Kinetics and Mechanism of the Acid–Base Equilibrium and the Subsequent Hydrolysis of 11b-Hydrogenbenzodiazepinooxazoles[1)]

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Oxazolidine ring-opening and ring-closing reactions of 11b-hydrogen(11b-nonsubstituted)benzodiazepinooxazoles (HBDOZ) and the subsequent hydrolysis of the diazepine ring were studied kinetically. The difference in the reactivities of the oxazolidine ring between *cis* and *trans* isomers (referring to substituents at the 2- and 11b-positions) for HBDOZs is small, compared with those for BDOZs having 11b-methyl (MBDOZ) and 11b-phenyl groups (PBDOZ) reported previously. The reactions of the oxazolidine ring for HBDOZ occur about 10 times faster than those for MBDOZ and PBDOZ. For HBDOZ, hydrolysis of the amide bond of the diazepine ring takes place rather than that of the iminium bond, in contrast to the cases of MBDOZs and PBDOZs.

Keywords Benzodiazepinooxazole; acid–base equilibrium; hydrolysis; kinetics; oxazolidine ring-opening, -closing; *cis–trans* isomer; diazepine ring; reaction pathway; iminium bond; amide bond

The acid–base equilibrium (oxazolidine ring-opening and ring-closing reactions) of benzodiazepinooxazoles (BDOZs) and the subsequent hydrolysis of the diazepine ring have been investigated kinetically from the standpoint of drug behavior after oral administration.[2-5)] The ring-opening and ring-closing reactions of the *cis* isomer (referring to the substituents at the 11b-position (methyl or phenyl group) and at the 2-position (methyl, ethyl or phenyl group)) are faster than those of the *trans* isomer.[2-4)] While hydrolysis of BDOZ having an 11b-phenyl group (PBDOZ) is irreversible, that of BDOZ possessing an 11b-methyl group (MBDOZ) is reversible.[5)] These reactivities of BDOZs seem to be greatly affected by the substituent at the 11b-position. It is of interest, therefore, to examine the reactivities of BDOZs having hydrogen alone at the 11b-position (see Table I). In this paper we describe the reactivities of 11b-hydrogen(11b-nonsubstituted)benzodiazepinooxazoles (HBDOZ) in comparison to those of MBDOZ and PBDOZ.

Experimental

Materials and Instruments Compounds **1**—**4** were synthesized by procedures similar to those reported by Deriege *et al.*,[6)] Miyadera *et al.*,[7)] and Lemke and Hanze.[8)] *N*-Acetyl-2-aminobenzaldehyde (**5**) was synthesized by acetylation of 2-aminobenzaldehyde-ethylene-diimine (**6**, Tokyo Kasei Kogyo, Tokyo) with acetic anhydride in dichloromethane and subsequent hydrolysis.[5,9,10)] The structures of these compounds were confirmed by the elemental analyses as well as proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) measurements. Melting points and the results of the elemental analyses are listed in Table I. Since isolation of hydrolyzates of compound **1** was difficult, compounds **5** and **6** were used as model compounds of them (see later). All other chemicals used were purchased commercially and were of reagent grade.

Ultraviolet (UV) spectra were measured with Shimadzu UV-260 and Hitachi UV-124 spectrophotometers. A stopped-flow spectrophotometer (Otsuka Denshi RA-401) was used for the measurement of the reaction rates. ^{1}H-NMR 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 the pH-rate profiles.

Kinetic Procedures The buffer systems were the same as those employed in the previous studies.[2-4)] The rates of the oxazolidine ring-opening and ring-closing were measured by the pH-jump method using stopped-flow instruments as reported previously.[2-4)] The pseudo first-order rate constants (k_{obs}) for the reactions were determined by the Guggenheim method[11)] for reactions having an unknown end point. These analyses were done directly by using a Sord microcomputer (M223 Mark III) linked to the stopped-flow instruments.

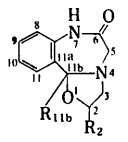
The hydrolysis of **1** was carried out at 70 °C. Aliquots of the sample solution were withdrawn at appropriate intervals and cooled to room temperature (25 °C). UV spectra of them were measured and compared with the spectra of the model compounds **5** and **6**.

