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Kinetics and mechanism of the acid-base equilibrium of mexazolam analogues: A modification of the mechanism proposed previously for mexazolam

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Summary

Oxazolidine ring-opening and ring-closing (acid-base equilibrium) reactions of 3-methyl-11*b*-hydrogen (**1**) and -11*b*-methyl (**2**) analogues of mexazolam (**3**) have been studied kinetically in solutions of various pH values and at 25°C. Compound **2** isomerizes to the *cis*/*trans* isomers (referring to substituents at the 3- and 11*b*-positions) in methanol-d_4 with a half-life of about 50 min, and at equilibrium the ratio of the *cis* to *trans* isomers is 6.9 : 1. The acid-base equilibrium reactions of **1** and **2** proceed via two steps due to the *cis* and *trans* isomers, the reactions of the *trans* isomer being faster than those of the *cis* isomer. The large difference between the *cis* and *trans* isomers of **2** is ascribed to their conformational differences (normal boat X_I and flat form Y_{II}, respectively). These results required a modification of the mechanism previously proposed for mexazolam (Kurono et al., *Chem. Pharm. Bull.*, 35 (1987) 3831–3837).

Introduction

The oxazolidine ring-opening and ring-closing (acid-base equilibrium) reactions of benzodiazepinooxazoles (BDOZs) have been investigated kinetically from the standpoints of drug behavior after oral administration (Kurono et al., 1985, 1987, 1988a,b, 1990). The reactions of the *cis* isomer (referring to the substituents at the 11*b*-position and at the 2-position) for oxazolam analogues are faster than those of the *trans* isomer (Kurono et al., 1985, 1988b). Mexazolam (**3**) which has 3-methyl and 11*b*-2′-chlorophenyl groups seemed to exist essentially as a single *cis* isomer (Miyadera et al., 1971; Kurono et al., 1987). Nevertheless, since mexazolam gave two-step reactions, we proposed previously a reaction mecha-

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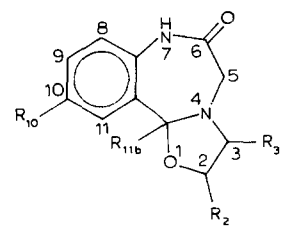


Scheme 1.

Compound	R_2	R_3	R_{10}	R_{11b}
1	H	CH_3	H	H
2	H	CH_3	H	CH_3
Mexazolam	H	CH_3	Cl	2'-ClC_6H_4
3				
4	H	CH_3	Cl	C_6H_5
Oxazolam	CH_3	H	Cl	C_6H_5

nism including an intermediate between oxazolidine ring-opened form and the ring-closed form (Kurono et al., 1987).

Ratios of the *cis*/*trans* isomers for various BDOZs have been estimated by using the lanthanide shift reagent ($Eu(fod)_3$-d_{27}) for nuclear magnetic resonance (NMR) spectroscopy (Kuwayama et al., 1990a). The ratio in $CDCl_3$ for mexazolam was 8.7:1 and that for the 11*b*-H and 3-CH_3 analogue (**1**, see Scheme 1 for the structure) was 3.4:1 (Kuwayama et al., 1990a), indicating the dependence of the ratio on the bulkiness of the 11*b* substituent. When the 3,11*b*-dimethyl compound (**2**) was synthesized, the *cis* and *trans* isomers were fractionally crystallized from ethanol (Hatano et al., 1991). The crystal structures were determined by X-ray diffraction techniques (Hatano et al., 1991).

The *cis*/*trans* ratio of **2** at equilibrium in methanol-d_4 was determined by NMR spectroscopy and was found to be about 6.9:1, irrespective of the starting *cis* or *trans* crystal. When the oxazolidine ring-opening and ring-closing reactions of **1** and **2** were studied by the pH-jump method, the two-step reactions due to the *cis* and *trans* isomers were observed similarly to the case of the oxazolam analogues having a 2-methyl group. These investigations required a modification of the mechanism previously proposed for mexazolam (Kurono et al., 1987). We report herein the circumstances of the affairs.

