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The Acid-Base Equilibrium Reaction of Benzodiazepinooxazoles[1]

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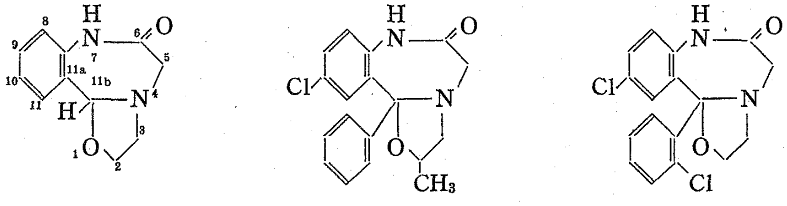
The acid–base equilibrium reactions of oxazolam (10-chloro-2,3,5,6,7,11b-hexahydro-2-methyl-11b-phenylbenzo[6,7]-1,4-diazepino[5,4-*b*]oxazol-6-one) and thirteen other derivatives of 1,4-benzodiazepinooxazole(BDOZ) were studied. Ultraviolet absorption and fluorescence spectroscopies were employed to obtain the equilibrium constants. In most BDOZs the protonated species were promptly cleaved at the oxazolidine ring fused to the diazepine nucleus, and equilibrated with the original species. A few exceptional compounds required an appreciable time lag for the equilibration. The effects of substituents on the pK_a value are discussed from the viewpoint of physical organic chemistry.

Keywords——oxazolam; benzodiazepine; acid–base equilibrium; pK_a; Hammett's σ-value

It is important to examine the chemical structures of drug species in the pH range close to physiological. Drug absorption in the gastrointestinal tract is greatly affected by the ratio of ionized to unionized species. Therefore, the pK_a of a drug compound is an important physicochemical property to be considered in pharmaceutical development.

A number of pK_a values have been reported for 1,4-benzodiazepines, which are commonly used tranquilizers (*e.g.* diazepam, chlordiazepoxide, and oxazepam)[2,3] The pK_a values were determined by ultraviolet(UV) absorption spectroscopy and polarography. The studies also revealed that the acid–base equilibrium reaction proceeded through a simple protonation–deprotonation process. Recently, Yoneda[4] and Nakano[5,6] separately investigated the equilibrium reactions of diazepam and its derivatives, and reported that a reversible cleavage of the diazepinone ring was involved in the equilibria.

In the present study, we attempted to investigate the acid–base equilibrium reaction of a new series of benzodiazepines, *i.e.* 1,4-benzodiazepinooxazoles(BDOZ). These drugs contain an oxazolidine ring fused to the seven-membered diazepinone nucleus as shown in Chart 1; they were synthesized by Tachikawa and Miyadera *et al*,[7] and their pharmacological activities were studied by Takagi *et al.*[8] On the other hand, no physicochemical studies have been reported.



1 oxazolam 2 cloxazolam

Chart 1. Benzodiazepinooxazoles

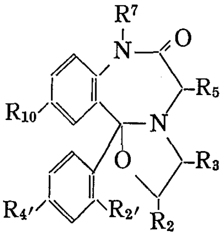
Experimental

Materials——Oxazolam (1) (10-chloro-2,3,5,6,7,11b-hexahydro-2-methyl-11b-phenylbenzo[6,7]-1,4-diazepino[5,4-*b*]oxazol-6-one) and cloxazolam (2) (10-chloro-11b-*ortho*-chlorophenyl-2,3,5,6,7,11b-hexahydrobenzo[6,7]-1,4-diazepino[5,4-*b*]oxazol-6-one) were obtained from manufacturing batches, and the other

Table I. pK_a Values of Benzodiazepinooxazoles

Compound	Substituents	pK_a	Wavelength analyzed (nm)	pK_a (Fl.)[a]
1[b]	2-CH_3, 10-Cl	5.74±0.04[c]	240	5.6
2[d]	10-Cl, 2′-Cl	7.27±0.17	243	7.4
3[e]	3-CH_3, 10-Cl, 2′-Cl	6.69±0.09	242	6.6
4	10-Br, 2′-F	6.18±0.02	245	6.2
5	2-CH_3, 7-CH_3, 10-Cl	4.52±0.03	244	—
6	2-CH_3	6.95±0.03	233	—
7	2-CH_3, 10-Cl, 2′-CH_3	8.30±0.10	243	—
8	2-CH_3, 10-Cl, 2′-F	5.56±0.07	243	—
9[e]	2-CH_3, 5-CH_3, 10-Cl	4.53±0.14	242	4.6
10	2-CH_3, 5-CH_3, 10-Cl, 4′-NO_2	3.63±0.07	245	—[f]
11	2-CH_3, 10-Cl, 4′-OCH_3	6.68±0.05	239	—
12	10-Cl	6.43±0.07	240	—
13	2-CH_3, 10-Br	5.67±0.03	240	—
14	2-CH_3, 10-NO_2	4.80±0.05	270	—

[a] pK_a values of fluorophotometry.
[b] Oxazolam.
[c] Range of experimental values.
[d] Cloxazolam.
[e] Compounds required significant time lags for equilibration.
[f] Fluorescence was observed in both acidic and basic solutions.



