Hydrogen-Bonded Complexes of Lumichrome

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Received: September 30, 2004; In Final Form: December 19, 2004

Hydrogen bonds were shown to play an important role in the lumichrome photophysics and photochemistry both in solutions and in the solid state. In solutions, lumichrome can form hydrogen-bonded complexes with a variety of molecules, such as acetic acid or methanol, as supported by spectral and equilibrium studies. Photoexcitation of some hydrogen-bonded complexes, having appropriate configuration, as in the case of acetic acid, may lead to excited-state proton transfer, resulting in formation of the isoalloxazinic structure, detectable by its characteristic emission, distinct from that of the intrinsically alloxazinic lumichrome. Theoretical calculations confirmed the role of the hydrogen-bonded complexes, yielding several stable eight-membered cyclic structures of such complexes characterized by spectral changes similar to those observed experimentally. Hydrogen bonds play an essential role in the formation of the lumichrome crystal structure, as follows from the X-ray diffraction results. Interestingly, the crystals studied included molecules of methanol used as solvent in crystal growth. The emission studies of polycrystalline samples, similar to the processes occurring in solutions, point to the importance of hydrogen-bonding interactions in crystal packing allowed by the symmetry of the hydrogen-bonded dimers.

Introduction

Lumichrome (Lch, 7,8-dimethylalloxazine = 7,8-dimethylbenzo[*g*]pteridine-2,4(1*H*,3*H*)-dione) is representative of alloxazines (benzo[*g*]pteridine-2,4(1*H*,3*H*)-diones), a class of nitrogen heterocycles related to lumazine and isoalloxazines. In the alloxazine molecules, including lumichrome (see Figure 1), there are several centers (oxygen atoms, nitrogen atoms, N−H groups), which may serve as hydrogen acceptors or hydrogen donors in the creation of hydrogen-bonded complexes. In the case of alloxazines, the interaction may involve N(1)−H, N(3)−H, N(10), and N(5) and both carbonyl oxygens. In alloxazines, and especially in lumichrome, proton-transfer reactions have been found to occur in the excited state. In this process, the proton from the N(1) nitrogen atom of the lumichrome molecule is transferred to the N(10) nitrogen atom, and the excited isoalloxazinic form is created.[1−11] It was shown that excited-state isomerization takes place in lumichrome and other N(1) unsubstituted alloxazines, in the presence of compounds that have proton donor and acceptor functions and are able to form hydrogen bonds of appropriate strength and conformation with the alloxazinic molecules, i.e., acetic acid.[12]

The topic of hydrogen interactions between isoalloxazines and the surrounding environments is very intensively studied.

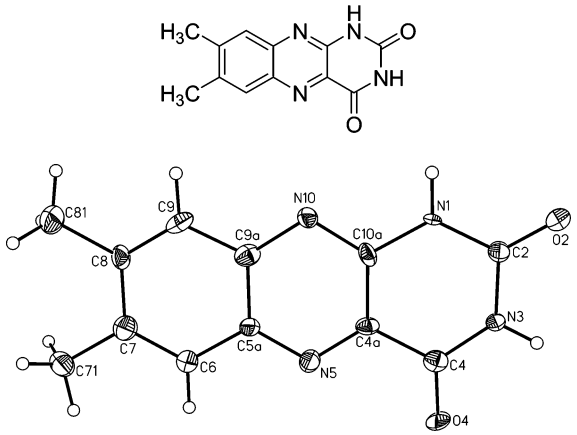

Figure 1. Structure and anisotropic-ellipsoid representation of lumichrome together with the numbering scheme. The ellipsoids are drawn at the 50% probability level; hydrogen atoms are represented by spheres of arbitrary radii.

Particularly interesting, also in the context of the present paper, are the studies by Yagi et al.,[13−15] based on quantum mechanical calculations of the effect of hydrogen bonding at the heteroatoms of flavins, along with many other aspects of hydrogen-bonding interactions in flavins that have been examined,[16] to mention only some of the recent experimental and theoretical studies.[17−21] Since the amount of data covering different ground- and excited-state properties of flavins and their hydrogen-bonding interactions with surrounding molecules is overwhelming, we shall only refer to the symposium proceedings, titled *Flavins and Flavoproteins*,[22] which illustrate both the wealth of the available

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10.1021/jp045550o CCC: $30.25
Published on Web 02/10/2005

information and the progress that has been made in the photochemistry, structure, and functionality of flavins.

In contrast to flavins, the studies into the effect of hydrogen-bonding interactions on alloxazines are very limited. No theoretical predictions are available for alloxazines in this respect, while the existing experimental results are also quite incomplete.[4,23] However, the hydrogen-bonding interaction between lumichrome and its surroundings is an important key to better understanding of, e.g., the biological role of lumichrome, excited-state proton-transfer reactions, and its photoactivity and chemical activity. These facts led us to undertake a theoretical study of the effects of the possible presence of hydrogen bonds between functional groups of lumichrome and surrounding molecules and in particular acetic acid, chosen as a convenient model compound, and the correlations between the structure and absorption spectra of the resulting complexes. Another objective involves studies of a possible lumichrome dimer structure. To the best of our knowledge, there are no known quantum-chemical calculations dealing with these issues. Apart from theoretical studies, we communicate experimental studies of the excited-state proton-transfer reaction of lumichrome in the presence of acetic acid. The X-ray structure of lumichrome crystals is reported.

Methods

Spectral and Photophysical Measurements. Time-resolved fluorescence measurements of lumichrome in ethanol were conducted using a model C-700 fluorometer from PTI. The system utilizes a nanosecond flashlamp for excitation and a stroboscopic detection system.[24] Some of the measurements were also conducted using the frequency-doubled output of a mode-locked, synchronously pumped, cavity-dumped argon-ion/DCM dye−laser system, with the emission detected with a Hamamatsu microchannel plate photomultiplier coupled to a time-correlated single-photon counting system. This measuring system has been described in detail previously.[25] Steady-state fluorescence spectra were obtained with a Jobin Yvon-Spex Fluorolog 3-11 spectrofluorometer, and UV−visible absorption spectra were obtained on a Varian Cary 5E spectrophotometer.

Laser-induced fluorescence (LIF) emission measurements of the powdered crystalline samples were performed at room temperature, in the front-face arrangement. A diagram of the system is presented in ref 26. The system uses the 337.1 nm pulse (suitable for lumichrome excitation) of a N_2 laser (Photon Technology Instruments, model 2000, ca. 600 ps fwhm, ~1.3 mJ/pulse) as the excitation source. The light arising from the irradiation of solid samples by the laser pulse is collected by a collimating beam probe coupled to a fused silica optical fiber and detected by a gated intensified charge-coupled device (ICCD, Oriel, model Instaspec V). The ICCD is coupled to a fixed imaging compact spectrograph (Oriel, model FICS 77441). The system can be used either by integrating all light emitted by the sample or in the time-resolved mode by using a delay box (Stanford Research Systems, model D6535) and a suitable gate width. The ICCD has high-speed (2.2 ns) gating electronics and an intensifier and covers the 200−900 nm spectral range. Time-resolved absorption and emission spectra are available in the nanosecond to second time range.[26−28]

DFT Calculations. Information on the electronic structure and geometry of lumichrome, its dimers, and complexes formed between lumichrome, acetic acid, and methanol was obtained using quantum-chemical calculations by means of the density-functional theory (DFT). The calculations were performed using the B3LYP functional[29] in conjunction with a modest 6-31G-(d) split-valence polarized basis set.[30] Excitation energies and oscillator strengths in the dipole length representation were calculated for the optimized ground-state geometries using the time-dependent (TD) approach as implemented in the Gaussian 98 package of ab initio programs.[31] The lowest-energy singlet−singlet transitions, $S_0 \rightarrow S_i$, have been calculated for the ground-state geometry. The excitation energies computed at the B3LYP/6-31G(d) level of theory are estimated to be accurate within 2000−3000 cm^{-1}, usually requiring a shift toward the red to reproduce experimental spectra. However, regarding the quality of our spectral predictions, it should be noted that the difference in the experimental transition energies in 1,4-dioxane solution between lumiflavin and lumichrome (22.7×10^3 and 26.4×10^3 cm^{-1}) is reproduced in the calculations (24.5×10^3 and 27.8×10^3 cm^{-1}) to within 0.5×10^3 cm^{-1},[32] with the shift of predicted values as compared to the experimental ones to the blue by less than 2.0×10^3 cm^{-1}.

