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Excited state proton transfer of methyl- and cyano- substituted alloxazines in the presence of acetic acid

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Abstract

The photoinduced excited state double proton transfer reaction of methyl- and cyano-substituted alloxazines was examined by steady state and time-resolved methods. The fluorescence decay times of the alloxazinic forms in 1,2-dichloroethane and 1,2-dichloroethane in the presence of acetic acid are similar and of the order of hundreds of picoseconds. The fluorescence decay times of the isoalloxazinic forms, created by excited state proton transfer, are of the order of nanoseconds. On the basis of the function describing the emission decay of the bands corresponding to the isoalloxazinic and alloxazinic forms and the lifetimes obtained, it was found that the excited alloxazinic form is a precursor of the excited isoalloxazinic form, and the rate constant of the proton transfer process is lower than 10^9 s^{-1}. These results and those of stationary studies, indicating that the isoalloxazinic and alloxazinic species derive from different forms in the ground state, have enabled a model of the proton transfer reaction of alloxazines to be proposed. In this model, the possible formation of alloxazine–acetic acid complexes in a conformation permitting proton transfer, in both the ground and excited states, was assumed.

Keywords: Excited state proton transfer; Methyl- and cyano- substituted alloxazines; Acetic acid

1. Introduction

A considerable number of studies on excited state proton transfer in organic molecules have been performed both experimentally and theoretically [1]. The excited state proton transfer in alloxazines containing no substituent at the N-1 position [2–4] was classified by Kasha [5] as an excited state strong catalysis double proton transfer. It proceeds only in the presence of certain compounds which possess proton donor and acceptor functions and are able to form hydrogen bonds of appropriate strength and conformation with the alloxazine molecules, i.e. some carboxylic acids and water [2–5].

Proton transfer in alloxazines in the presence of acetic acid has been the subject of many studies [2–14]; however, the mechanism of this process has not yet been fully explained. As postulated by Song et al. [3], proton transfer takes place in cyclic complexes in which the same acetic acid molecule is simultaneously hydrogen bonded at the N-1 and N-10 nitrogen atoms of alloxazine. On excitation, the electron density at N-10 increases and that at N-1 decreases, creating the driving force for the concerted double proton transfer.

The basic controversies which arise from the literature data concern the rate of the proton transfer process. Some studies based on time-resolved methods have revealed a relatively high rate constant (of the order of 10^{12} s^{-1}) [6]. Such results lead to the conclusion that alloxazine–acetic acid complexes with a structure permitting proton transfer are created in the ground state. The static catalysis of double proton transfer in alloxazines was corroborated by results of steady state studies [5,7] and by the persistence of the isoalloxazinic emission at 77 K in a rigid solvent matrix [5].

However, Choi et al. [8], on the basis of steady state and time-resolved studies, have estimated the rate constant of proton transfer in the lumichrome (7,8-dimethylalloxazine)–acetic acid system to be of the order of 10^8 s^{-1}. Dzugan [9] reported three different values of the proton transfer rate constants depending on the type of complex between lumichrome and acetic acid involved in the reaction. The relatively slow rates of proton transfer reported in these papers may indicate that alloxazine–acetic acid complexes with a structure permitting proton transfer are created after excitation. In addition, the dependence of the phototautomeric efficiency on the viscosity and temperature [4] testify to such a possibility.

To learn more about the kinetics of excited state proton transfer in alloxazines, we have undertaken a study using

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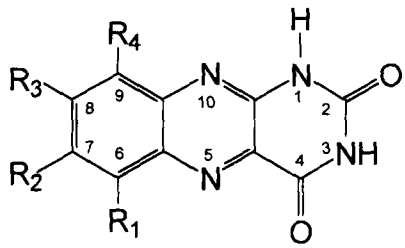


R_1=CH_3,	R_2=H,	R_3=H,	R_4=H	6-methylalloxazine, 6MAll
R_1=H,	R_2=CH_3,	R_3=H,	R_4=H	7-methylalloxazine, 7MAll
R_1=H,	R_2=H,	R_3=CH_3,	R_4=H	8-methylalloxazine, 8MAll
R_1=H,	R_2=H,	R_3=H,	R_4=CH_3	9-methylalloxazine, 9MAll
R_1=H,	R_2=CN,	R_3=H,	R_4=H	8-cyanoalloxazine, 7CNAll
R_1=H,	R_2=H,	R_3=CN,	R_4=H	7-cyanoalloxazine, 8CNAll

Fig. 1. Structures of the alloxazines studied.

steady state and time-resolved methods. As the phototautomerism efficiency in alloxazines depends on the substituent in the benzene ring [4,10,11], we have chosen a series of methyl- and cyano-substituted alloxazines for investigation (Fig. 1). The results obtained permit a more complete scheme of the excited state reaction to be proposed. In this model, we postulate that it is possible to create alloxazine–acetic acid complexes in the ground and excited states.

