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Kinetics and Mechanism of Reversible Hydrolysis of 11b-Methylbenzodiazepinooxazoles[1)]

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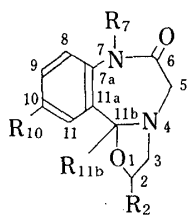
Reversible hydrolyses of benzodiazepinooxazoles having an 11b-methyl group and of oxazolam having a 7-methyl group were investigated kinetically in buffers of various pH's and at 25 °C, in the light of the difference in the reversibility of the hydrolysis between 11b-methyl- and 11b-phenyl- (*e.g.*, oxazolam itself) benzodiazepinooxazoles. The irreversibility of the hydrolysis of 11b-phenylbenzodiazepinooxazoles is considered to arise from the larger size of the phenyl group than the methyl group in addition to the intramolecular hydrogen bonding between carbonyl oxygen of benzophenone and amide hydrogen at the *ortho* position. Substituents at the 2-position of 11b-methylbenzodiazepinooxazole do not greatly affect the reversible hydrolysis.

Keywords——benzodiazepinooxazole; reversible hydrolysis; kinetics; intramolecular hydrogen bonding; oxazolam; 7-methyl oxazolam; pH-rate profile; oxazolidine ring; diazepine ring

Oxazolidine ring-opening and ring-closing reactions of benzodiazepinooxazoles and the subsequent hydrolysis of the diazepine ring were studied kinetically[2–5)] from the viewpoint of the drug behavior after oral administration. It is considered that the central nervous system activity observed for 1,4-benzodiazepines is inherent only in the closed seven-membered ring.[6–8)] During the course of the kinetic studies, the benzodiazepinooxazoles having a methyl group at the 11b-position were found to be hydrolyzed considerably more rapidly than those having a phenyl group (*e.g.*, oxazolam and haloxazolam). Furthermore, although the hydrolyses of oxazolam and haloxazolam were irreversible, those of the 11b-methyl compounds were reversible. In this paper, we describe the results of reaction rate measurements for compounds **1**—**4** (see Chart 1 for the chemical structures) and we discuss the reason for the difference in the reversibility of the hydrolysis between the 11b-methyl and 11b-phenyl compounds.



Compound	R_2	R_7	R_{11b}	R_{10}
1	H	H	CH_3	H
2	CH_3	H	CH_3	H
3	C_6H_5	H	CH_3	H
4	CH_3	CH_3	C_6H_5	Cl
oxazolam	CH_3	H	C_6H_5	Cl
haloxazolam	H	H	2-FC_6H_4	Br

Chart 1

Experimental

Materials and Apparatus——Compounds **1**—**3** were the same as those used previously.[5)] Compound **4** (7-

methyloxazolam) was synthesized by procedures similar to those reported by Deriege *et al.*,[9] Miyadera *et al.*,[6] and Lemke and Hanze.[10] Hydrolyzates of compounds **1** (**5**) and **3** (**6**) were isolated from **1** and **3**, respectively, by methods similar to those used previously.[11,12] *Anal.* Calcd for $C_{12}H_{16}N_2O_3 \cdot HCl$ (**5**): C, 52.84; H, 6.29; N, 10.27. Found: C, 52.72; H, 6.07; N, 10.05. mp 165—170 °C (dec.) *Anal.* Calcd for $C_{18}H_{20}N_2O_3 \cdot HCl$ (**6**): C, 61.97; H, 6.08; N, 8.03. Found: C, 61.87; H, 6.07; N, 7.99. mp 181—184 °C (dec.). Although we attempted to isolate the hydrolyzates of compounds **2** and **4**, the results of the elemental analyses were not within ±0.3% of the calculated values. Thus, *N*-acetyl-2-amino-acetophenone (**7**), *N*-acetyl-*N*-methyl-2-amino-5-chlorobenzophenone (**8**), and *N*-acetyl-2-amino-benzophenone (**9**) were prepared by procedures similar to those of Walker *et al.*[3,13] Compounds **7** and **8** were used as model compounds of the hydrolyzates of **2** and **4**, respectively. The choice of the model compound (*e.g.*, compound **7**) may be reasonable, because the ultraviolet (UV) spectrum of **7** was almost identical with that of **5** and the chemical structure of **5** differs only in the absence of the methyl group (R_2) from the hydrolyzate of **2**. All other chemicals were purchased and were of reagent grade.

UV spectra were measured with a Hitachi UV-200 spectrophotometer and a Shimadzu UV-260 spectrophotometer. The pH values were measured with a Hitachi-Horiba F-7_{LC} pH meter.

Kinetic Procedures——The buffer systems were the same as those used previously.[3] The experiments were carried out at 25 °C in the buffer containing 4% (v/v) ethanol with $\mu=0.1$ (NaCl).