Materials and Methods

Materials

Compounds **1** and **2** were the same as those used previously (Kuwayama et al., 1990a; Hatano et al., 1991), which were originally synthesized according to methods similar to those reported by Deriege et al. (1971), Miyadera et al. (1971) and Lemke and Hanze (1971). The *cis* and *trans* isomers of **2** were fractionally crystallized from ethanol case by case (Hatano et al., 1991). Identification of the crystals were achieved by X-ray crystallography and IR spectroscopy (Hatano et al., 1991). Mexazolam was supplied by Sankyo Co., Ltd (lot 2) and was used after recrystallization from ethanol. All other chemicals used were purchased commercially and were of reagent grade.

Apparatus

Ultraviolet (UV) spectra were recorded with Shimadzu UV-260 and UV-2200 spectrophotometers. A stopped-flow spectrophotometer (Otsuka Denshi RA-401) was used for the measurements of the reaction rates and also of the absorbance changes due to the fast reaction. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL JNM-FX 100 spectrometer at 400 and 100 MHz, respectively. IR spectra were recorded on a Perkin-Elmer FTIR-1600 as a KBr pellet. An NEC microcomputer (PC-9800E) was used for the analyses of the pH-rate profiles.

Kinetic runs

The buffer systems were the same as those employed in the previous studies (Kurono et al., 1985, 1987, 1988a,b, 1990). Unless otherwise noted, the reaction medium contained 4% (v/v) ethanol; for compound **2**, however, 4% (v/v) methanolic medium was employed. The rates of oxazolidine ring-opening and ring-closing were measured by the pH-jump method using stopped-flow instruments as reported previously (Kurono et al., 1985, 1987, 1988b, 1990). The

pseudo first-order rate constants (k_{obs}) for the reactions were determined by ordinary first-order analysis and also by the Guggenheim method (1926) in the case where the reaction end-point is unknown. These analyses were performed directly by using a Sord microcomputer (M223 Mark III) linked to the stopped-flow instruments.

The *cis / trans* isomerization rates of compound **2** were measured in methanol-d_4 by monitoring the 3-methyl protons, i.e., the integrated areas of the 3-CH_3 signals due to the *cis* and *trans* isomers were compared with the aromatic hydrogen signal area as a function of time. The pseudo first-order rate constant (k_{obs}) for the isomerization was calculated by using Eqn 1 (Kurono et al., 1989):

$$\log|CP - CP_\infty| = -k_{obs}t/2.303 + \text{constant} \quad (1)$$

where CP and CP_∞ are the percent of the *cis* or *trans* isomer at time t and at infinity, respectively.

Determination of equilibrium constant

The apparent equilibrium constant (K_{eq}) of BDOZs was determined spectrophotometrically based on Eqn 2 (Kurono et al., 1985, 1987, 1988b, 1990).

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

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

cis / trans Isomerization rate of compound 2 in methanol-d_4

Fig. 1 shows the time courses of the proton signal changes measured for **2** in methanol-d_4. The spectra were obtained after dissolving the *trans* isomer crystals which were determined by X-ray crystallography and IR spectroscopy (Hatano et al., 1991). The assignments of each of the signals have been reported previously (Hatano et al., 1991). The signals at around 1.42 and 1.52 ppm are due to the 11*b*-methyl protons of the *trans* and *cis* isomers, respectively, and diminish gradually, indicating an 11*b*-methyl proton exchange reaction with deuterium in methanol-d_4 (Kuwayama et al., 1988, 1990b; Kurono et al., 1992). The doublets at around 1.06 and 1.14 ppm are due to the 3-methyl protons of the *trans* and *cis* isomers, respectively. The *trans* 3-methyl proton signals at around 1.06 ppm decrease and the *cis* signals at 1.14 ppm increase. At equilibrium the ratio of the *cis / trans* isomers is 7.1 : 1. Signals obtained after dissolving the *cis* crystals showed an identical pattern at equilibrium, i.e., the ratio was 6.6 : 1. The pseudo first-order rate constant (k_{obs}) for this *cis / trans* isomerization was determined to be 2.30×10^{-4} s^{-1} (half-life = 3.01×10^3 s) by using Eqn 1, irrespective of the starting crystals. Complete explanations for the isomerization rate and the proton exchange reaction are reported elsewhere (Kurono et al., 1992).

