Excited-state Reactions of Coumarins in Aqueous Solutions. I. The Phototautomerization of 7-Hydroxycoumarin and Its Derivative

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The kinetics and mechanism of the reaction in the photo-excited state of 7-hydroxycoumarin and its derivative have been studied in aqueous solutions by means of measuring the fluorescence spectra and the fluorescence lifetime. The four fluorescence bands have been identified as emissions from the neutral, anionic, tautomeric, and cationic forms of 7-hydroxycoumarins. The quantitative analysis was performed by using the rate equation which included the term for the direct conversion of the excited neutral molecule to its tautomer in addition to the usual reaction term of dissociation. The rate constants of the tautomerization and dissociation were discussed in detail, and it was revealed that the tautomerization in aqueous solutions was mainly caused by the transfer of a proton between two active sites in the molecule. Such an intramolecular proton-transfer of the coumarins, found first in this paper, is considered to be promoted by water molecules with a hydrogen bond surrounding the excited molecule.

In recent years there have appeared many experimental works on the fluorescence behavior of solutions in which there had been dissolved various coumarin derivatives, which are used practically as fluorescennt indicators, and as laser-dye solutions.[1-3] The greater part of the experiments have been performed only on the solutions under particular conditions, and most investigators have used rather qualitative methods for the analysis of fluorescence phenomena. In regard to coumarins with a 7-hydroxyl group as a substituent, complex fluorescence spectra have been observed in a variety of organic solvents and in acidic, neutral, and basic aqueous solutions. It has been revealed that, typically, four fluorescent species exist in the photo-excited state according to the solvent conditions; they have been tentatively identified as neutral, anionic, tautomeric, and protonated species of the excited molecule.

Concerning this 7-hydroxy compound, several authors have studied the reaction kinetics of these molecular species using quantitative or semiquantitative methods;[4-7] they have derived some reaction-rate constants from the fluorescence spectra and the fluorescence lifetime. Although the results obtained by the former workers may be valid under restricted solvent conditions, it will be shown later in this article that an important point is missing in their conclusions and that the total fluorescence data cannot be explained without including a new mechanism in the reaction process to generate a phototautomer. Therefore, we wish to report here the detailed fluorescence data which enabled one to analyze quantitatively the reaction mechanism of the photo-excited 7-hydroxycoumarins in an aqueous solution, and to show a generalized method to treat the reaction kinetics over a very wide range of hydrogen-ion concentrations in solution. The way of analysis performed below should also be applicable to various 7-hydroxycoumarin derivatives and essentially to other types of hydroxy- and amino-substituted coumarins.

Experimental

The 7-hydroxycoumarin (**1**) was obtained from Wako Pure Chemical Ind., and the 7-hydroxy-4-methylcoumarin (**2**), from Tokyo Kasei; they were both recrystallized from ethanol and then washed with diethyl ether. The purity was checked by means of differential thermal analysis (DTA), which could rigorously monitor the melting point of the reagents: **1**, mp 233.5 °C; **2**, mp 186.3 °C. Solutions for the measurement of the absorption and fluorescence spectra were prepared by diluting an aqueous solution of a known concentration with distilled water; their pH (or H_0[8]) value was controlled over wide ranges by the use of $HClO_4$ for the pH (or H_0) −4.3—5 and by utilizing the following buffer solutions for the pH 5—11: pH 5—5.8, succinic acid–borax; pH 5.8—9.2, KH_2PO_4–borax; pH 9.2—11, Na_2CO_3–borax. All the chemicals used for the pH control were analytical or spectrograde reagents. The ionic strength of the buffer solutions was suppressed as much as possible within the limit of effective buffer action in order to avoid the influence of various ions on the excited-state reactions of the molecular species being observed. The absorption spectra were obtained on a Hitachi 323 spectrophotometer, and the fluorescence spectra, on a Hitachi MPF 4 fluorescence spectrophotometer with an S-5 type photomultiplier tube. Usually the output response was not corrected for instrumentaf response, since the correction did not affect the analysis ol the reaction kinetics. The lifetimes of the photo-excited state of each molecular species were measured by using a time-correlated single-photon-counting fluorometer similar to the one described in Ref. 9. When a repetitive short-pulsed light at 337 nm from an air-flash lamp was used as the excitation source, a fluorescence lifetime as short as 1 ns could be measured. In order to observe the steady-state fluorescence, the excitation was effected at the isosbestic point of the absorption spectra, *i.e.*, at 337 nm for 7-hydroxycoumarin and at 334 nm for 7-hydroxy-4-methylcoumarin. The concentrations of the fluorescent molecules in the solution were $\approx 6.1 \times 10^{-5}$ mol dm^{-3} for 7-hydroxycoumarin and $\approx 6.3 \times 10^{-5}$ mol dm^{-3} for 7-hydroxy-4-methylcoumarin; these values are most appropriate for the reliable and stable recording of the fluorescence spectra.[10] The pH value of solutions was measured directly with a Radiometer PHM 64 pH meter equipped with a G2401C combined glass electrode. Much care was taken for each measurement run to set the quartz cell containing the sample solution at exactly the same position, thus ensuring the reproducibility of the recorded fluorescence intensity. The temperature of the solution could be controlled by a cell holder with water circulation. Usual spectral measurements were performed at 20 °C otherwise mentioned.

