[Chem. Pharm. Bull. 36(2) 732—740 (1988)]

Kinetics and Mechanism of the Acid–Base Equilibrium of Benzodiazepinooxazoles (Oxazolam Analogs)[1)]

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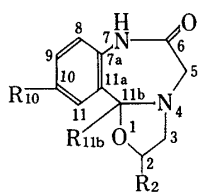
(Received July 13, 1987)

Oxazolidine ring-opening and ring-closing reactions of oxazolam analogs were investigated kinetically by a pH-jump method in order to elucidate the detailed reaction mechanism focusing on *cis* and *trans* isomers. Seven compounds having substituents (methyl, ethyl or phenyl group) at the 2- and 11b-positions were synthesized and examined. Coupled (parallel) reversible reactions due to the isomers were confirmed by analog computer analyses. The ring-opening and ring-closing reactions due to the *cis* isomer are both faster than those due to the *trans* isomer, although in the alkaline region, the ring-closing reaction due to the *trans* isomer could not be followed spectrophotometrically. The reason for the rate differences between the *cis* and *trans* isomers and the effects of the substituents on the intrinsic rate constants for the ring-opening and ring-closing reactions are discussed.

Keywords——benzodiazepinooxazole; oxazolam; *cis–trans* isomer; oxazolidine ring-opening, -closing; kinetics; pH-rate profile; acid–base equilibrium; analog computer simulation; coupled reversible reaction; pH-jump

The kinetics and mechanism of the acid–base equilibrium (oxazolidine ring-opening and ring-closing reactions) of oxazolam itself were reported previously.[2)] The ring-opening of the *cis* isomer (referring to the 2-methyl group and 11b-phenyl group) of oxazolam was faster than that of the *trans* isomer, and these rate measurements were valuable to distinguish between the *cis* and *trans* isomers. Such kinetic studies are also necessary to obtain an understanding of the drug behavior after oral administration. In order to elucidate in more detail the mechanism of the oxazolidine ring-opening and ring-closing reactions based on the *cis* and *trans* isomers, we synthesized oxazolam analogs having substituents at the 2- and 11b-positions as shown in Chart 1, and carried out kinetic studies of them, similarly to the case of oxazolam. These investigations required a slight modification of the mechanism proposed for



Compound	R_2	R_{11b}	R_{10}
1	CH_3	C_6H_5	H
2	C_2H_5	C_6H_5	H
3	CH_3	CH_3	H
4	C_2H_5	CH_3	H
5	C_6H_5	CH_3	H
6	H	C_6H_5	H
7	H	CH_3	H
Oxazolam	CH_3	C_6H_5	Cl

Chart 1

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TABLE I. Physical and Analytical Data for Oxazolam Analogs

Compd.	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found)		
				C	H	N
1	185—187 (lit.[4] 174—176)	Ethanol	$C_{18}H_{18}N_2O_2$	73.45 (73.19	6.16 6.07	9.52 9.51)
2	165—167	Ethanol	$C_{19}H_{20}N_2O_2$	74.00 (74.10	6.54 6.45	9.08 9.09)
3	165—167	Ethanol–ether	$C_{13}H_{16}N_2O_2$	67.22 (67.14	6.94 6.96	12.06 11.85)
4	127—129.5	Ether	$C_{14}H_{18}N_2O_2$	68.27 (68.39	7.37 7.53	11.37 11.46)
5	185—187	Ethanol	$C_{18}H_{18}N_2O_2$	73.45 (73.41	6.16 6.04	9.52 9.34)
6	181.5—182	Ethanol	$C_{17}H_{16}N_2O_2 \cdot C_2H_5OH$	69.92 (69.75	6.79 6.53	8.58 8.48)
7	164—166	Methanol	$C_{12}H_{14}N_2O_2$	66.04 (65.89	6.47 6.42	12.84 12.85)

oxazolam. Although the ring-closing rates of the *cis* and *trans* isomers of oxazolam were considered previously to be identical,[2] the rate of the *cis* isomer was found to be larger than that of the *trans* isomer in this study.