TABLE 1: Crystal Structure and Structure Refinement Parameters of Lumichrome[a]

chemical formula	$(C_{12}H_{10}N_4O_2)\cdot(CH_3OH)$
formula weight	274.28
crystal system	triclinic
space group	$P\bar{1}$
A	5.6902(11) Å
B	10.423(2) Å
C	10.706(2) Å
α	94.084(16)°
β	92.045(16)°
γ	99.749(16)°
volume, *V*	623.5(2)Å^3
Z	2
calculated density, D_x	1.46 g cm^{-3}
M	0.11 mm^{-1}
reflections:	
collected	4489
independent [R_{int}]	2138 [0.055]
R [$I > 2\sigma(I)$]	0.047
w$R2$ (all data)	0.108
goodness of fit, *S*	0.78
max/min $\Delta\rho$	0.27/−0.26 e Å^{-3}

[a] Crystallographic data and tables of atomic coordinates, thermal parameters, bond lengths, and bond angles have been deposited with the Cambridge Crystallographic Data Centre (CCDC) with the deposition no. CCDC 238588. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Rd., Cambridge CB2 1EZ, UK (fax +44 1223 336 033, E-mail deposit@ccdc.cam.ac.uk; http://www.ccdc.cam.ac.uk).

X-ray Diffraction Analysis. A colorless platelike crystal (0.3 × 0.1 × 0.02 mm) was analyzed at 100(1) K on an Oxford Diffraction KM4CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å).The data were collected using the ω-scan technique to a maximum θ value of 25° and corrected for Lorentz and polarization effects. The structure was solved with SHELXS97[33] and refined by the full-matrix least-squares method with SHELXL97.[34] Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were put in idealized positions and refined isotropically using the riding model with U_{iso} values set at 1.2 (1.4 for methyl groups) times U_{eq} of the appropriate carrier atom. Crystal structure and structure refinement parameters of the lumichrome are given in Table 1.

Results and Discussion

Excited-State Proton Transfer in Ethanol. Spectroscopic properties of lumichrome and other alloxazines in different solvents have been the subjects of a number of previous studies.[2,32,35−39] The two strong longer-wavelength absorption

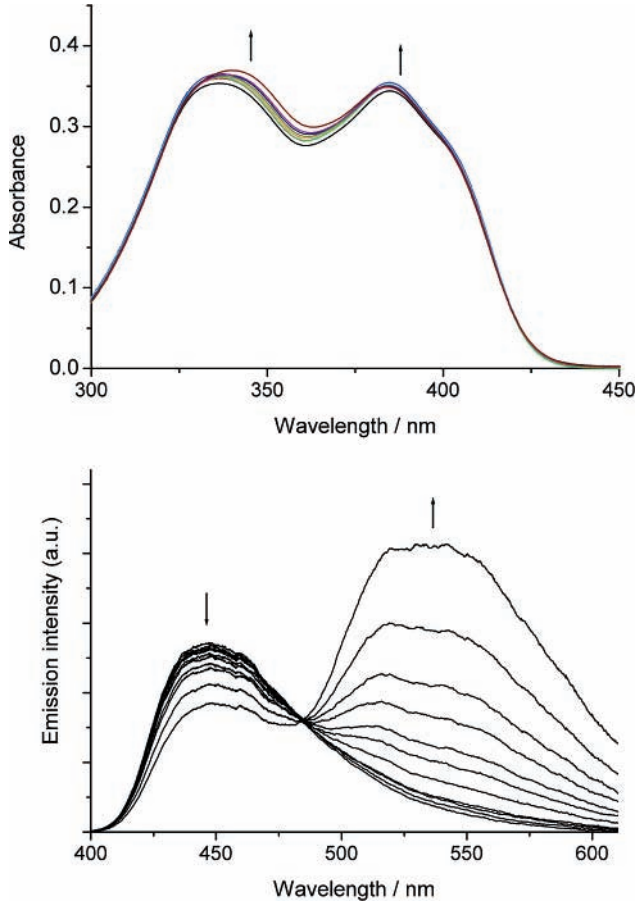


Figure 2. (Top) Effect of varying acetic acid concentration on the absorption spectra of lumichrome in ethanol for 0, 0.07, 0.14, 0.28, 0.42, 0.56, 0.84, 1.12, and 1.68 mol dm^{-3} of acetic acid. (Bottom) Effect of increasing acetic acid concentration on the fluorescence emission spectra of lumichrome (λ_{exc} = 385 nm) for 0, 0.07, 0.14, 0.28, 0.42, 0.56, 0.84, 1.12, 1.68, and 2.66 mol dm^{-3} of acetic acid. Arrows indicate increasing concentration of the acetic acid.

bands of these compounds, with maxima at about 330 and 380 nm, have been assigned to two independent π,π^* transitions.[2,40,41] The exact position and energy of the n,π^* state have not been determined exactly; however, it is generally believed that the weak fluorescence emission of alloxazines relative to isoalloxazines may reflect a close spacing of n,π^* and π,π^* excited singlet states, with the lowest-energy state being of n,π^* character. It is very desirable to learn more about the role of n,π^* states in the spectroscopy and photophysics of lumichrome, which we will refer to later in discussing the results of DFT calculations. Koziołowa[2] demonstrated that, with increasing solvent polarity, both of the longer-wavelength maxima show a red shift accompanied by a hypochromic effect on the lower-energy maximum and a hyperchromic effect on the higher-energy maximum. Koziołowa demonstrated that the positions of the two maxima exhibit a linear correlation with the solvent polarity expressed in Z values.[2] Deviations from the linear correlation observed for acetic acid, pyridine, and water had been interpreted in terms of specific solute−solvent interactions. The specific solute−solvent interactions occurring between lumichrome and acetic acid are especially interesting for many reasons. Namely, the structure and interactions present in the ground state are central to the mechanism of the excited-state proton transfer in alloxazines.[1,11,39,42]

The absorption spectrum of lumichrome in ethanol exhibits two absorption bands (see Figure 2). In the presence of acetic acid, only small changes in the absorption spectra are observed, with an increase of absorbance for both low-energy bands. As the presence of acetic acid in ethanol caused relatively small changes in the absorption spectra of lumichrome, we used two other solvents, acetonitrile and 1,2-dichloroethane, which revealed better-defined changes. The analysis of changes in the absorption spectra of lumichrome in the presence of acetic acid allows determination of the equilibrium constants of complexation, K. It is well known that the self-association of acetic acid may become an important process at higher concentrations. The analysis of spectral changes in 1,2-dichloroethane is particularly interesting from this point of view, because the dimerization constant for acetic acid in 1,2-dichloroethane is known to be equal to 154 mol^{-1} dm^3.[43]

