Proton Transfer Equilibria in The Excited State of Piroxicam and Its Analog in Aqueous Solution

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The pH dependence of the absorption and fluorescence of 4-hydroxy-2-methyl-1,2-benzothiazinenecarboxylates, piroxicam and HMBDC have been measured and compared with the solvent dependence of the spectra reported previously. Four different prototropic species are observed in both ground and excited states of piroxicam; the cation, the neutral, the anion and the dianion, while three different species such as the cation, the neutral and the anion are observed in HMBDC. The pK_a and pK_a^* have been determined by absorptiometric titration and Förster cycle method, respectively. The probable structure of each species has been proposed on the basis of the intramolecular phototautomerism.

Introduction

Piroxicam(4-hydroxy-2-methyl-N-2-pyridyl-2H-1,2-zothiazine-3-carboxamide-1,1-dioxide), a nonsteroidal and anti-inflammatory drug which acts as a cutaneous photosensitizer in some patients.[1,2] In the previous paper[3], we presented evidences of the excited state intramolecular proton transfer (ESIPT) (intramolecular phototautomerism) from the hydroxyl group of the benzothiazine ring to the carbonyl group of the amide group of the side chain through the intramolecular hydrogen bonding, even though these evidences can not rule out the possibility of intermolecular (biprotonic) phototautomerism completely. This observation has caused a great deal of interest in the exploration of the proton transfer mechanisms in the excited state of multifunctional compounds, since the piroxicam has four functional groups which can play as a proton acceptor as well as a proton donor. Actually the excited state proton transfer reactions of several bifunctional molecules such as *o*-quinoline carboxylic acids[4] and *o*-hydroxy aromatic acids[5,6] have been studied well, but not much work has been carried out in molecules having more than two functional groups except a few cases.[7,8]

We report here further details of the previous study with extension of the work to the pH dependence of the absorption and fluorescence spectra of piroxicam and the acidity constants of the different prototropic equilibria of piroxicam in aqueous solution. The same studies have been also performed with a skeletal precursor of piroxicam, HMBDC (4-hydroxy-2-methyl-1,2-H-benzothiazine-1,1-dioxide-3-methyl carboxylate) for the comparison purpose.

Experimental

Piroxicam was a generous gift from Yu Han Pharmaceutical Co. in Korea and further purified by recrystallizing three times from methanol. HMBDC was synthesized by modifying the methods described elsewhere[9] (See also ref. 3 for the details). Water used for the preparation of aqueous solutions was triply distilled in the presence of $KMnO_4$. Buffer solutions of known pH were prepared by mixing Anala *R* grade acid or base with its salt; KCl-HCl (pH, 1-3), HAc-NaAc (pH, 3-5), KH_2PO_4-Na_2HPO_4(pH, 6-8), NH_4Cl-NH_4OH (pH, 9-13). A modified Hammett's scale[10] for the H_2SO_4-H_2O mixture and Yagil's basicity scale[11] for the KOH-H_2O mixture were used for the solutions below pH 1 and above pH 13, respectively. The pH measurements were made on a Crison Model 501 digital pH/mV mether. Absorption spectra were recorded on a Beckman UV-5260 spectrometer. Fluorescence measurements were made on a scanning SLM-Aminco-4800 spectrofluorometer which makes it possible to obtain corrected spectra using Rhodamine B as a quantum counter. A narrow excitation slit (4 nm) was used to minimize the photolysis of the samples. The excitation wavelengths were chosen from isobestic points of the absorption spectra of different prototropic species. 2.0×10^{-5}M solutions were prepared for the spectral measurements by dluting 0.2 m*l* of a 1.0×10^{-3}M to 10 m*l* using different Ho/pH/H solutions. The stock solutions were prepared in 40 vol.% methanol-60 vol% water for the pH study becase of the low solubility of piroxicam or HMBDC.