BDOZs listed in Table I were from the sources mentioned in the acknowledgements. The compounds, which have already been reported,[7] were used without further purification.

Buffer Solutions——Buffer solutions used were 0.1 N hydrochloric acid (pH 1.2), glycinate (pH 2—4), acetate (pH 3.5—6), phosphate (pH 6—8), and borate (pH 8—10.5). Concentrations of the buffer solutions were 0.005—0.01 M, and the ionic strength was adjusted to $\mu=0.1$ by addition of sodium chloride. All the reagents were of reagent grade, and distilled water was used.

Apparatus——UV absorption spectra were obtained on a Hitachi 323 spectrophotometer. Fluorescence measurements were carried out with a Hitachi 203 fluorescence spectrophotometer, and a Hitachi-Horiba F-7 pH meter was used for pH measurements.

Procedure——A stock solution of each compound was prepared by dissolving the compound in ethanol at 200 μg/ml concentration, and kept in a refrigerator. The stock solution was diluted to 10 μg/ml (3.04×10^{-5} M for oxazolam) with appropriate buffers to give experimental solutions, which consequently contained 5% (v/v) ethanol. The range from about pH 1 to 10 was scanned for each compound in increments of 1 pH unit to determine the approximate position of the pK_a value by observation of spectral changes. The pH region around the approximate pK_a was then studied in more detail by using buffers differing by increments of about 0.2 pH units. From the spectra obtained, the pK_a values were calculated by employing the Henderson–Hasselbelch equation.[9] UV scanning was carried out within 5 min after diluting the stock solution with the buffer to minimize hydrolytic decomposition of the sample compound. The cases of compound **3** and **9** were exceptional, since it took a significant time for the reaction to reach equilibrium in those cases. Therefore, UV measurements were carried out after the equilibria had been reached, by leaving experimental solutions to stand at room temperature for a sufficient period; equilibration was detected by following the development of fluorescence in the experimental solutions as described below.

Fluorescence Measurements——Fluorescence measurements were carried out in each experimental solution at constant wavelengths (380 nm for exciting, 500 nm for analyzing). Reading of the intensity was continued until it reached a constant level for the determination of the equilibrium value. No temperature control was used during the measurements.

Results and Discussion

pH Dependency of UV Absorption Spectra

The UV spectra of **1** and **4** in aqueous solutions with various pH values are shown in Figs. 1 and 2. The preliminary study indicated that the spectral changes were most prominent at

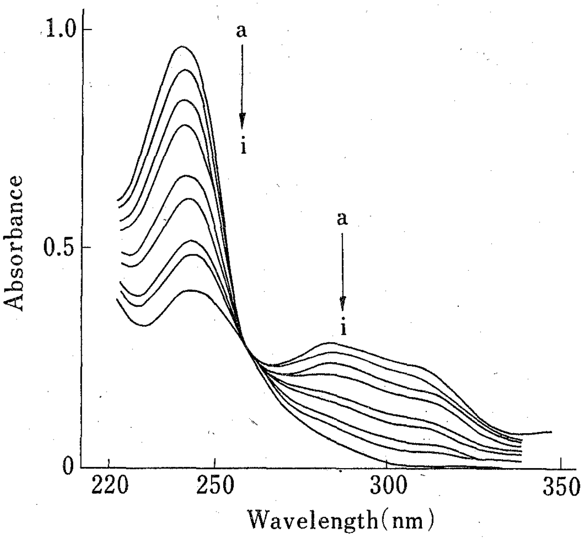


Fig. 1. UV Absorption Spectra of Oxazolam in Aqueous Solution at Various pH_s

Concentration; 3.04×10^{-5} M.
a, pH 1.20; b, 4.85; c, 5.15; d, 5.38; e, 5.79; f, 5.89; g, 6.33; h, 6.48; i, 9.08.