The equilibrium constant for the reaction of complexation,

$$\mathrm{Lch} + n\mathrm{AA} \rightarrow \mathrm{Lch}\cdots\mathrm{AA}_n \qquad (1)$$

is given by the equation

$$K_n = \frac{[\mathrm{Lch}\cdots\mathrm{AA}_n]}{[\mathrm{Lch}][\mathrm{AA}]^n} \qquad (2)$$

The number of acetic acid molecules engaged in complexation with a single lumichrome molecule, n, was determined on the basis of the following plot, cf. ref 44, and taking into account the dimerization of the acetic acid:

$$\ln\left[\frac{A - A_0}{A_\infty - A}\right] = n \ln [\mathrm{AA}] + b \qquad (3)$$

The results indicate that the stoichiometry of lumichrome−acetic acid complexes in 1,2-dichloroethane varies from 1:1 to 1:2, as a function of the acetic acid concentration. The equilibrium constants K_1 and K_2 and the extinction coefficients of the two complexes at 350 nm, where the measurements were taken, are 11 and 81 mol^{-1} dm^3, and 1.9×10^4 and 8.9×10^3 cm^{-1} mol^{-1} dm^3, respectively. These results were obtained using the nonlinear least-squares fitting of the measured absorbance data at 350 nm as a function of acetic acid concentration. The results indicate that in the range of acetic acid concentrations used, a mixture of Lch···AA_1 and Lch···AA_2 complex species is present, with the Lch···AA_2 predominant at higher concentrations. This result is understandable considering the structure of Lch, which can form complexes with two acetic acid molecules, with a total of four hydrogen bonds created.

In acetonitrile, a more polar solvent, it is expected that the relatively strong lumichrome−solvent and acetic acid−solvent interactions would restrict the reaction between lumichrome and the acetic acid. Thus, in acetonitrile, the apparent lumichrome−acetic acid complexation constant is much lower than that in 1,2-dichloroethane.[39] In fact, the kinetics of the excited-state proton-transfer reaction in the lumichrome−acetic acid system was found to be strongly dependent on the solvent when acetonitrile and 1,2-dichloroethane were used.

The experimental spectral data suggest that, depending on the concentration of acetic acid, the stoichiometry of the complexes formed between lumichrome and acetic acid varies from 1:1 to 1:2, with the stability constant depending on the solvent. Previous authors have proposed that these spectral changes are due to hydrogen bonding at the N(10) of the lumichrome. This conclusion was mainly based on the comparison of the changes in the absorption spectra for 9-methyl-substituted alloxazine and 1-methyl- and 3-methyl-substituted lumichromes in the presence of the acetic acid. It was shown that the methyl groups at the positions N(1) and C(9) restrict

TABLE 2: Fluorescence Lifetimes for Alloxazinic and Isoalloxazinic Forms of Lumichrome for Various Acetic Acid Concentrations in Ethanol[a]

acetic acid concn/ mol dm^{-3}	425 nm		580 nm		
	τ_F/ns	χ^2	τ_F^1/ns (a^1)	τ_F^2/ns (a^2)	χ^2
0	0.86	1.444	0.95 (0.95)	6.34 (0.05)	1.063
0.28	0.83	2.140	0.87 (0.56)	7.42 (0.44)	0.879
0.56	0.81	1.612	0.90 (0.24)	7.47 (0.76)	1.301
0.84	0.87	1.129	−	7.42	1.237
1.12	0.83	1.097	0.42 (−0.50)	7.29 (0.50)	1.156
2.40	0.74	1.498	0.44 (−0.48)	6.88 (0.52)	

[a] τ_F^1, τ_F^2 are the fluorescence lifetimes, and a^1, a^2 are the pre-exponential factors ($|a^1| + |a^2| = 1$).

the possibility of hydrogen bond formation at N(10), without preventing it completely.

The emission spectra of lumichrome in ethanol exhibit one broad band with a maximum at about 453 nm, with a new band appearing in the presence of acetic acid with a maximum at about 520 nm (see Figure 2). The new emission is similar to the emission spectrum of lumiflavin, a compound with isoalloxazinic structure, and has been identified as the emission of the isoalloxazinic form resulting from the excited-state proton transfer from N(1) to N(10).[2] The intensity of alloxazinic emission decreased and the intensity of isoalloxazinic emission increased with increasing acetic acid concentration. Clear isoemissive points in the spectra were observed, indicative of only two emissive species present. The apparent equilibrium constants for the complexation between lumichrome and acetic acid can be determined from the changes in the emission spectra of lumichrome in the presence of acetic acid, given that the isoalloxazinic emission only originates from the lumichrome molecules involved in such complexes. The equation for the emission intensity may be presented in the form

$$\frac{1}{I_F - I_F^0} = \frac{1}{a[\mathrm{Lch}]_0} + \frac{1}{a[\mathrm{Lch}]_0 K^*}\frac{1}{[\mathrm{AA}]} \qquad (4)$$

where I_F^0 and I_F are the fluorescence intensity monitored at the isoalloxazinic emission band without and in the presence of acetic acid, a is a proportionality constant, $[\mathrm{Lch}]_0$ is the concentration of lumichrome, and [AA] is the acetic acid concentration. The apparent equilibrium constant K^* obtained in ethanol using eq 1 is 0.6 ± 0.2 mol^{-1} dm^3. The analogous value obtained in acetonitrile is 2.4 ± 0.2 mol^{-1} dm^3. In the much less polar 1,2-dichloroethane, the equilibrium constants are 93 ± 2 mol^{-1} dm^3 for the 0−0.022 mol dm^{-3} concentration range and 68 ± 2 mol^{-1} dm^3 for the 0−0.69 mol dm^{-3} concentration range.[39]

The excitation spectra of iso- and alloxazinic forms of lumichrome are slightly different. In the excitation spectrum of the isoalloxazinic form, a small red shift is observed for the higher-energy band as compared to that of the normal alloxazinic form. Additionally, the emission spectrum is slightly dependent on the excitation wavelength. In the presence of acetic acid, the ratio of the emission intensity of iso- to alloxazinic forms achieves a maximum at an excitation wavelength of about 350 nm. This observation suggests that the excited allo- and isoalloxazinic forms have different precursors in the ground state, those of the excited isoalloxazinic form being the ground-state hydrogen-bonded lumichrome−acetic acid complexes.

The decay of the alloxazinic emission of lumichrome in ethanol with and without acetic acid is single-exponential in the whole range of the acetic acid concentrations studied (see Table 2). The decay of the isoalloxazinic emission of lumichrome for the acetic acid concentrations up to 0.56 mol dm^{-3} could be described by a sum of two single-exponential decays with respective lifetimes of hundreds of picoseconds and several nanoseconds. It seems that the faster decay component observed for isoalloxazinic emission is due to overlapping emission spectra of the two forms. As a result, the growth of isoalloxazinic emission is completely compensated by the decay of alloxazinic emission at a certain acetic acid concentration (0.84 mol dm^{-3}). The decay of isoalloxazinic emission at higher acetic acid concentrations is described by the sum of a single-exponential rise on the subnanosecond time scale and a single-exponential decay on the nanosecond time scale. These results allow us to conclude that there is a kinetic relationship between the excited alloxazinic and isoalloxazinic forms of lumichrome in the presence of acetic acid, with the excited alloxazinic form being the precursor of the excited isoalloxazinic form. Moreover, single-exponential decay of the alloxazinic form suggests that there is no equilibrium between the two tautomeric forms in the excited state. The observation of the rise times of the isoalloxazinic emission of several hundreds of picoseconds suggests that the excited-state proton transfer is a relatively slow process.