Experimental

Materials——Compounds **1**—**7** were synthesized by procedures similar to those reported by Deriege *et al.*,[3] Miyadera *et al.*,[4] and Lemke and Hanze.[5] 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 for oxazolam analogs are listed in Table I. Oxazolam (lot. No. 8) was supplied by Sankyo Co., Ltd. and was used after recrystallization from ethanol[6] or dichloromethane.[3] The ratios of *cis* to *trans* isomers of oxazolam recrystallized from ethanol and dichloromethane were about 1 : 3 and 1 : 4, respectively, as estimated from ^{1}H-NMR measurements immediately after dissolving the compounds in dimethylformamide-d_7. All other chemicals were purchased commercially and were of reagent grade.

Instruments——Ultraviolet (UV) absorption spectroscopy was carried out with a Shimadzu UV-260 spectrophotometer and a Hitachi UV-124 spectrophotometer. 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. A Hitachi-Horiba F-7_{LC} pH meter was used for pH measurement. A Hitachi Denshi analog computer (ALS-20M) and an NEC microcomputer (PC-9801E) were employed for the simulation of time courses of the reactions and for the analyses of the pH-rate profiles, respectively.

Kinetic Runs——The buffer systems were the same as those employed in the previous studies.[2,7] The rates of the oxazolidine ring-opening and ring-closing were measured by the pH-jump method employing the stopped-flow apparatus as reported previously.[2,7] The ring-opening reaction was initiated by the rapid mixing of a diluted basic buffer (pH about 9) containing benzodiazepinooxazole and the appropriate acid buffer at a volume ratio of 1 : 1. The ring-closing reaction was carried out in a similar manner: a diluted acid buffer (pH 3) containing the compound was mixed with the alkaline buffer. The data of successive measurements on the same reaction mixture were accumulated at least 5 times to enhance the signal-to-noise ratio. The pseudo first-order rate constant (k_{obs}) was determined by the ordinary analysis which was done directly by using a Sord microcomputer (M223 Mark III) linked to the stopped-flow apparatus.

Determination of Equilibrium Constant——The apparent equilibrium constants and dissociation constants of benzodiazepinooxazoles were determined by methods similar to those reported previously.[2,7,8]

Results

Figure 1 shows the pH-rate profile for the oxazolidine ring-opening and ring-closing

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

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}	$k^{OH^-}_{Cl,A}$ $s^{-1}M^{-1}$	$K'_{a,2}$ ($pK'_{a,2}$) M^{-1}	$K_{a,2}$ ($pK_{a,2}$) M^{-1}	K^{UV}_{eq} (pK^{UV}_{eq}) M^{-1}
1	9.52×10^4	1.35×10^0	5.64×10^6	4.20×10^0	4.58×10^3	3.25×10^{-10} (9.49)	$<1\times10^{-12}$ (>12)[b)]	2.00×10^{-7} (6.7)
	5.94×10^3	—	—	—	—	—		
2	2.68×10^5	4.09×10^{-1}	9.10×10^6	1.24×10^1	6.34×10^3	8.08×10^{-10} (9.09)	1.26×10^{-12} (11.9)	3.98×10^{-7} (6.4)
	3.36×10^3	—	—	—	—	—		
3	8.42×10^4	1.06×10^0	3.76×10^6	1.32×10^1	9.84×10^3	1.69×10^{-10} (9.77)	1.00×10^{-12} (12.0)	3.16×10^{-7} (6.5)
	1.81×10^4	—	—	—	—	—		
4	6.75×10^4	1.84×10^0	9.56×10^6	8.98×10^0	1.26×10^4	4.16×10^{-10} (9.38)	2.51×10^{-13} (12.6)	6.31×10^{-7} (6.2)
	1.97×10^4	—	—	—	—	—		
5	6.08×10^4	3.75×10^0	1.85×10^7	1.30×10^1	1.22×10^5	2.59×10^{-10} (9.59)	1.58×10^{-12} (11.8)	5.01×10^{-7} (6.3)
	8.31×10^3	—	—	—	—	—		
6	7.90×10^4	5.83×10^0	5.42×10^7	0	1.20×10^6	4.14×10^{-10} (9.38)	$<1\times10^{-12}$ (>12)[b)]	6.31×10^{-8} (7.2)
7	c.n.d.[c)]	c.n.d.[c)]	1.81×10^7	0	6.34×10^5	1.73×10^{-10} (9.76)	c.n.d.[c)]	3.16×10^{-8} (7.5)
Oxazolam[d)]	2.08×10^4	5.58×10^{-1}	1.31×10^7	2.75×10^1	3.88×10^3	1.95×10^{-9} (8.71)	3.16×10^{-13} (12.5)	3.16×10^{-6} (5.5)
	1.19×10^3	—	—	—	—	—		

