Kinetics and Mechanism of Tautomerism of a Hydroxy Schiff Base, *N*-[2-{2-Hydroxyethylimino(methyl)methyl}phenyl]-2-chlorpropamide, in Solution[1)]

Yukihisa KURONO,*[,a] Hiroshi TAMAKI,[a] Yasunari YOKOTA,[a] Mitsuyasu IDA,[a] Chiaki SUGIMOTO,[a] Tomonari KUWAYAMA,[b] Ken IKEDA,[a] and Tamotsu YASHIRO[a]

Faculty of Pharmaceutical Sciences, Nagoya City University,[a] 3–1 Tanabe-dori, Mizuho-ku, Nagoya 467, Japan and Pharmacy, NTT Tokai General Hospital,[b] 2–17–5 Matsubara, Naka-ku, Nagoya 460, Japan.
Received July 26, 1993; accepted October 1, 1993

The tautomerism between a hydroxy Schiff base (I), the titled compound, and the corresponding ring-closed oxazolidine (II) was kinetically studied in solution. In chloroform, the rates of tautomerism (I $\underset{k_2}{\overset{k_1}{\rightleftarrows}}$ II) were determined using NMR and UV spectroscopies, and both methods gave the almost identical pseudo first-order rate constants ($k_{obs}=k_1+k_2$) of 5.4×10^{-4} s^{-1}. The ratio of I to II at equilibrium, estimated by an NMR method in chloroform-d_1, was 1:1 and 7:1 in methanol-d_4.

The molecular species of compound I in various pH buffers were deduced by NMR, UV and other spectroscopies. In an acid solution (*e.g.*, pH 3.0) compound I existed as the protonated Schiff base (IH^+) at the imine nitrogen atom, and in the alkaline region (*e.g.*, pH 9.0) as the oxazolidine form II. The tautomerism rates in the aqueous solutions were measured by a pH-jump method using a stopped-flow apparatus. The rate (I $\rightleftarrows$ II) in the alkaline region was faster than that ($IH^+ \rightleftarrows IIH^+$) in the acid region, where IIH^+ represents an N-protonated oxazolidine form of II.

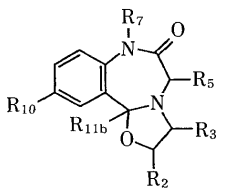
Keywords tautomerism; hydroxy Schiff base; oxazolidine; kinetics; benzodiazepinooxazole; stopped-flow

The degradation kinetics and mechanisms of benzodiazepinooxazoles (BDOZs, see Chart 1) have been investigated in terms of drug behavior after oral administration. The oxazolidine ring-opening and ring-closing (acid-base equilibrium) reactions[2–6)] and hydrolysis[7–10)] have been examined in aqueous solutions. The *cis*/*trans* isomerization (referring to the substituents at the 11b-position and 2- or 3-position) in organic solvents[11,12)] and the proton exchange reactions of the 11b-methyl group with methanol-d_4[12,13)] have also been studied mainly by NMR spectroscopy. In the course of the synthesis of 5,11b-dimethyl-BDOZ, compound I (see Chart 1) was isolated from the reaction mixtures. When the kinetic behaviors of compound I were examined in order to compare them with those of BDOZs previously reported,[5,6,10–13)] compound I was found to exist in equilibrium (tautomerism) with an oxazolidine (II, see Chart 2) in solution. In aqueous solution, there has been no kinetic study of the tautomerism of the hydroxy Schiff base. In this paper, we describe the kinetics and mechanism of the tautomerism in chloroform and aqueous solutions.