Results and Discussion

Effect of pH on the absorption spectra. Figure 1 shows four different absorption spectra of piroxicam as a function of pH, indicating that four different prototropic species can be formed. The spectrum at pH 2 has a maximum absorption band at 340 nm, which resembles that in moderately polar organic solvents[3]. No changes of this spectrum are observed between pH 1 and pH 2.40 Thus, it can be inferred that piroxicam stays in neutral form in this pH region. Below pH 1, the position of absorption maximum is slowly blue-shifted to 331 nm that is believed to be due to protonation of the molecule. In piroxicam, there are three most possible candidates protonation sites; hydroxyl group of benzothiazine ring, and nitrogen atoms of amide group and pyridine ring. The nitrogen of pyridine is known to be a charge-transfer acceptor in the excited state,[12] and protonation of its non-bonded electron pair enhances the acceptor property of this group so that it results in stabilization of the excited states relative to the ground state.[12] If this is the case, one should expect that protonation of pyridine nitrogen produces red-shifted absorption spectra in contrast to the actually observed blue-shifted one. Therefore, the pyridine nitrogen can be ruled out as the protonation site. On the

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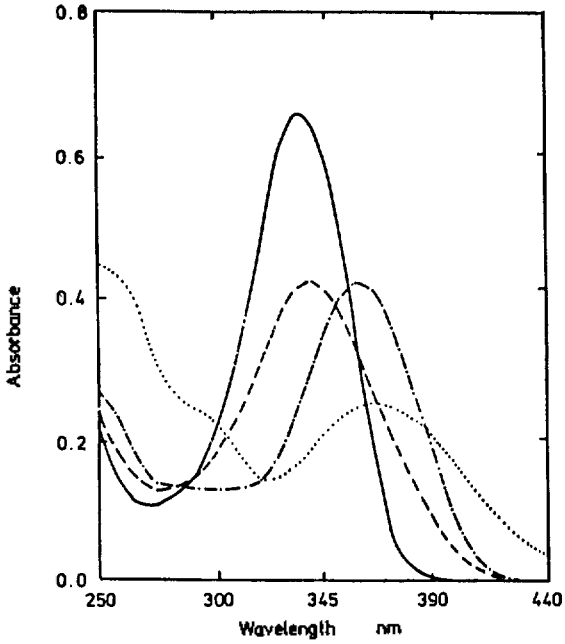

Figure 1. Absorption spectra of different species of piroxicam at 298K: (— at H_0-6.56) cation; (···· at pH 2) neutral; (- - - - at pH 7) anion; (···· at H_17.14) dianion.

other hand, the hydroxyl group and amide -NH are charge-transfer donors, and protonation of these functional groups would produce the blue-shift of the absorption maximum. Because of difference in electronegativities of oxygen and nitrogen, the first protonation would take place at the amide -NH and not at oxygen of hydroxyl group, forming a cation form of piroxicam. The pK_a for the equilibrium between the neutral molecule and cation, determined spectrophotometrically, is found to be 1.46. This is slightly greater than that for diphenyl amine (1.15)[13], which is probably due to different substituents around nitrogen as compared to diphenyl amine. The second protonation is not observed under the present experimental conditions.

In solutions above pH 3, the positions of the absorption maxima are observed at wavelength longer than 340 nm. The absorption spectrum between pH 3 and H_ 16.58 show the maximum band at 357 nm which further moves to 370 nm in basic solutions aboe H_ 17.14. These spectral changes are attributed to deprotonation leading to the formation of mono- and dianion species of piroxicam. In piroxicam, two possible deprotonation sites can be considered; one is hydroxyl group and the other is amide -NH. This is in line with the theory that charge-transfer donors such as these groups cause the red-shift of the absorption spectrum by deprotonation[12]. The first deprotonation would take place at the hydroxyl group because the electronegativity of oxygen is smaller than that of nitrogen. The pK_a for such a deprotonation is found to be 3.33, which is much lower than that for phenol[14]. The low pK_a could be due to more electron-withdrawing groups around the hydroxyl group and more extended ring system as compared to phenol. The pK_a for the second deprotonation at amide -NH is found to be 17.10 which is consistent with the fact that most of secondary amines have pK_a larger than 16.00[7]. From the absorption spectral results as discussed above, the ground state equilibrium reactions of the prototropic species of piroxicam are