a) At 25 °C; in the presence of 4% (v/v) ethanol. *b*) The absorbance change due to the deprotonation of the 7 nitrogen atom was not observed from pH about 10 up to pH 12. *c*) c.n.d. means "could not be determined" because of relatively fast subsequent hydrolysis. *d*) Reported previously.[2)]

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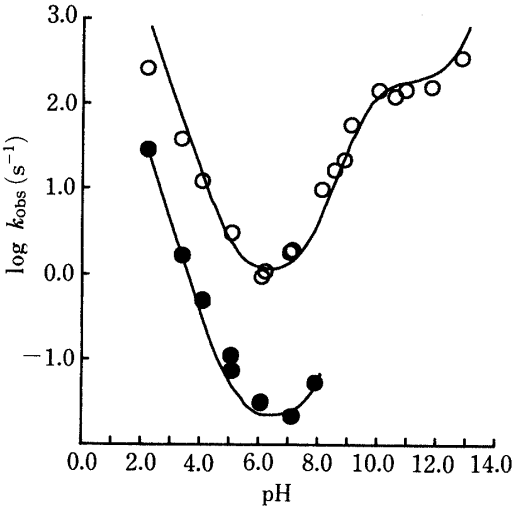

Fig. 1. The pH-Rate Profiles for Oxazolidine Ring-Opening and Ring-Closing Reactions of Compound **1** at 25 °C

○, k^{L}_{obs}; ●, k^{S}_{obs}.

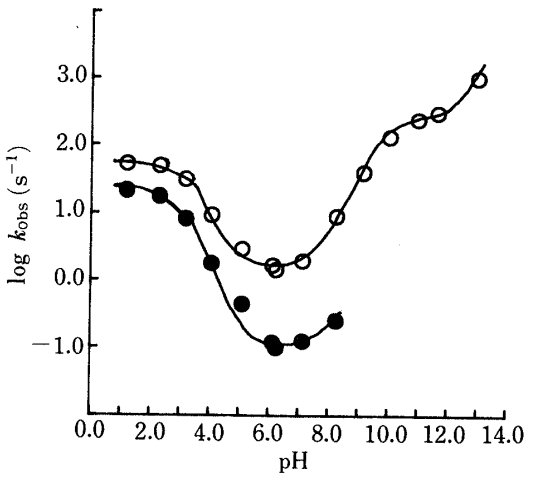

Fig. 2. The pH-Rate Profiles for Oxazolidine Ring-Opening and Ring-Closing Reactions of Compound **5** at 25 °C

○, k^{L}_{obs}; ●, k^{S}_{obs}.