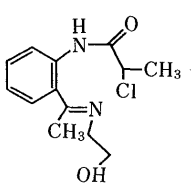
Experimental

Instruments ^{1}H- and ^{13}C-NMR spectra were recorded using a JEOL JNM GSX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard. IR spectra were recorded using a Perkin-Elmer FTIR-1600 spectrometer as a KBr pellet. A crystal was examined using an Enraf-Nonius CAD4 Kappa goniometer using MoK_α radiation.[14)] Ultraviolet (UV) spectra were measured with Shimadzu UV-260 and UV-2200 spectrophotometers. A stopped-flow spectrophotometer (Otsuka Denshi RA-401) was used for measuring the reaction rates and also the absorbance changes due to the tautomerism. The analyses for the rates were performed directly using an NEC microcomputer (PC-9801E) linked to the stopped-flow instruments.[5,6)]

Materials Compound I (*N*-[2-{2-hydroxyethylimino-(methyl)-methyl}phenyl]-2-chlorpropamide) was synthesized from *N*-(2-acetyl-



BDOZ



I

Chart 1

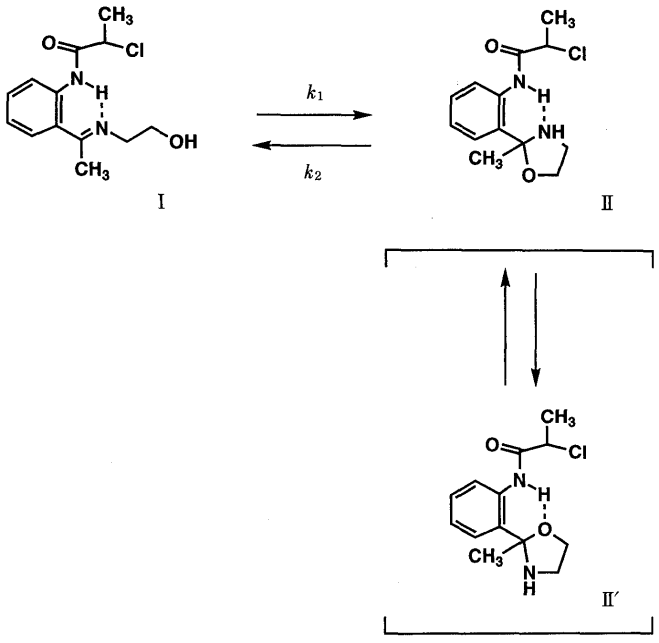


Chart 2

phenyl)2-chlorpropamide and 2-aminoethanol. The procedures were similar to those reported by Deriege *et al.*,[15] Miyadera *et al.*,[16] and Lemke and Hanze.[17] Compound I was recrystallized from ethanol. The chemical structure was confirmed by elemental analysis, IR spectroscopy, ^{1}H- and ^{13}C-NMR spectroscopies, and X-ray crystallography.

I: mp 97—98 °C. *Anal.* Calcd for $C_{13}H_{17}ClN_2O_2$: C, 58.10; H, 6.38; N, 10.42. Found: C, 58.03; H, 6.40; N, 10.43. IR ν_{max}^{KBr} cm^{-1}: 3482 (OH), 3342 (NH), 1676 (NCO), 1654 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 13.40 (1H, br s, NH), 8.59 (1H, d, ArH_6), 7.40 (1H, t, ArH_4 or H_5, J=8.0 Hz), 7.13 (1H, t, ArH_4 or H_5, J=8.0 Hz), 7.63 (1H, d, ArH_3, J=8.0 Hz), 4.54 (1H, q, –CHCl–, J=6.0 Hz), 4.04 (2H, t, OCH_2, J=5.0 Hz), 3.72 (2H, t, NCH_2, J=5.0 Hz), 2.37 (3H, s, CH_3=), 1.77 (3H, d, CH_3CHCl, J=6.0 Hz), 2.04 (1H, br s, OH). X-Ray crystallography indicated a hydrogen bond between the amide proton and the imine nitrogen for compound I (detailed data will be shown elsewhere).

Compound II in solution was identified by ^{13}C-NMR spectroscopy. Signals for the carbon atom at the 2-position of the oxazolidine ring were found at 96.5 ppm[18] and 97.3 ppm in $CDCl_3$ and methanol-d_4, respectively.

All other chemicals were commercially purchased and were of reagent grade.