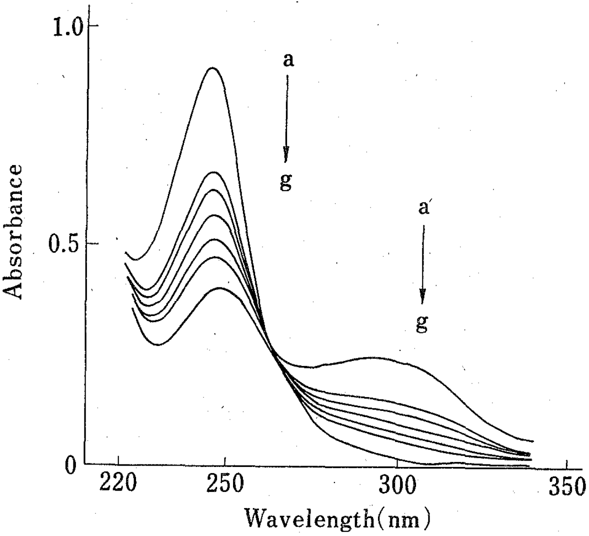
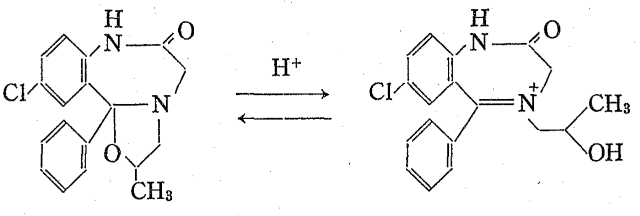


Fig. 2. UV Absorption Spectra of Compound **4** in Aqueous Solution with Various pHs

Concentration; 2.65×10^{-5} M.
a, pH 1.19; b, 6.08; c, 6.26; d, 6.48; e, 6.68; f, 6.88; g, 9.40.

around pH 5.5 and pH 6.0 for **1** and **4**, respectively. Absorption curves (a) and (i) in Fig. 1 correspond to the compounds **15** and **1** in Chart 2, respectively, and others represent equilibrium mixtures of these two.

The protonation of 1,4-benzodiazepines was reported[2,3,10] to take place on the nitrogen at position 4. In the case of oxazolam (**1**), the protonated species at the nitrogen atom further reacted to open the five-membered (oxazolodine) ring, forming a benzodiazepinium ion (**15**) as shown in Chart 2.[11] The reaction pathway was assumed on the basis of the experimental result that the UV absorption spectrum in acidic media, which was similar to that of protonated desmethyldiazepam, was greatly changed from the original. This assumption was confirmed by infrared (IR) spectroscopy of **15** isolated from chloroform solution of oxazolam by introducing dry hydrogen chloride. The IR spectrum suggested the presence of a hydroxyl group (broad absorption around 3100—3400 cm^{-1}) and the absence of ether oxygen because no strong absorption peak was observed at around 1100 cm^{-1} attributable to carbon-oxygen stretching.



1 oxazolam **15**

Chart 2. Acid-Base Equilibrium Reaction of Oxazolam

Other BDOZs in Table I exhibited similar UV spectral curves and shifts with change of medium pH. Therefore the acid–base equilibrium reaction was considered to follow the same pathway as shown in Chart 2 for all 14 compounds.

pK_a Value of BDOZ

Although the equilibrium reaction was not a simple protonation–deprotonation, the equilibrium constant could be considered as a kind of acid-dissociation constant, and a pK_a was defined through K_a described by equation (1).

$$AH^+ \rightleftarrows A + H^+$$

$$K_a = \frac{[A]\cdot[H^+]}{[AH^+]} \qquad (1)$$

where AH^+ is a diazepinium ion species such as **15**, A is an original molecule such as oxazolam, and H^+ is a hydrogen ion. The pK_a values were obtained from the pH dependencies of UV spectra through the procedure described in the experimental section, and the results are shown in Table I with the analytical wavelengths used.

Since the diazepinium ions exhibited fluorescence which was not observed in the original molecules with the exception of **10**, the pK_a values were also derived from the relationships between equilibrium fluorescence intensity and pH value of the experimental solution. The relationship is given by equation (2).[12)]

$$\mathrm{pH} = \mathrm{pK_a} + \log\frac{F_0 - F}{F} \qquad (2)$$

where F and F_0 are the fluorescence intensities of the experimental solution and the solution in 0.1 N hydrochloric acid, respectively. Thus, F_0 is the maximum intensity which is developed in the solution of 100% diazepinium ion species. By introducing the term relative intensity $I_{rel}=F/F_0$, equation (2) can be reformulated as equation (3).

$$I_{rel} = \frac{1}{10^{(\mathrm{pH}-\mathrm{p}K_a)}+1} \qquad (3)$$

This equation predicts a sigmoidal curve for a plot of I_{rel} *versus* pH, and when I_{rel} is 0.5 the pH value is equal to the pK_a of the compound.