TABLE III. Estimated Rate Constants and Dissociation Constants[a)]

Compound	$k^{0}_{Op,C}$ $M^{-1}S^{-1}$	$K_{a,1}$ ($pK_{a,1}$) M^{-1}	$K'_{a,1}$ ($pK'_{a,1}$) M^{-1}
3	1.51×10^{1}	4.49×10^{-4} (3.35)	6.31×10^{-2} (1.2)
	3.84×10^{0}	1.00×10^{-3} (3.00)	
4	1.73×10^{1}	6.65×10^{-4} (3.18)	1.00×10^{-1} (1.0)
	6.46×10^{0}	1.52×10^{-3} (2.82)	
5	6.67×10^{1}	1.80×10^{-3} (2.75)	6.31×10^{-2} (1.2)
	6.92×10^{0}	3.16×10^{-3} (2.50)	

a) At 25 °C; in the presence of 4% (v/v) ethanol.

reactions of compound **1**. The two profiles observed from acid to neutral regions are due to *cis* and *trans* isomers of **1** as described in the previous paper.[2)] Above pH 8 only one process (fast reaction due to the *cis* isomer) was observed, and the slow reaction could not be found spectrophotometrically (see Discussion). pH-Rate profiles similar to those in Fig. 1 were obtained for **2** and oxazolam,[9)] that is, for the compounds having a phenyl group at the 11b position. Compound **6** having an 11b-phenyl group gave, of course, only one profile, because **6** does not have *cis* and *trans* isomers ($R_2=H$).

Figure 2 illustrates the pH-rate profiles for the reactions of **3**. The plateau portion is found in the acid region, suggesting the involvement of protonation of the 7 nitrogen atom in the ring-opening rate. Compounds **4** and **5** showed pH-profiles similar to those in Fig. 2. Compounds **3**—**5** possess a methyl group at the 11b-position.

Table II lists rate constants and equilibrium constants obtained from the pH-rate profiles by means of analytical procedures similar to those used previously for oxazolam.[2)] The superscripts and subscripts of the rate constants in Tables II and III 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 indicates the free form (F) at the 7 nitrogen atom of the compound, the

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anionic form (A) or cationic form (C). $K'_{a,2}$ and $K_{a,2}$ are the dissociation constants of AF (acid (A) form of a compound having the free (F) form at the 7 nitrogen atom, that is, oxazolidine ring-opened (iminium) form) and BF (base (B) form, that is, oxazolidine ring-closed form), respectively. K_{eq}^{UV} is the apparent acid–base equilibrium constant (([BF$_{cis}$]+[BF$_{trans}$])[H$^+$]/[AF]) of the oxazolidine ring determined by UV spectroscopy.

Table III lists the parameters for compounds **3**—**5** in the acid region, $K'_{a,1}$ and $K_{a,1}$ in Table III are the dissociation constants of AC (acid (A) form of the compound having the cationic (C) form at the 7 nitrogen atom) and BC (basic (B) form having the cationic (C) form at the 7 nitrogen atom), respectively. The parameters in the upper row and the lower row for each compound correspond to those for *cis* and *trans* isomers, respectively.

Discussion

Kinetic Aspect of Reactions (Acid–Base Equilibrium) based on *cis–trans* Isomers

The two step reactions shown in Fig. 1 (expressed as large rate constant k_{obs}^{L} and small constant k_{obs}^{S}) are considered, similarly to the case of oxazolam reported previously,[2] to result from the *cis* and *trans* isomers of **1** as shown in Chart 2. In Chart 2, k_i indicates the respective first-order or second-order rate constants. The fast and slow reactions seem to be due to the *cis* and *trans* isomers, respectively.[10] Each rate constant (k_i) in Chart 2 can be estimated as follows, and then the time courses of the reaction species can be simulated by an analog computer using the k_i values.