Kinetic Measurements in Organic Solvents The tautomerism rates of compound I were measured in $CDCl_3$ by monitoring the methyl protons; *i.e.*, the integrated areas of the methyl signals due to the Schiff base (I) and oxazolidine (II) forms were compared with the aromatic hydrogen signal area as a functiin of time. The pseudo first-order rate constant (k_{obs}) for the tautomerism was calculated using Eq. 1[12]

$$\log|A-A_\infty| = -k_{obs}t/2.303 + \text{const.} \quad (1)$$

where A and A_∞ are the percentage of the Schiff base or oxazolidine formed at time t and at infinity, respectively.

The tautomerism rate in $CHCl_3$ was also measured by UV spectroscopy.

Kinetic Runs in Aqueous Solution The buffer systems were the same as those used in previous studied.[5,6,10] The reaction medium contained 4% (v/v) ethanol unless otherwise noted. The tautomerism rates were measured by the pH-jump method using the stopped-flow instrument as previously reported.[5,6] The reaction was, for example, carried out by the rapid mixing of an appropriate acid buffer and a diluted basic buffer (pH *ca.* 9) containing compound I (2.00×10^{-4} M) and 8% (v/v) ethanol at a volume ratio of 1 : 1. The diluted compound I solution had been prepared from an ethanol stock solution of compound I (2.50×10^{-3} M).[19] The pseudo first-order rate constants (k_{obs}) for the reactions were determined by ordinary first-order analysis and also by the Guggenheim method in the case where the reaction endpoint is unknown.

Determination of Equilibrium Constant The apparent equilibrium constant (K_{eq}^{UV}) of compound I was spectrophotometrically determined based on Eq. 2.[5,6]

$$\log((A-A_B)/(A_A-A)) = pK_{eq}^{UV} - pH \quad (2)$$

where A_A, A_B, and A are the absorbances at an appropriate wavelength for the acid form, base form, and their mixture, respectively.

Results and Discussion

Tautomerism between Schiff Base (I) and Oxazolidine (II) in Organic Solvents Figure 1 shows the time course of changes in proton signals measured for compound I in chloroform-d_1 at 27 °C. The spectra were recorded after dissolving the crystals of compound I. The singlets at 2.37 and 1.67 ppm are assigned to methyl groups on the imine carbon atom of compound I and on the oxazolidine ring, respectively.[13,20,21] The signals at 13.5 and 11.8 ppm and assigned to amide protons on I and II, respectively. In solution, therefore, compound I exists as the tautomer of the oxazolidine, as shown in Chart 2. The imine methyl signal at 2.37 ppm and the amide proton signal at 13.5 ppm decreased in intensity while the oxazolidine methyl signal at 1.67 ppm and the amide proton signal at 11.8 ppm increased. At equilibrium, the ratio of Schiff base I to the oxazolidine form II is approximately unity.