Results and Discussion

pH Dependence of Absorption and Fluorescence Spectra.

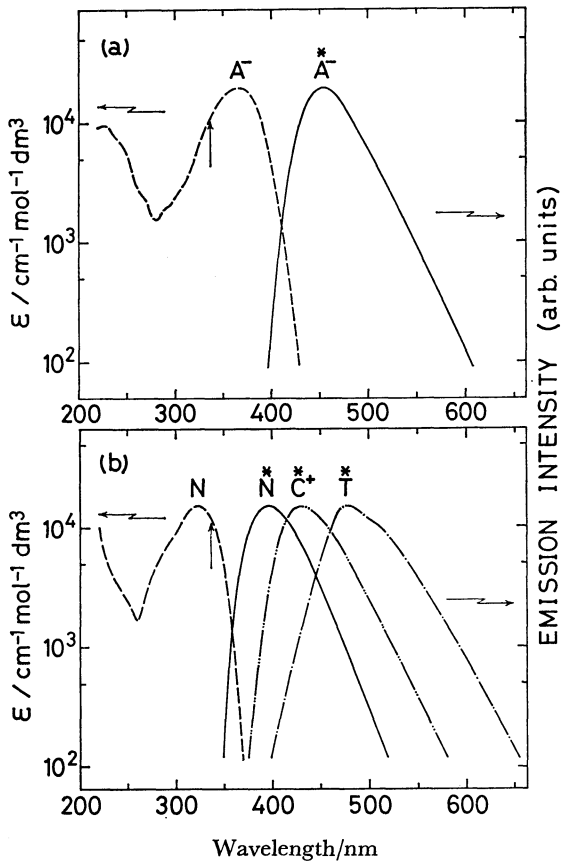
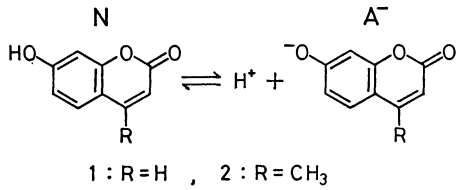


Fig. 1. Absorption and fluorescence spectra of 7-hydroxycoumarin (**1**) in aqueous solutions with various pH values. (a) Spectra at pH$\geqslant$11; (b) spectra at pH$\leqslant$5. A^- and N indicate the absorption spectra, and $\overset{*}{A}^-$, $\overset{*}{N}$, $\overset{*}{T}$, and $\overset{*}{C}^+$ the fluorescence spectra. The fluorescence bands are separated each other by the procedure described in the text and normalized to the same peak intensity. In (b) the fluorescence spectrum of $\overset{*}{A}^-$ is omitted since this is the same as in (a). The little arrow in the figure shows the isosbestic point.

In the electronic ground-state of 7-hydroxycoumarins in aqueous solutions, only two molecular species are spectroscopically detectable. One is a neutral molecule (N), and the other is an ionized molecule (A^-), in which the 7-hydroxyl group is ionized as is shown below:



1 : R=H , 2 : R=CH_3

Figures 1 and 2 show the absorption spectra of 7-hydroxycoumarin (**1**) and 7-hydroxy-4-methylcoumarin (**2**) respectively. The absorption maxima of the neutral and anionic forms of **1** and **2** are 324 nm and 366 nm, and 321 nm and 361 nm, respectively. The pK values of the 7-hydroxyl substituent were 7.75 (**1**) and 7.84 (**2**). From these values, it is readily shown that, in the ground state, almost all molecules

