Journal of Photoscience, Vol. 2, No. 1, pp. 13–18, 1995
Printed in the Republic of Korea.

1225-8555/95

EXCITED-STATE INTRAMOLECULAR PROTON TRANSFER IN DICOUMAROL, A CH_2-BRIDGED DIMER OF 4-HYDROXYCOUMARIN

DAE WON CHO[1], SEONG GWAN KANG[1], YONG HEE KIM[1], MINYUNG LEE[2]
DONGHO KIM[3], AND MINJOONG YOON[1]*
[1]Department of Chemistry, Chungnam National University, Taejon 305-764, Korea
[2]Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea
[3]Spectroscopy Laboratory, Korea Research Institute of Standards and Science, Taejon 305-606,Korea

(Received 22 December 1994; *accepted* 10 *March* 1995*)*

Abstract — The steady-state emission spectra of dicoumarol (DC) in ethanol and EPA have been examined at various temperatures (77-298 K). At room temperature, a fluorescence spectrum of DC in ethanol shows a emission maximum at 350 nm. In EPA a Stokes-shifted emission band appears around 470 nm in addition to the 350 nm emission, and its intensity is enhanced as temperature decreases. This emission is attributed to a zwitterionic tautomer of DC formed by a single excited-state intramolecular proton transfer (ESIPT) along the internal hydrogen-bonding. The fluorescence lifetimes have been measured at 350 and 450 nm as a function of temperature. The fluorescence decay at 350 nm is single exponential at any temperature, whereas the one at 450 nm becomes biexponential at temperatures below 250 *K*. These results are discussed in terms of a conformational change followed by the ESIPT. The activation energy barrier for the conformational change has been determined to be 3.7 ± 0.2 kJ/mole.

INTRODUCTION

Dicoumarol (DC), 3,3'-methylene bis(4-hydroxycoumarin) - a CH_2-bridged dimer of 4-hydroxycoumarin (4HC) - has been identified as a hemorrhagic agent in the spoiled sweet clover which causes a disease in cattle.[1] It is also known as an effective uncoupler of mitochondrial oxidative phosphorylation.[2] DC is susceptible to photobiological and photochemical behaviors in addition to the above biological activities.[3-5] Thus, much interest in the spectroscopic properties of DC has arised because of the relationship between its structure and biological significance.

The ^{1}H NMR and IR spectroscopic measurements by Hutchinson and Tomlinson[6] have confirmed the presence of an intramolecular hydrogen-bonded structure for DC. The formation of the intramolecular hydrogen-bonded structure has been suggested to play an important role in assisting DC to attain a suitable configuration for biological activities. Also, the intramolecular proton transfer phototautomerism can take place along the intramolecular hydrogen bonds as the most possible photochemical process in DC. Nevertheless this process has not been investigated extensively, because the fluorescence emission is weak at room temperature like other coumarin derivatives.[7-9] The weak fluorescence of coumarin derivatives is simply attributable to the thermally enhanced radiationless transition such as intersystem crossing. However, the excited-state intramolecular proton transfer can not be ruled out as one of the major radiationless transitions. Thus, being interested in the phototautomerism of DC, we have investigated the photophysical properties of DC in polar solvents and EPA by using the steady-state and time-resolved fluorescence spectroscopic methods at various temperatures.

MATERIALS AND METHODS

Coumarin derivatives were purchased from Sigma Chemical Co. (DC) and Aldrich chemical Co. (4HC). The purity of DC and 4HC was checked by the melting point measurement (292 and 214°C, respectively).[10] All organic solvents were spectrograde quality (Merck Co.). Buffer solutions of known pH were prepared by mixing AR grade acid and 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

* To whom all correspondence should be addressed.
† *Abbreviations* : ESIPT, excited-state intramolecular proton transfer; DC, dicoumarol; 4HC, 4-hydroxycoumarin; EPA, 5:5:2 volume mixture of ether, isopentane and ethanol.