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

Results and Discussion

Oxazolidine Ring-Opening and -Closing Reactions Fig-

TABLE I. Physical and Analytical Data for 11b-Hydrogenbenzodiazepinooxazoles



Compound	R_{11b}	R_2	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
1	H	H	166—168	Ethanol	$C_{11}H_{12}N_2O_2$	64.69	5.92	13.72	64.84	5.81	13.78
2	H	CH_3	174—176 (lit.[7)] 172—173)	Ethanol	$C_{12}H_{14}N_2O_2$	66.04	6.47	12.84	66.31	6.46	12.98
3	H	C_2H_5	147—149	Methanol–ether	$C_{13}H_{16}N_2O_2$	67.22	6.94	12.06	67.40	6.90	12.20
4	H	C_6H_5	173—175.5	Ethanol	$C_{17}H_{16}N_2O_2$	72.84	5.75	9.99	72.86	5.78	10.02
5[a)]	—	—	65—68	Ethanol	$C_9H_9NO_2$	65.78	5.56	8.41	66.24	5.57	8.59

a) Compound **5** is *N*-acetyl-2-aminobenzaldehyde and not HBDOZ.

ure 1 illustrates the pH-rate profiles for the oxazolidine ring-opening and ring-closing reactions of compound **2**. The two reaction steps (Fig. 1) (expressed as large rate constant k^{L}_{obs} and small constant k^{S}_{obs}) are considered, similarly to the cases of MBDOZ and PBDOZ reported previously,[2-4] to be due to the *cis* and *trans* isomers of **2** as shown in Chart 1.[12] In Chart 1, k_i indicates the respective first-order or second-order rate constants. Since the ratio of k^{L}_{obs} to k^{S}_{obs} seems to be less than 10, however, both k^{L}_{obs} and k^{S}_{obs} are hybrid rate constants consisting of all k_i values.[4,13,14] Only approximate analyses can thus be made on the oxazolidine ring reactivities for HBDOZs.

Table II lists the rate constants and equilibrium constants obtained from the pH-rate profiles by analytical procedures similar to those employed previously for oxazolam analogs.[2,4] The superscripts and subscripts of the rate constants in Table II 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.

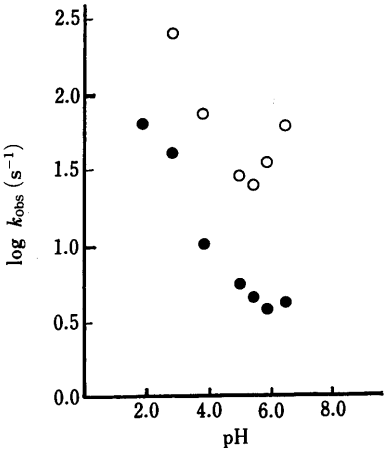


Fig. 1. The pH-Rate Profiles for Oxazolidine Ring-Opening and Ring-Closing Reactions of Compound **2** at 25°C

○, k^{L}_{obs}; ●, k^{S}_{obs}.

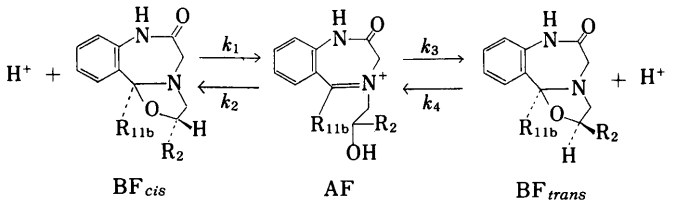


Chart 1

TABLE II. Estimated Rate Constants and Equilibrium Constants

Compound	$k^{H^+}_{Op,F}$ ($s^{-1}M^{-1}$)	$k^{0}_{Op,F}+k^{0}_{Cl,F}$ (s^{-1})	$k^{OH^-}_{Cl,F}$ ($s^{-1}M^{-1}$)	$k^{0}_{Cl,A}$ (s^{-1})	K^{UV}_{eq} (pK^{UV}_{eq}) (M)
1	1.45×10^{6}	7.16×10^{1}	5.87×10^{9}	8.83×10^{8}	5.89×10^{-7} (6.23)
2	5.00×10^{5}	1.39×10^{1}	2.50×10^{9}	3.12×10^{8}	1.74×10^{-6} (5.76)
	1.40×10^{5}				
3	4.59×10^{5}	1.45×10^{1}	2.82×10^{9}	1.00×10^{9}	1.74×10^{-6} (5.76)
	2.20×10^{5}				
4	4.43×10^{5}	2.63×10^{1}	6.61×10^{9}	5.96×10^{8}	2.69×10^{-6} (5.57)
	2.24×10^{5}				