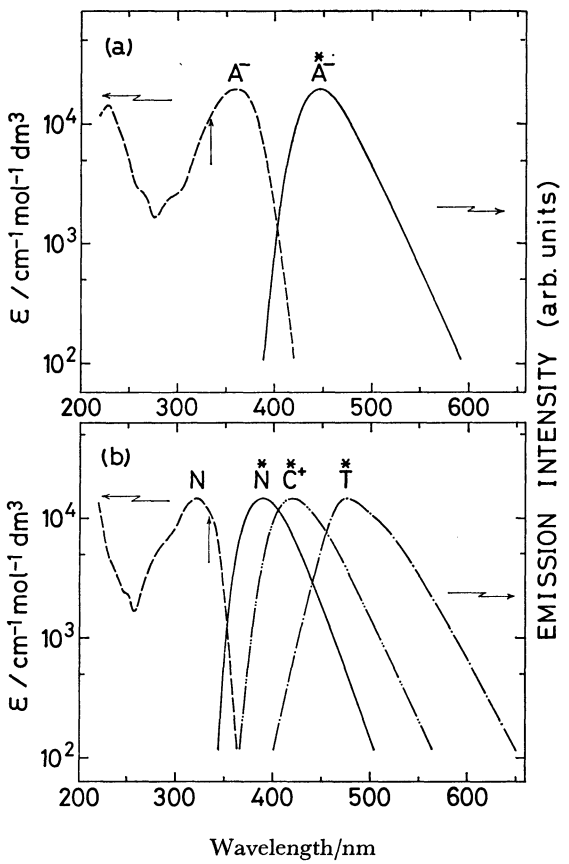


Fig. 2. Absorption and fluorescence spectra of 7-hydroxy-4-methylcoumarin (**2**) in aqueous solutions with various pH values. (a) Spectra at pH$\geqslant$11; (b) spectra at pH$\leqslant$5. The notations are the same as in Fig. 1.

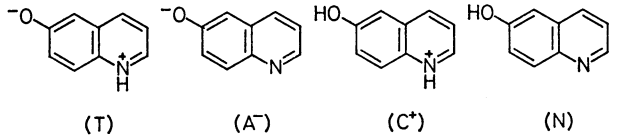
exist as anions at pH$\geqslant$10 and as neutral molecules at pH$\leqslant$6. The isosbestic points of the absorption spectra were 337 nm (**1**) and 334 nm (**2**).

When only the anion was excited at a high pH, the observed fluorescence band was due simply to the anionic form with emission peaks at 453 nm (**1**) and 447 nm (**2**). The interrelation between the corresponding absorption and emission spectra may be read from Figs. 1(a) and 2(a). In contrast to this, when the neutral molecule was excited at a low pH, three kinds of fluorescence bands with emission peaks at about 397 nm, 478 nm, and 431 nm for **1**, and at about 390 nm, 475 nm, and 419 nm for **2**, were observed in addition to the emission from the anion. In many cases, these fluorescence bands overlapped each other to a greater or lesser extent; therefore, we must make an effort to separate each spectral band from the others. In the course of this separation procedure, it is very convenient to plot the emission intensity of the fluorescence spectrum with a logarithmic scale, as is shown in Figs. 1 and 2, because the spectral shape of each band can readily be recognized by shifting the plotted spectral band upward or downward in the graph. At first, each band was roughly separated by the inspection of the graphs obtained for solutions with a variety of pH values; then, the band spectrum was improved by a numerical treat-

ment until a self-consistent relation became realized between every fluorescence band. The emission-band spectra thus separated are shown in Figs. 1 and 2.

From the energy positions of the absorption and emission bands, the fluorescence designated by $\overset{*}{A}^-$ may naturally be thought to originate from an anionic form, and $\overset{*}{N}$, from a neutral form, of the photo-excited molecule. This is supported by the fact that the spectrum of the long-wavelength absorption band of the neutral molecule (N) and the anionic molecule (A^-) coincides very well with a mirror image of their corresponding emission bands, $\overset{*}{N}$ and $\overset{*}{A}^-$, which can easily be read from Figs. 1 and 2. The fluorescence measurement in various solvents also supported this conclusion.[11] When organic solvents without a hydroxyl group, *e.g.*, dioxane, chloroform and acetonitrile, were used, simply an emission band which corresponded to $\overset{*}{N}$ in Figs. 1(b) and 2(b) was observed in the neutral solution; apparently, this originated from the neutral molecule. Further, the luminescence experiment using the method of Beens *et al.*[12] revealed that, on the addition of alkylamines to the solution, a contact ion-pair between –OH and NR_3 as well as an ionized anionic species could exist in the photo-excited state for the solvent without a hydroxyl group, while in basic alcoholic and aqueous solutions only an ionized anionic species was detected because of the higher ionizing ability of such solvents as possessed a hydroxyl group. Therefore, the formerly proposed model of Zinsli,[4] which assumed the existence of a bound ion-pair of the excited molecule in ethanol–water mixtures, is highly improbable.