which shifts very little with the solvent polarity, indicating that the absorption band is attributed to the π,π^* transition. The vibrational fine structures were also observed in these organic solvents suggesting little solvent-solute interaction and existence of a single conformer in ground-state. Theoretically, the optimized conformer of DC in ground-state can be predicted by AM1 calculation, which is "face-to-face" conformer 1 (see Scheme 1). In conformer 1, the intramolecular hydrogen-bonding is geometrically favorable. In accordance with this, Hutchinson and Tomlinson[6] previously confirmed the intramolecular hydrogen-bonded protons in DC by observing a single NMR signal at -1.31τ. They also reported that the IR frequency of the carbonyl stretching is lowered by about 30 cm^{-1} due to the intramolecular hydrogen-bonding. Considering the NMR and IR results and the theoretical calculation, it is suggested that the conformer 1 is the most stable structure in ground-state. The intramolecular hydrogen-bonding seems to form a rigid cyclic molecular structure, being consistent with the fact that the vabrational fine structure is clearly observed even in protic solvents.

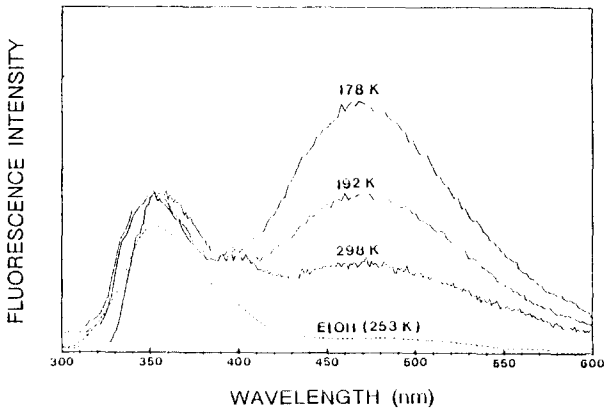

Figure 2. Fluorescence spectra of DC (2.0×10^{-5} *M*) in EPA and ethanol solution observed at various temperatures. They are normalized arbitrarily at 350 nm. The excitation wavelength was 280 nm.

In contrast to the absorption spectra, the fluorescence spectra of DC in ethanol at room temperature exhibit a single emission band around 350 nm with structureless non-mirror image to the absorption spectra (Fig. 2). These results imply that the low energy torsional vibration of 4HC moiety with poor Franck-Condon overlap seems to couple to the electronic transitions. In other words, upon excitation the molecular geometric change takes place by internal rotation around the central $-CH_2-$ group from the "face-to-face" conformer 1. The molecular rotation in excited-state results in a formation of a twisted conformer which is incapable of the intramolecular hydrogen bonding. It is also important to note that in addition to the 350 nm emission a Stokes-shifted (11,000 cm^{-1}) emission band around 470 nm is observed for DC fluorescence in EPA. Though EPA contains small amount of ethanol, its hydrogen-bonding ability is relatively weaker than ethanol. Thus, the long wavelength emission observed in EPA should be related to the formation of a valence isomerized product through relaxation process such as the excited-state intramolecular proton transfer (ESIPT) along the intramolecular hydrogen-bonding of conformer 1.

In order to confirm the ESIPT process, it is important to dertermine the acidity and basicity changes of photoexcited DC. Since DC is insoluble in water, we could not evaluate the ground-state and excited-state pK_a values for the functional groups of DC. Therefore, we tried to measure the pK_a values of 4HC, the half moiety of DC, by spectrophotometric titration and Förster cycle method.[14] Figure 3 shows the absorption and fluorescence emission spectra of 4HC (2×10^{-5} *M*) in different pH solutions at room temperature. The absorption maxima were observed at 285, 280 and 295 nm at pH=11, 2.4 and -8.9, respectively, with two isosbestic points, indicating the existence of three molecular species; the 280 nm band is due to a neutral molecular species (N), and the 285 and 295 nm bands are due to anionic and cationic ones, respectively. Similarly, three distinct fluorescence spectra were observed with the same variation of pH values as in the case of absorption spectra. Those three fluorescence bands at 386, 348, and