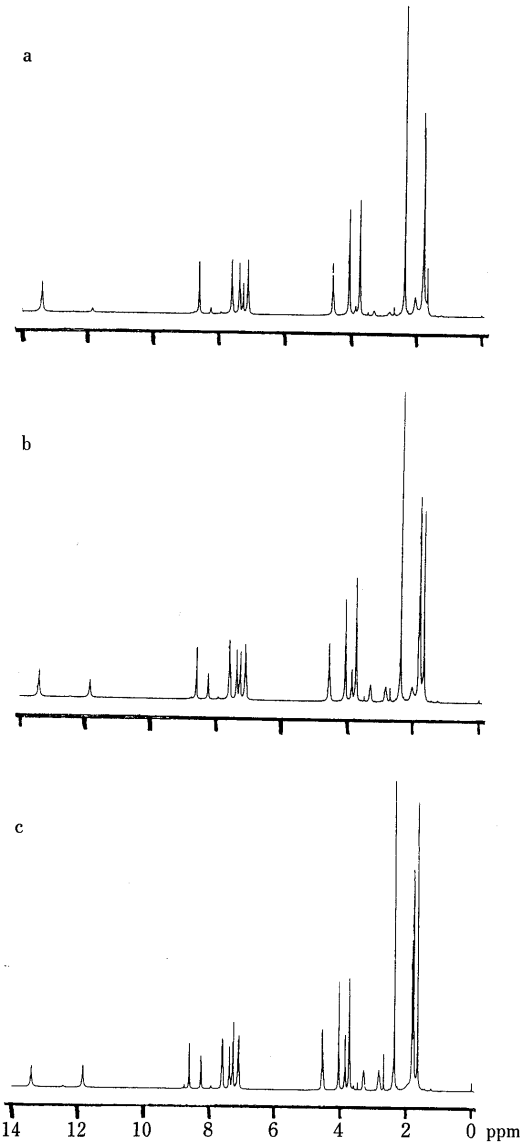


Fig. 1. ^{1}H-NMR Spectral Changes of Compound I in $CDCl_3$ as a Function of Time at 27°C

a, 0 min; b, 40 min; c, 1 d.

The pseudo first-order rate constants (k_{obs}) for this tautomerism were determined according to Eq. 1. According to Chart 2, K_{obs} is expressed by Eq. 3.[11,12]

$$k_{obs} = k_1 + k_2 \quad (3)$$

The equilibrium constant, K_T, is defined by Eq. 4.

$$K_T = [I]_{eq}/[II]_{eq} = k_2/k_1 \quad (4)$$

From Eqs. 3 and 4, the individual rate constants were calculated and then listed in Table I.

To confirm the rate constants for the tautomerism and to examine the difference in UV spectra between I and II, the reaction was also followed by UV spectroscopy. Figure 2 shows the time course of spectral changes for the tautomerism.

Spectrum 1 in this figure is due to compound I, and spectrum 6 is due to the approximate 1 : 1 mixture of I and II. The k_{obs} (in s^{-1}) value of 5.45×10^{-4} ($t_{1/2}$ = 1.26×10^3 s) was obtained from these spectral changes,

TABLE I. The Rate Constants for Tautomerism of Compound I in Chloroform

Spectroscopy	$k_{obs} \times 10^4$ (s^{-1})	$k_1 \times 10^4$ (s^{-1})	$k_2 \times 10^4$ (s^{-1})
NMR from 13.5 and 11.8 ppm[a)]	5.6	2.8	2.8
NMR from 2.37 and 1.67 ppm[a)]	5.1	2.6	2.6
UV[b)]	5.45	2.73[c)]	2.73[c)]

a) At 27 °C. *b*) At 25 °C. *c*) K_T was assumed to be 1.

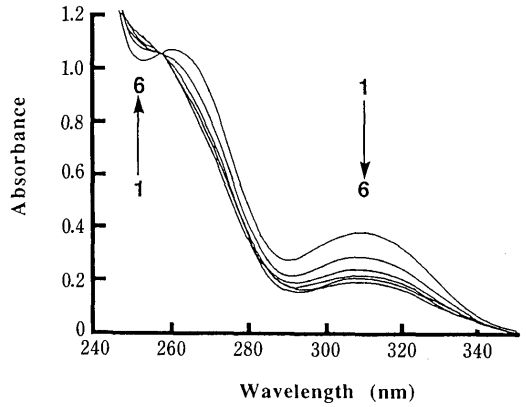


Fig. 2. UV Spectral Changes for Tautomerism (I⇄II) in $CHCl_3$ at 25 °C

The concentration of compound I was 1.00×10^{-4} M. Spectrum 1, 0 min; 2, 20 min; 3, 40 min; 4, 60 min; 5, 80 min; 6, 180 min.

which is almost equal with those obtained from ^{1}H-NMR spectroscopy.

In methanol-d_4, the ratio of I to II measured by ^{1}H-NMR spectroscopy was about 7 : 1, and the tautomerism seemed to occur quickly (that is, the isomers were at equilibrium (7 : 1) soon after the dissolution of I in methanol-d_4 for the rate measurements). The ratio and rate appear to significantly depend on the solvents. The difference in the ratio of I to II between methanol-d_4 (7 : 1) and chloroform-d_1 (1 : 1) may be explained as follows. The intensity of the hydrogen bond for compound I is larger than that for compound II because of the flatter structure of the six-membered ring of I than of II. In methanol-d_4 (protic solvent), the hydrogen bond for compound II may be broken by the solvent, leading to an increase in the ratio of I to II. In the non-polar solvent (chloroform-d_1), on the other hand, the hydrogen bond for compound II still exists,[18] resulting in the ratio of 1 : 1.