Although the effect of ethanol on the reaction rates is not clear at present, a stock solution of a sample (benzodiazepinooxazole or its hydrolyzate, 5.00×10^{-4} M) was prepared in ethanol for experimental convenience, that is, for easy solubilization of the samples. The solution was diluted to 2.00×10^{-5} M with the buffer solution. Aliquots were withdrawn at appropriate intervals and the absorbances at fixed wavelength were measured.

The pseudo first-order rate constant (k_{obs}) was calculated from the slope of a linear plot of $\log|A_t - A_\infty|$ against time, where A_t and A_∞ are the absorbances at time t and at infinity, respectively. From the k_{obs} value determined experimentally, the rate constant (k_1 in $s^{-1}M^{-1}$) for diazepine ring-opening (hydrolysis) and the rate constant (k_2 in s^{-1}) for the diazepine ring-closing were determined as follows. The equilibrium constant K is obtained from Eq. 1.

$$K = k_1/k_2 = [OF]_{eq}/([CF]_{eq}[H_2O]_{eq}) \quad (1)$$

where $[OF]_{eq}$, $[CF]_{eq}$, and $[H_2O]_{eq}$ are the equilibrium concentrations of ring-opened form (OF), ring-closed form (CF), and water, respectively. Since the concentration of water is constant (55.6 M), Eq. 1 can be written as Eq. 2.

$$[OF]_{eq}/[CF]_{eq} = K[H_2O]_{eq} = K' = k_1[H_2O]_{eq}/k_2 = k'_1/k_2 \quad (2)$$

The concentrations of the respective molecular species were calculated by using the absorbance of the equilibrium solution, the molar absorptivities (ε in $M^{-1}cm^{-1}$) of the respective species, and the initial concentration of the compound examined. The individual rate constants were estimated by using Eqs. 1—3.[3]

$$k_{obs} = k'_1 + k_2 = k_2(K' + 1) \quad (3)$$

Since the equilibrium for the reaction of **4** in the alkaline region was displaced toward the ring-closed form, the k_{obs} values were obtained as follows. An ethanol stock solution of **4** (1.00×10^{-3}M) was diluted to 4.00×10^{-5} M with a diluted acid buffer solution (1/100 of the ordinary buffer concentration), and after the completion of the reaction (mainly ring-opened form) the pH of the reaction mixture was brought to an alkaline pH with a concentrated alkaline buffer containing 4% (v/v) ethanol (twice the ordinary buffer concentration). Then, the reaction rate of the alkaline solution (2.00×10^{-5} M) was measured, and the kinetic analysis described above was applied.

Results and Discussion

Figure 1 shows the first-order plots for the reactions of compounds **1**—**4**, from which the pseudo first-order rate constants k_{obs} were determined (see Experimental).

Figure 2 shows the pH-rate profiles for the reaction of compound **1**, that is, the pseudo first-order rate constant (k_{obs} in s^{-1}), pseudo first-order rate constant (k'_1 in s^{-1}) for the hydrolysis of **1**, and first-order rate constant (k_2 in s^{-1}) for the ring-closure. It is reported that benzodiazepinooxazoles having a phenyl group at the 11b-position (oxazolam, haloxazolam and so on) hydrolyze irreversibly because of the intramolecular hydrogen bonding between carbonyl oxygen of benzophenone and amide hydrogen at the *ortho*-position.[3,14] Because the hydrolyzate of **1** (**5**) also seems to possess similar hydrogen bonding,[15] the observation of the reversible reactions (k'_1 and k_2 in Fig. 2) over the whole pH range is of great interest.

In order to reconfirm that the intramolecular hydrogen bonding is responsible for the irreversibility of the reaction of oxazolam reported previously,[3,14] we examined the reaction of an amide hydrogen-deficient compound (**4**, 7-methyloxazolam), where the hydrolyzate can

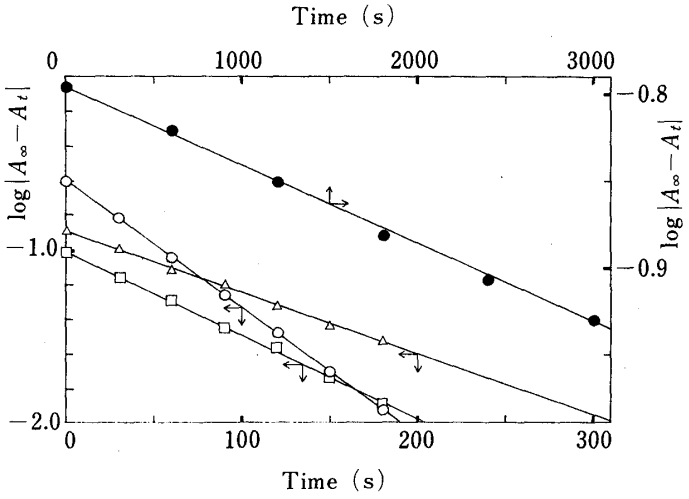


Fig. 1. First-Order Plots for the Reactions of Compounds **1**—**4** at pH 7.0 and 25 °C
○, **1**; △, **2**; □, **3**; ●, **4**.