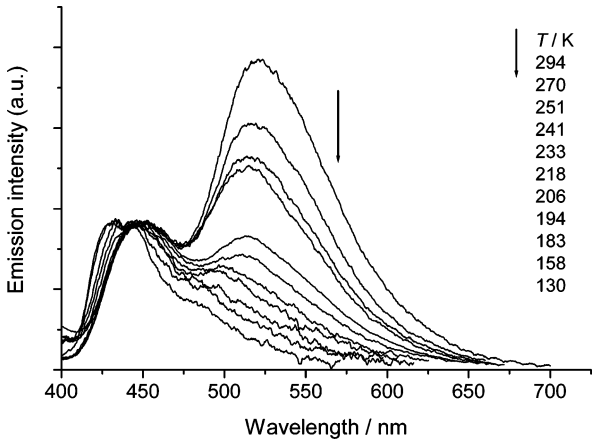


Figure 3. Temperature effect on the emission spectra of lumichrome in the presence of acetic acid.

Temperature and isotopic effects on the emission spectra of lumichrome in the presence of acetic acid were studied. At lower temperatures, the intensity of the isoalloxazinic emission band decreases, accompanied by an enhanced alloxazinic emission. None or a very weak isoalloxazinic emission was observable below 150 K. Figure 3 shows the dependence of the intensity ratio of the isoalloxazinic to alloxazinic emission on temperature in the presence of 2.4 mol dm^{-3} acetic acid.

DFT Calculations. To test the influence of the solvent on the absorption spectra of lumichrome, we chose to quantify theoretically the interactions between lumichrome and methanol and acetic acid. In the model used, molecules of methanol or acetic acid were added to a molecule of lumichrome at the locations considered important for hydrogen bond formation. The corresponding optimized structures may be used as models for lumichrome in methanol or acetic acid solutions. Of course, this model is very simplified because in reality many molecules of solvent are present; thus, it would be necessary to average over all possible configurations of the solvent molecules. However, we believe that even such simplified models can reveal the fundamental trends.

The electronic structure of lumichrome and its possible complexes with methanol was studied by means of time-dependent density-functional theory in singlet states. The structures of the possible complexes between lumichrome and

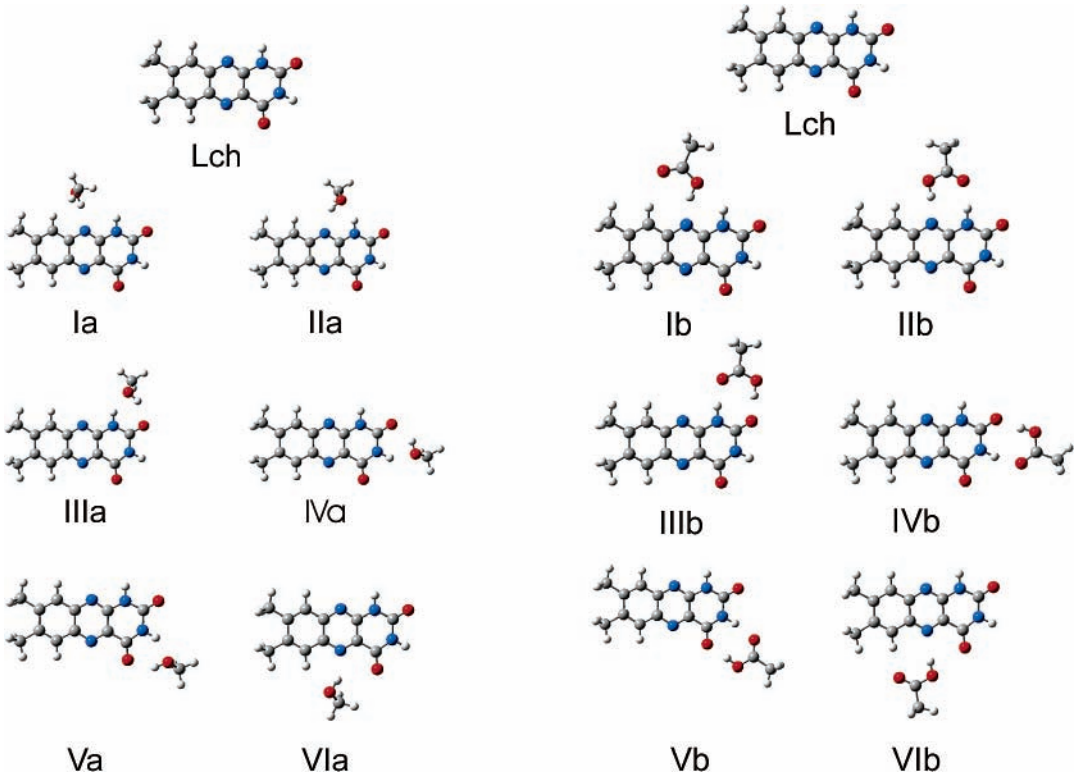


Figure 4. Possible structures of lumichrome and its complexes with methanol (left) and acetic acid (right).

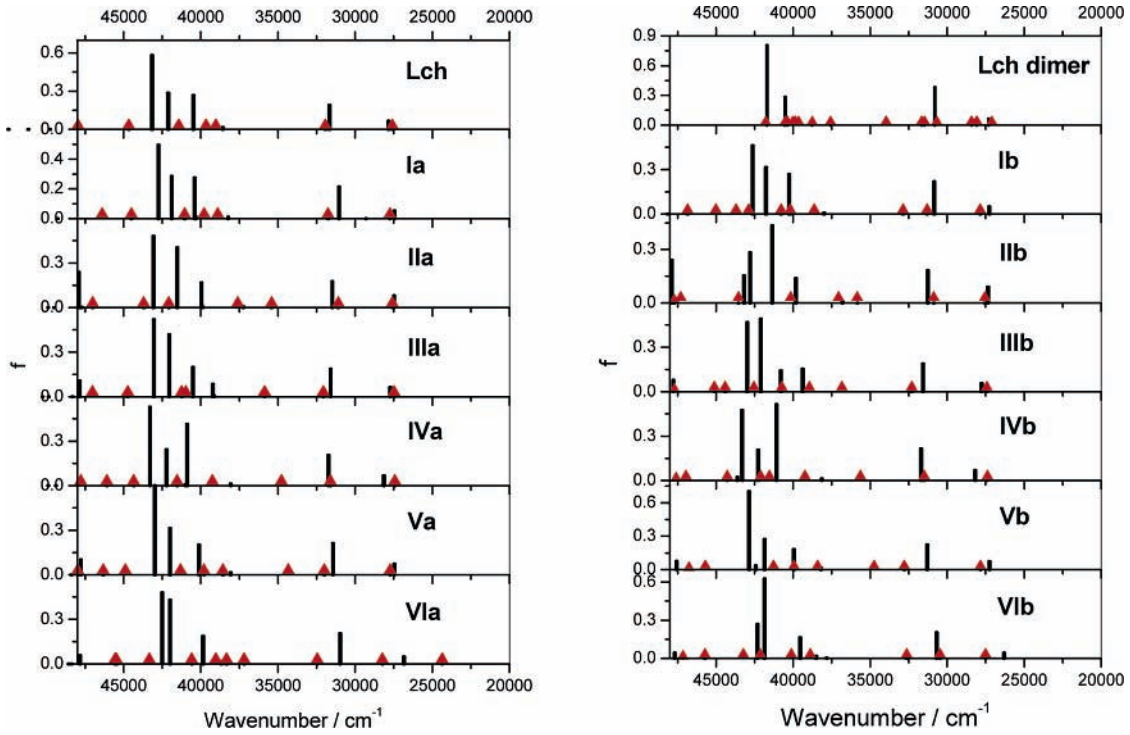


Figure 5. Predicted transition energies and oscillator strengths of lumichrome and its various hydrogen-bonded complexes with methanol (left) and acetic acid (right). Triangles mark locations of the weak n,π^* transitions.