Since in the neutral region the fast step is 50—100 times faster than the slow step, k_{obs}^{L} and k_{obs}^{S} according to Chart 2 are approximately represented by Eqs. 1 and 2.[11,12]

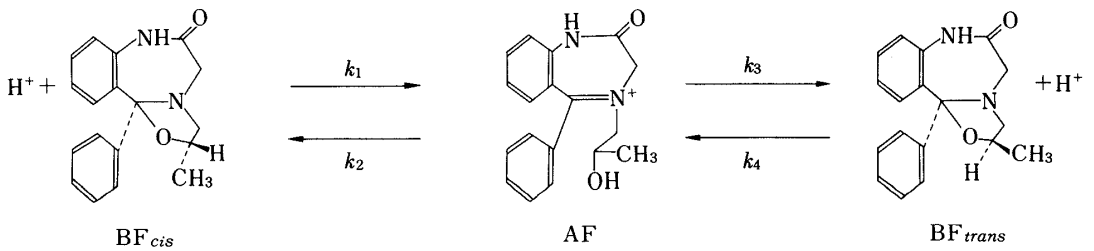
$$k_{obs}^{L}=k_1[H^+]+k_2=k'_1+k_2 \tag{1}$$

$$k_{obs}^{S}=\frac{k_3}{1+(k_2/k'_1)}+k_4[H^+]=\frac{k_3}{1+(k_2/k'_1)}+k'_4 \tag{2}$$

where $k'_1=k_1[H^+]$ and $k'_4=k_4[H^+]$. Since we always use buffer solution for the reactions, k'_1 and k'_4 are apparently constant during the reactions. The acid–base equilibrium constant (K_{eq}^{UV}) defined by Eq. 3 can be determined from the UV spectra of the compound in various pH buffer solutions as reported previously,[2,7] and is given in Table II.

$$K_{eq}^{UV}=\frac{([BF_{cis}]_{eq}+[BF_{trans}]_{eq})[H^+]}{[AF]_{eq}} \tag{3}$$

where $[BF_{cis}]_{eq}$, $[BF_{trans}]_{eq}$, and $[AF]_{eq}$ represent the equilibrium concentrations of the respective species. As reported previously for oxazolam[2] and as can also be judged for compounds **1**—**5** from the ratio of the absorbance change (increment) due to the fast reaction to that due to the slow reaction (both reactions being initiated by the pH-jump from the alkaline to the acid region), the concentration of the *cis* isomer in the aqueous alkaline region



BF$_{cis}$ AF BF$_{trans}$

Chart 2

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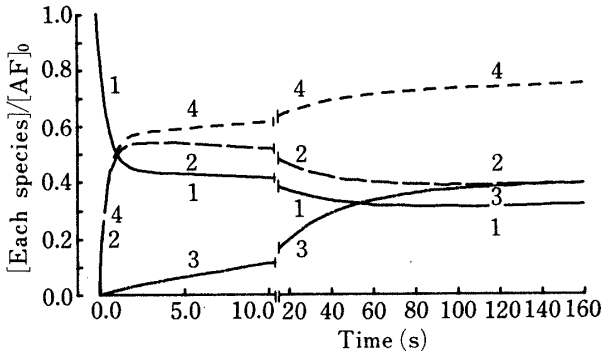

Fig. 3. Analog Computer Simulation Curves for Compound **1** at pH 7.06 and 25 °C

$k_1'=8.46\times10^{-1}\,s^{-1}$; $k_2=1.07\,s^{-1}$; $k_3=2.50\times10^{-2}\,s^{-1}$; $k_4'=2.00\times10^{-2}\,s^{-1}$; —— and 1, [AF]; - - - - and 2, [BF_{cis}]; - - - - and 3, [BF_{trans}]; ------ and 4, [BF_{cis}]+[BF_{trans}]; the reaction is assumed to be started by the pH-jump from pH about 3 to pH 7.06.