The first subscript indicates whether ring-opening (Op) or ring-closing (Cl) occurs, and the second one represents the free form (F) at the 7 nitrogen atom of the compound or the anionic form (A). K^{UV}_{eq} is the apparent acid–base equilibrium constant (([BF_{cis}]+[BF_{trans}])[H^+]/[AF]) of the oxazolidine ring determined by UV spectroscopy. The rate constants in Table II allow us to reach the following conclusions. (a) There seem to be no systematic effects of the substituent at the 2-position on the rate constants. (b) The rate constants are about 10 times larger than those for MBDOZ and PBDOZ reported previously.[2,4] (c) The ratios of $k^{H^+}_{Op,F}$ for the *cis* isomer to that for the *trans* isomer are small (1.98 for **4** and 3.57 for **2**), compared with the corresponding ratios for MBDOZ and PBDOZ reported previously.[2,4]

These observations may be interpreted as follows. Since compounds **2**—**4** are diastereoisomers, they have four isomers, that is, 2*R*-*cis*, 2*R*-*trans*, 2*S*-*cis*, and 2*S*-*trans* isomers.[2,4,15-17] Each individual isomer is considered to have three possible conformations. Two conformations concern the relative arrangement of the oxazolidine ring and diazepine ring, *i.e.*, planar (conformation X) and skewed (conformation Y).[2,4,18] The conformation X is divided into two subgroups concerning the 11b-substituent and the lone pair of the N_4 atom, that is, the lone pair on the same side as the 11b-group (conformation X_I) and on the opposite side (conformation X_{II}). For the ring-opening reactions ($k^{H^+}_{Op,F}$) of MBDOZ and PBDOZ we proposed previously[2,4] that an approach of a proton to the lone pair resulting in ring-opening occurs mainly in conformation X_{II}. Because of the steric effect of the substituent at the 2-position, the *cis* isomer reacts faster than the *trans* isomer (see Refs. 2 and 4 for details). For HBDOZ (compound **1**—**4**) the ring-opening reaction may also occur in conformation X_I in addition to conformation X_{II} because of the small H atom at the 11b-position. The reactions due to both conformations (X_I and X_{II}) may be the reason for the 10-times larger rate constants, and also may account for the small difference in the $k^{H^+}_{Op,F}$ values among compounds **1**—**4** (small substituent effect at the 2-position). In the case of the

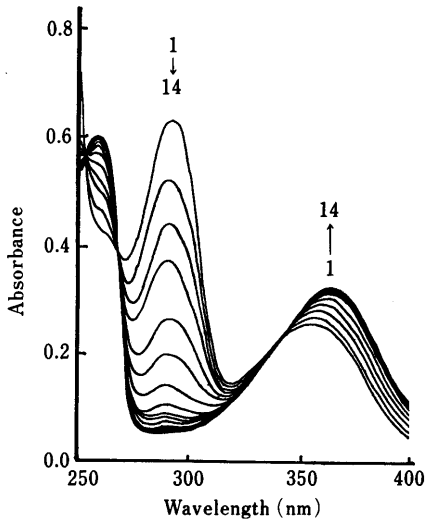


Fig. 2. Typical Spectral Changes for Hydrolysis of Compound **1** in Aqueous Buffer Solution (pH 2) at 70°C

1, 0; 2, 11; 3, 23; 4, 34; 5, 57; 6, 78; 7, 114; 8, 140; 9, 168; 10, 188; 11, 219; 12, 236; 13, 291; 14, 320 h. The concentration of **1** was 1.0×10^{-4} M.