2. Materials and methods

The alloxazine derivatives with methyl monosubstituted at the C-6, C-7, C-8 and C-9 positions in the benzene ring (6MAll, 7MAll, 8MAll, 9MAll) and cyano monosubstituted at the C-7 and C-8 positions (7CNAll, 8CNAll) were synthesized in our laboratory according to the methods of Kühling [15], Goldner et al. [16] and Panek-Janc and Kozioł [17,18]. All compounds were repeatedly crystallized from methanol until chromatographically (TLC) and spectrophotometrically pure.

1,2-Dichloroethane was chosen as solvent since, as shown earlier [7], it is of relatively low polarity, moderately active in hydrogen bond formation and dissolves alloxazines well. 1,2-Dichloroethane was of p.a. grade and was distilled over phosphorus pentoxide from an all-glass apparatus. Acetic acid (p.a. grade) was used without further purification; the purity was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer.

Absorption spectra were recorded on a Cary 118C spectrophotometer (Varian, Palo Alto, CA). Steady state corrected fluorescence emission and excitation spectra were taken using an MPF-44A/E fluorescence spectrophotometer (Perkin-Elmer, Norwalk, CT). The samples were excited at a wavelength of 360 nm to record the emission spectra and were monitored at the emission maximum to record the excitation spectra.

The fluorescence quantum yields Φ_S were determined by a relative method, using lumichrome in water ($\Phi_R = 0.088$ [19]) as reference

$$\Phi_S = \Phi_R \frac{F_S(1-10^{A_R})n_S^2}{F_R(1-10^{A_S})n_R^2} \quad (1)$$

where F_S and F_R are the integrated fluorescence intensities of the samples and reference solutions measured under identical conditions, A_S and A_R are the absorbances of the samples and reference solutions at 360 nm and n_S and n_R are the solvent refractive indices. All measurements were made at an excitation wavelength of 360 nm in solutions with optical densities below 0.05. The accuracy in the emission quantum yields is $\pm 10\%$.

The fluorescence decay curves were measured using a time-correlated single-photon counting method on the commercially available IBH model 5000U fluorescence lifetime spectrometer. Time-resolved fluorescence data were fitted to a single exponential or to a sum of exponentials by iterative convolution of trial decay curves with the instrument response function employing a least-squares fitting procedure. A good fit was determined by the reduced χ^2 criterion $0.8 < \chi^2 < 1.2$ and by the Dubrin–Watson parameter $DW > 1.7$–1.8 [20]. The accuracy in the lifetime data is $\pm 10\%$. The concentration of the alloxazine solutions for lifetime measurements was about 2×10^{-5} mol dm^{-3}, while the concentration of acetic acid was kept at 0.8 mol dm^{-3} throughout this study.

3. Results

3.1. Spectral properties of alloxazines and their phototautomers

In Fig. 2, as an example, the absorption spectra of 7-methylalloxazine in 1,2-dichloroethane and 1,2-dichloro-

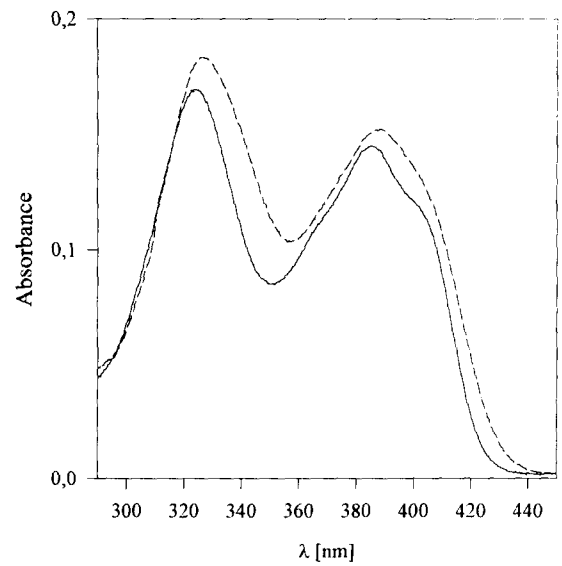


Fig. 2. Absorption spectra of 7-methylalloxazine in 1,2-dichloroethane (full line) and 1,2-dichloroethane in the presence of acetic acid (0.8 mol dm^{-3}) (broken line).