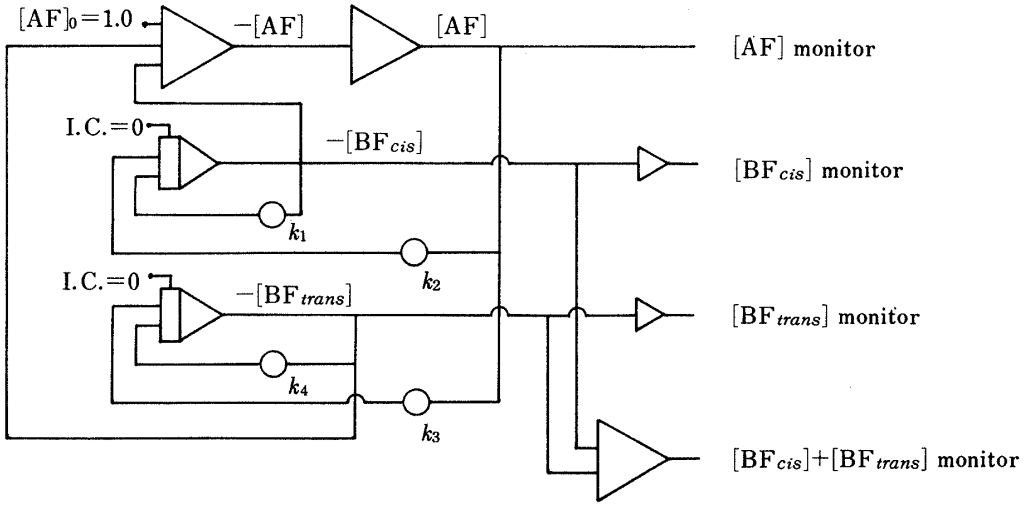

Fig. 4. Analog Circuit Diagram Applicable to the Reaction Scheme in Chart 2

is almost equal to that of the *trans* isomer (that is, $[BF_{cis}]_{eq}=[BF_{trans}]_{eq}$).[2] Eq. 3 is, thus, written in Eq. 4

$$K_{eq}^{UV}=2[BF_{cis}]_{eq}[H^+]/[AF]_{eq}=2[BF_{trans}]_{eq}[H^+]/[AF]_{eq}$$
$$=2k_2/k_1=2k_3/k_4$$
$$=2K_{eq}^{cis}=2K_{eq}^{trans} \quad (4)$$

where K_{eq}^{cis} and K_{eq}^{trans} are the acid–base equilibrium constants for the *cis* and *trans* isomers, respectively. From Eqs. 1—4, therefore, the individual k_i value can be calculated. At pH 7.06 ($[H^+]=8.71\times10^{-8}$ M), for example, we obtained the following values: $k_1'=8.46\times10^{-1}\,s^{-1}$, $k_2=1.07\,s^{-1}$, $k_3=2.50\times10^{-2}\,s^{-1}$, and $k'_4=2.00\times10^{-2}\,s^{-1}$.

Figure 3 shows the time course of each species simulated by the analog computer, using the estimated k_i values at pH 7.06. The analog circuit diagram is illustrated in Fig. 4. The details of the programming have been described.[13,14] In Fig. 3, experimentally we can follow only the decrease of AF and /or the increase of the sum of BF_{cis} and BF_{trans}, because the UV spectroscopic properties of BF_{cis} and BF_{trans} are virtually the same. It is of interest that BF_{cis} (curve 2) increases initially and then decreases to reach a final equilibrium state, at which the concentration of BF_{cis} is equal to that (curve 3) of BF_{trans}. This time course of [BF_{cis}] can be understood by a close scrutiny of the interrelation of the magnitudes of k_i values, Eqs. 1—4, and Eqs. 5—7 in the next paragraph.