Oxazolidine ring-opening and ring-closing reactions

Fig. 2 shows the UV spectra of compound **2** in various pH buffer solutions containing 4% (v/v) methanol. These spectra are attributable to equilibrium mixtures of the ring-opened iminium form (AF) in acid solution and the ring-closed form (BF) in weakly alkaline solution (Kurono et al., 1985, 1987, 1988a,b, 1990). From the spectral data the apparent pK_{eq}^{UV} value ($-\log([BF][H^+]/[AF])$) was determined to be 6.23 by using Eqn 2.

The ring-opening reaction of compound **2** was followed by the pH-jump method from the methanol solution of **2** at equilibrium of *cis / trans* ratio (6.6 : 1) to pH 3.0 aqueous buffer. The reaction medium thus consisted of 50% (v/v) methanolic buffer solution having an apparent pH value of about 3.6. Two-step reactions (expressed as a fast reaction having a large rate constant k_{obs}^L and a slow reaction having a small constant k_{obs}^S) were observed. The k_{obs}^L and k_{obs}^S values were about 2.50×10 and 1.26×10^{-1} s^{-1}, respectively. The ratio of the absorbance increment (ΔA_L) at 280 nm due to the fast reaction to that ($\Delta A_S = \Delta A_{total} - \Delta A_L$) due to the slow reaction was about 1 : 7. Between the equilibrium methanol solutions made from the *cis* and *trans*

crystals, identical rate constants (both k^{L}_{obs} and k^{S}_{obs}) and identical absorbance increments (both ΔA_L and ΔA_S) were obtained.

The results of ^{1}H-NMR spectroscopy (previous section) and the pH-jump kinetic analyses indicate that the two-step reactions are due to the *cis* and *trans* isomers as shown in Scheme 2, similarly to the case of oxazolam analogues which have a 2-CH_3 group. In Scheme 2, k_i represents the respective first-order or second-order rate constants. Moreover, from the agreement between the *cis* / *trans* ratio in methanol-d_4 at equilibrium determined by ^{1}H-NMR spectroscopy and the $\Delta A_S/\Delta A_L$ ratio determined by the pH-jump method, it is deduced that the slow (k^{S}_{obs}) step is due to the *cis* isomer and the fast (k^{L}_{obs}) step to the *trans* isomer.

To further confirm that the two-step reactions derive from the *cis* / *trans* isomers, the following numerical analysis was attempted. The apparent acid-base equilibrium constants of compound **2** are expressed as Eqn 3.

$$K^{UV}_{eq} = (([BF]_{trans} + [BF]_{cis})[H^+])/[AF]$$
$$= ([BF]_{trans}[H^+]/[AF])$$
$$+ ([BF]_{cis}[H^+]/[AF]) = K^{trans}_{eq} + K^{cis}_{eq} \quad (3)$$

where $K^{trans}_{eq} = [BF]_{trans}[H^+]/[AF]$ and $K^{cis}_{eq} = [BF]_{cis}[H^+]/[AF]$. The absorbance increment due to the fast reaction measured by the pH-jump method from pH 9.0 to the strong acid solution reflects the concentration of the *trans* isomer at equilibrium in the pH 9.0 buffer ($\Delta A_L \alpha [AF]$ from the *trans* isomer). From the dependence of