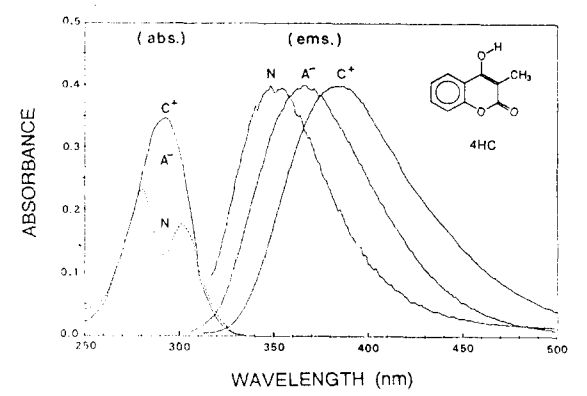

Figure 3. Absorption (abs.) and fluorescence (ems.) spectra of 4HC (2.0×10^{-5} *M*) in aqueous solutions of different pH: C^+(cationic)=-8.9; A^-(anionic)=11; N(neutral)=2.4. Fluorescence spectra are normalized. The excitation wavelength was 280 nm.

367 nm are originated from the cationic, neutral and anionic species, respectively. The anionic species are formed by deprotonation of 4-hydroxy group while the cationic species are formed by protonation of 2-carbonyl group. From the spectrophotometric titraion, the pK_a values of 4-hydroxy and 2-carbonyl groups were determined to be 4.8 and -2.8,respectively. The excited-state pK_a values (pK_a*) determined by the Förster cycle method[14] are 2.6 and 6.0 for 4-hydroxy and 2-carbonyl groups, respectively. The change in pK_a values upon excitation infers that deprotonation of 4-hydroxy group and protonation of 2-carbonyl are facilitated in excited-state 4HC. This suggests that protonation and deprotonation of those functional groups in DC are also faciliated upon exciation, supporting the possibility of the ESIPT in DC.

Now a question arises whether the ESIPT is single or double processes, because there exist two intramolecular hydrogen-binding sites. If double proton transfer occurs, the electronic structure of the phototautomer is the same as that of 4HC because DC is a symmetric dimer of 4HC. Then the phototautomer emission of DC should be the same as that of 4HC. However, the Stokes-shifted emission was not observed for 4HC phototautomer in EPA at any temperatures, in contrast to the case of DC. This indicates that no intramolecular proton transfer occurs in the excited-state of 4HC even through the hydroxy and carbonyl groups in 4HC become more acidic and basic upon excitation. This may be due to less feasibility of biprotonic ESIPT in 4HC compared to monoprotonic ESIPT expected for DC. This comparison releases an evidence that the phototautomerization of DC is a result of single ESIPT. If the single proton transfer takes place, the phototautomer formed *via* ESIPT at room temperature probably exists as charged species such as zwitterion (Scheme 1). This may be one of the reasons why the ESIPT emission is so weak at room temperature. Since a geometric change of DC is expected in normal excited-state as stated above, the phototautomerization in the ESIPT state should be followed by the rotation of 4HC moiety around the -CH_2- bond. Thus, the possible structure of zwitterion is an open twisted conformer as shown in Scheme 1.

If this is the case, the 470 nm emission as well as the 350 nm emission should be sensitive to temperature variation. Thus, we measured the temperature dependence of the fluorescence spectra of DC in EPA. In fact, as shown in Figure 2, the relative intensity ratio of the ESIPT emission at 470 nm to the 350 nm emission increases as temperature decreases. This infers that the conformer 1 is maintained in excited-state at low temperatures to facilitate the ESIPT. In order to further investigate the temperature dependence of the ESIPT emission in EPA solution, we measured the time-resolved fluorescence decay of DC at low temperatures. Figure 4 shows the typical temperature-dependent

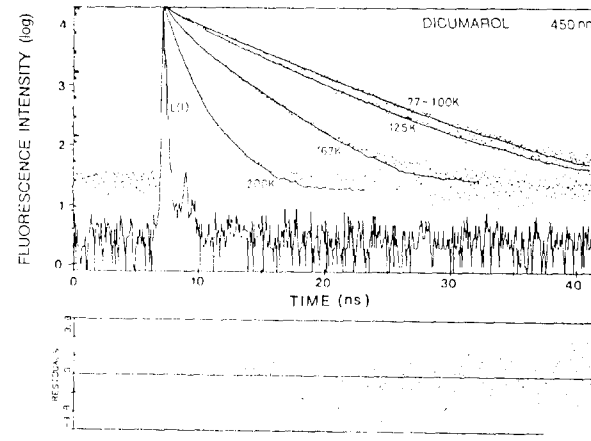

Figure 4. Fluorescence decay profiles of DC in EPA solutions observed at 450 nm as a function of temperature.

fluorescence decay profiles at 450 nm. The fluorescence lifetimes at various temperatures are summarized in Table 1.

