Mechanism of Intramolecular Excited-State Proton Transfer and Relaxation Processes in the Ground and Excited States of 3-Hydroxyflavone and Related Compounds

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Two components of fluorescence rise due to the phototautomer generated in the excited-state proton transfer were observed in 3-methylpentane solutions of 3-hydroxyflavone (3-HF) and its derivatives, while only a fast rise was observed in 3-hydroxychromone (3-HC) which lacks a 2-phenyl group. The shorter rise times are independent of temperature and substituent at the para position in the 2-phenyl group of 3-HF, whereas the longer rise times are dependent on each of them. As a result, the former and latter were ascribed to excited-state proton transfer in the Franck–Condon state (probably a twisted form of the phenyl group to the γ-pyrone ring) and to that in the vibrationally and/or torsionally relaxed excited state (a coplanar form of the phenyl group to the pyrone ring), respectively. The transient absorption of these compounds exhibits absorption bands at 430–530 nm with lifetimes of 7–30 μs at room temperature, which were attributable to the ground-state tautomers of these compounds. The two-step laser excitations (TSLE) of these transient absorption bands show TSLE fluorescence spectra being identical with the steady-state fluorescence spectra. Further, variable delay plots of TSLE fluorescence intensity afford lifetimes of the ground-state tautomers. From the temperature dependence of these lifetimes, the activation free-energy changes for the reverse proton transfer in the ground state were determined. Taking into account the reaction constant (ρ) of Hammett's plots of the activation free-energy change vs. substituent constants (σ), it was concluded that the electron-attracting group at the phenyl para position may assist the reverse proton transfer to the parent molecules and that the phenyl group in 3-HF may act as an electron-donating group to the γ-pyrone ring to decrease the reaction rate of the reverse proton transfer in comparison with 3-HC.

Introduction

Numerous investigations of excited-state proton transfer and photoisomerization in hydrogen-bonding systems by nano- and picosecond fluorescence spectroscopies have been reported.[2-7] The intramolecular excited-state proton transfer in some hydrogen-bonding systems has been suggested to take place at an unresolvably rapid rate ($k_{PT} > 10^{11}$ s^{-1}) even at low temperature, placing upper limits on the potential barrier height for the transfer of ~1 kcal/mol.[4-12] For 3-hydroxyflavone (3-HF), one of the intramolecular excited-state proton-transfer systems, several reports for the rate and potential barrier of the intramolecular excited-state proton transfer in aprotic solvents have been made for some years. Sengupta and Kasha,[13] Woolfe and Thistlethwaite,[11] and Itoh et al.[14] suggested some amount of barrier in this proton transfer. Itoh et al. reported the time-resolved and steady-state fluorescence studies of the excited-state proton transfer in 3-HF in 3-methylpentane and 2-methyltetrahydrofuran in comparison with 3-hydroxychromone (3-HC) which lacks the 2-phenyl group in 3-HF. They suggested an existence of a barrier for the excited-state proton transfer only in 3-HF, which may be attributable to the electronic and torsional effect of the phenyl group of 3-HF. Barbara et al.[15] reported the rise of the tautomer fluorescence in methylcyclohexane which consists of two components (10 and 140 ps) at 260.4 K. They interpreted that the fast component of two rise times means a proton transfer from the initially excited state (i.e., a kind of Franck–Condon state) and the other slow one from the vibrationally "relaxed" excited state.[15,16]

Recently, in contrast with the above results, McMorrow et al.[17] have observed the rapid rise time of the yellow–green fluorescence of the proton transfer to be 40 ps (*n*-heptane or *n*-octane solution) at 77 K and 26 ps (3-methylpentane) at 142 K, and they have suggested the absence of the barrier in the exited-state proton transfer in 3-HF. In spite of these investigations, the kinetics and dynamics of the intramolecular excited-state proton transfer in 3-HF, especially, the presence of the potential barrier in the excited process and the role of a phenyl ring in 3-HF are almost completely obscure. On the other hand, Itoh et al. have reported two-step

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laser excitation (TSLE) fluorescence as one of the recent developments of laser spectroscopies.[18-22] They have demonstrated the existence and dynamic properties of unexpectedly stable ground-state tautomers which are generated in the excited-state proton transfer and fluorescent relaxation processes of some hydrogen-bonding systems by the new laser technique.

In these circumstances, we have investigated the dynamic process of excited-state proton transfer in several substituted 3-HFs, which have electron-donating and -attracting groups on the 2-phenyl group, by nano- and picosecond laser spectrocopies. Further, relaxation processes of the labile ground-state tautomers which are formed from excited-state proton transfer and fluorescent relaxation, i.e., the reverse proton transfer in the ground state to the parent molecule, have been studied by transient absorption and two-step laser excitation fluorescence spectroscopies. In the picosecond laser spectroscopy, two rise times of the tautomer fluorescence of 3-HF and derivatives in MP solutions have been observed. The slower rise is dependent on the substituents on the phenyl group and on temperature, and the fast one is independent of them. 3-HC was observed to show only one fast rise time of the fluorescence. Judging from these experimental facts and the structures of these 3-hydroxyflavones, it seems that the faster rise time is attributable to a proton-transfer rate in a kind of Franck–Condon state which remains to keep the torsional angle (40°) of the C_2–$C_{1'}$ bond in the ground state and that the slower one is attributable to a proton-transfer rate in the vibrationally and torsionally "relaxed" excited state. In addition to these investigations on the excited state, the rate and activation free-energy changes of the ground-state reverse proton transfer from the tautomer to the parent molecule of 3-HF and their derivatives were obtained by means of the TSLE fluorescence technique. Judging from Hammett's plots of activation free-energy change vs. substituent constant (σ),[23] it was found that a phenyl group of 3-HF plays the role of an electron-donating group to the γ-pyrone ring which decreases the rate of the reverse proton transfer in the ground state, in comparison with 3-HC, and that the electron-attracting substituents at the 2-phenyl para position may assist the reverse proton transfer to the parent molecules.