Table 1
Spectral data for alloxazines in 1,2-dichloroethane

Compound	Absorption data			Emission data			
	λ_{II} (nm)	λ_{I} (nm)		λ_{all} (nm)	$\Phi_{all} \times 10^2$	λ_{iso} (nm)	F_{iso}/F_{all}
6MAll	336	375		455	2.9	505	3.8
7MAll	323	384		442	3.4	518	8.1
8MAll	348	368(s)		425	2.4	502	17.2
9MAll	336	380		450	2.8	505	1.7
7CNAll	322	372	386(s)	420	2.2	508	6.8
8CNAll	320	380	398(s)	430	1.6	520	3.8

s, shoulder; λ_{II}, λ_{I}, absorption band maxima in 1,2-dichloroethane; λ_{all}, fluorescence maximum of alloxazinic form in 1,2-dichloroethane with and without acetic acid; λ_{iso}, fluorescence maximum of isoalloxazinic form in 1,2-dichloroethane in the presence of acetic acid; Φ_{all}, quantum yield of alloxazinic emission in 1,2-dichloroethane; F_{iso}/F_{all}, ratio of intensity at the fluorescence maxima of isoalloxazinic forms in 1,2-dichloroethane in the presence of acetic acid.

ethane in the presence of acetic acid are presented. The spectral properties of alloxazines are summarized in Table 1.

The absorption spectra of alloxazines in 1,2-dichloroethane exhibit two absorption bands in the region 300–400 nm. As predicted from theoretical calculations, these two long- wavelength bands in the absorption spectra of alloxazines reflect two independent π–π^* transitions [21–23].

In the fluorescence emission spectra of alloxazines in 1,2-dichloroethane, a single band appears with a maximum at about 435 nm, depending on the derivative (Fig. 3). The quantum yield of this emission for all the compounds studied is of the order of $(2–3) \times 10^{-2}$. The excitation spectra monitored at the maximum of this emission band agree well with the absorption spectra.

The addition of acetic acid to 1,2-dichloroethane causes small characteristic changes in the shape of the absorption bands: an increase in absorptivity and a bathochromic shift of both long- wavelength maxima positions by several nanometres (Fig. 2). Analogous changes in the absorption spectra

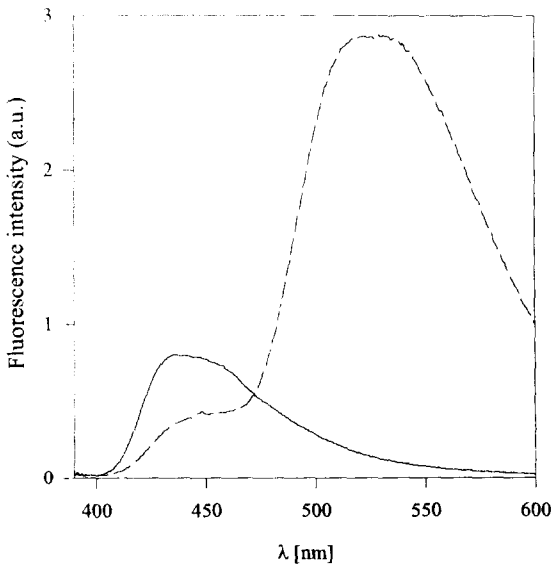


Fig. 3. Fluorescence emission spectra of 7-methylalloxazine, $\lambda_{em} = 360$ nm, in 1,2-dichloroethane (full line) and 1,2-dichloroethane in the presence of acetic acid (0.8 mol dm^{-3}) (broken line).

of alloxazines induced by the addition of acetic acid were observed by Koziołowa [4] and Szafran et al. [7], and for isoalloxazines by Yagi et al. [24], and have been ascribed to hydrogen bond formation between acetic acid and alloxazine molecules.