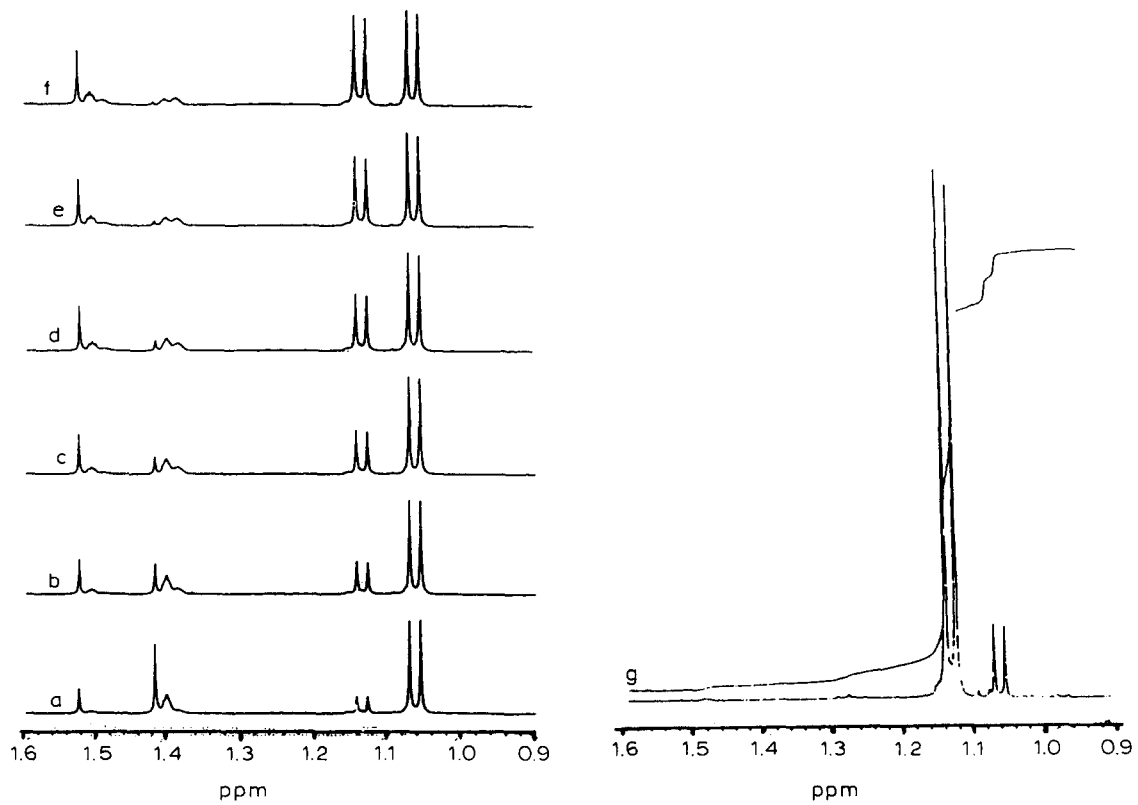
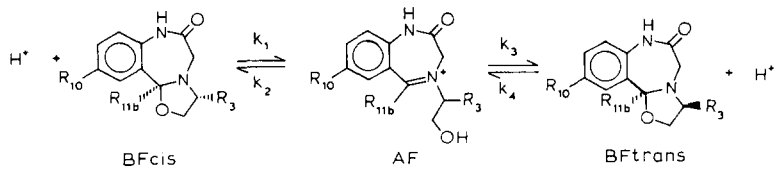


Fig. 1. ^{1}H-NMR spectral changes of compound **2** in methanol-d_4 with time at 23°C. (a) 0, (b) 9, (c) 16, (d) 23, (e) 32, (f) 42, (g) 4320 min.



Scheme 2.

ΔA_L on pH (Fig. 3a), pK_{eq}^{trans} can be determined by using Eqn 2 (Fig. 3b). From K_{eq}^{UV} and K_{eq}^{trans}, K_{eq}^{cis} is calculated using Eqn 3 ($K_{eq}^{cis} = K_{eq}^{UV} - K_{eq}^{trans}$). The equilibrium constants thus obtained are listed in Table 1. The ratio of $K_{eq}^{trans}/K_{eq}^{cis}$ becomes the ratio of the *trans / cis* isomers ($[BF]_{trans}/[BF]_{cis}$) in the alkaline buffer solution. The ratio for compound **2** is found to be about 1 : 7 which is almost the same as that in methanol-d_4. The agreement of the *cis / trans* isomer ratio calculated from K_{eq}^{cis} and K_{eq}^{trans} with the ratio of $\Delta A_L/(\Delta A_{total} - \Delta A_L)$ obtainable experimentally implies that the reaction scheme shown in Scheme 2 is reasonable for the kinetic analyses of the oxazolidine ring-opening and ring-closing reactions of mexazolam analogues having the 3-methyl group.