Experimental Section

Materials. 3-Hydroxyflavone (3-HF, Tokyo Kasei Co.) was purified by recrystallization several times from aqueous ethanol and by vacuum sublimation (5 mmHg, 114 °C), mp 175 °C. 3-Hydroxychromone (3-HC)[24] was prepared from 4-chromanone (Aldrich Chemical Co.) and purified by several vacuum sublimations (5 mmHg, 105 °C), mp 177 °C. Anal. Calcd for $C_9H_6O_3$: C, 66.67; H, 3.73. Found: C, 66.60; H, 3.63. 3-Methoxyflavone (3-MeOF) was synthesized by methylation of 3-HF with diazomethane. The purification was performed by column chromatography (SiO_2, $CHCl_3$) and recrystallization several times from aqueous ethanol, mp 115 °C. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 75.70; H, 4.79. Five 3-HF derivatives 2′-methyl-3-hydroxyflavone (2′-Me-3-HF); 2′,3′,4′,5′,6′-pentamethyl-3-hydroxyflavone (PMB-3-HF), 4′-(dimethylamino)-3-hydroxyflavone (4′-DMA-3-HF), 4′-(trifluoromethyl)-3-hydroxyflavone (4′-CF_3-3-HF), and 4′-chloro-3-hydroxyflavone (4′-Cl-3-HF): were prepared by essentially the same procedure, the first step being Claisen–Schmidt reaction and further oxidation by hydrogen peroxide.[25-27] The purification was carried out by column chromatography (SiO_2, $CHCl_3$), recrystallization from ethanol several times, and the vacuum sublimation. The results of these analyses are as follows. 2′-Me-3-HF: mp 193–195 °C. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.12; H, 4.72. PMB-3-HF: mp 283 °C. Anal. Calcd for $C_{20}H_{20}O_3$: C, 77.92; H, 6.49. Found: C, 78.16; H, 6.52. 4′-DMA-3-HF: mp 194–196 °C. Anal. Calcd for $C_{17}H_{15}NO_3$: C, 72.60; H, 5.34; N, 4.98. Found: C, 72.53; H, 5.38; N, 5.13. 4′-CF_3-3-HF: mp 163 °C. 4′-Cl-3-HF: mp 204 °C. Anal. Calcd for $C_{15}H_9ClO_3$: C, 66.06; H, 3.30. Found: C, 66.10; H, 3.20. The purity of all these compounds was also confirmed spectroscopically. The solvent 3-methylpentane (MP) (Aldrich) was refluxed over lithium aluminum hydride (Nakarai Chemical Co.) for several hours and rendered free of fluorescent impurities by

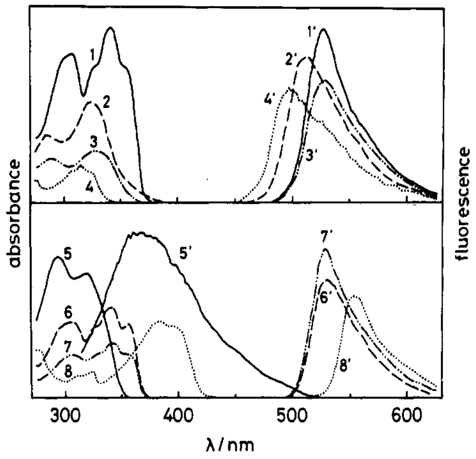

Figure 1. Absorption and fluorescence spectra of deaerated MP solutions of 3-HF[1] and its derivatives[2-8] at room temperature: 3-HF, 1; PMB-3-HF, 2; 2′-Me-3-HF, 3; 3-HC, 4; 3-MeOF, 5; 4′-CF_3-3-HF, 6; 4′-Cl-3-HF, 7; 4′-DMA-3-HF, 8. Primed numbers are corresponding fluorescence spectra.

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(33) McMorrow et al. (ref 17) have reported a single rapid rise of tautomer fluorescence in heptane (77 K) and MP (142 K) solutions of 3-HF. Further, McMorrow and Kasha (ref 32) have reported several papers of the excited-state proton transfer in 3-HF and suggested that the further slow rise of tautomer fluorescence in the MP solution of this compound at 150–200 K reported in our previous paper (ref 14) and also reported by Barbara et al. (ref 15 and 16) may be attributable to the H-bonding impurities in the solvent. Further, they have reported that the variation of details of proton-transfer spectroscopy on the 3-HF and 3-HC is the result of varying traces of H-bonding impurities in the solvents. (ref 32) However, no indication of the fluorescence slow rise in alcoholic solvent nor in the solvent containing H-bonding impurities has been presented by them. The solvent (MP) used here was purified very carefully as mentioned in the Experimental Section and has no H-bonding impurities (OH and H_2O). This can be easily understood by observation of the deuterium substitution effect of the 3-OH group to the excited-state proton transfer. If the solvent contains any H-bonding impurities in this solvent, no deuterium substitution effect can be observed because of the rapid H–D exchange in the solvent, as reported in the previous paper (ref 21) and will be also mentioned later.

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redistillation. The solvent was kept in the bottle, with a potassium mirror in the inside wall of the bottle, connected to a vacuum line. The solutions of samples in a rectangular quartz cell (cell length, 10 or 5 mm) with graded seals were prepared by the following way: the cell containing the compound was completely deaerated by vacuum line, and the solvent was transferred to this sample cell from the solvent bottle through the vacuum line without contacting open air. The solution was degassed by freeze–pump–thaw cycles (4 times) and sealed off from the vacuum line (~10^{-5} Torr). The deuterium substitutions of the 3-hydroxy hydrogen atom of 3-HF were performed by CH_3OD or C_2H_5OD (CEA, deuterium grade 99%) in a vacuum system. After CH_3OD or C_2H_5OD was removed, MP pretreated by potassium mirror was added through a vacuum system.