The fluorescence emission of alloxazines in the presence of acetic acid yields two bands with separated maxima (Fig. 3). The intensity of the alloxazinic emission band decreases and a new, intense emission band appears with a maximum at longer wavelengths. The new band is assigned to the emission of the isoalloxazinic form. The appearance of the isoalloxazinic form is interpreted as a result of excited state proton transfer from the N-1 to the N-10 position in the alloxazine molecule [3,4]. The relative intensity of the alloxazinic and isoalloxazinic emission bands depends on the concentration of acetic acid (not shown), and on the substitution in the alloxazine molecule.

The excitation spectra monitored at the emission maxima of the alloxazinic and isoalloxazinic forms in 1,2-dichloroethane in the presence of acetic acid are slightly different (Fig. 4). The excitation spectrum monitored at the emission maximum of the alloxazinic form in 1,2-dichloroethane in the presence of acetic acid also differs from that monitored in pure 1,2-dichloroethane. Similar relations between the excitation spectra monitored at the alloxazinic and isoalloxazinic bands were observed by Szafran et al. [7].

3.2. Photophysical properties of alloxazines and their phototautomers in the excited state

3.2.1. Fluorescence lifetimes

To determine the fluorescence lifetimes of the alloxazinic and isoalloxazinic forms, we have measured the fluorescence emission decays in both pure 1,2-dichloroethane and 1,2-dichloroethane in the presence of acetic acid ($c = 0.8$ mol dm^{-3}). The fluorescence decay data were analysed using single-exponential functions and a sum of exponentials. The results are presented in Table 2.

The fluorescence emission decays monitored at the emission maxima of the alloxazines in 1,2-dichloroethane can be

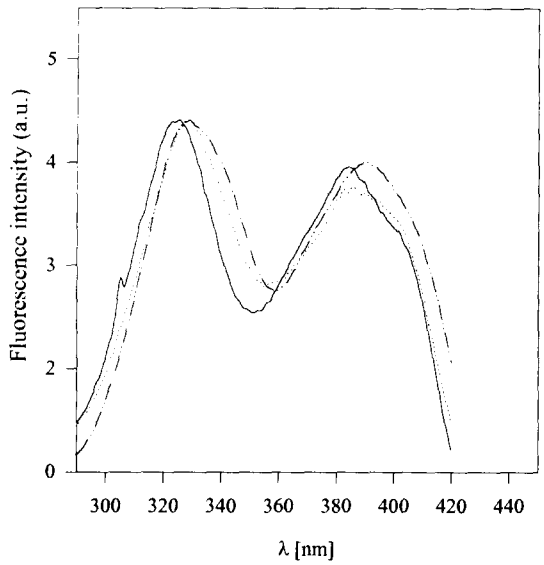


Fig. 4. Fluorescence excitation spectra of 7-methylalloxazine in 1,2-dichloroethane monitored at the emission maximum of the alloxazinic form (λ_{em} = 442 nm) (full line), and in 1,2-dichloroethane in the presence of acetic acid monitored at the emission maxima of the alloxazinic (λ_{em} = 442 nm) (· · ·) and isoalloxazinic (λ_{em} = 518 nm) (–· ·–) forms.

fitted well by single-exponential functions. The lifetimes obtained from these decays, τ_{all}^{0}, are of the order of hundreds of picoseconds and are attributed to the decay of alloxazinic species.

In 1,2-dichloroethane in the presence of acetic acid, the emission decays of the alloxazinic and isoalloxazinic forms cannot be described by a single-exponential function. In 1,2-dichloroethane in the presence of acetic acid, the alloxazinic fluorescence emission decay is well characterized as a sum of two exponentials (with positive pre-exponential factors). From these two decays observed at the maximum of the alloxazinic emission band, we obtained two distinctly different lifetimes. Shorter lifetimes, τ_{all}^{1}, which were close to the decay times of alloxazines in pure 1,2-dichloroethane and longer lifetimes, τ_{all}^{2}, of the order of nanoseconds. The fluorescence emission decays measured at the isoalloxazinic emission band (monitored on the long-wavelength slope of the emission band) can be fitted well by a sum of two components: one exponential rise (the negative pre-exponential factor) and one exponential decay (the positive pre-exponential factor). From the decay components, we have obtained lifetimes τ_{iso}^{2} which are equal to the longer lifetimes τ_{all}^{2} obtained from the decays measured at the alloxazinic emission band. The rise times of the isoalloxazinic species, τ_{iso}^{1}, determined from the rise components of the isoalloxazinic decay data agree well with the shorter alloxazinic decay times τ_{all}^{1}.