Similar analyses were, therefore, applied to the case of mexazolam itself. The results are listed in Table 1. There is an approx. 2-fold difference in the ratios estimated from the equilibrium constants and from the absorbance changes. This difference may be inevitable because the concentration of the *trans* isomer is very low (below 10% in methanol-d_4 (Kuwayama et al., 1990a)) and the reaction of the *trans* isomer of mexazolam is fast, i.e., experimental error may occur. Although concrete numerical values for compound **4** were not determined, similar results to those of mexazolam were obtained. Consequently, the oxazolidine ring-opening and ring-closing reactions of mexazolam appear to occur according to the reaction scheme shown in Scheme 2 instead of the scheme proposed previously (Kurono et al., 1987).

Fig. 4 shows the pH-rate profiles for the oxazolidine ring-opening and ring-closing reactions of compound **2**. The shapes of the profiles and the large difference between k_{obs}^{L} and k_{obs}^{S} are similar to those for mexazolam reported previously (Kurono et al., 1987). Fig. 5 shows the pH-rate profiles for the reactions of compound **1**. The shapes of the profiles and the small difference between k_{obs}^{L} and k_{obs}^{S} are similar to those for the 11*b*-H and 2-CH_3 oxazolam analogue reported previously (Kurono et al., 1990). These profiles illustrated in Figs 4 and 5 suggest that the reaction scheme shown in Scheme 3 is applicable, similarly to the case of oxazolam analogues. In Scheme 3, AA and BA represent acid anionic N_7 form and basic anionic N_7 form, respectively. The superscripts and subscripts of the rate constants in Scheme 3 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

TABLE 1

Acid-base equilibrium constants estimated and the ratio of trans to cis isomers

Compound	K_{eq}^{UV} (M) (pK_{eq}^{UV})	K_{eq}^{trans} (M) (pK_{eq}^{trans})	K_{eq}^{cis} (M) (pK_{eq}^{cis})	$K_{eq}^{trans}/K_{eq}^{cis}$	Absorbance change ratio *trans / cis*
2 [a]	5.83×10^{-7} (6.23)	7.53×10^{-8} (7.12)	5.08×10^{-7} (6.29)	1 : 6.75	1 : 7.53
Mexazolam **3** [b]	2.29×10^{-7} (6.64)	3.63×10^{-8} (7.44)	1.93×10^{-7} (6.71)	1 : 5.32	1 : 11.5

[a] Containing 4% (v/v) methanol.
[b] Containing 4% (v/v) ethanol.

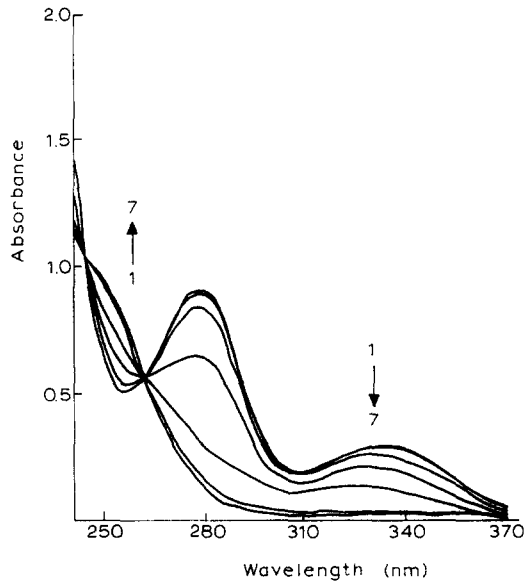


Fig. 2. UV absorption spectra of compound **2** at various pH values. Concentration of **2** was 1.00×10^{-4} M; the buffer system contained 4% (v/v) methanol. pH values: (1) 2.97, (2) 3.93, (3) 4.98, (4) 5.82, (5) 6.99, (6) 8.15, (7) 8.99.