Measurements. Picosecond measurements were made on the apparatus reported previously[45] using the fourth (266 nm) harmonic of a mode-locked Nd^{3+} YAG laser as a light source, in order to determine fluorescence rise times of 3-HF and its derivatives in comparison with 3-HC which has no absorption band at the third harmonic wavelength. A single pulse was selected and amplified. The fluorescence rise curves were obtained by detecting the fluorescence signals with a streak camera (Hamamatsu C797) and averaging the signals about 5 times. The excitation was made with the fourth harmonic of the laser (pulse width, fwhm 11 ps), and a 532-nm prepulse was used to provide a reference mark on the time axis for the signal averaging. The rise signals were processed with a minicomputer system (Hamamatsu C1000) and were handled with suitable programs for obtaining the rise curves. The ethanol solution of Rhodamine 6G was confirmed to exhibit a very fast rise of fluorescence by these equipments and procedures. The nanosecond fluorescence lifetimes were determined by using a single-photon counting system with a nanosecond light pulser (PRA 510).[20,28]

Transient absorption spectra were determined by using an excimer laser (Lambda Physik, EMG-50E, 308 nm) and a monitoring steady Xe lamp with synchronously operated shutter (COPAL).

For the TSLE fluorescence, the excimer laser was used as the first excitation light pulse, and the second N_2 laser pumped dye laser (Molectron UV-12 and DL-14) was synchronously operated with the excimer laser by a pulse generator and a variable-delay circuit. The detection and signal analyses were performed by a method similar to those reported previously,[20] except using a storagescope (Iwatsu TS8123). The TSLE time-resolved fluorescence spectra were constructed by way of a point-by-point by changing the delay time between the first and second laser pulses.[18-22]

Results and Discussion

Fluorescence Spectra and Lifetimes. Figure 1 shows the absorption and fluorescence spectra of the MP solutions of 3-HF and 3-HC and its derivatives at room temperature. The large Stokes-shifted fluorescence spectra of these compounds at 490–650 nm may be attributable to the excited-state tautomer generated by the intramolecular proton transfer as assigned in the previous papers.[11,13,14] On the other hand, the lowest singlet excited state of flavone, which lacks a 3-OH group, is attributable to an $n\pi^*$ transition,[29] while the longest absorption band (350 nm) of 3-HF is known to be a $\pi\pi^*$ transition.[30,31] The excited-state proton transfer may take place from this $\pi\pi^*$ singlet excited state. Further, Figure 1 also shows the absorption and fluorescence spectra of the MP solution of 3-methoxyflavone (3-MeOF) at room temperature. The MP solution of 3-MeOF exhibits only a shorter wavelength fluorescence whose intensity is much smaller than 3-HF. Although the spectral differences between OH and OCH_3 substituents of aromatic molecules are usually not so dramatic, the spectral difference of absorption bands between 3-HF (λ_{max} 355 nm) and 3-MeOF (λ_{max} 319 nm) is significant. Therefore, the intramolecular hydrogen bonding in the MP solution of 3-HF may take an important role in the transition character of the lowest electronic excited state and then in the intramolecular excited-state proton transfer. In these MP solutions of 3-HF, 3-HC, and their derivatives, no normal form fluorescence was observed at 300–180 K, though two components of fluorescence rise of the tautomer (rise time, <50 and ~100–200 ps) were detected, as will be mentioned later. These similar fluorescence behaviors are usually encountered in the other intramolecular excited-state proton transfer.[2,5,6,10,12] In these intramolecular excited-state proton transfers, a rapid nonradiative process as well as rapid proton transfer may be responsible for the no normal form fluorescence in spite of the detection of the tautomer form fluorescence rise.

TABLE I: Tautomer Fluorescence Rise Time of Deaerated MP Solutions of Flavonols through 295–172 K. Errors of Fluorescence Rise Times Were Estimated Approximately 10–15 ps, taking into account Pulse Width (fwhm 11 ps)

compd	temp, K	rise time (fast), ps	rise time (slow), ps
3-HF	286	22 ± 15	82 ± 15
	273	29	105
	251	39	132
	233	52	201
	214	51	208
	192	60	287
3-HC	295	~20	~20
	193	~20	~20
2′-Me-3-HF	287	17	104
	273	25	121
	254	30	132
	235	32	169
	214	41	289
	192	45	270
PMB-3HF	294	14	38
	271	19	69
	251	38	155
	232	48	194
	211	46	224
	193	54	344
4′-DMA-3-HF	291	16	40
	273	23	84
	235	19	96
	213	42	147
	193	36	187
	172	34	138
4′-CF_3-3-HF	295	19	19
	273	19	40
	254	16	58
	230	28	77
	212	41	169
	193	30	187
3-DF	295	22	44
	276	19	95
	252	21	116
	232	24	148
	213	33	159
	193	51	226
3-HF[a]/ CH_3OH	295	14	14

[a] In a deaerated methanol solution.

The fluorescence (526 nm) of 3-HF in MP solution exhibits a single-exponential decay throughout 300–120 K, whose lifetimes are very temperature dependent. The temperature dependence of the tautomer fluorescence lifetimes seems to be mainly attributable to a radiationless process including partially an internal molecular motion of 3-HF: most probably torsional motion of the phenyl and γ-pyrone rings.

On the other hand, the observation of rise of these tautomer fluorescences is instructive for investigation of the primary process of the proton transfer and/or isomerization in the excited state. The rises of the tautomer fluorescence of MP solutions of 3-HC and several substituted 3-HFs were measured by picosecond fluorescence spectroscopy, though nano- and picosecond studies have been reported in previous papers.[11,14-16] Figure 2 (and Table I) shows the rise of the tautomer fluorescence of all compounds

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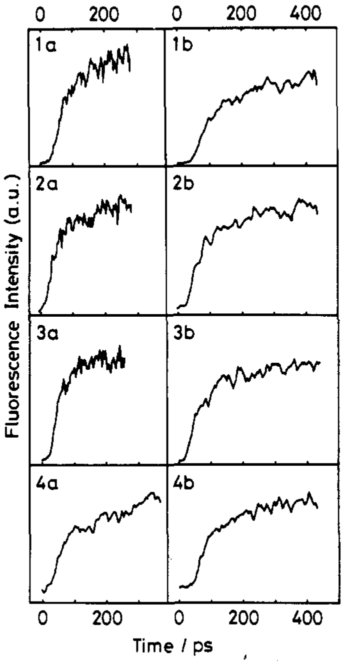


Figure 2. Rise curves of tautomer fluorescences in several flavonols following pulsed picosecond 266-nm laser excitation. Curves a and b show rises at 273 and 216 K, respectively. PMB-3-HF, 1; 4'-DMA-3-HF, 2; 4'-CF$_3$-3-HF, 3; 3-DF, 4.