3.2.2. Decay rate constants of excited alloxazines

In order to determine the excited state decay kinetics of the alloxazinic forms in pure 1,2-dichloroethane, we calculated the radiative k_r and non-radiative k_{nr} rate constants using the equations

$$k_r = \Phi_{all} \times (\tau_{all}^{0})^{-1} \quad (2)$$

$$k_{nr} = (1 - \Phi_{all}) \times (\tau_{all}^{0})^{-1} \quad (3)$$

where τ_{all}^{0} and Φ_{all} are the fluorescence decay time and fluorescence quantum yield of alloxazines in pure 1,2-dichloroethane. The combination of low quantum yields and short lifetimes for alloxazines gave k_{nr} values of the order of 10^9 s^{-1} and k_r values two orders of magnitude lower, 10^7 s^{-1} (Table 3). These values testify to the occurrence of an efficient non-radiative process in the deactivation of excited alloxazines.

4. Discussion

4.1. Kinetic scheme for the excited state reaction

To explain the observed decay kinetics of isoalloxazinic and alloxazinic species, we may consider the following scheme for the excited state proton transfer reaction of alloxazines

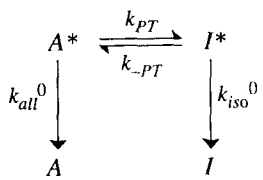
Table 2
Fluorescence decay times of alloxazines in pure 1,2-dichloroethane and in 1,2-dichloroethane in the presence of acetic acid

Compound	τ_{all}^{0} (ns)	τ_{all}^{1} (a_{all}^{1}) (ns)	τ_{all}^{2} (a_{all}^{2}) (ns)	τ_{iso}^{1} (a_{iso}^{1}) (ns)	τ_{iso}^{2} (a_{iso}^{2}) (ns)
6MAll	0.87	0.83 (0.40)	2.46 (0.60)	0.69 (−0.63)	2.35 (1.63)
7MAll	0.49	0.51 (0.58)	4.07 (0.42)	0.50 (−2.58)	4.49 (3.58)
8MAll	0.32	0.39 (0.79)	2.78 (0.21)	0.30 (−3.09)	2.76 (4.09)
9MAll	0.74	0.56 (0.84)	2.07 (0.16)	0.53 (−1.78)	2.17 (2.78)
7CNAll	0.18	0.18 (0.79)	4.21 (0.21)	0.18 (−0.69)	3.80 (1.69)
8CNAll	0.16	0.16 (0.87)	2.75 (0.13)	0.15 (−4.20)	3.34 (5.20)

τ_{all}^{0}, decay time measured at the emission band of alloxazinic forms in 1,2-dichloroethane from single-exponential functions; τ_{all}^{1}, τ_{all}^{2}, decay times measured at the emission band of alloxazinic forms in 1,2-dichloroethane in the presence of acetic acid from a sum of two exponentials; the pre-exponential factors normalized to unity ($a_{all}^{1} + a_{all}^{2} = 1$) are given in parentheses; τ_{iso}^{1}, τ_{iso}^{2}, decay times measured at the emission band of isoalloxazinic forms in 1,2-dichloroethane in the presence of acetic acid from a sum of two exponentials; the pre-exponential factors normalized to unity ($a_{iso}^{1} + a^{iso2} = 1$) are given in parentheses.

Table 3
Radiative k_r and non-radiative k_{nr} decay rate constants of the studied alloxazines in 1,2-dichloroethane

Compound	$k_r \times 10^{-8}$ (s^{-1})	$k_{nr} \times 10^{-9}$ (s^{-1})
6MAll	0.33	1.12
7MAll	0.69	1.97
8MAll	0.75	3.05
9MAll	0.38	1.31
7CNAll	1.22	5.43
8CNAll	1.00	6.15



where

$$k_{all} = k_{all}^0 + k_{PT} \quad (4)$$

$$k_{iso} = k_{iso}^0 + k_{-PT} \quad (5)$$

and k_{all}^0 and k_{iso}^0 are the sums of the rate constants for all processes of S_1 depopulation in excited alloxazinic (A*) and isoalloxazinic (I*) forms respectively, except the reactions of forward k_{PT} and back k_{-PT} proton transfer.