methanol (**Ia**−**VIa**) are presented in Figure 4. Figure 5 presents a summary of the calculation results, showing the predicted transition energies and oscillator strengths indicated by solid vertical lines. The binding energies of lumichrome with methanol in these complexes were also calculated, and are listed in Table 5. The transitions presented in the Figure 5 are of the π,π^* character for all the species considered, with the two calculated lowest-energy transitions located at approximately 316 nm (31.9×10^3 cm^{-1}) and 359 nm (27.8×10^3 cm^{-1}). However, both low-lying π,π^* transitions of lumichrome are accompanied by two closely located n,π^* transitions at 313 nm (31.7×10^3 cm^{-1}) and 362 nm (27.6×10^3 cm^{-1}) of very low oscillator strengths. Such forbidden n,π^* transitions are marked by small triangles in Figure 5 for better visualization. Like many aza-aromatics, lumichrome possesses close-neighboring n,π^* and π,π^* (calculated $\Delta E = 0.2 \times 10^3$ cm^{-1}) singlet excited states, the lowest excited singlet state having the n,π^* character. Recently, we have shown that the photophysics of lumichrome

TABLE 3: Calculated (B3LYP/6-31G(d)) Singlet Excitation Energies Starting from the Ground State and Corresponding Oscillator Strengths, *f*, for Lumichrome and Its Complexes with Methanol Ia−VIa

	lumichrome		**Ia**		**IIa**		**IIIa**		**IVa**		**Va**		**VIa**	
$S_0 \rightarrow S_i$	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f
$\rightarrow S_1$	27.6	0.002	27.5	0.052	27.5	0.080	27.5	0.002	27.4	0.002	27.5	0.072	24.4	<0.001
$\rightarrow S_2$	27.8 26.2[a] 26.0[b]	0.066	27.7	0.001	27.6	0.009	27.7	0.063	28.1	0.068	27.7	0.002	26.8	0.048
$\rightarrow S_3$	31.7 29.1[a] 28.6[b]	0.190	29.3	<0.001	31.1	<0.001	31.6	0.186	31.6	<0.001	31.4	0.212	28.2	0.001
$\rightarrow S_4$	31.9	<0.001	31.0	0.214	31.5	0.177	32.1	<0.001	31.7	0.204	32.0	<0.001	31.0	0.204
$\rightarrow S_5$	38.6	0.015	31.7	<0.001	35.4	<0.001	35.9	<0.001	34.8	0	34.3	0	32.4	<0.001
$\rightarrow S_6$	39.1	0	38.2	0.010	37.3	0.010	39.1	0.005	38.1	0.011	38.1	0.014	37.2	<0.001
$\rightarrow S_7$	39.7	0	38.9	0	37.6	0	39.2	0.084	39.2	0	38.6	<0.001	38.3	0.002
$\rightarrow S_8$	40.5	0.266	39.7	0	39.9	0.006	40.5	0.197	40.9	0.412	39.8	<0.001	38.7	0.015
$\rightarrow S_9$	41.4	0	40.3	0.015	40.0	0.167	41.0	0	41.0	0.006	40.1	0.203	39.0	0
$\rightarrow S_{10}$	42.1	0.284	40.4	0.275	41.5	0.405	41.2	<0.001	41.5	<0.001	41.3	0	39.9	0.184
$\rightarrow S_{11}$	43.2	0.581	41.0	<0.001	42.1	<0.001	42.0	0.416	42.2	0.241	42.0	0.313	40.6	<0.001
$\rightarrow S_{12}$	44.7	<0.001	41.9	0.284	43.1	0.480	43.0	0.517	43.3	0.527	43.0	0.655	42.0	0.427
$\rightarrow S_{13}$	48.0	0	42.7	0.495	43.7	<0.001	44.7	<0.001	44.3	<0.001	44.9	0	42.5	0.479
$\rightarrow S_{14}$	48.1	0.139	44.5	<0.001	47.0	<0.001	47.0	0	46.1	<0.001	46.3	<0.001	43.3	0
$\rightarrow S_{15}$	49.8	<0.001	46.4	0	47.7	0.003	47.9	0.106	47.9	0	47.8	0.102	45.5	<0.001

[a] In 1,2-dichloroethane. [b] In acetic acid, experimental results.

TABLE 4: Calculated (B3LYP/6-31G(d)) Singlet Excitation Energies Starting from the Ground State and the Corresponding Oscillator Strengths, *f*, for Lumichrome and Its Complexes with the Acetic Acid Ib−VIb

	lumichrome dimer		**Ib**		**IIb**		**IIIb**		**IVb**		**Vb**		**VIb**	
$S_0 \rightarrow S_i$	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f	$E \times 10^{-3}$/ cm^{-1}	f
$\rightarrow S_1$	27.1	0	27.2	0.050	27.3	0.089	27.4	0.002	27.4	0.002	27.3	0.073	26.3	0.043
$\rightarrow S_2$	27.3	0.062	27.9	0.001	27.6	<0.001	27.8	0.057	28.2	0.067	27.8	0.002	27.5	<0.001
$\rightarrow S_3$	28.1	0	30.8	0.218	30.9	<0.001	31.6	0.188	31.5	<0.001	31.3	0.225	30.5	<0.001
$\rightarrow S_4$	28.1	0.003	31.3	<0.001	31.3	0.182	32.3	<0.001	31.7	0.214	32.8	<0.001	30.7	0.203
$\rightarrow S_5$	28.4	<0.001	32.9	<0.001	35.8	<0.001	36.8	0	35.6	0	34.7	0	32.6	<0.001
$\rightarrow S_6$	28.4	0	38.0	0.009	36.8	0.009	38.9	0	38.1	0.012	38.2	0.015	37.8	0.003
$\rightarrow S_7$	30.7	0	38.6	0	37.1	0	39.4	0.153	39.2	0	38.4	0	38.5	0.017
$\rightarrow S_8$	30.8	0.381	40.2	<0.001	39.8	0.138	40.7	0	41.1	0.514	39.9	<0.001	38.9	0
$\rightarrow S_9$	31.4	0	40.3	0.268	40.2	0	40.8	0.142	41.5	0	40.0	0.179	39.5	0.162
$\rightarrow S_{10}$	31.5	0.024	40.8	0.009	41.3	0.435	42.1	0.490	42.1	0	41.3	0	40.1	0
$\rightarrow S_{11}$	31.6	0	41.8	0.314	42.8	0.282	42.5	0	42.2	0.206	41.9	0.271	41.9	0.625
$\rightarrow S_{12}$	31.7	<0.001	42.6	0.461	43.2	0.002	43.0	0.468	43.3	0.474	42.4	0.036	42.1	0
$\rightarrow S_{13}$	33.9	0	42.9	0	43.2	0.153	44.4	0.002	43.6	0.023	42.9	0.702	42.3	0.268
$\rightarrow S_{14}$	34.0	0	43.7	0	43.6	<0.001	45.1	<0.001	44.3	<0.001	45.7	0	43.2	0
$\rightarrow S_{15}$	37.6	<0.001	45.0	0	47.3	0	47.7	0	47.0	0	46.8	0	45.7	<0.001