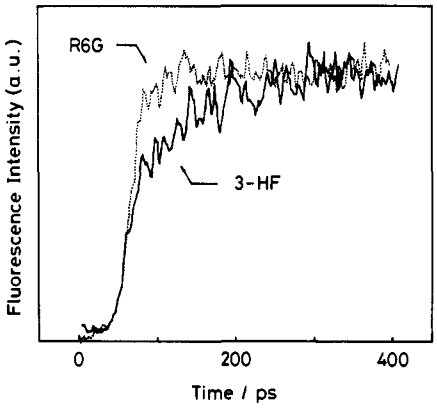


Figure 3. Time evolution of the fluorescence of the methylpentane solution (—) of 3-HF in comparison with the ethanol solution (···) of rhodamine 6G at 295 K.

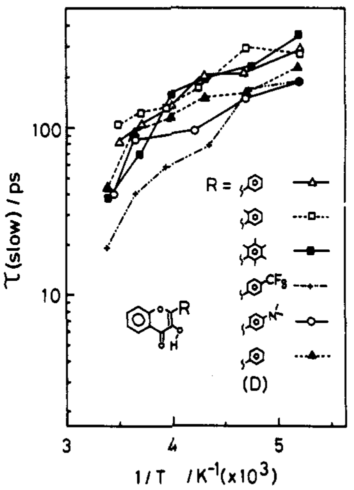


Figure 4. Plots of only *slow rise times* vs. the inverse of temperature (295–193 K). (△) 3-HF; (□) 2'-Me-3-HF; (■) PMB-3-HF; (+) 4'-CF$_3$-3-HF; (○) 4'-DMA-3-HF; (▲) 3-DF.

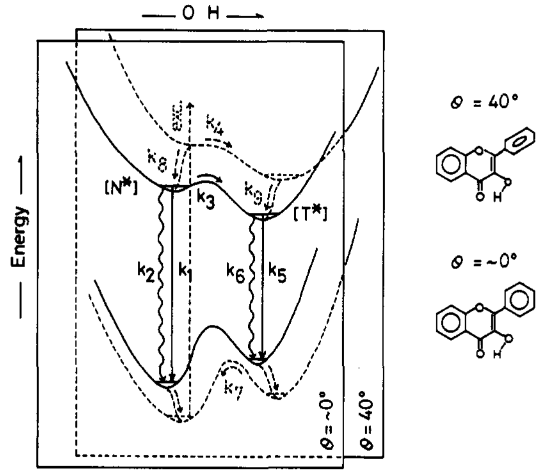


Figure 5. Schematic dual-coordinate potential energy diagram for the ground and excited states. The angle θ illustrates an angle where the γ-pyrone and phenyl planes in the flavonols make a C$_2$–C$_{1'}$ bond to each other.

in MP solutions at 273 and 216 K. The fast and slow components of fluorescence rise times were observed for these flavonols at 295–172 K, though the fast rise times obtained may contain approximately 10–15-ps uncertainty. However, only a very fast rise of fluorescence was observed in the MP solution of 3-HC even at low temperature (193 K). Figure 3 shows the fluorescence curves of the MP solution of 3-HF in comparison with the ethanol solution of rhodamine 6G. The difference between 3-HF and 3-HC is attributable to the presence and absence of a phenyl ring. Further, in these rise times observed in several flavonols, the slower rise time (>50 ps) seems to be dependent on the substituents of the phenyl ring, as shown Figure 4. Judging from the fast and slow fluorescence rise times in 3-HF and its derivatives and only a very fast rise in 3-HC, it seems that the occurrence of the slower fluorescence rise time is associated with the torsional motion of the phenyl group in the γ-pyrone ring of flavonols. On the other hand, since only one fast rise time (14 ps) of the tautomer fluorescence was observed in methanol solution in 3-HF, the slower one in MP cannot be probably attributable to protic solvent effect, though McMorrow et al.[17,32,33] pointed out that the slow rise of tautomer fluorescence of 3-HF in MP is ascribed to the effect of alcoholic impurity in the solvent, as reported in the previous paper. Therefore, it is likely that the faster rise time may be attributable to the excited-state intramolecular proton transfer from the upper vibrationally excited state, which remains to keep a torsional angle (40°)[34] of a phenyl ring to a γ-pyrone ring in the ground state (i.e., a kind of Franck–Condon state), as suggested first by Strandjord and Barbara.[15,16] On the other side, the slower one seems to be attributable to the proton transfer from the vibrationally "relaxed" excited state, where the 2-phenyl group become closer to coplanar in comparison with the torsional angle (40°) in the ground state and also in the Franck–Condon state, as will be mentioned later.