Equilibrium of Compound I in Aqueous Solution Figure 3 shows the UV spectra of compound I in various pH buffer solutions. There are two isosbestic points at about 245 and 260 nm. In order to deduce the molecular species of compound I in the aqueous solutions from the spectra shown in Fig. 3, UV spectra of compound I in various solvents, whose species had been already identified by NMR spectroscopy and the other methods, were measured, and are illustrated in Fig. 4. The explanations of the species corresponding to each spectrum are given in the caption to Fig. 4. Spectrum 4 was calculated according to Eq. 5.

$$A = 2(A_\infty - 0.5A_0) \quad (5)$$

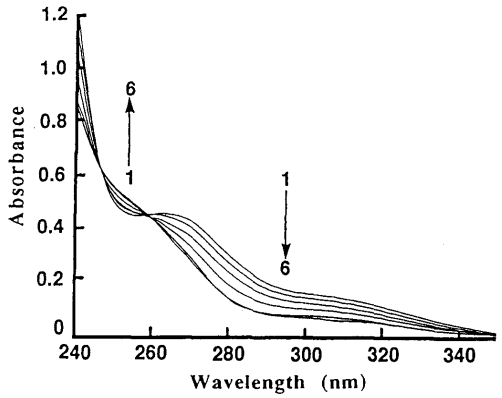


Fig. 3. UV Spectra of Compound I at Various pH Values

The concentration of Compound I was 1.00×10^{-4} M. Spectrum 1, pH 3.0; 2, pH 5.5; 3, pH 6.0; 4, pH 6.5; 5, pH 8.0; 6, pH 9.0.

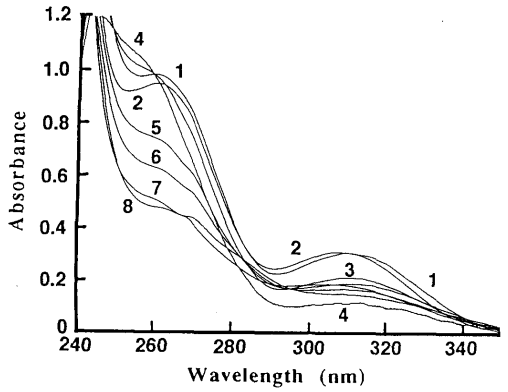


Fig. 4. UV Spectra of Various Species of Compound I in Various Solvents

Concentration of compound I was 1.00×10^{-4} M. Spectrum 1, I in $CHCl_3$; 2, I : II = 7 : 1 in methanol; 3, I : II = 1 : 1 in $CHCl_3$; 4, II in $CHCl_3$ (calculated by Eq. 5 using spectra 1 and 3); 5, I in $CHCl_3$ containing 5.0×10^{-4} M trifluoroacetic acid (TFA); 6, I in $CHCl_3$ containing 1.0×10^{-3} M TFA; 7, I in $CHCl_3$ containing 1.0×10^{-2} M TFA; 8, I in methanol containing 1.0×10^{-4} M TFA.

where A_∞ and A_0 are the absorbances at each wavelength due to the species at the equilibrium state (I : II = 1 : 1) and at time zero (I alone) in chloroform, respectively. Spectrum 4, constructed from the absorbance A, is therefore considered to be due to II alone in chloroform.[22]

The absorbances at around 320 nm decrease with a decrease in the ratio of I to II (an increase in the concentration of II) as observed from spectra 1 through 4 in Fig. 4. In Fig. 3, since the absorbance at 320 nm at pH 3.0 is larger than that at pH 9.0, spectrum 1 at pH 3.0 consequently seems to be due to the Schiff base I and spectrum 6 at pH 9.0 to the oxazolidine form II.