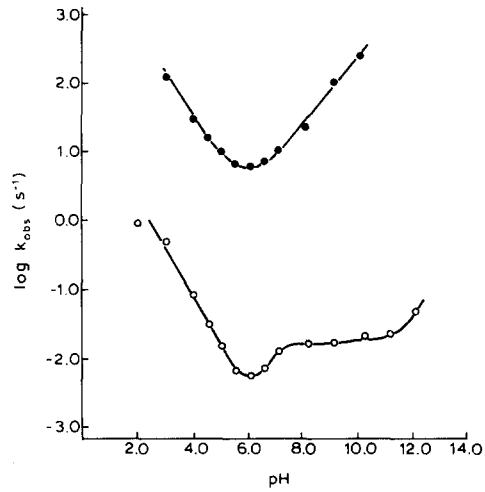


Fig. 4. The pH-rate profiles for oxazolidine ring-opening and ring-closing reactions of compound **2** at 25°C. The buffer system contained 4% (v/v) ethanol; (●) k^{L}_{obs}, (○) k^{S}_{obs}.

(Cl) occurs, and the second one represents the free form (F) or the anionic form (A) at the 7-nitrogen (N_7) atom of the compound. $K_{a,2}$ and $K'_{a,2}$ are the dissociation constants of the respective forms, and the dissociation processes are in general much faster than the processes of ring-opening and ring-closing (Kurono et al., 1985, 1988b).

The rate constants and equilibrium constants in Scheme 3 were determined using procedures similar to those employed previously (Kurono et

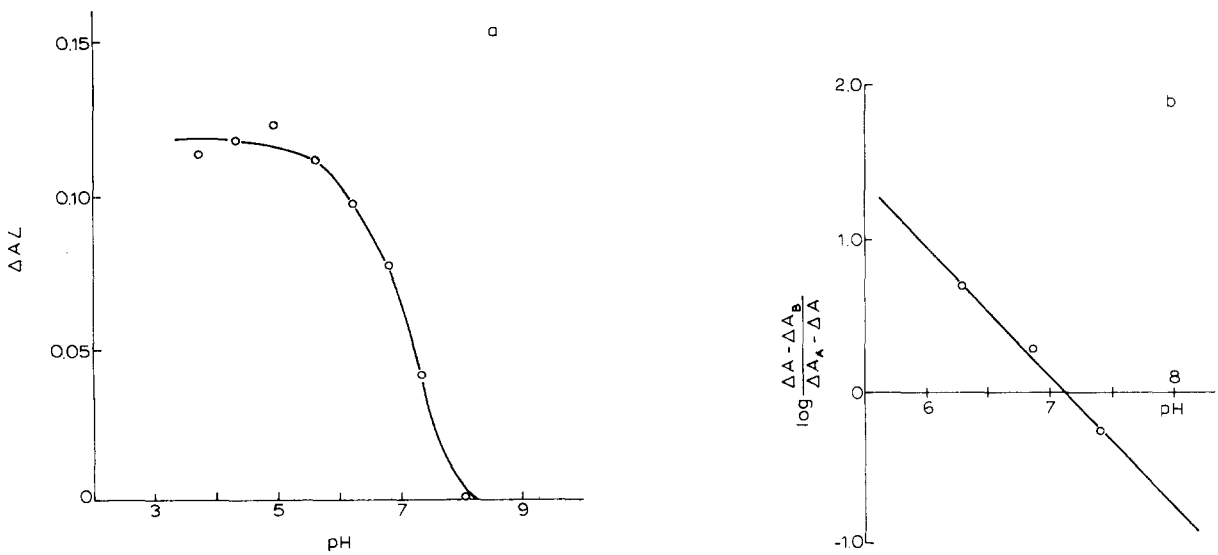


Fig. 3. (a) Plot of absorbance changes at 280 nm vs pH for compound **2**. The buffer system contained 4% (v/v) methanol. (b) Plot of $\log[(\Delta A-\Delta A_B)/(\Delta A_A-\Delta A)]$ vs pH for compound **2**. Data from panel a.