Calculation by Song et al. for the structurally similar pelargonidin suggests an increase in bond order of the phenyl linkage upon excitation.[35] This calculation supports that the torsional angle of the phenyl and γ-pyrone rings is nearly close to zero in the excited state. If it is the case, a rapid relaxation to this coplanar conformation seems to take place in the excited state followed by the proton transfer. The rate constants for torsional motion of this type of phenyl group in excited molecules have been reported. Kaminska et al.[36] predicted the rate (9.1 × 10^{11} s^{-1}) from the planar form to the twisted form of *m*-methyl-*p*-cyano-*N*,*N*-dimethylaniline in an acetonitrile solution. Siemiarczuk suggested a rate constant of >1.9 × 10^{10} s^{-1} for 4-(9-anthryl)-*N*,*N*-dimethylaniline.[37] Further, Wang et al. also showed 19 ± 3 ps for the rate of the similar torsional motion in *p*-(dimethylamino)benzonitrile.[38]

Figure 5 shows three-dimensional energy curves in the excited state. Assuming neglect of intramolecular reverse proton transfer in the excited state, the following equations for the time-dependent concentrations of normal and tautomer forms of the fluorescent

TABLE II: Temperature Dependence of the Tautomer Fluorescence Lifetime in Deaerated MP Solvents

compd	τ, ns[a]	k_5, s^{-1}	A, s^{-1}	E_a, kcal/mol
3-HF	4.4	1.3×10^8	5.1×10^{13}	7.6
3-HC	0.4	0.9×10^8	4.9×10^{12}	4.9
2'-Me-3-HF	3.1	1.3×10^8	3.4×10^{10}	3.0
PMB-3-HF	1.9	1.2×10^8	4.0×10^{13}	6.5
4'-DMA-3-HF	1.3	2.3×10^8	2.3×10^{13}	6.2
4'-CF$_3$-3-HF	2.6	1.3×10^8	7.4×10^{12}	5.9
4'-Cl-3-HF	4.3	1.3×10^8	5.1×10^{13}	7.6
3-DF	5.1	1.3×10^8	4.3×10^{12}	6.3

[a] Lifetime of the tautomer fluorescence at room temperature.

states (vibrationally relaxed coplanar forms) were obtained (N* = normal and T* = tautomer forms)

$$[\mathrm{N}^*] = A(\exp(-(k_1 + k_2 + k_3)t) - \exp(-(k_4 + k_8)t)) \quad (1)$$

$$[\mathrm{T}^*] = B \exp(-(k_5 + k_6)t) - C \exp(-(k_4 + k_8)t) - D \exp(-k_9 t) - E \exp(-(k_1 + k_2 + k_3)t) \quad (2)$$

where k_1 and k_5 are radiative rate constants, k_2 and k_6 are nonradiative rate constants, k_8 and k_9 are rate constants for torsional motion, k_4 and k_3 are rate constants for proton transfer in the Franck–Condon state and in the vibrationally relaxed state, respectively, and A, B, C, D, and E are preexponential factors (Appendix section).

Equation 2 indicates three components of the fluorescence rise of T* and one component of decay. However, $k_4 + k_8$ and k_9 may be approximately same order of magnitude. It is because k_4 and k_8 are rate constants of competitive processes of proton transfer and vibrational (torsional) relaxation from the Franck–Condon state and also because k_8 and k_9 are both concerned with torsional motion from the twisted to the coplanar conformers. Therefore, the second and third terms of eq 2 seem to be observed as only one rapid rise of fluorescence. Further, according to eq 2, another rise of fluorescence, whose rate constant $k_1 + k_2 + k_3$ seems to be smaller than $k_4 + k_8$ (or k_9), may be observed. Therefore, it is likely that the fast and slow rises of tautomer fluorescence mentioned above are attributable to these terms owing to $k_4 + k_8$ (k_9) and $k_1 + k_2 + k_3$, respectively. If it is the case, the fast rise time $1/(k_4 + k_8)$ (or $1/k_9$) does not seem to be temperature dependent very much from room temperature to 172 K, though the torsional motion may be temperature dependent at very low temperature. On the other hand, the slow rise of fluorescence due to $k_1 + k_2 + k_3$ is a sum of radiative (k_1) and nonradiative (k_2) rate constants of the vibrationally relaxed state (normal) and of the proton transfer (k_3) from this state. In the vibrationally and rotationally relaxed excited state, a phenyl group in 3-HF becomes coplanar to the γ-pyrone ring so that the relaxed excited state (normal) may be stabilized to some degree by the extention of the π-resonance system. As a result, the appearance of a barrier of the proton transfer and hence a temperature dependence of k_3 are expected. Further, the potential barrier of the proton transfer and then k_3 may be dependent on the para substitution of the phenyl ring. These arguments are consistent with the temperature and also substituent effects of the longer rise time of the tautomer fluorescence as shown in Figure 4 and Table I, though the nonradiative rate constant (k_2) may be temperature dependent to some extent.

The temperature dependence of the fluorescence lifetimes of 3-HF, its derivatives, and 3-HC may be analyzed by the simplified assumptions of temperature-dependent nonradiative (rate constant k_6) and temperature-independent radiative (k_5) processes. Assuming Arrhenius behavior, we obtain

$$\tau_T^{-1} = k_5 + k_6 \quad (3)$$

$$k_6 = A \exp(-E_a/RT) \quad (4)$$

where A for the Arrhenius frequency factor, E_a is the activation energy for the temperature-dependent radiationless process, and τ_T is the tautomer fluorescence decay time. In accordance with the above equations, E_a for 3-HF was calculated (Table II) from temperature dependence of the fluorescence lifetimes.[14] The obtained E_a's for 3-HC and other 3-HF derivatives in MP solution also were calculated. The obtained data are summarized in Table II.

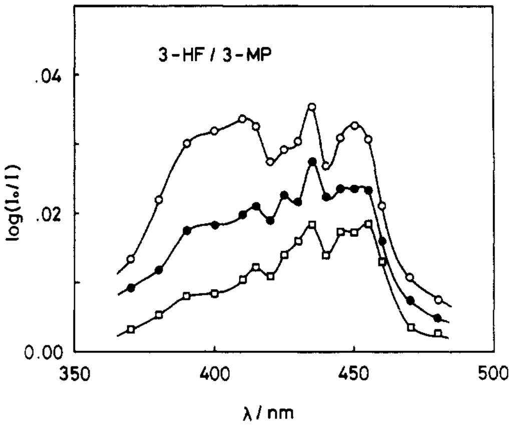


Figure 6. Transient absorption spectra of a deaerated MP solution of 3-HF (1.0×10^{-5} M) at room temperature. Delay times from 308-nm XeCl laser pulse excitation are 0.3 (○), 4.2 (●), and 12.5 μs (□).