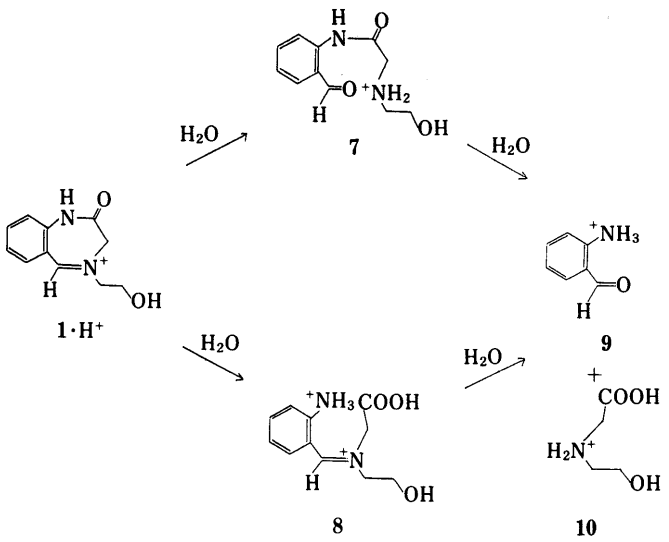


Chart 2

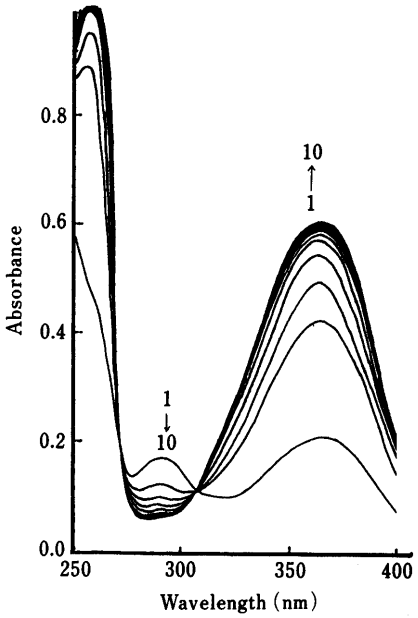


Fig. 3. Typical Spectral Changes for Hydrolysis of Compound **6** at pH 2.0 and 25°C

1, 0; 2, 1; 3, 2; 4, 3; 5, 4; 6, 5; 7, 6; 8, 10; 9, 11; 10, 12 h. The concentration of **6** was 1.0×10^{-4} M.

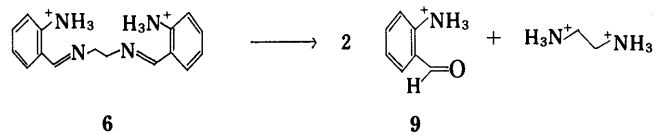


Chart 3

reaction due to conformation X_I, the *cis* isomer may react more slowly than the *trans* isomer because of the steric effect, leading consequently (overall) to the small difference in the $k^{H^+}_{Op,F}$ values between the *cis* and *trans* isomers.

Hydrolysis of 11b-Hydrogenbenzodiazepinooxazoles Since hydrolysis of HBDOZ at 25 °C was slow, the reaction was examined at 70 °C. Figure 2 shows successive UV spectra of compound **1** in buffer solution at 70 °C. The initial and final pH of the reaction solution after cooling to 25 °C was 2.0, that is, the pH of the solution seemed to be unchanged during the reaction. The first-order plot of the spectral changes in Fig. 2 is linear up to 5 half-lives, giving the rate constant $k_{obs} = 4.97 \times 10^{-7}\,s^{-1}$. The initial spectrum in Fig. 2 is due to the oxazolidine ring-opened structure (**1**·H$^+$). The final spectrum, however, seems to be due to 2-aminobenzaldehyde (**9**), in contrast to the case of **5**. For the interpretation of the spectral changes in Fig. 2, the reaction pathway shown in Chart 2 can be considered. To determine whether the reaction proceeds *via* intermediate **7** or **8**, the following experiments using the model compounds **5** and **6** were carried out. Compound **5**, which is the model compound of **7**, was stable and unchanged under experimental conditions similar to those used for Fig. 2. Figure 3 shows successive UV spectra of compound **6**, which reflect the reaction shown in Chart 3. The final spectrum in Fig. 3 was identical with the final one in Fig. 2. The k_{obs} value obtained from Fig. 3 is $1.49 \times 10^{-4}\,s^{-1}$ (25°C) which is much larger than $k_{obs} = 4.97 \times 10^{-6}\,s^{-1}$ (70 °C) obtained from Fig. 2. These results obtained with compounds **5** and **6** suggest that the reaction occurs *via* intermediate **8**; the reaction of **8**→**9** is faster than that of **1**·H$^+$→**8**, that is, the intermediate **8** is not accumulated in the reaction mixtures. The spectra in Fig. 2 are due to only compounds **1**·H$^+$, **9**, and **10** (negligible).

The ^{1}H- and ^{13}C-NMR spectra of the reaction mixtures started individually from **1** and **6** in 0.5 N DCl and at 25 °C supported the reaction pathway **1**·H$^+$→**8**→**9**+**10** shown in Chart 2. For HBDOZs, therefore, the iminium bond is more stable than the amide bond of the diazepine ring in the acidic region, leading to breakdown *via* intermediate **8** rather than intermediate **7**, in contrast to the cases of MBDOZs and PBDOZs.

Acknowledgments We are grateful to Mr. M. Saburi, Director of NTT Tokai General Hospital Pharmacy and to Miss S. Kato of our University for kind encouragement and NMR spectral measurements, respectively.

References and Notes

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12) Although the nomenclature of *cis* and *trans* becomes reversed when R_{11b}=H, we still adopt the terms for convenience of comparison of

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