Several examples of the relationship are shown in Fig. 3. F_0 values were 2.55, 1.81, 1.94, and 0.84 for compounds **1**, **2**, **3**, and **4**, respectively, when represented by the ratio to the fluorescence intensity of a quinine sulfate 0.1 μg/ml 0.1 N sulfuric acid solution (350 nm for excitation, and 450 nm as the analytical wavelength). The pK_a values were graphically obtained as the pH values at the half relative intensity, regarding the curves as straight lines in a narrow range. The values determined by this method are also given in Table I, and are in good accord with those obtained by UV spectroscopy.

In most samples the development of the fluorescence was instantaneous upon preparation of the experimental solutions. However, it took a significant time to reach the equilibrium intensity for two of the compounds as specified in Table I. Figure 4 shows the process of the

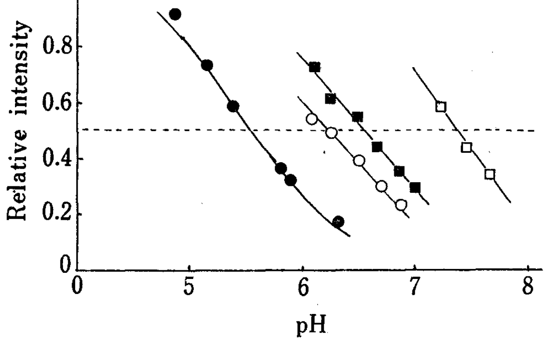


Fig. 3. Relationship between Fluorescence Intensity and Medium pH in Benzodiazepinooxazoles

●, oxazolam; ○, compound **4**; ■, compound **3**; □, cloxazolam.

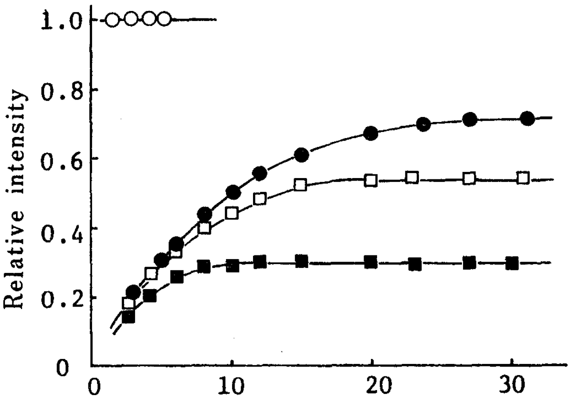


Fig. 4. Development of Fluorescence in Aqueous Solution of Compound 3 at Room Temperature

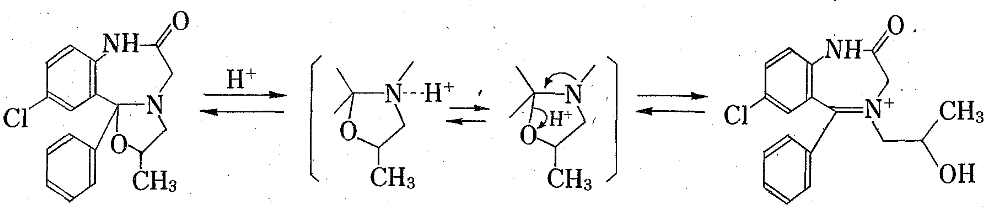
○, pH 1.18; ●, pH 6.08; □, pH 6.48; ■, pH 6.98.

fluorescence development in **3**, as an example, in four kinds of solution with different pH values.

Mechanism of the Equilibrium Reaction

The fluorescence study revealed that a significant time was required for equilibration in the above two compounds. The required time depended on the compounds and also on the pH of buffer solutions. A rapid equilibration occurred in strong acid solution, *i.e.* 0.1 N hydrochloric acid, pH 1.2, for all 14 compounds. On the other hand, slow equilibration was observed in a solution with pH close to the pK_a value of the corresponding compound, or more basic.

Although no detailed kinetic investigation was carried out in the present study, reaction pathway described in Chart 3 is feasible. The protonation on a tertiary nitrogen might be the slowest step in the forward reaction, since strongly acidic media prompted the development of fluorescence. This assumption is also supported by the fact that the methyl substitution at position 3 or 5 markedly delayed the equilbration. This could be due to steric interference of the methyl group with proton approach to the nitrogen.



1 **15**

Chart 3. Possible Mechanism for the Acid–Base Equilibrium Reaction of Benzodiazepinooxazoles (exemplified by Oxazolam)

Effect of Substituents on the pK_a

Fourteen compounds in the present study possessed the same fundamental chemical structure, so the differences of the pK_a values were considered to depend on the substituents. The results in Table I can thus be interpreted from the viewpoint of electronic and steric effects of the substituents.