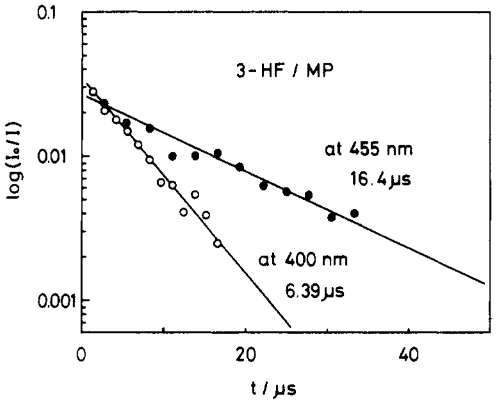


Figure 7. Logarithmic plots of the transient absorption of a deaerated MP solution of 3-HF at room temperature. The short (τ = 6.39 μs at 400 nm, ○) and long (τ = 16.4 μs at 455 nm, ●) decay components are due to the triplet-state and the ground-state tautomers of 3-HF, respectively. The concentration is 1.0×10^{-5} M.

Transient Absorption. Transient absorption spectra of the deaerated MP solutions of 3-HF, 3-HC, and their derivatives were measured by laser flash photolysis at room temperature. The transient absorption spectra exhibit absorption bands at λ_{max} 410 and 435 nm for 3-HF, as shown Figure 6. The decay curves of the transient absorption bands are shown in Figure 7. Although the transient absorption band (λ_{max} 410 nm) of 3-HF exhibits a single-exponential decay of τ = 6.39 μs at room temperature, the decay time remarkably decreases both in intensity and decay time on aeration of the solution. Therefore, the band seems to be ascribed to the $T_n \leftarrow T_1$ absorption. On the other hand, the band ($\lambda_{max} \sim$ 435 nm) exhibiting a single-exponential decay of τ = 16.4 μs is ascribed to the absorption band of the ground-state tautomer generated by the excited-state intramolecular proton transfer. This assignment is confirmed by the fact that the same fluorescence spectra and lifetime are obtained by the excitation of the transient band ($\lambda_{max} \sim$ 435 nm) by the TSLE method, as will be mentioned later. This absorption band decreases in decay time by ca. 1 μs on aeration, which is ascribed to the photoreaction of the ground-state tautomer of 3-HF.[39-41] Table III shows the maximum wavelengths and lifetimes of $T_n \leftarrow T_1$ and the tautomer absorption bands in MP solutions of the respective compounds, together with lifetimes of the ground-state tautomers obtained by the TSLE technique. They are in fair agreement with each other within experimental error. However, since the transient absorption has less accuracy than the TSLE fluorescence technique, the effect of the substitutents on the phenyl ring to the lifetimes of the ground-state tautomers should be discussed in terms of the data obtained by the TSLE technique.

TABLE III: Wavelengths (λ_{max}) and the Decay Times of the $T_n \leftarrow T_1$ Transient Absorption Band of the Ground-State Tautomers of Flavonols in Deaerated MP Solvents at Room Temperature

	$T_n \leftarrow T_1$[a]		tautomer[a]		
compd	λ_{max}, nm	τ, μs	λ_{max}, nm	τ, μs	TSLE[b] τ (N ← T), μs
3-HF	400	6.39	~435	16.4	13.9
3-HC	390	1.93	~430	7.00	10.0
2′-Me-3-HF	410	4.79	~440	16.7	13.2
PMB-3-HF	395	4.22	~435	15.7	24.3
4′-DMA-3-HF	430	17.9	~530	27.8	32.8
4′-CF_3-3-HF	395	1.19	~440	9.29	9.90
4′-Cl-3-HF	400	9.55	~450	16.0	13.7

[a] Determined by the laser flash photolysis. [b] Determined by the variable-delay technique of the TSLE fluorescence.

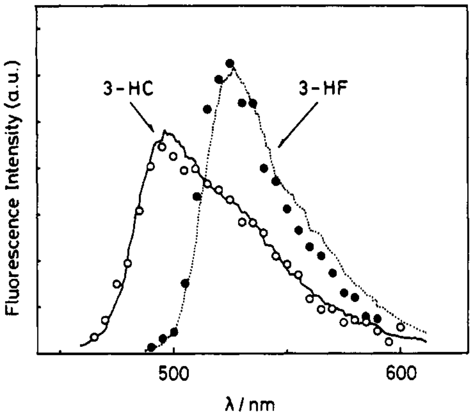


Figure 8. TSLE (●) and steady-state (- - -) fluorescence spectra of 3-HF and TSLE (○) and the steady-state (—) spectra of 3-HC in deaerated MP solutions (1.0×10^{-5} M). The delay time after the first excimer laser excitation (308 nm) of both solutions is 4 μs. The second laser wavelength is 440 nm.

Two-Step Laser Excitation Fluorescence and Variable-Delay Technique. The TSLE fluorescence spectra and lifetimes were measured in order to confirm the relation between transient absorption and fluorescence spectra. The transient species generated by excitation of the first excimer laser pulse can be excited again by the second dye laser pulse within the lifetime. If the transient absorption band ($\lambda_{max} \sim 435$ nm) of the ground-state tautomer is exclusively excited within the lifetime by the second laser pulse of the absorption wavelength, the TSLE fluorescence spectra and the emission lifetime of the tautomer should be observed,[18-22] while no TSLE fluorescence was observed by the second laser excitation of the $T_n \leftarrow T_1$ absorption band (380–420 nm). The TSLE fluorescence spectra of 3-HF and 3-HC in deaerated MP solutions are shown in Figure 8. These spectra and fluorescence lifetimes are well consistent with the respective steady-state fluorescence spectra and lifetimes of these tautomers. The TSLE fluorescence spectra and lifetimes of other compounds are also in agreement with the respective steady-state ones.