Comparing spectra 1, 5, 6 and 7 in Fig. 4, the absorbances at 260 nm due to I in chloroform decreased with an increase in the concentration of trifluoroacetic acid (TFA). The increase in TFA concentration is considered to lead to protonation at the nitrogen atom of the imine bond of compound I, causing a decrease in absorbance at around 260 nm. From these considerations of the UV spectra shown in Figs. 3 and 4, the overall species of compound I in the aqueous solutions are proposed to be those shown in Chart 3. In an acid solution, compound I exists as IH^+, and in an alkaline solu-

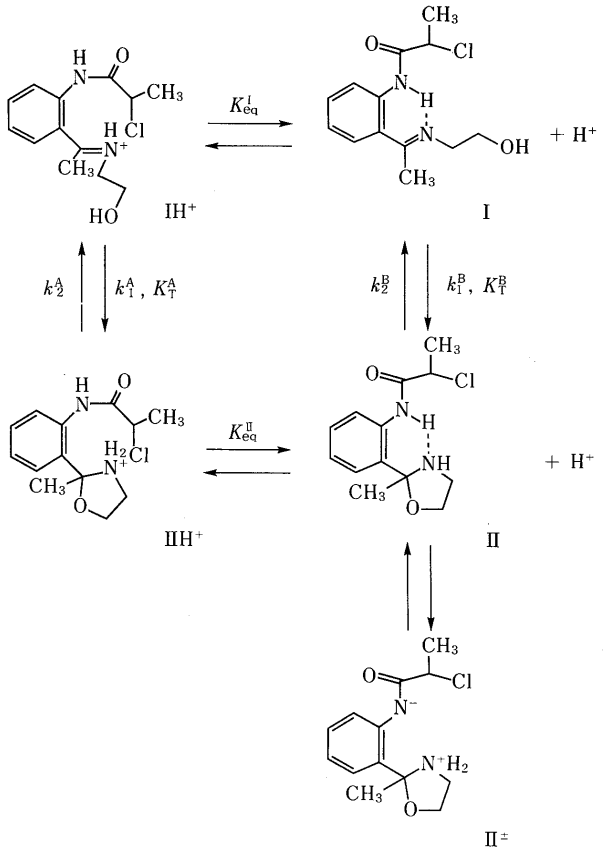


Chart 3

tion as II. Structure $II^{\pm}$ was considered to be the limiting-formula of II, in order to explain why the absorbance at around 260 nm at pH 9.0 in Fig. 3 is smaller than that in spectrum 4 (II in chloroform) in Fig. 4 (*i.e.*, because of the necessity for the protonation of the nitrogen atom).

The UV spectra in Fig. 3 are attributable to the equilibrium mixtures, as shown in Chart 3. From the spectral data, the apparent pK_{eq}^{UV} value ($-\log(([I]+[II]$ (or $[II^{\pm}]))[H^+]/([IH^+]+[IIH^+])$) was determined to be 6.06 using Eq. 2.

Tautomerism Rate of Compound I in Aqueous Solution

When the rate of absorbance change at 277 nm was measured by the pH-jump method (*e.g.*, from pH 9 to 3.0) using the stopped-flow apparatus, the observed incremental change in the absorbance (0.02) was only about 10% of those (0.40—0.20=0.20) shown in Fig. 3. The decrement in the absorbance (0.02) by the pH-jump from pH 3 to 9.0 also was about 10% of the absorbance change (0.20) illustrated in Fig. 3. The deficiency in the absorbance changes measured by the stopped-flow method is proposed to be as follows. The rates of the processes $IH^+ \rightleftarrows H^+ + I$ and $IIH^+ \rightleftarrows H^+ + II$ are too fast to measure using the stopped-flow method because of the protonation and deprotonation rates.[5,18] The absorbance changes measured by the pH-jump method, therefore, were due to the processes of the tautomerism ($I \rightleftarrows II$ and/or $IH^+ \rightleftarrows IIH^+$). The equilibrium constant for the tautomerism at pH 3.0 ($K_T^A=[IH^+]/[IIH^+]=k_2^A/k_1^A$ in Chart 3) is probably larger than that at pH 9.0 ($K_T^B=[I]/[II]=k_2^B/k_1^B$), because the pH-jump from pH 9 to 3.0 increases the absorbance ($IIH^+ \rightleftarrows IH^+$), and that from pH 3 to 9.0 decreases the absorbance ($I \rightleftarrows II$).

