each conformation: R_{I} form, the substituent at 12b-position and the lone pair of N_5 are *gauche*, and the oxazine ring and diazepine ring are skewed with each other; R_{II} form, the substituent at 12b-position and the lone pair of N_5 are *gauche*, and the oxazine ring and diazepine ring are skewed with each other; the conformation $\mathrm{R}_{\mathrm{III}}$ cannot be formed for the diazepine ring, so this conformation is ignored; R_{IV} form, the substituent at 12b-position and the lone pair of N_5 is *anti*, and the oxazine ring and diazepine ring are approximately coplanar with each other. In the R_{IV} conformation the lone pair of N_5 is most sterically unhindered by 12b-substituent. The oxazine ring and diazepine ring in R_{IV} are almost coplanar and thus less crowded sterically in the vicinity of N_5. Therefore, the majority of the conformations may be R_{IV} in solution or, at least, the ring-opening reaction may occur through the conformation R_{IV}.

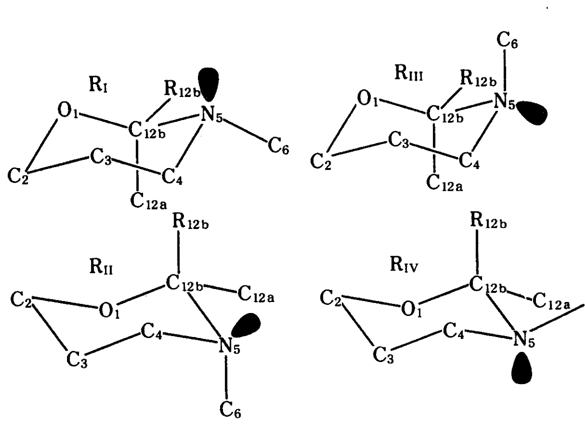


Chart 3

Hydrolyses of BZINs Similar to compounds **7** and **8**,[5,11)] hydrolyses of compounds **2** and **3** proceed through cleavage of N_5–$\mathrm{C}_{12\mathrm{b}}$ bond (cleavage of diazepine ring), and subsequent hydrolyses through cleavage of amide bond (C_7–N_8 bond of the original numbering). The cleavage of diazepine ring for **2** is about 10 times faster than that for **7** at neutral to alkaline regions.[5)] The cleavage for **3** is also faster than that for **8** by a factor of 10—100.[5)]

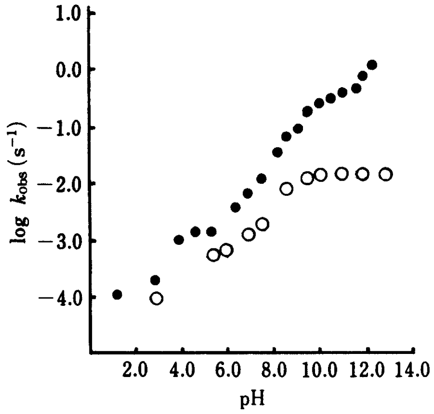


Fig. 3. The pH-Rate Profiles for the Hydrolyses of Compounds **3** and **4** at 25 °C

●, compound **3**; ○, compound **4**.

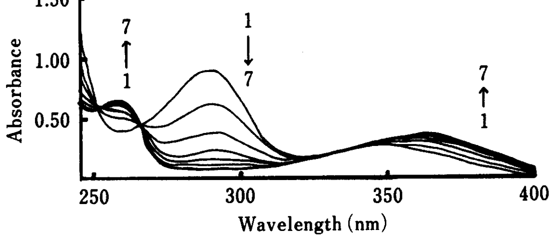


Fig. 4. Typical UV Spectral Changes for Hydrolysis of Compound **1** at pH 3.9 and 70 °C

Concentration of **1** was 1.00×10^{-4} M. 1, 0; 2, 2; 3, 5; 4, 9; 5, 15; 6, 21; 7, 47 d.