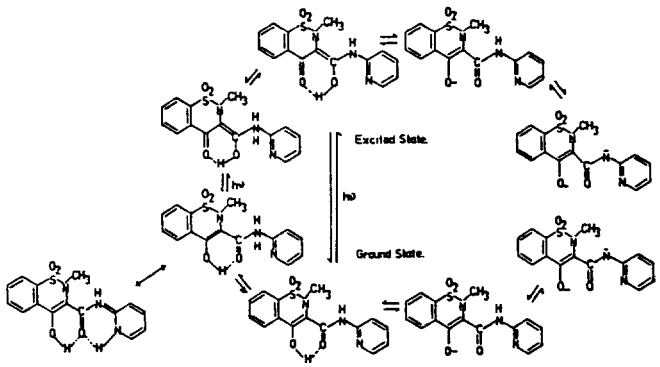

Figure 2. Acid-base equilibria scheme for piroxicam in the ground and excited states.

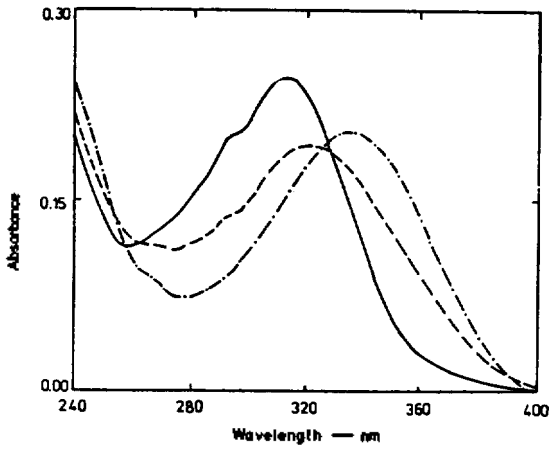

Figure 3. Absorption spectra of different species of HMBDC at 298K: (— at pH 1) cation; (---- at pH 6) neutral; (- - - - at pH 10) anion.

summarized in Figure 2.

The absorption spectrum of HMBDC, which has the same skeletal structure in benzothiazine ring but has a methoxy group in stead of 2-aminopyridine of piroxicam, is also sensitive to variation of H^+ concentration. Three different spectra are observed in the regions of pH 5~H_0-5.04, pH 5~pH 7 and pH 7~H_17.14 (Figure 3), indicating that three different prototropic species such as cationic, neutral and anionic forms can be formed. In contrast to the case of piroxicam, no dianionic form is observed. This supports above argument that the second deprotonation in piroxicam occurs at the amide -NH.

Effect of pH on the fluorescence spectra. The fluorescence spectra of piroxicam and HMBDC were observed in aqueous solution in the range H_0-0.56 to H_18.23 and shown in Figure 4 and 5, respectively. From the spectra of piroxicam, it can be inferred that four prototropic species are present in the excited state: the cation in the region H_0-0.56 to pH 1, the neutral form in the pH range 1.2-3.0, the monoanion in the range pH 3.2 to H_16.0 and the dianion in the range H_17.0 to H_18.2. The spectra of HMBDC also show three different species formed in aqueous solution: the cation in the range H_0-0.56 to pH 0.5, the neutral form in the range pH 1 to H_13.0 and the anion in the range H_14.0 to H_18.2. The cation fluorescence of piroxicam is blue-shifted as compared with the neutral and dianionic forms, being consistent with the absorptional spectral changes (Table 1). This in-

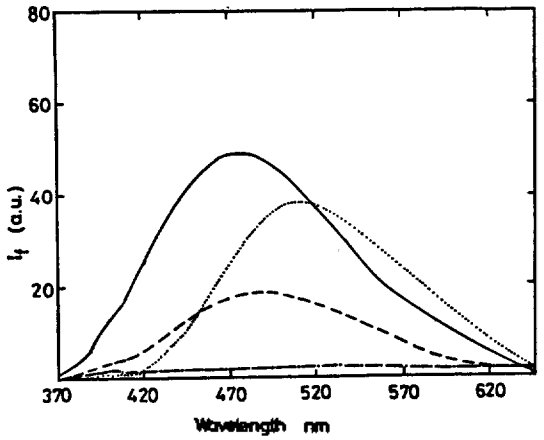

Figure 4. Fluorescence spectra of different species of piroxicam at 298K: (— at H_0-6.56) cation; (— at pH 1) neutral; (- - - - at pH 7) anion; (···· at H_17.66) dianion. λ_{ex} = 350nm.