Since the concentrations of the chemical species are low enough in the present study, the equilibrium constant K_a in Eq. (1) can be represented by Eq. (4) in thermodynamic terms.

$$K_a = \frac{a_A \cdot a_{H^+}}{a_{AH^+}} \qquad (4)$$

where a_{AH^+}, a_A, a_{A^+} are activities of chemical species AH^+, A, and H^+ respectively. In the next step, Eq, (4) can be converted into Eq. (5) in free energy terms.[13]

Table II. Substituent Effects on pK_a of Benzodiazepinooxazoles

Substituent	ΔpK_a	Compounds in comparison
10-Cl	−1.21	**1** and **6**
10-Br	−1.28	**6** and **13**
10-NO_2	−2.15	**6** and **14**
2-CH_3	−0.69	**1** and **12**
3-CH_3	−0.58	**2** and **3**
5-CH_3	−1.21	**1** and **9**
7-CH_3	−1.22	**1** and **5**
2′-F	−0.18	**1** and **8**
2′-Cl	+0.84	**2** and **12**
2′-CH_3	+2.56	**1** and **7**
4′-OCH_3	+0.94	**1** and **11**
4′-NO_2	−0.90	**9** and **10**

$$K_a = \exp\left(-\frac{\Delta G}{RT}\right)$$
$$= \exp\left\{-\frac{1}{RT}(G_A + G_{H^+} - G_{AH^+})\right\} \tag{5}$$

where G_{AH^+}, G_A, and G_{H^+} are the molar free energies of AH+, A, and H+, respectively, and ΔG is the overall difference of the free energy in the system. Since G_{H^+} is constant a at given pH, the K_a changes with the energy difference between G_{AH^+} and G_A, which depends on the substituents.

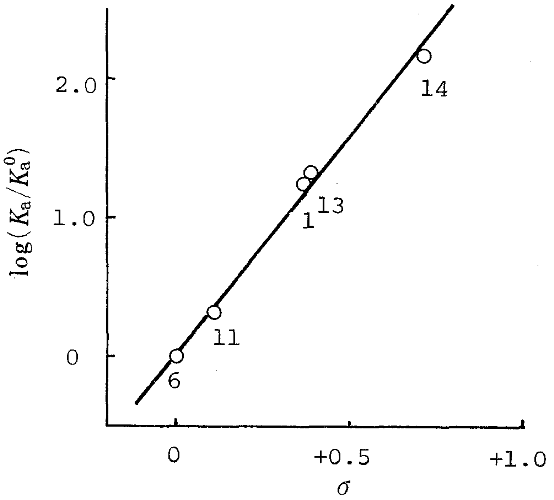


Fig. 5. Relationship between Hammett's σ-Value and the Logarithm of the Relative Dissociation Constant

K_a°, dissociation constant of compound **6**;
K_a, dissociation constant of the compound corresponding to the indicated number.
The regression equation derived by the least-squares method was:
$\log (K_a/K_a^\circ) = 3.10\sigma + 0.003$, $r = 0.998$.

Table II summarizes the substituent effect on pK_a by comparing pairs of compounds with hydrogen and a substituent at the corresponding position. Although only one pair of compounds was compared for each substituent, the trend and the approximate extent of the pK_a shift could be estimated. The substituents at R_{10}, $R_{2'}$, and $R_{4'}$ could electronically affect the energy level of the diazepinium ion (G_{AH^+}) through stabilizing or destabilizing the carbon-nitrogen double bond. In other words, electron-donating substituents stabilized AH+ and raised the pK_a value, whereas electron-withdrawing groups lowered it. Methyl substitution at R_2, R_3, and R_5 markedly lowered the pK_a value. Since it was difficult to explain the effect on pK_a on the basis of the electronic properties of the methyl group, the steric effect may be involved in this case. The group might interfere with proton approach to the tertiary nitrogen, or prevent solvation (hydration) of the diazepinium ion species that stabilizes the ions in solution.[14]

Figure 5 shows the relationship between Hammett's σ-value for substituents and log (K_a/K_a^0) for the five compounds, which were suitable for plotting because no steric effects are anticipated. The figure showed an excellent linear free energy relationship with a correlation coefficient of 0.998, and therefore the result supports the organo-electronic interpretation of the substituent effect at positions $R_{4'}$ and R_{10}.

The effect of the methyl group at position R_7 which greatly lowered the pK_a was difficult to interpret either electronically or sterically.

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