The analogous kinetic scheme has been analysed for the excited state reaction [25]. In general, the decays of the excited normal (A*) and tautomeric (I*) species are given by: (1) decay of excited alloxazinic species

$$[A^*] = \frac{[A^*]_0}{(\lambda_2 - \lambda_1)} [\lambda_2 - k_A) \times \exp(-\lambda_1 t) + (k_A - \lambda_1) \exp(-\lambda_2 t)] \quad (6)$$

(2) decay of excited isoalloxazinic species

$$[I^*] = \frac{k_{PT}[A^*]_0}{(\lambda_2 - \lambda_1)} [\exp(-\lambda_1 t) - \exp(-\lambda_2 t)] \quad (7)$$

$$\lambda_{1,2} = 1/2\{k_{all} + k_{iso} \pm [(k_{iso} - k_{all})^2 + 4k_{PT}k_{-PT}]^{1/2}\} \quad (8)$$

where $[A^*]_0$ is the initial concentration of the excited alloxazinic form after excitation with a Dirac δ-shape pulse.

If the equilibrium is not established within the lifetime of the excited state, then

$$[A^*] = [A^*]_0 \exp(-k_{all} t) \quad (9)$$

$$[I^*] = \frac{k_{PT}[A^*]_0}{(k_{all} - k_{iso})} [\exp(-k_{iso} t) - \exp(-k_{all} t)] \quad (10)$$

From the kinetic equations (Eqs. (6) and (7)), the excited alloxazinic species undergoing excited state reaction decay with two different rate constants, which are equal to the rate constant of rise and rate constant of decay of the isoalloxazinic species.

As shown in Table 2, and according to Eqs. (6) and (7) presented above, the experimentally obtained fluorescence decay data of the alloxazinic species are well described by the sum of two single-exponential decays, while the fluorescence decay data of the isoalloxazinic species are described by the sum of a single-exponential decay and a single-exponential rise. The shorter decay times of the alloxazinic forms, τ_{all}^1, and the rise times of the isoalloxazinic forms, τ_{iso}^1, are equal. Similarly, the longer decay times measured at the alloxazinic emission bands, τ_{all}^2, are equal to the decay times measured at the isoalloxazinic emission bands, τ_{iso}^2. The agreement between the experimental data and those predicted by the equations derived from the excited state reaction scheme indicates that the excited isoalloxazinic and alloxazinic species are kinetically related. The excited alloxazinic form is a precursor of the isoalloxazinic form.

It follows from the kinetic equations that the observed decay times of the alloxazinic species and the rise of the isoalloxazinic species depend on the rate constants of deactivation of the alloxazinic (k_{all}^0) and isoalloxazinic (k_{iso}^0) forms as well as on the rate constants of proton transfer, k_{PT} and k_{-PT}. The experimentally determined decay times of the alloxazinic forms in 1,2-dichloroethane in the presence of acetic acid, τ_{all}^1, are very close to the decay times of alloxazines in 1,2-dichloroethane without acetic acid, τ_{all}^0. This means that the rate constant of the decay of the alloxazinic species, k_{all}^0, has the highest value of all the considered constants also in the presence of acetic acid, and this value determines the observed decay time of the alloxazinic species and the observed rise time of the isoalloxazinic species. Therefore the observed rise time of the isoalloxazinic emission permits us to conclude that the proton transfer occurs at a rate constant k_{PT} smaller than 10^9 s^{-1}.

4.2. Proposed mechanism of phototautomerism in alloxazines

For systems undergoing proton transfer, the appearance of dual fluorescence may be due to the coexistence of different conformers in the ground state and, in this case, there is no direct kinetic relation between the normal and proton transfer fluorescing forms [1,26,27]. In alloxazines, as proposed previously [3,6], an analogous situation may take place, i.e. proton transfer occurs only in the alloxazine molecules appropriately bonded with acetic acid (Fig. 5) in the ground state, while the remaining molecules with no proton transfer emit alloxazinic fluorescence. Changes in the absorption spectra in the presence of acetic acid and the different excitation spectra from the alloxazinic and isoalloxazinic emission bands may support this conclusion.