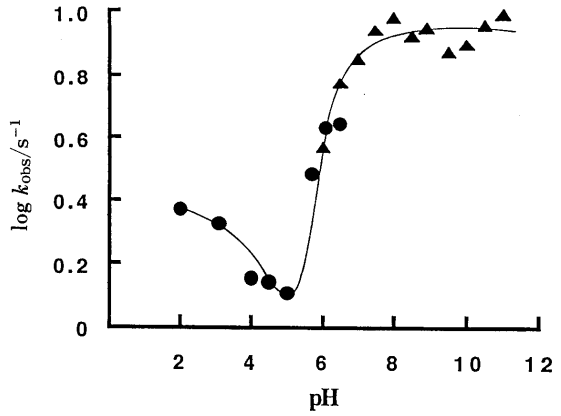


Fig. 5. The pH-Rate Profile for Tautomerism of Compound I at 25°C

●, pH-jump from pH 9 to the respective pH buffer; ▲, pH-jump from pH 3 to the respective pH buffer.

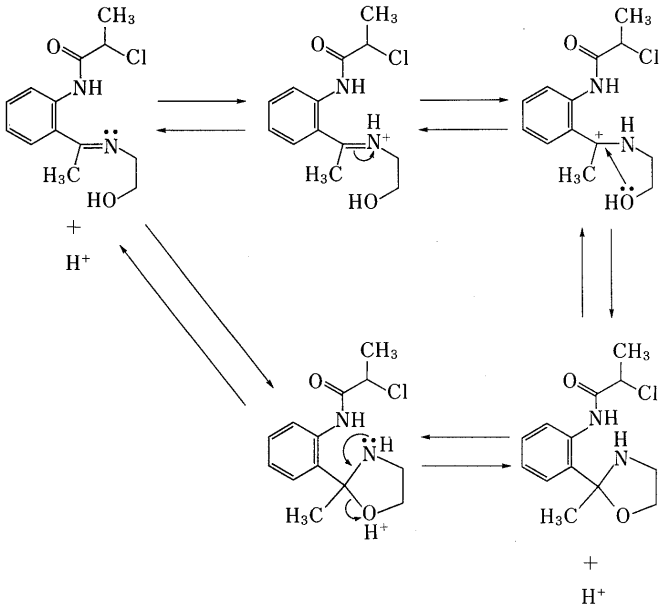


Chart 4

Figure 5 shows the pH-rate profile for the tautomerism of compound I obtained from such the pH-jump method. The tautomerism in the alkaline region ($k_1^B+k_2^B$) is slightly faster than that in the acid region ($k_1^A+k_2^A$). In the strongly acidic region, a specific acid catalysis seems to be involved in the tautomerism.

The rates of the tautomerism of compound I in protic solvents (water and methanol) were much larger than in an aprotic solvent (chloroform). This may be due to a cationic intermediate ($=N^+H-$) involved in the reaction produced by the addition of a proton of the imine nitrogen atom, as shown in Chart 4.

As described above, the reactions and their kinetic behaviors involved in compound I are quite different from those in the benzodiazepinooxazoles previously reported,[5,6,10-13] because compound I has no diazepine ring in its molecule.

Acknowledgments We wish to thank Prof. T. Yotsuyanagi, Depart-

ment of Pharmaceutics, Nagoya City University, for the reading of this manuscript, and also Mr. A. Matsuura, Director of the Department of Pharmacy, NTT Tokai General Hospital, for his encouragement.