From the weakly alkaline to the alkaline region ($pH>pK_{eq}^{cis}(=pK_{eq}^{UV}+0.3)+1$), it was difficult to determine the k_{obs}^{S} value, because the observed UV spectral change due to the slow reaction becomes very small. The small spectral change can be readily predicted from Eqs. 1—

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4 and also from the simulation curves shown in Fig. 3. An approximate equilibrium due to the process of $BF_{cis}+H^+ \rightleftarrows AF$ is attained initially, the process showing the large UV spectral change. At the initial apparent equilibrium state, the concentration of AF, $[AF]^i_{eq}$, is approximately represented by Eq. 5.

$$[AF]^i_{eq}=\frac{1}{(K^{cis}_{eq}/[H^+])+1}[AF]_0 \tag{5}$$

where $[AF]_0$ is the initial concentration of AF. Subsequently, the complete (final) equilibrium as shown in Chart 2 is attained gradually, the concentration of AF, $[AF]^f_{eq}$, being given by Eq. 6.

$$[AF]^f_{eq}=\frac{1}{(2K^{cis}_{eq}/[H^+])+1}[AF]_0 \tag{6}$$

The difference $\Delta[AF]_{eq}$ between $[AF]^i_{eq}$ and $[AF]^f_{eq}$, represented by Eq. 7, causes the UV spectral change due to the slow step reaction.

$$\begin{aligned}\Delta[AF]_{eq}&=[AF]^i_{eq}-[AF]^f_{eq}\\&=\frac{1}{([H^+]/K^{cis}_{eq})+3+(2K^{cis}_{eq}/[H^+])}[AF]_0\end{aligned} \tag{7}$$

It is clear from Eq. 7 that in the alkaline region ($[H^+] \ll K^{cis}_{eq}$), $\Delta[AF]_{eq}$ becomes very small.

Basis of the Rate Differences between *cis* and *trans* Isomers

Oxazolam and its analogs used in this study are diastereoisomers, since the carbons at 2- and 11b-positions are asymmetric ones. Oxazolam has, for example, four isomers, that is, 2*R-cis*, 2*R-trans*, 2*S-cis*, and 2*S-trans* isomers, whose registry numbers in *Chemical Abstracts* are [102916-80-3], [102916-81-4], [102916-82-5], and [102916-83-6], respectively.[15,16] As described above and previously,[2] therefore, the reactions due to the 2*R-cis* and 2*S-cis* isomers of oxazolam are faster than those due to the 2*R-trans* and 2*S-trans* isomers.

Here the reason for the rate differences between the *cis* and *trans* isomers should be discussed. Each individual isomer of the benzodiazepinooxazole can be considered theoretically to possess the following three conformations. There are two conformations with respect to the benzodiazepine ring and the oxazolidine ring, that is, relatively plane form (conformation X) and skewed form (conformation Y). Conformation X rather than conformation Y seems to be favorable for the ring-opening and ring-closing reactions, since the skewed form is more crowded sterically than the plane form. In conformation X, there exist two conformations with respect to the 11b-substituent and the lone pair of the 4-nitrogen atom, that is, the lone pair occupying the same side as the 11b-substituent (conformation I) and the opposite side (conformation II).[17]

For the ring-opening reaction we proposed in the previous paper[2] that an approach of a proton to the lone pair resulting in ring-opening occurs mainly in conformation II, and because of the steric effect of the methyl group at the 2 position, the *cis* isomer of oxazolam reacts more quickly than the *trans* isomer (see Ref. 2 for details).

The ring closure, on the other hand, may be achieved in the form of conformation I, since conformation I is considered to be more stable than conformation II on the basis of the CPK model. In conformation I, the axial methylene protons at the 3- and 5-positions and the methyl group at the 2-position of the *trans* isomer (the two substituents attached at the 2- and 11b-positions are *trans*) are more crowded than those of the *cis* isomer, and consequently the *cis* isomer seems to react more quickly than the *trans* isomer.