Table 1. The lifetime data of DC (2.0×10^{-5} *M*) with various temperatures in EPA solution.

Temp. (*K*)	Fluorescence lifetimes (ns)		
	at 340 nm	at 450 nm	
77	0.60	5.40 (78%)	0.90 (22%)
83	0.60	5.50 (79%)	0.90 (21%)
100	0.60	5.40 (77%)	0.90 (23%)
125	0.52	5.00 (72%)	1.00 (28%)
167	0.24	3.30 (41%)	1.00 (59%)
200	0.08	1.90 (10%)	0.70 (90%)
250	0.02	0.80 (3%)	0.50 (97%)

The fluorescence decay at 350 nm is single exponential at any temperatures, indicating that the 350 nm emission is attributable to the conformer 1. As temperature is lowered, the fluorescence lifetime increases from 0.02 ns to 0.6 ns. This must be due to the restriction of the internal rotation of 4HC moiety. The fluorescence lifetime of 0.02 ns at room temperature is probably comparable to the internal rotation time. The fluorescence decay at 450 nm is almost single exponential at temperatures above 250 *K*, which has a decay time of 0.5 ns being longer than the lifetime of 350 nm emission. This supports again that the 450 nm emission at room temperature is originated from single phototautomer species. On the other hand, as the temperature decreases, the fluorescence decay at 450 nm becomes biexponential

with long (2.0-5.5 ns) and short (0.5-1.0 ns) decay components. The amplitude of the long component increases whereas that of the short component decreases with decreasing temperature. This suggests the existence of the two different phototautomer species as shown in Scheme 1. As discussed already, the first phototautomer is the face-to-face zwitterion formed by the ESIPT along the internal hydrogen bond of the conformer 1. At room temperature this would be immediately converted into the twisted zwitterion as the second photo-tautomer. This is the reason why only one short-decay component was observed at room temperature. However, at low temperatures the internal rotation of 4HC moiety is restricted, and the first phototautomer is relatively stabilized and consequantly detected as a long-decay component.

The fluorescence lifetimes approached constant values below 110 *K*, indicating no internal rotation in excited-state. Thus, the internal rotation of the 4HC moiety seems to be the only thermally activated decay process for the long decay emission originated from the first phototautomer. Then, by setting $k_R=k_R°\exp(-E_R/RT)$, the observed fluorescence lifetime at room temperature can be written as $\tau_f=1/(k_f+k_R)$, where k_f and $k_R$are the fluorescence decay constant and internal rotational constant, respectively. Thus, from the plot of $\ln(1/\tau_f-1/\tau_f°)$ vs. 1/T we can evaluate the E_f and k_R values. The intrinsic fluorescence lifetime at 450 nm is 5.50 ns for the long lived component. Since the long lived component at 450 nm is attributed to the proton transferred form, from the plot of $\ln(1/\tau_f-1/\tau_f°)$ *vs* 1/T can be calculated the intrinsic internal rotational time constant ($k_f°$) which is 1.79×10^8 s^{-1} (a kind of Arrhenius frequency factor) (see Fig. 5). From the slope of above plot the internal rotational energy (E_R) can be derived as 3.7 ± 0.2 kJ/mole in EPA.

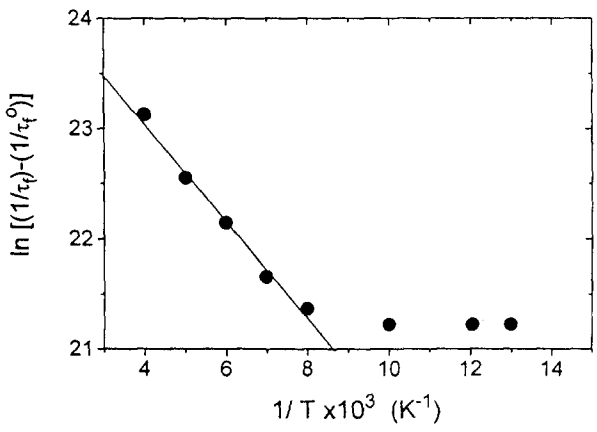

Figure 5. The plot of $\ln(1/\tau_f-1/\tau_f°)$ *vs* the reciprocal temperature.

$$k_R = 1.79 \times 10^8\ s^{-1} \exp(-E_R/RT)$$

In summary, as the temperature decrease, the internal rotation becomes slower and the fluorescence emission at 450 nm assigned as the face-to-face zwitterion becomes more intense.