On the other hand, the TSLE fluorescence intensities of 3-HF, 3-HC, and their derivatives in deaerated MP solutions were plotted vs. delay times between two laser pulses, as shown in Figure 9. The lifetimes of the ground-state tautomers of these compounds were obtained from slopes of these variable-delay plots of TSLE fluorescence intensity, as shown in Figure 9. These lifetimes of the ground-state tautomers are summarized in Table III and are consistent with those by the transient absorption as mentioned in the last section. Therefore, it was confirmed that the fluorescent species are ascribed to the excited states of these tautomers whose ground states show transient absorption bands. These situations are illustrated in the potential energy diagram as shown in Figure 5.

The lifetimes of the ground-state tautomers show a remarkable temperature dependence. Therefore, an activation energy for the ground-state reverse proton transfer (k_7 in Figure 5) may be obtained by measuring this temperature dependence, if the lifetime obtained reflects the reaction rate to the parent molecules. According to the transition-state theory, the reaction rate constant (k_7) of the reverse proton transfer is expressed as

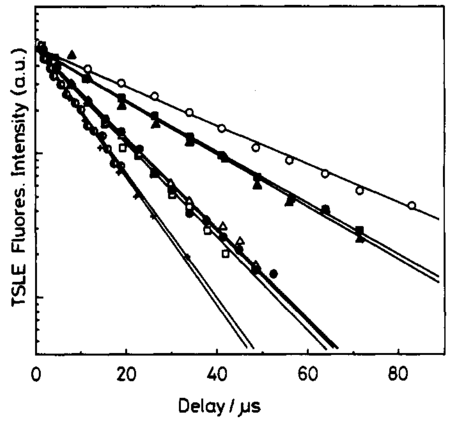


Figure 9. Decay of TSLE fluorescence of 1.0×10^{-5} M MP solutions of 3-HF and its derivatives at room temperature. Logarithmic plots of TSLE fluorescence intensities against delay times of the second laser pulse (440 nm) and the first one (308 nm). (△) 3-HF; (□) 2′-Me-3-HF; (■) PMB-3-HF; (◐) 3-HC; (○) 4′-DMA-3-HF; (+) 4′-CF_3-3-HF; (●) 4′-Cl-3-HF; (▲) 3-DF.

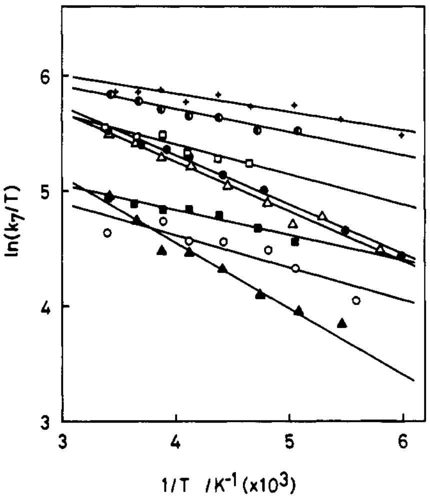


Figure 10. Plots of ln (k_7/T) vs. $1/T$ for deaerated 1.0×10^{-5} M MP solutions of 3-HF and its derivatives. (-△-△-): 3-HF; (-□-□-): 2′-Me-3-HF; (-■-■-): PMB-3-HF; (-◐-◐-): 3-HC; (-○-○-): 4′-DMA-3-HF; (-+-+-): 4′-CF_3-3-HF; (-●-●-): 4′-Cl-3-HF; (-▲-▲-): 3-DF.

TABLE IV: Changes of Activation Enthropy, Activation Enthalpy, and Activation Free Energy at 298 K of Flavonols in Deaerated MP Solvents

compd	$\Delta S^\ddagger$, cal/(mol deg)	$\Delta H^\ddagger$, cal/mol	$\Delta G^\ddagger_{298K}$, kcal/mol
3-HF	−33.39	847.8	10.80
3-HC	−34.28	398.4	10.61
2′-Me-3-HF	−34.42	516.7	10.77
PMB-3-HF	−35.89	430.6	11.13
4′-DMA-3-HF	−35.83	553.4	11.23
4′-CF_3-3-HF	−34.35	313.6	10.55
4′-Cl-3-HF	−33.29	843.6	10.76
3-DF	−33.62	1140.2	11.16

$$k_7 = (k_B T/h) \exp(\Delta S^\ddagger/R) \exp(-\Delta H^\ddagger/(RT)) \quad (5)$$

where k_B, h, T, and R are the Boltzmann constant (1.38×10^{-23} J K^{-1}), the Planck constant (6.63×10^{-34} J s), the temperature (K), and the gas constant (1.987 cal deg^{-1} mol^{-1}), respectively. Equation 6 is obtained from eq 5. Therefore,

$$\ln (k_7/T) = \ln (k_B/h) + \Delta S^\ddagger/R - \Delta H^\ddagger/(RT) \quad (6)$$

$\Delta S^\ddagger$ and $\Delta H^\ddagger$ are obtained from a y-axis intercept and the slope of a straight line of ln (k_7/T) plotted vs. $1/T$, respectively. Figure

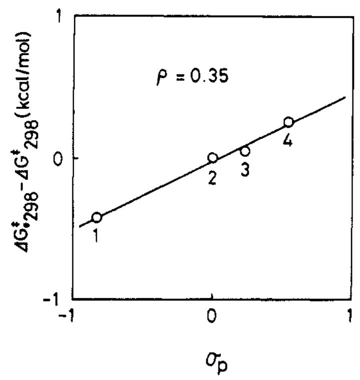


Figure 11. Activation free-energy difference at 298 K, $\Delta G_0{}^\ddagger{}_{298} - \Delta G^\ddagger{}_{298}$, plotted against the para-substituent constant (σ_p) of 3-HF and its derivatives. Here, $\Delta G_0{}^\ddagger$ denotes the activation free energy of 3-HF. 4'-DMA-3-HF, 1; 3-HF, 2; 4'-Cl-3-HF, 3; 4'-CF_3-3-HF, 4.