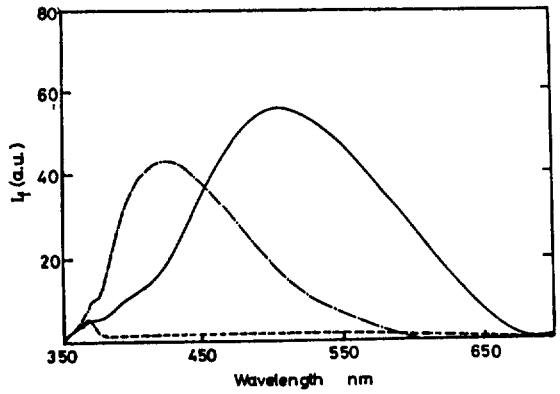

Figure 5. Fluorescence spectra of different species of HMBDC at 298K: (—at H_0-5.04) cation; (---- at pH 7) neutral; (· · · · at H_17.95) anion. λ_{ex} = 330nm.

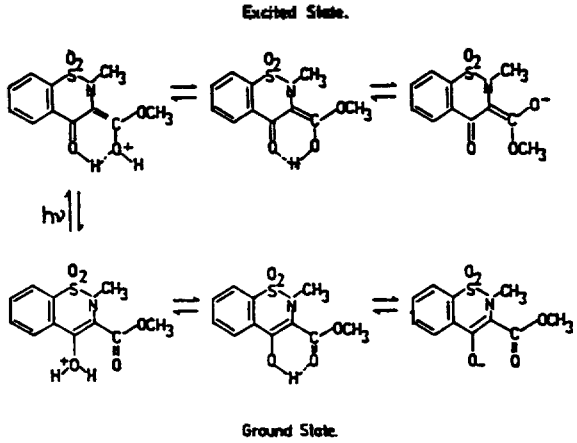

Figure 6. Acid-base equilibria scheme for HMBDC in the ground and excited states.

dicates that the protonation and deprotonation take place at the same sites as those in the ground state (Figure 1). However, the cation fluorescence of HMBDC is strongly red-shifted in comparison with anionic and neutral forms. This is quite opposite against the case of the absorption spectra, indicating that the protonation site in the excited state is different from the ground state. As shown in Figure 6, the protonation in HMBDC in the ground state would take place at -OH which is a charge transfer donor in the excited state as stated earlier for piroxicam. However, in the excited state $-CO_2CH_3$ would be more favorable than -OH, since the $-CO_2CH_3$ is a charge transfer acceptor and the protonation at this functional group is expected to cause the red shift of the fluorescence spectrum[12]. This also suggests the existence of intramolecular phototautomerism which corresponds to the excited state intramolecular proton transfer between -OH and $-CO_2CH_3$ (see Figure 6). The intramolecular phototautomerism leads to an abnormally large Stokes' shift (over 9,000 cm^{-1}) of emission maximum.[3,15] Actually the emission maximum of the cation of HMBDC shows the extraordinarily large Stokes' shift (ca. 12,300 cm^{-1}) (Table 1), in contrast to that of the anion. Even though the magnitude is smaller than the case of HMBDC, the emission maxima of cationic and neutral species of piroxicam also show the abnormally large Stokes' shift (over 9,000 cm^{-1}), indicatitive of the same intramolecular phototautomerism as in the case of HMBDC (see Figure 6).

Table 1. Absorption and Fluorescence Maximum Wavelengths of Piroxicam and HMBDC

Piroxicam (HMBDC)	λ_{ab}^{max},nm	λ_{fl}^{max},nm	Stokes' shift, cm^{-1}
cation	330(310)	475(500)	9159(12258)
neutral	340(320)	495(-)	9209(-- --)
anion	365(335)	- (420)	- (6041)
dianion	370(-)	515(-)	7609(-)

*( ) represents the value for HMBDC.