and its 1- and 3-methyl and 1,3-dimethyl derivatives is controlled by close-lying n,π* and π,π* states.[32] The *proximity effect* (see ref 45 for a review) is believed to be a consequence of vibronic interaction between close-lying n,π* and π,π* singlet states and is responsible for strongly solvent-dependent photophysical properties, mainly due to significant variations in the nonradiative decay rates. Such an interpretation based on the proximity effect is also supported by our present TD-DFT calculations for the hydrogen-bonded complexes of lumichrome. For all the complexes examined, the $^1\pi,\pi^*-^1$n,π* electronic energy gap is relatively small, with the highest values corresponding to the complex **VIa** with methanol (2.4×10^3 cm^{-1}) and to the complex **VIb** with acetic acid (1.2×10^3 cm^{-1}) (see Tables 3 and 4). However, those highest-gap complexes have the lowest binding energy of 9.7 and 10.9 kcal/mol, for methanol and acetic acid, respectively. Note that all binding energies of the lumichrome−methanol complexes are lower than those obtained for the lumichrome−acetic acid complexes. In the complexes **III** and **IV**, the lowest excited singlet state has the n,π* character. However, in the case of the lumichrome-acetic acid complexes **I**, **II**, **V**, and **VI**, the lowest excited singlet state was predicted to have the π,π* character.

To theoretically investigate the solvent effect on the absorption spectra, we considered a number of possible complexes between lumichrome and acetic acid. Naturally, the real situation in a solution can be far more complex; nevertheless, the simple complexes considered can give a first insight into the interactions occurring in real systems.

The acetic acid may act as both a hydrogen donor and acceptor agent; thus, the lumichrome−acetic acid complexes may have various structures. Special attention has been given to the possible interaction of acetic acid with the active centers of lumichrome, namely N(1)−H, N(3)−H, N(10), and N(5) and both carbonyl oxygens. The structures of eight-membered cyclic complexes considered are shown in Figure 4.

On the basis of the calculation results presented in Table 4 and Figure 5, it is expected that the changes in the range between 222 nm (45.0×10^3 cm^{-1}) and 250 nm (40.0×10^3 cm^{-1}) should be most informative with regard to the nature and type of the hydrogen-bonding interactions. However, this region of the lumichrome spectrum is masked by strong absorption of the acetic acid, making the analysis very difficult. Thus, the changes in the region of the two lower-energy absorption bands are much more convenient to monitor. As can be seen from

TABLE 5: Binding Energy and Hydrogen Bond Data of Lumichrome with Methanol (Ia−VIa) and Acetic Acid (Ib−VIb) in the Complexes Studied[a]

complex	D−H···A	D−H distance (Å)	H···A distance (Å)	D···A distance (Å)	D−H···A angle (°)	total energy, hartrees	binding energy, kcal/mol
Ia	C9−H9···O	1.080	2.450	3.320	138	−948.551735	7.7
	O−H···N10	0.970	1.990	2.930	166		
IIa	N1−H1···O	1.031	1.880	2.810	149	−948.558410	11.8
	O−H···N10	0.980	2.000	2.850	144		
IIIa	N1−H1···O	1.030	1.900	2.790	144	−948.559791	12.7
	O−H···O2	0.980	1.920	2.780	146		
IVa	N3−H3···O	1.031	1.910	2.810	146	−948.558890	12.2
	O−H···O2	0.970	1.940	2.780	146		
Va	N3−H3···O	1.030	1.900	2.800	146	−948.559276	12.4
	O−H···O4	0.980	1.930	2.790	146		
VIa	C6−H6···O	1.088	2.420	2.940	139	−948.554969	9.7
	O−H···N5	0.970	2.060	3.310	152		
Ib	C9−H9···O	1.080	2.480	3.510	160	−1061.924561	11.4
	O−H···N10	1.000	1.780	2.740	159		
IIb	N1−H1···O	1.030	1.820	2.840	172	−1061.931377	15.7
	O−H···N10	1.000	1.790	2.780	174		
IIIb	N1−H1···O	1.034	1.810	2.820	168	−1061.933415	17.0
	O−H···O2	0.990	1.710	2.690	176		
IVb	N3−H3···O	1.033	1.830	2.840	169	−1061.932054	16.1
	O−H···O2	0.990	1.730	2.710	176		
Vb	N3−H3···O	1.030	1.820	2.830	169	−1061.932506	16.4
	O−H···O4	0.990	1.730	2.710	176		
VIb	C6−H6···O	1.080	2.120	3.212	171	−1061.923707	10.9
	O−H···N5	0.980	2.130	3.110	177		

[a] The DFT total energies of individual molecules were Lch = −832.825119 hartrees, MeOH =−115.714407 hartrees, and AA =−229.081205 hartrees.

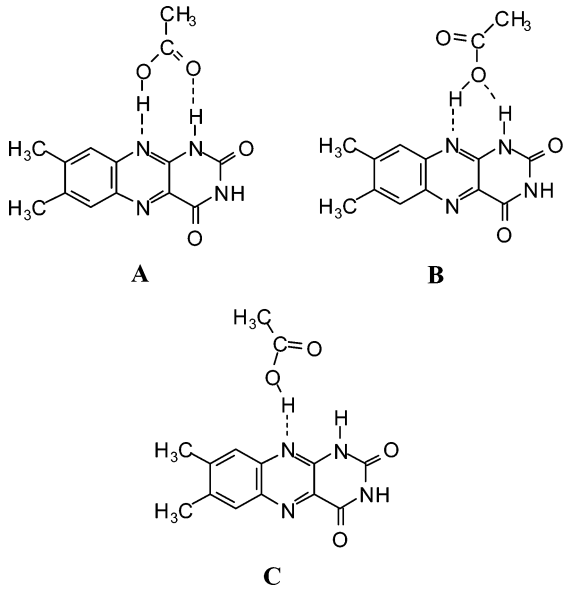
Table 4 and Figure 5, both absorption bands shift to the red upon complex formation of type **Ib**, due to both transition energy reduction and the intensity redistribution between the two lowest-energy transitions, as compared to free lumichrome. The electronic structure of complex type **IIb** shows a similar red shift of those two bands, but the shift is smaller than in the case of complex **Ib** and is accompanied by the hyperchromic effect for the lowest-energy band and the hypochromic effect for the second band, as compared to lumichrome. Complex **IIIb** demonstrates hardly any spectral shift, and for the complex **IVb**, to the others, we predict a blue shift of the lowest-energy absorption band. Complexes **Vb** and **VIb**, similar to **Ib** and **IIb**, demonstrate a red shift of both lower-energy absorptions bands.