However, the appearance of dual fluorescence may be due to excited state reaction between the alloxazinic and isoalloxazinic forms separated by an energy barrier. The results of the lifetime measurements reported here clearly show that the excited alloxazinic form is the precursor of the isoalloxazinic tautomer. Moreover, the results indicate that the rate constant

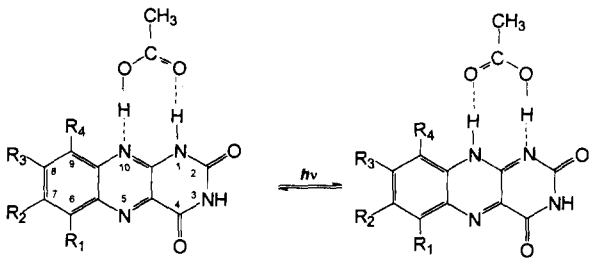


Fig. 5. Mechanism of excited state proton transfer in alloxazine–acetic acid complex proposed by Song et al. [3].

of proton transfer is lower than 10^9 s^{-1}. These results may be explained by assuming a two-stage model of reaction in the excited state: the first stage involves the formation of an acetic acid–alloxazine complex with an appropriate structure permitting proton transfer, and the second stage consists of proton transfer in the complex formed. The first stage (involving diffusion or reorientation) may be slow and may determine the observed reaction rate. The participation of diffusion or reorientation in the excited state is also indicated by the dependence of the yield of alloxazine phototautomerism on the viscosity and temperature [4].

Moreover, the relation between the phototautomeric efficiency, expressed as the ratio between the isoalloxazinic and alloxazinic fluorescence intensities (F_{iso}/F_{all}), and the transition energy difference between the alloxazinic and isoalloxazinic forms [28], as well as the good correlation between the phototautomeric efficiencies and electron density differences on the N-10 and N-1 nitrogen atoms found by Koziołowa et al. [11], suggest that the excited alloxazinic and isoalloxazinic forms are separated by an energy barrier. The height of the intrinsic energy barrier is determined by the energy differences between the tautomeric forms and by the charge redistribution on the N-1 and N-10 nitrogen atoms.

In summary, the results of steady state studies, especially the difference between the excitation spectra of the tautomeric forms, indicate that the excited alloxazinic and excited isoalloxazinic forms have different ground state precursors, but the results of the time-resolved studies indicate that the excited isoalloxazinic forms are created from excited alloxazinic forms. These apparent discrepancies may be explained by assuming that alloxazine molecules can exist in a wide range of hydrogen bonded complexes with acetic acid and that only some of these possess an appropriate structure for proton transfer to occur in the ground state. It is possible that the cyclic complexes tautomerize immediately after excitation. It follows from the proton transfer rates reported in the literature [26,27,29] that, in such a favourable hydrogen bonding arrangement, this process is rapid and occurs on a picosecond time scale. The time resolution of our equipment did not allow us to monitor this rise time, but the difference between the fluorescence excitation spectra of the isoalloxazinic and alloxazinic forms testifies to such a possibility. Thus the differences between the excitation spectra of the alloxazinic and isoalloxazinic bands may reflect differences between unrelated and/or related "incorrectly" and "correctly" hydrogen bonded (with acetic acid) alloxazine molecules. The alloxazine molecules either unrelated to acetic acid or related incorrectly produce, in the excited state, "correct" complexes. Thus the observed overall reaction rate reflects both the rate at which the "correct" structure is formed and the rate of intrinsic proton transfer for the "correct" structure. The "formation" stage seems to be a slower process and may determine the overall proton transfer rate; as a result, the observed rate constant of the process is lower than 10^9 s^{-1}.

5. Conclusions

It can be concluded from the results discussed in this work that the alloxazine–acetic acid complexes with an appropriate structure for proton transfer may be created either in the ground or excited state. In the latter case, the excited state reaction consists of two steps: creation of complexes with an appropriate structure followed by proton transfer. Such a model requires further studies to establish which is the rate-determining process in the excited state. The mechanism of phototautomerism in alloxazines seems to be analogous to the two- step mechanism of excited state proton transfer discussed in the case of 7-azaindol–alcohol or water complexes [30,31], but different from the process observed in 7-azaindol–acetic acid complexes [32].

The study of alloxazine derivatives differing in the form and position of their substituents enables the effect of the intrinsic properties of the molecules on the proton transfer process to be determined [28].

The results presented in this paper may constitute a starting point for more detailed studies on the kinetics and mechanism of proton transfer in alloxazines. We intend to continue these studies.

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