References and Notes

1) This report constitutes Part XX of the series entitled "The Behavior of 1,4-Benzodiazepine Drugs in Acidic Media." Part XIX: Y. Kurono, S. Kato, T. Kuwayama, H. Tamaki, K. Hatano, T. Yashiro, K. Ikeda, H. Bundgaard, *Int. J. Pharmaceut.*, **81**, 179 (1992).
2) T. H. Fife, J. E. C. Hutchins, *J. Org. Chem.*, **45**, 2099 (1980).
3) R. A. McClelland, R. Somani, *J. Org. Chem.*, **46**, 4345 (1981).
4) M. Ikeda, T. Nagai, *Chem. Pharm. Bull.*, **30**, 3810 (1982).
5) Y. Kurono, T. Kuwayama, Y. Jinno, K. Kamiya, E. Yamada, T. Yashiro, K. Ikeda, *Chem. Pharm. Bull.*, **36**, 732 (1988).
6) Y. Kurono, H. Tamaki, T. Kuwayama, T. Ichii, H. Sawabe, T. Yashiro, K. Ikeda, H. Bundgaard, *Int. J. Pharmaceut.*, **80**, 135 (1992).
7) H. Bundgaard, M. Johansen, *Int. J. Pharmaceut.*, **10**, 165 (1982).
8) M. Johansen, H. Bundgaard, *J. Pharm. Sci.*, **72**, 1294 (1983).
9) M. Ikeda, T. Nagai, *Chem. Pharm. Bull.*, **32**, 1080 (1984).
10) Y. Kurono, Y. Jinno, T. Kuwayama, T. Yashiro, K. Ikeda, *Chem. Pharm. Bull.*, **36**, 2582 (1988).
11) Y. Kurono, Y. Jinno, T. Kuwayama, N. Satoh, T. Yashiro, K. Ikeda, *Chem. Pharm. Bull.*, **37**, 1044 (1989).
12) Y. Kurono, S. Kato, T. Kuwayama, H. Tamaki, K. Hatano, T. Yashiro, K. Ikeda, H. Bundgaard, *Int. J. Pharmaceut.*, **81**, 179 (1992).
13) T. Kuwayama, Y. Kurono, H. Sawabe, S. Kato, T. Yashiro, K. Ikeda, *Yakugaku Zasshi*, **110**, 133 (1990).
14) K. Hatano, Y. Kurono, T. Kuwayama, H. Tamaki, T. Yashiro, K. Ikeda, *J. Chem. Soc., Perkin Trans.* 2, **1992**, 621.
15) M. E. Deriege, J. V. Earley, R. I. Fryer, R. J. Lopresti, R. M. Schweiniger, L. H. Sternach, H. Wharton, *Tetrahedron*, **27**, 2591 (1971).
16) T. Miyadera, A. Terada, M. Fukunaga, Y. Kuwano, T. Kamioka, C. Tamura, H. Takagi, R. Tachikawa, *J. Med. Chem.*, **14**, 520 (1971).
17) T. L. Lemke, A. R. Hanze, *J. Heterocycl. Chem.*, **8**, 125 (1973).
18) In $CDCl_3$, precisely, two signals based on the carbon atom at the 2-position of the oxazolidine ring are observed at 96.53 and 96.58 ppm with an approximately equal intensity. The two carbon atoms (signals) probably result from hydrogen bonds of the amide proton with the nitrogen atom of the oxazolidine ring and also with the oxygen atom of the ring (see Chart 2). Compound II: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 11.83 (1H, br s, NH), 1.66, 1.67 (3H, 2s, ) 1.81, 1.84 (3H, 2d, CH_3CHCl).
19) Y. Kurono, T. Kuwayama, K. Kamiya, T. Yashiro, K. Ikeda, *Chem. Pharm. Bull.*, **33**, 1633 (1985).
20) T. Kuwayama, S. Kato, Y. Kurono, T. Yashiro, K. Ikeda, *Yakugaku Zasshi*, **108**, 641 (1988).
21) M. E. A. Astudillo, N. C. J. Chokotho, T. C. Jarvis, C. D. Johnson, C. C. Lewis, P. D. McDonnell, *Tetrahedron*, **41**, 5919 (1985).
22) These calculations were done directly using a computer linked to the Shimadzu UV-2200 spectrophotometer.