We performed additional calculations using the 6-31G(d,p) basis set in order to verify if that would improve the results. We found that the inclusion of the polarization p functions for the hydrogen atoms does not affect significantly the complex binding energies. In particular, we obtained the binding energies for the complexes with the strongest/weakest hydrogen bonds to be **IIIa** 12.7 (12.7), **Ia** 7.8 (7.7), **IIIb** 17.3 (17.0), and **VIb** 10.9 (10.9) kcal/mol. The results obtained with the 6-31G(d) basis set are given in parentheses for comparison (see Table 5). The largest difference amounts to 0.3 kcal/mol and is certainly negligible in comparison to the uncertainty of calculations at the B3LYP/6-31G(d) level of theory. Our calculations similarly indicate that use of the 6-31G(d,p) basis set does not affect significantly the electronic excitation energies, while the oscillator strengths are affected very weakly, if at all (see the Supporting Information).

Direct comparison of the TDF calculations with experiments is not trivial, as the situation in real solvents should be quite complex. Complexes of other than 1:1 stoichiometry may form; additionally, both lumichrome and acetic acid may produce various aggregates between identical molecules. Nevertheless, it is interesting to compare our calculation results with the situation in real solvents. Recently in ref 39 we have discussed the effects of added acetic acid on the ground-state absorption spectra of lumichrome in 1,2-dichloroethane and acetonitrile. In the presence of acetic acid in 1,2-dichloroethane, a red shift and a hyperchromic effect are observed for the band with a maximum at 344 nm. A hyperchromic effect is also observed for the band at 382 nm. Similar although smaller changes in the absorption spectra of lumichrome are observed in the presence of acetic acid in acetonitrile. These changes in the absorption spectra of lumichrome and other alloxazines have been studied previously and ascribed to the formation of hydrogen-bonded ground-state complexes between lumichrome and acetic acid.[2] The small red shift of the absorption bands indicates a larger stabilization of the excited state due to the hydrogen-bonding interaction. Investigations by Koziołowa[2] and Szafran[4] suggest that the observed changes in the absorption spectra are a result of acetic acid binding at the N(10) nitrogen atom of the lumichrome molecule. A straightforward comparison to the DFT calculations suggests the presence of the type **IIb** complex.

The present TD-DFT calculations predict that the complex **IIb** is quite feasible, as its calculated binding energy is virtually equal to the binding energies of three other eight-membered complexes, **IIIb**, **IVb**, and **Vb**, given the typical precision estimate of such calculations of a few kilocalories per mole. The calculations predict the correct sign of spectral changes for complex **IIb** and the majority of the other configurations, apart from complex **IIIb**, in that a red shift is observed accompanied by an intensity growth.

Considering the data presented in Table 5, we can note a correlation between the binding energy and geometry of a single *isolated* hydrogen bond, D−H···A. Of the three bond parameters, the D···A distance and the D−H···A angle are particularly indicative of the hydrogen bond strength. However, it is worth noting that, in the present complexes, the methanol/acetic acid molecule is involved simultaneously in two hydrogen bonds, being simultaneously a donor and an acceptor of hydrogen.



A **B** **C**

Figure 6. Structure of eight- (**A**) and six- (**B**) membered cyclic complexes between lumichrome and acetic acid, together with the structure of an open complex between two molecules (**C**).

These two hydrogen bonds should be equally important; therefore, the observed D−H···A angles result from the equilibrium geometry of the respective six-membered ring for the methanol complexes, or that of the eight-membered ring for the acetic acid complexes. Thus, the presently observable correlations may not coincide with those observed for single hydrogen bonds.

It has to be noted that there has been some controversy about the possible structure of complexes between lumichrome and acetic acid, with hydrogen bonds at N(1)−H and N(10) of lumichrome. Previously, two such structures had been proposed, shown in Figure 6. The first structure assumes the formation of 1:1 eight-membered cyclic complexes between lumichrome and acetic acid with hydrogen bonds at N(1)−H and N(10) in the lumichrome molecule, **A**. These eight-membered cyclic complexes between lumichrome and acetic acid had been proposed by Kozioł, Koziołowa, Song, and co-workers.[1,2,42] The increase in the basicity of the N(10) nitrogen atom and an increase in the acidity of N(1)−H group after excitation provide the driving force for a proton shift between these two nitrogen atoms and are a source of excited-state proton-transfer reaction in the lumichrome−acetic acid system.[1,9,39] However, Kasha proposed an analogous mechanism, postulating a six-membered complex between lumichrome and acetic acid, **B**.[3]

Our calculations show that the six-membered complex between lumichrome and acetic acid, **B**, does not correspond to an energy minimum, and used as a starting configuration in the geometry optimization, it quickly rearranges itself into the structure of the complex **A**, equivalent to **Ib**. Another important point of our calculations was to investigate the stability of the open complex between lumichrome and acetic acid with a single hydrogen bond at N(1)−H, **C**. As we found for the complex **B**, energy minimization of this structure resulted in the eight-membered cyclic complex, **A**.

X-ray Analysis. Crystallographic data of lumichrome are summarized in Table 1. Figure 1 shows an ORTEP drawing of lumichrome with the numbering scheme. Molecular dimensions are well within the typical values. The molecule as a whole is almost planar, the maximum deviation from the least-squares plane calculated for all 14 ring atoms is 0.025(3)Å, and the dihedral angles between almost perfectly planar six-membered rings are very small, up to 1.5(3)°. In the crystal structure, the molecules of lumichrome are arranged into molecular tapes by means of intermolecular hydrogen bonds (Figure 7). The details of the hydrogen bonds are given in Table 6. The hydrogen bonds N1−H1···N10($-x,1-y,2-z$) connect molecules into centrosymmetric dimers, additionally strengthened by C9−H9···O2($-x,1-y,2-z$) hydrogen bonds. These dimers are further connected with other dimers by pairs of centrosymmetric N3−H3···O4($1-x,1-y,1-z$) hydrogen bonds. The tapes of molecules are stacked onto one another with an interplanar distance of ca. 3.25 Å. There is also the solvent−methanol molecule in the crystal structure. It also takes part in the hydrogen-bonding system: a relatively strong O1S−H1S···N5 hydrogen bond connects the hydroxyl group of the methanol molecule with the only hydrogen-bond-acceptor atom of lumichrome not engaged in any intermolecular interactions. The solvent molecule fits perfectly—thanks to both its shape and its hydrogen-bonding properties—within the voids in the tape structure of lumichrome.

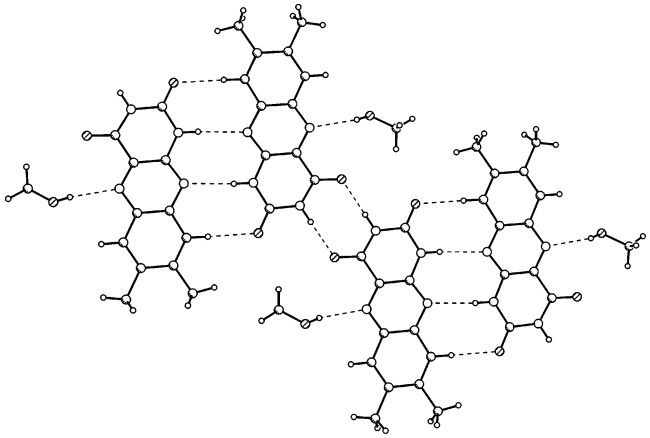


Figure 7. Hydrogen-bonded molecular tape in the crystal structure of lumichrome−MeOH. Hydrogen bonds are depicted by dashed lines.