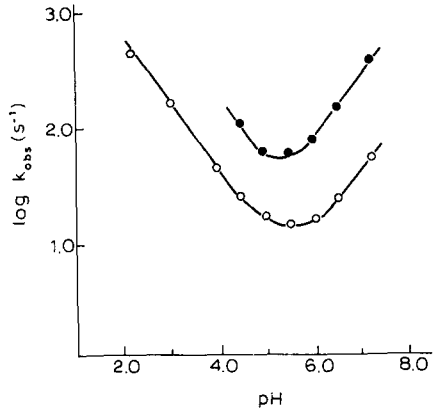
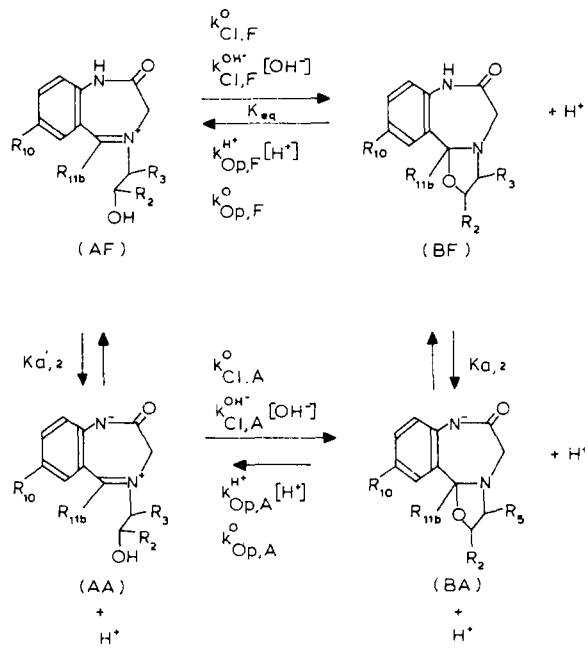


Fig. 5. The pH-rate profiles for oxazolidine ring-opening and ring-closing reactions of compound **1** at 25°C. The buffer system contained 4% (v/v) ethanol; (●) k^{L}_{obs}, (○) k^{S}_{obs}.

al., 1985, 1987, 1988b, 1990) and are summarized in Table 2. Table 2 lists the values for mexazolam (**3**), compound **4** and oxazolam obtained previously (Kurono et al., 1985, 1987) for comparison.



Scheme 3.

TABLE 2

Estimated rate constants and equilibrium constants at 25°C [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^{UV}_{eq} (M) (pK^{UV}_{eq})	$K'_{a,2}$ (M) ($pK'_{a,2}$)	$K_{a,2}$ (M) ($pK_{a,2}$)
1 [b]	4.95×10^{6}	3.76×10^{0}	2.32×10^{9}			1.15×10^{-6}		
	2.09×10^{5}	1.16×10^{0}	3.84×10^{8}			(5.94)		
2 [c]	2.01×10^{5}	7.20×10^{0}	1.73×10^{7}	-	- [d]	5.83×10^{-7}	- [d]	cnd [e]
	1.02×10^{3}	1.71×10^{-3}	1.49×10^{5}	neg. [f]	5.12×10^{0}	(6.23)	2.51×10^{-8} (7.60)	
3 [b]	5.36×10^{5}	1.15×10^{1}	1.20×10^{7}	1.69×10^{0}	1.43×10^{4}		8.55×10^{-10}	
Mexazolam						2.29×10^{-7}	(9.07)	6.31×10^{-13}
	1.93×10^{2}	2.87×10^{-3}	2.97×10^{3}	1.98×10^{-3}	7.43×10^{0}	(6.64)	1.94×10^{-9} (8.72)	(12.2)
4 [b]	8.12×10^{4}	2.35×10^{0}	1.30×10^{8}	neg. [f]	4.40×10^{3}		1.10×10^{-8}	
						6.31×10^{-7}	(7.96)	1.00×10^{-12}
	1.82×10^{2}	1.76×10^{-3}	5.09×10^{4}	neg. [f]	1.00×10^{1}	(6.20)	5.03×10^{-8} (7.30)	(12.0)
Oxazolam	2.08×10^{4}	5.58×10^{-1}	1.31×10^{7}	2.75×10^{1}	3.88×10^{3}		1.95×10^{-9}	
						3.16×10^{-6}	(8.71)	3.16×10^{-13}
	1.19×10^{3}					(5.50)		(12.5)