TABLE V: Para-Substituent Constants and Differences of Activation Free-Energy Change at 298 K between 3-HF and Other Flavonols

compd	$\Delta G_0{}^\ddagger{}_{298K} - \Delta G^\ddagger{}_{298K}$, kcal/mol	σ_p
3-HF	0	0
3-HC	0.19	
2'Me-3-HF	0.03	
PMB-3-HF	-0.33	
4'-DMA-3-HF	-0.43	-0.83
4'-CF_3-3-HF	0.25	0.54
4'-Cl-3-HF	0.04	0.23
3-DF	-0.36	

10 and Table IV exhibit $\Delta S^\ddagger$, $\Delta H^\ddagger$, and $\Delta G^\ddagger{}_{298K}$ at 298 K in MP solutions of all compounds. As shown in Table IV, $\Delta S^\ddagger$ values for all compounds were just the same as one another within experimental error. The fact seems to indicate that the site which has a steric obstacle is away from the reaction sites concerned with the ground-state reverse proton-transfer reaction, i.e., a carbonyl oxygen and a hydroxy group. Since no difference was observed in $\Delta S^\ddagger$ of these compounds for this ground-state reverse proton transfer, Hammett's rule (eq 7) is able to be applied to this reaction[42]

$$\log (K/K_0) = \rho\sigma \qquad (7)$$

where K and K_0 are equilibrium constants of the reaction of substituted and unsubstituted molecules, respectively, ρ is the proportional reaction constant, and σ is the substituent constant. K is related to a free-energy change ΔG as follows:

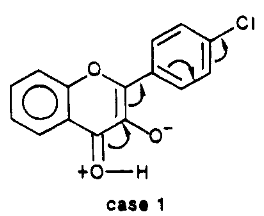
$$\Delta G = -RT \ln K \qquad (8)$$

Then, we obtained an equation (eq 9) of the free-energy relationship by applying eq 7 and 8 to a transition-state theory where

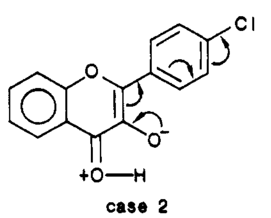
$$\Delta G^\ddagger{}_0 - \Delta G^\ddagger = 2.303RT\rho\sigma \qquad (9)$$

$\Delta G^\ddagger$ and $\Delta G^\ddagger{}_0$ are the free-energy change between a starting material and an activated complex in the reaction of substituted and unsubstituted (3-HF) compounds, respectively.

Table V and Figure 11 show the relationship between $\Delta G^\ddagger{}_0 - \Delta G^\ddagger$ and σ_p (a para-substituted constant) in 298 K. The plots for 3-HF, 4'-DMA-3-HF, 4'-CF_3-3-HF, and 4'-Cl-3-HF show the linear relationship, and the slope was obtained as a positive value ($\rho = 0.35$). The positive value of the reaction constant ρ implies that the electron-attracting effect of substituent at the para-phenyl position assists this reaction.[43] Hence, two mesomeric effects can be considered in the flavonols:



case 1



case 2

If it is the case, case 1 may assist the ground-state reverse proton transfer because of a decrease of electron density on the carbonyl oxygen by an electron-attracting group at the phenyl para position. Therefore, the electron-attracting effect of the substituents of the phenyl ring works predominantly on the carbonyl oxygen rather than the hydroxyl group.

Further, information for the effect of the phenyl ring of 3-HF in comparison with 3-HC can be obtained by use of the Hammett's relationship of this reaction, since $\Delta S^\ddagger$ for 3-HC is the same as that of 3-HF within experimental error as mentioned before. If the lifetime of the ground-state tautomer reflects the rate of the reverse proton transfer to the parent molecule, the rate of the reverse proton transfer at room temperature is greater in 3-HC (1/10.0 μs) than in 3-HF (1/13.9 μs) as seen in Table IV. Furthermore, taking into account the positive value of the reaction constant ρ, the phenyl ring of 3-HF may donate an electron to the γ-pyrone ring because an increase of electron density on the carbonyl oxygen decreases the rate of the reverse proton transfer as mentioned above. For 2'-Me-3-HF and PMB-3-HF, the rate of the reverse proton transfer is decided by competitive contributions of an electron-donating effect of methyl groups and a torsional angle about the C_2–$C_{1'}$ bond.

If any photochemical reaction and any other decay processes other than the reaction to the parent molecule in deaerated solutions do not seem to be involved, the observed deuterium isotope effect ($\Delta H^\ddagger{}_{OH}$ = 848 cal/mol; $\Delta H^\ddagger{}_{OD}$ = 1140 cal/mol) on the decay of the ground-state tautomer may really reflect the reaction rate constant of "tautomer" → "normal". The difference of the activation enthalpy of this reaction between –OH and –OD was estimated to be ca. 300 cal/mol, which is smaller than that of the zero-point energy between O–H and O–D vibrations of the initial state of the ground-state tautomer.[44] This means that an initial O–H bonding (O–D) character in an activated complex of this reaction seems to still remain. It is likely that a proton in this activated complex exists in the middle of the carbonyl and hydroxyl oxygens in the reverse proton transfer.

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Appendix

According to the photokinetic scheme (Figure 5), one can obtain ordinary differential equations for the time-dependent concentrations in the excited-state molecules of flavonols,

$$\frac{d[N_F^*]}{dt} = -(k_8 + k_{10})[N_F^*]$$

$$\frac{d[N^*]}{dt} = k_{10}[N_F^*] - (k_1 + k_2 + k_3)[N^*]$$

$$\frac{d[T_F^*]}{dt} = k_8[N_F^*] - k_{11}[T_F^*]$$

$$\frac{d[T^*]}{dt} = k_{11}[T_F^*] + k_3[N^*] - (k_5 + k_6)[T^*]$$

where N and T show the normal and tautomer forms, respectively. The subscript F predicts that the molecule exists in the Franck–Condon excited state (a torsional form).

Registry No. 3-HF, 577-85-5; 3-HC, 13400-26-5; 2'-Me-3-HF, 95911-70-9; PMB-3-HF, 104197-21-9; 4'-DMA-3-HF, 101442-35-7; 4'-CF_3-3-HF, 104197-22-0; 3-DF, 104197-23-1; 3-HF-CH_3OD, 6665-82-3.