TABLE 6: Hydrogen Bond Data[a]

D−H···A[b]	D−H distance (Å)	H···A distance (Å)	D···A distance (Å)	D−H···A angle (°)
N1−H1···N10^{i}	0.88 (1.02)	2.22 (2.14)	3.096(4) (3.17)	174 (179)
N3−H3···O4^{ii}	0.88	1.98	2.847(3)	167
C9−H9···O2^{i}	0.95 (1.08)	2.25 (2.12)	3.195(4) (3.21)	176 (177)
O1S−H1S···N5	0.84	2.19	2.917(4)	145

[a] The results of DFT calculations for lumichrome dimer are given in parentheses. [b] Symmetry codes: (i) $-x, 1-y, 2-z$; (ii) $1-x, 1-y, 1-z$.

Spectroscopy and Photophysics of Lumichrome Crystals. The very different emission spectrum of a polycrystalline sample of lumichrome, as compared to the corresponding spectra in solutions or adsorbed from solvent onto microcrystalline cellulose, have led us previously to believe that the new type of emission of the polycrystalline sample has its origin in lumichrome isoalloxazinic tautomers in the excited state of the crystals, or perhaps from stacked dimers.[46,47] The previously proposed structure admitted the possibility of double excited-state proton transfer, provided that a center of symmetry exists in the crystal structure at the geometric center of a planar arrangement of four nitrogen atoms {N(10), N(1), N′(10), N′(1)} from two adjacent molecules. However, the lack of clear confirmation from the lifetime data that the emission originates predominately from a single longer-lived species which could be identified with the excited isoalloxazine, and our past inability to produce single crystals for X-ray analysis, made such a simple

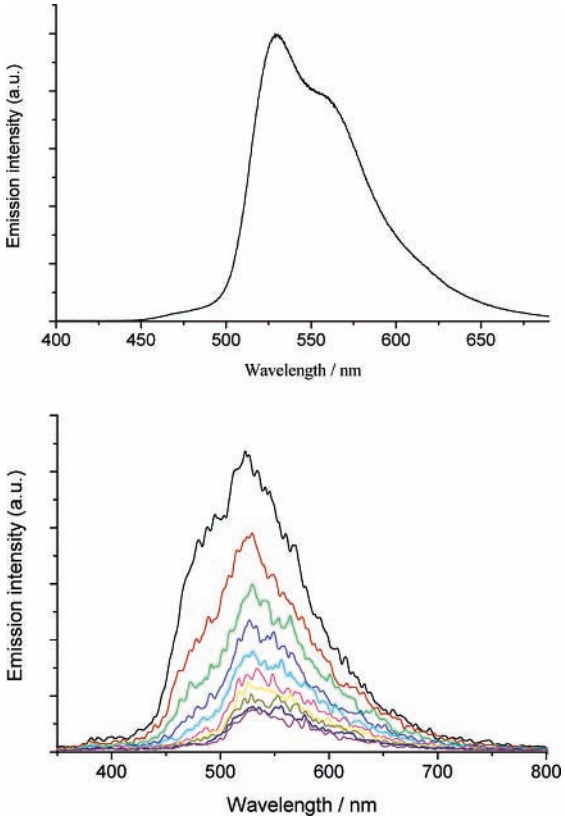


Figure 8. Steady-state fluorescence emission spectrum of lumichrome polycrystals (top), and time-resolved spectra of lumichrome polycrystals (bottom). Excitation was at 337 nm, and spectra were recorded with the time step of 1 ns.

interpretation problematic. Now, with the X-ray data in hand, we can corroborate our previous suggestion.

The emission spectrum of polycrystalline samples of lumichrome is markedly different from the spectra of lumichrome in solutions (compare Figure 8 and Figure 2). The similarity between the new emission band at lower energies and the emission of the excited isoalloxazinic tautomer of lumichrome in common solvents corroborates the assignment of the new emission to the isoalloxazinic tautomers, formed upon photoexcitation of lumichrome dimers existing in polycrystalline samples. Such dimer emission had never been reported in solutions. Assignment of emission of polycrystalline samples as originating from dimers is also supported on the basis of the comparison with the emission of a mechanical mixture of β-CD and lumiflavin (Lfl, 7,8,10-trimethylisoalloxazine, $\lambda_{max} = 526$ nm) and literature data.[39,47] All these data and the fact that a similar spectrum was obtained for lumichrome polycrystals mechanically mixed with KBr confirm that this new emission may arise from doubly hydrogen-bonded lumichrome dimers undergoing double proton transfer in the excited state. The existence of photoinduced double proton transfer in hydrogen-bonded dimers in the solid state has been reported for other compounds, such as 1-azacarbazole,[48] 7-azaindole,[49,50] and salicylic acid.[51] "In search for phototautomerisation in solid...",[52] the lumichrome is a very attractive object, especially in view of the X-ray structure reported. Further tests of the validity of this suggestion are in progress in our laboratories, as presently we are unable to rule out other possible explanations of the very different emission existing in the solid and in solution. Indeed, the importance of hydrogen bonds in the crystal packing of lumichrome in the solid state is evident from our X-ray data. Note that only a limited number of X-ray crystal structures of alloxazines are known, namely those of 1-methyl- and/or 9-methyl-substituted alloxazines. On the basis of the X-ray data available for alloxazines and isoalloxazines, it has been proposed that the chemical properties of neutral alloxazines should correspond to those of cationic isoalloxazines.[53] Now, in view of the new X-ray data for lumichrome and the results on spectroscopy and photophysics studies of cationic isoalloxazines,[54] this idea should also be considered. In this alternative explanation, the new emission would be the result of the hydrogen-bonding interactions in the crystal packing of lumichrome in the solid state, with the double proton transfer in the excited state becoming redundant.

We also recorded the fluorescence decay kinetics for the polycrystalline samples of lumichrome. None of the data could be satisfactorily described by a single-exponential function; thus, we used biexponential functions of the form

$$I(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) \qquad (5)$$

The values of the fitted lifetimes (and amplitudes) are 3.87 ns (0.58) and 0.43 ns (0.42). The fits are not perfect; however, the existence of two equivalent exponential contributions provides a rationale for seeking a mechanism with two distinct emitting species. Since the lifetimes are not too different from those found for alloxazines and isoalloxazines (in neutral or cationic form) excited separately in solutions, it might be tempting to assign the shorter-lived component to the excited alloxazine and the longer-lived component to a separate excited species, perhaps formed as the result of intermolecular proton transfer within the lumichrome dimer or as a result of hydrogen-bonding interactions in the crystal packing. The presently recorded time-resolved spectra of emission of lumichrome polycrystals clearly indicate the presence of at least two emitting species: a short-lived one with a maximum at about 475 nm, and a second emitting species with an emission maximum at about 530 nm and a lifetime of about 3.5 ns. This is consistent with the presence of dimers in the polycrystalline solid sample, with the longer-wavelength emission resulting from the products of the excited-state proton-transfer reaction occurring in the solid sample or, more likely, reflecting the hydrogen-bonding interactions in the crystal structure. Clearly, further work is needed to clarify the lumichrome photophysics in the solid state.

Acknowledgment. The grant from Polish State Committee for Scientific Research, No. 2P06T 112 26, is gratefully acknowledged. The quantum chemical calculations were performed at the Poznań Supercomputer Centre (PCSS).

Supporting Information Available: Calculated (B3LYP) singlet excitation energies starting from the ground state using the basis of 6-31G(d,p) and 6-31G(d) of Lch−Me **IIIa** complex and Lch−AA **IIIb** complex, with the corresponding oscillator strengths, *f*. This material is available free of charge via the Internet at http://pubs.acs.org.

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