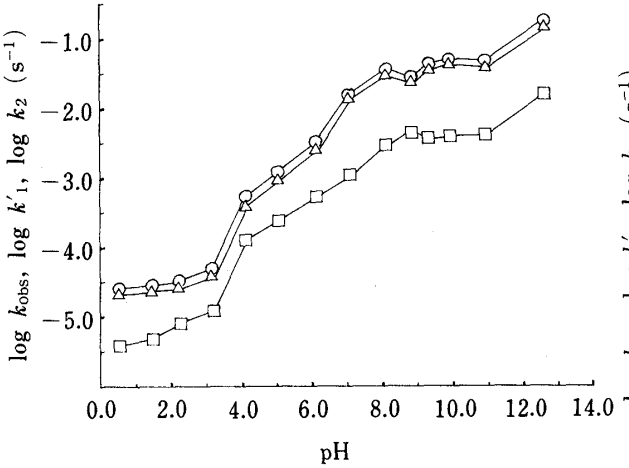


Fig. 2. pH-Rate Profiles for the Reactions of Compound **1** at 25 °C
○, k_{obs}; △, k'_1; □, k_2.

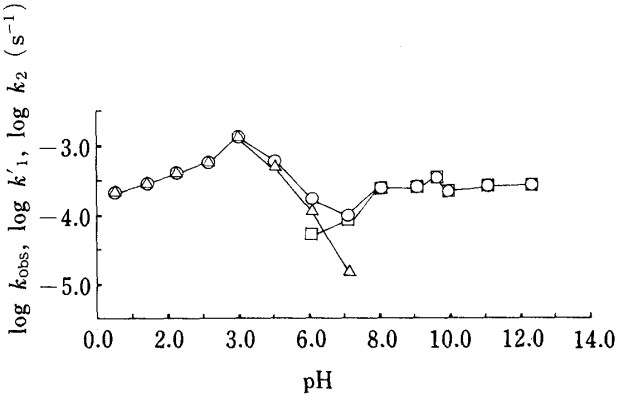


Fig. 3. pH-Rate Profiles for the Reactions of Compound **4** at 25 °C
○, k_{obs}; △, k'_1; □, k_2.

not form the intramolecular hydrogen bonding. The results are shown in Fig. 3, indicating the reactions to be reversible. Thus, the hydrogen bonding is certainly one of the factors responsible for the irreversibility in the case of oxazolam. In Fig. 3 the equilibrium for the reaction of compound **4** in the acid region is displaced markedly to the ring-opened form, and in the alkaline region, to the ring-closed form.

It is conceivable that the difference in the reversibility betweeen compound **1** and oxazolam, *etc.*, arises from substituent effects. First we examined the substituents at R_2, and measured the reaction rates of compounds **2** and **3**. The results for **3** are shown in Fig. 4. Here again the reversible reactions (k'_1 and k_2) are observed, and the shapes of the profiles are similar to those in Fig. 2. The results for compound **2** were also similar to those in Figs. 2 and 4. The magnitudes of the individual rate constants for **2** were approximately intermediate between those of compounds **1** and **3**. The difference in the reversibility is, thus, considered not to be due to the difference in the substituents at the 2-position (R_2). In addition, the substituents at R_2 appear not to affect largely the reaction rates.

A chlorine-deficient derivative of oxazolam (R_{10}=H on oxazolam **10**) was hydrolyzed irreversibly at pH's 2, 7, and 11.[16] Consequently, the difference in the reversibility must arise from the difference in the substituents at the 11b-position, that is, the methyl and phenyl groups. Three different effects on the reaction (that is, diazepine ring-closure) may be

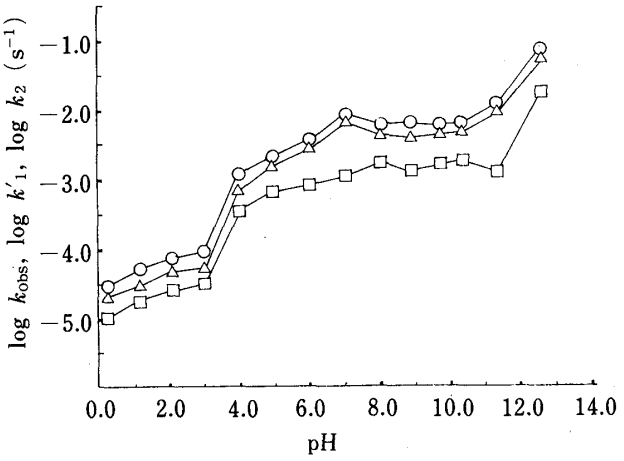


Fig. 4. pH-Rate Profiles for the Reactions of Compound **3** at 25 °C

○, k_{obs}; △, k'_1; □, k_2.