CONCLUSION

The fluorescence spectra of DC in EPA or ethanol at low temperatures exhibit dual emission bands at 350 and 470 nm in contrast to the single emission band at 350 nm at room temperature. These two emissions are interpreted by the normal and ESIPT emissions, respectively, because of a large Stokes-shift of the 450 nm emission. The picosecond time-resolved fluorescence of DC in EPA at 450 nm exhibits a double exponential decay. This can be explained in terms of the formation of two phototautomers: one is a face-to-face zwitterion and the other is a twisted zwitterion. The formation of twisted zwitterion is the only thermally activated decay process of the face-to-face zwitterion generated *via* the ESIPT process from photoexcited DC. The twisted zwitterion is formed by the internal rotation of 4HC moiety around the $-CH_2-$ bond with an activation energy of 3.7±0.2 kJ/mole.

Acknowledgement — This work has been partially supported by the Basic Science Research Institute Program, Ministry of Education (BSRI-95-3432) and the Korea Science and Engineering Foundation through the Special Aiming Basic Studies Project (94-0501-05-01-3) and Center for Molecular Catalysis.

REFERENCES

1. Campbell, H. A. and K. P. Link (1941) Hemorrhagic sweet clover disease (IV) isolation and crstallization of the hemorrhagic agent. *J. Biol. Chem.* **138,** 21.
2. Biggs, R. and K. G. Macfarlane (1962) Human blood coagulation and its disorders. Blackwell.
3. Henry, B. R. and E. A. Lawler (1973) Substituent effects on the triplet-triplet absorption spectra of coumarin and its derivatives. *J. Mol. Spec.* **48,** 117-123.
4. Henry, B. R. and R. V. Hunt (1971) Triplet-triplet absorption studies on coumarin and related derivatives. *J. Mol. Spec.* **39,** 466-70.
5. Otagiri, M., R. Yamamichi, T. Imal, Y. Imamura and A. Takadake (1988) Study on the binding of dicoumarol to 1-acid glycoprotein using circular dichroism spectroscopy. *Chem. Pharm. Bull.* **36,** 4958-4962.
6. Hutchinson, D. W. and J. A. Tomlinson (1969) The structure of dicoumarol and related compounds. *Tetrahedron* **25,** 2531-2537.
7. Song, P.-S. and W. H. Gordon III, (1970) A spec-

troscopic study of the excited state of coumarin. *J. Phys. Chem.* **74**, 4234-4240.
8. Graber, D. R., M. W. Grimes and A. Haug (1969) Electron paramagnetic resonance studies of the triplet state of coumarin and related compounds. *J. Chem. Phys.* **50**, 1623-1626 .
9. Gallivan, J. B. (1970) Spectroscopic studies on coumarin. *Mol. Photochem.* **2**, 191-211.
10. Budavari, S. (1989) The merck index. 11th edition, published by merck & Co., Inc. Rahway N. J. USA.
11. Harris, D. C. (1982) *Quantitative Chemical Analysis*, W. H. Freeman and company, New York, pp242-243
12. Yagil. G., (1967) The effect of ionic hydration on equilibria and rates in concentrated electrolyte solutions. III. The H_ scale in concentrated hydroxide solutions *J. Phys. Chem.* **71**, 1034-1044
13. Cho, D. W., Y. H. Kim, S. G. Kang, M. Yoon, and D. Kim, (1994) Cyclodextrin effects on excited-state geometry change and intramolecular charge transfer of 4-biphenylcarboxylic acid. *J. Phys. Chem.* **97**, 554-562
14. Schulman, S. G., (1977) Fluorescence and phosphorescence spectroscopy: Physicochemical principles and practice. *International Series in Analytical Chemistry*, vol 59 (Ed. Belcher, R. and H. Freiser) Pergamon Press, Oxford, pp. 83-91