[a] The upper values in each column represents the fast reaction and the lower value the slow reaction.
[b] Containing 4% (v/v) ethanol.
[c] Containing 4% (v/v) methanol.
[d] Not observed.
[e] Could not be determined due to rapid hydrolysis.
[f] Negligible.

The solid curves in Figs 4 and 5 were calculated by applying the values in Table 2 to equations which were derived in a similar way to that used previously (Kurono et al., 1985, 1988b, 1990). The observed data points fit the calculated lines well. The determined kinetic parameters in Table 2 can be read as the following characteristics. For compounds **2–4** the differences in the rates between the fast (k^{L}_{obs}) and slow (k^{S}_{obs}) reactions, which are represented by the parameters in the first three columns from the left, are very large compared with those for compound **1**, oxazolam and its analogues (Kurono et al., 1988b, 1990). This large difference is probably ascribed to the k^{S}_{obs} values for **2–4** (due to their *cis* isomers) being very small.

Here the reason for the rate difference between the *cis* (k^{S}_{obs}) and *trans* (k^{L}_{obs}) isomers of **2** is described. As proposed previously (Kurono et al., 1988b; Hatano et al., 1991), there are four possible conformations for the BDOZ ring system, i.e., there are two elements of conformational motion. One is ring inversion characterized by a motion of C_5 and this is designated symbolically as the conformational conversion from X to Y. The other element is a Walden-type inversion of the N_4 atom and this is distinguished by a *syn* or *anti* relationship between the 11*b*-substituent and the lone pair of the N_4 atom. These *syn* and *anti* orientations are expressed by subscripts I and II, respectively. Thus, four conformations, X_I (normal boat), X_{II} (twisted boat), Y_I (skewed system) and Y_{II} (flat form) are introduced as a plausible stable conformation of the BDOZ model. X-ray analyses of the *cis* and *trans* isomer crystals of **2** illustrate the X_I and Y_{II} conformations, respectively (Hatano et al., 1991). Examination of the ^{1}H-NMR spectra of the *cis* and *trans* isomers of **2** by phenyl ring current effects (Johnson and Bovey, 1958) indicated that the conformations in the crystal are retained in solution (Hatano et al., 1991).

The oxazolidine ring-opening reaction needs an approach of a proton to the lone pair of the N_4 atom. The substituents at the 11*b*-position in the *cis* isomers of **2–4** (all X_I conformations (Hatano et al., 1991)) prohibit the approach of the proton and thus give very low k^{S}_{obs} values. The *trans* isomer (Y_{II} conformation (Hatano et al., 1991)) of **2** readily accepts the proton due to the flat conformation and gives the k^{L}_{obs} value. The restriction of the 11*b*-hydrogen in the *cis* isomer of **1** is relatively small, and thus the difference between k^{S}_{obs} (due to the *cis* isomer) and k^{L}_{obs} (due to the *trans* isomer (Y_{II} conformation (Hatano et al., 1991))) is small. For oxazolam analogues which have substituents at the 2-position, the restriction due to the 2-substituents is smaller than that due to the 3-substituent (CH_3) because of its greater distance (C_2–$N_4 > C_3$–N_4) to the lone pair of the N_4 atom, and thus the difference between k^{L}_{obs} and k^{S}_{obs} is small compared with the case of the 3-methyl analogues (**2–4**).

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