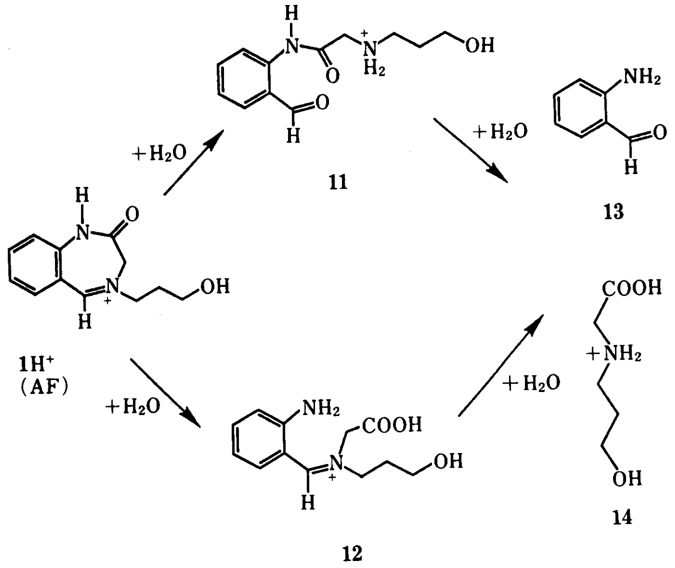


Chart 4

Figure 3 shows the pH-rate profiles for the cleavage of diazepine ring of compounds **3** and **4**. The shapes of the pH-profiles are very complicated. Although buffer concentration effects were not corrected, the shape of the profile may be a result of a reaction mechanism involving many molecular species, as shown in Chart 2, proton and hydroxide ion catalyses for each step, transition of the rate-determining step of the reaction,[13,14] and so on. The difference between the shapes at alkaline for compounds **3** and **4** may reflect the involvement of N_8 deprotonation of **3** in the cleavage of diazepine ring. Complete quantitative analyses of these pH-profiles are difficult at present because of the complexity of the system.

Since hydrolysis of compound **1** at 25 °C was very slow, the reaction was followed at 70 °C. Figure 4 shows the time course of the UV spectral changes for **1** in buffer solution. The initial and final pH of the reaction solution after cooling to 25 °C was the same at 3.9, *i.e.*, the pH of the solution seemed to be unchanged during the reaction. The pseudo first-order rate constant (k_{obs} in s^{-1}) of 2.32×10^{-6} was obtained from this spectral change. The spectral change shown in Fig. 4 is very similar to those for **6** in pH 2.0 at 70 °C, where the k_{obs} value for **6** was $4.97 \times 10^{-6}\ s^{-1}$.[6] The close values suggest that the reaction scheme for the hydrolysis of **1** is considered to be the same as that for **6**. As shown in Chart 4, two branching routes of hydrolysis of AF are now proposed.[6] The hydrolysis of **1**, therefore, proceeds *via* the intermediate **12** rather than **11** which is formed first in compounds **2**—**5**, and both result in the ultimate formation of **13** and **14**.

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References and Notes

1) This report constitutes Part XVII of the series entitled "The Behavior of 1,4-Benzodiazepine Drugs in Acidic Media," Part XVI; T. Kuwayama, S. Kato, Y. Kurono, K. Hatano, T. Hayazaki, T. Yashiro, and K. Ikeda, *Yakugaku Zasshi*, **110**, 764 (1990).
2) Y. Kurono, T. Kuwayama, K. Kamiya, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **33**, 1633 (1985).
3) Y. Kurono, K. Kamiya, T. Kuwayama, Y. Jinno, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **35**, 3831 (1987).
4) Y. Kurono, T. Kuwayama, Y. Jinno, K. Kamiya, E. Yamada, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **36**, 732 (1988).
5) Y. Kurono, Y. Jinno, T. Kuwayama, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **36**, 2582 (1988).
6) Y. Kurono, H. Sawabe, T. Kuwayama, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **38**, 193 (1990).
7) M. E. Deriege, J. V. Earley, R. I. Fryer, R. J. Lopresti, R. M. Schweiniger, L. H. Sternach, and H. Wharton, *Tetrahedron*, **27**, 2519 (1971).
8) T. Miyadera, A. Terada, M. Fukunaga, Y. Kuwano, T. Kamioka, C. Tamura, H. Takagi, and R. Tachikawa, *J. Med. Chem.*, **14**, 520 (1971).
9) T. L. Lemke and A. R. Hanze, *J. Heterocycl. Chem.*, **8**, 125 (1973).
10) E. A. Guggenheim, *Phil. Mag.*, **2**, 538 (1926) [A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., Wiley International, New York, 1961, p. 49].
11) T. Kuwayama, Y. Kurono, T. Muramatsu, T. Yashiro, and K. Ikeda, *Chem. Pharm. Bull.*, **34**, 320 (1986).
12) G. N. Walker, A. R. Engle, and R. J. Kempton, *J. Org. Chem.*, **37**, 3755 (1972).
13) E. M. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 832 (1962).
14) E. M. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **85**, 2843 (1963).