Effects of 2- and 11b-Substituents on the Oxazolidine Ring-Opening and Ring-Closing Rates

There is a maximally 4.4-fold difference in the $k^{H^+}_{Op,F}$ values for the *cis* isomers between

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compounds **1**—**5**, and there exists a 5.9-fold difference for the *trans* isomers. Although these differences in $k_{Op,F}^{H^+}$ are not large, the difference in ratios of $k_{Op,F}^{H^+}$ for the *cis* isomer to $k_{Op,F}^{H^+}$ for the *trans* isomer (that is, values of $k_{Op,F}^{H^+}$ for the *cis* isomer divided by $k_{Op,F}^{H^+}$ for the *trans* isomer) becomes large. There exists, for example, about a 23-fold difference in the ratios between compounds **2** (79.8) and **4** (3.43), reflecting the sterical effect of the combination of the 2- and 11b-substituents on the ring-opening rates.

A maximally 4.9-fold difference in $k_{Cl,F}^{OH^-}$ for the *cis* isomers between compounds **1**—**5** was found. These relatively small differences in $k_{Cl,F}^{OH^-}$ may support the proposed mechanism for the ring-closing reaction: ring closure occurs in the form of conformation I and the *cis* isomer reacts more quickly than the *trans* isomer. The $k_{Cl,F}^{OH^-}$ value for *trans* isomers would be more affected by the substituents at the 2- and 11b-positions than those for *cis* isomers because of the steric influence described above. Unfortunately, we could not obtain the $k_{Cl,F}^{OH^-}$ value for *trans* isomers owing to the very small UV spectral changes.

The values of $k_{Cl,A}^{OH^-}$ may be slightly unreliable, since a few data points were used for the determination of the parameter in the strongly alkaline region (pH > 12).

Acknowledgments We are grateful to Mr. M. Kurita, director of Tokai Teishin Hospital Pharmacy, and to Miss S. Kato, Faculty of Pharmaceutical Sciences, Nagoya City University, for encouragement and NMR spectral measurements, respectively. Thanks are also due to Sankyo Co., Ltd. for the gift of oxazolam.

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9) Careful and repeated examinations of the rates for oxazolam from pH 5.0 to pH 8.0 required modification of the k_{obs}^{S} values at pH 5.0 and 5.5 reported previously[2] (*i.e.*, 3.80×10^{-1} and $4.79 \times 10^{-1}\,s^{-1}$, respectively). The corrected values of k_{obs}^{S} at pH 5.0 and 5.5 are 3.03×10^{-2} and $1.26 \times 10^{-2}\,s^{-1}$, respectively.
10) This conclusion was confirmed experimentally as follows using oxazolam, in addition to the results reported previously.[2] From ^{1}H-NMR measurements the ratio of the *cis* isomer to the *trans* isomer of oxazolam (recrystallized from dichloromethane) immediately after dissolving in dimethylformamide-d_7 was found to be about 1 : 4 (*cis* isomer is about 20%). When equilibrium was attained (about 3 h after dissolving[4]), the ratio was about 2 : 3 (*cis* isomer is about 40%). The process (*cis–trans* isomerization in dimethylformamide) had a half-life of about 30 min. By using the stopped-flow apparatus, the rates of the fast and slow reactions and also the absorbance increments (at 250 nm) based on the oxazolidine ring-opening reactions were measured in dimethylformamide solution containing an appropriate concentration of hydrogen chloride. The ratio of the absorbance increment due to the fast reaction to that due to the slow one was 23 : 77 for a sample immediately after being dissolved in pure (not containing HCl) dimethylformamide. For the sample standing for 3 h after being dissolved in pure dimethylformamide, the absorbance increment ratio was 35 : 65. These ^{1}H-NMR and stopped-flow studies lead to the conclusion that the fast reaction is due to the *cis* isomer of oxazolam and the slow one to the *trans* isomer. Although the conclusion was derived from the results in dimethylformamide, we assume that the order of the reaction rates (that is, fast or slow reaction) is the same between dimethylformamide and aqueous solution.
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17) Conformation Y consists of conformation I. Conformation II for conformation Y is identical with conformation II for conformation X. Each isomer, therefore, possesses three conformations.

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