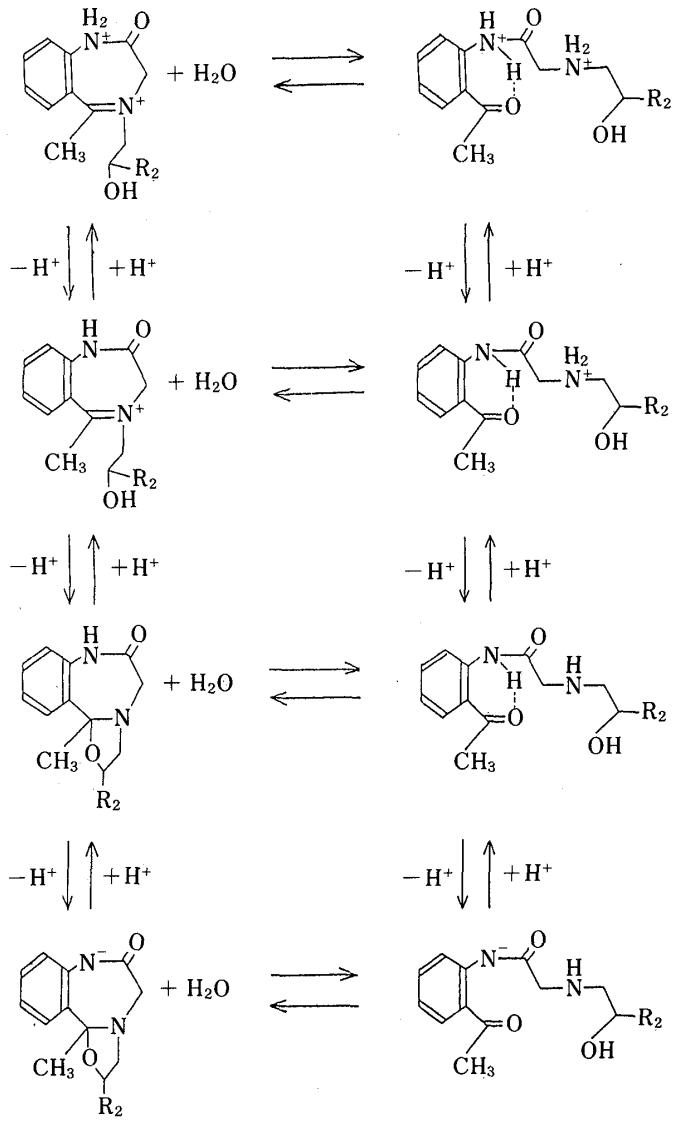


Chart 2

considered: inductive, resonance, and steric effects. The influence of the inductive effect on the diazepine ring-closure may be small, since the formation of an azomethine bond (Schiff's base formation) is little affected by the inductive effect.[17] The resonance effect of the phenyl group on the reaction should be small, since the benzophenone carbonyl group and 11b-phenyl

group are not coplanar.[18] The difference in the steric effect (the phenyl group is larger than the methyl group) is, therefore, considered to be the major reason for the difference in the reversibility of the reaction between 11b-methyl- and 11b-phenylbenzodiazepinooxazoles.

The shapes of the pH-profiles shown in Figs. 2 and 4 are very complicated. Although buffer concentration effects were not corrected, the shape of the profiles may be a result of reaction mechanisms 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,[19,20] and so on. Quantitative analyses of these pH-profiles are impossible at present because of the complexity of the system.

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References and Notes

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15) In chloroform-*d*, the free base of compound **6** and compound **7** showed ^{1}H-NMR spectra consistent with hydrogen bonding between the amide hydrogen and the acetophenone carbonyl oxygen. These compounds are, thus, considered to possess the hydrogen bonding in aqueous solution, similarly to the cases of the hydrolyzates of oxazolam[11] and haloxazolam.[3]
16) Compound **10** was the same as that used previously.[5] By using compound **9** as a model compound of the hydrolyzate of **10**, the irreversibility for **10** was confirmed by the UV spectral method as follows. The UV spectrum of the reaction solution of **10** after completion of the spectral change due to the hydrolysis was almost identical with that of compound **9** solution equimolar with compound **10**. The k_{obs} values at pH's 2, 7, and 11 were 1.66×10^{-5} (s^{-1}), 2.33×10^{-4} (s^{-1}), and 2.72×10^{-4} (s^{-1}), respectively.
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