Table 2. pK_a and pK_a^* Values of Piroxicam and HMBDC

Equilibrium (piroxicam)	pK_a	$pK_a^*(ab)$	$pK_a^*(fl)$
cation-neutral	1.46	-0.27	-2.5
neutral-anion	3.33	-0.61	1.30
anion-dianion	17.10	-15.03	-17.30
Equilibrium (HMBDC)	**pK_a**	**$pK_a^*(ab)$**	
Cation-neutral	5.48	3.55	
neutral-anion	6.53	3.81	

*pK_a^* have been calculated by Förster cycle method, using absorption and fluorescence data.

The pK_a^* for the excited-state equilibria of piroxicam and HMBDC have been calculated by Förster cycle method[16], using the fluorescence and absorption data. These results are listed together in Table 2. These observations show that pK_a^* of each equilibrium is much lower than pK_a, being consistent with the fact that $-NH_2^+$, $-OH_2^+$, -OH and -NH in each species become more acidic in the excited state than in the ground state as in the case of similar groups in other compounds.[17,18] This suggests that the proton transfer in the excited state of piroxicam or HMBDC is more feasible than in the ground state, as discussed above. Such a proton transfer can cause the presence of different structures in the excited state as compared with those in the ground state, *i.e.* phototautomerized structures as shown in Figure 6. Furthermore, the data of Table 2 show that the pK_a^*'s obtained from fluorescence data are different from those obtained from ab-

sorption data, supporting the possibility of the structural differences in the two states.

Acknowledgement. This work was supported by the grant for the Basic Research Institute Program of the Ministry of Education of the Republic of Korea (1988–1989).

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Properties of Sodium Dodecyl Sulfate / Triton X-100 Mixed Micelle

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The cmc's of sodium dodecyl sulfate (SDS)/Triton X-100 surfactant mixtures were determined by surface tension measurement at various surfactant compositions. The cmc values were lower than those predicted from ideal mixture. The regular solution theory was applied to calculate the interaction parameter, micellar composition, and the activity coefficients of surfactants in the mixed micelle. The interaction parameter (β) was -2.1. The nonideality arised largely from decreased activity of SDS in the mixed micelle. The mean aggregation numbers ($\bar{n}$) and micropolarity of hydrocarbon region of the mixed micelles were determined by luminescence probe techniques. The total aggregation number ($\bar{n}_{SDS} + \bar{n}_{TX}$) in mixed micelles showed little dependency on the composition of the micelle. The apparent dielectric constant of the hydrocarbon region of the micelle *vs* micellar composition plot showed positive deviation from linearity. Emission and emission quenching of excited tris(2,2'-bipyridine)ruthenium(II) cation, ($Ru(bpy)_3^{2+}$), by methylviologen (MV^{2+}) were also investigated in the mixed micellar solutions. The quenching rate was lowest when the mole fraction of SDS in the surfactant mixtures (α_{SDS}) is about 0.25 and highest at $\alpha_{SDS} = 0.85$. This was explained in terms of combined effects of binding of the cations with the micelle and mobility of the bound cations on the surface of the micelles.

Introduction

The physico-chemical properties of micelle-solubilized or bound substrates are strikingly different from those in homogeneous media. The properties are critically dependent on microenvironment of the substrates in the micellar pseudophase. In view of increasing interest in the chemistry of micellar system,[1] it is desirable to design the micellar systems of particular characteristics. Since homomicellar systems which can be practically used are limited, mixed surfactant systems are required to obtain the desired properties. In fact, surfactants used in practical applications are essentially mixed surfactant systems. In many applications, they exhibit superior properties and are less expensive than single-compound surfactant systems. Therefore, there is increasing interest in understanding the strucutre and properties of mixed micelles.[2-13] However, most of work on mixed surfactant systems are focused on the critical micelle concentration (cmc) and thermodynamics of formation of mixed micelles. Little attention has been paid on the microscopic characteristics of the micelles.

In this paper, we describe the results of studies on the formation and physico-chemical properties of mixed micelle formed between sodium dodecyl sulfate (SDS) and Triton X-100 (TX-100:polyoxyethylene glycol *p*-isooctyl phenyl ether with about 10 oxyethylene units), which are the most