As for the determination of the origin of the remaining two emission bands, *i.e.*, $\overset{*}{T}$ and $\overset{*}{C}^+$, the case of quinolinols and its analog offers a very similar example which was fully discussed in Refs. 13 and 14. It has been shown that the long-wavelength absorption and fluorescence maxima of quinolinols generally lie at wavelengths, dependent on the ionic species, in the order of: tautomer ($\overset{*}{T}$)>anion ($\overset{*}{A}^-$)>cation ($\overset{*}{C}^+$)>



neutral molecule ($\overset{*}{N}$).[14] This conclusion is derived essentially by the use of the general concept that, in MO calculations, the Coulomb integral and the electronegativity of the conjugated atom are roughly proportional to one another, and that the electronegativity is ordered: oxygen>nitrogen>carbon, and positively charged atom>neutral atom>negatively charged atom. By coupling these facts with the one-electron charge densities in the highest-occupied and lowest-unoccupied π-orbitals of the molecule, we are able to account qualitatively for the relative positions of the absorption and emission maxima of the various ionic species formed in the solution. From the similarity of the fluorescent behavior of the 7-hydroxycoumarins with that of quinolinols, and also from the MO calculations of the tautomeric form of the molecules,[15,16] $\overset{*}{N}$, $\overset{*}{A}^-$, $\overset{*}{C}^+$, and $\overset{*}{T}$, in Figs. 1 and 2 may naturally be identified as fluorescence from the neutral, anionic, cationic, and tautomeric forms of 7-hydroxycoumarins respectively.

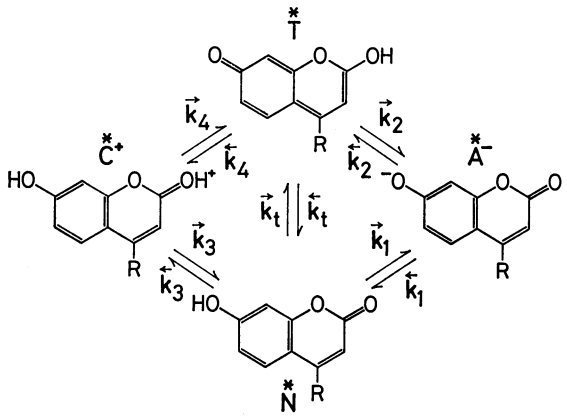


Fig. 3. Reaction scheme of the photo-excited states of 7-hydroxycoumarins. $\overset{*}{N}$, $\overset{*}{A}^-$, $\overset{*}{T}$, and $\overset{*}{C}^+$ represent the excited states of the neutral, anionic, tautomeric and cationic molecular forms, respectively. $\vec{k}$'s and $\overleftarrow{k}$'s are the reaction rate constants between them.

In the most basic solution (pH$\geqslant$11), the anionic form ($\overset{*}{A}^-$) is the only existing species, while in the most acidic solution ($H_0\leqslant -4$), the cationic form ($\overset{*}{C}^+$), probably protonated on the carbonyl oxygen in the molecule, is also generated. For the intermediate pH range, three of the emission bands, *i.e.*, $\overset{*}{N}$, $\overset{*}{A}^-$, and $\overset{*}{T}$, appear simultaneously, and the role of the emission band $\overset{*}{T}$ becomes very important in the reaction kinetics of the excited species, as will be discussed fully below. We concentrated our attention especially on this pH range and took the luminescence data as carefully as possible, because the former workers[4-7] did not notice the coexistence of the $\overset{*}{T}$ band with the $\overset{*}{N}$ and $\overset{*}{A}^-$ bands, which caused much confusion in the interpretation of the phenomenon.

Rate-equation Analysis of Reactions in the Excited State. We have shown in Fig. 3 a generalized total scheme for the excited-state reactions which is different from the previously proposed model in the point that there exist the terms $\vec{k}_t$ and $\overleftarrow{k}_t$ representing a reaction pathway that does not involve a step-by-step protonation or deprotonation process, *i.e.*, a "nondissociative" reaction pathway. $\vec{k}_1$ and $\overleftarrow{k}_1$ are the usual forward and backward reaction rates of the $\overset{*}{N}\rightleftarrows\overset{*}{A}^-+H^+$ dissociative process respectively; k_2—k_4 have the same meaning. As for the region of pH$\leqslant$6, only the neutral molecule exists in the electronic ground-state, as has been mentioned before, so only the neutral molecule can be excited by light absorption in the first instance. Therefore, this excited molecule in the solution is converted in its lifetime into the anionic or cationic species,

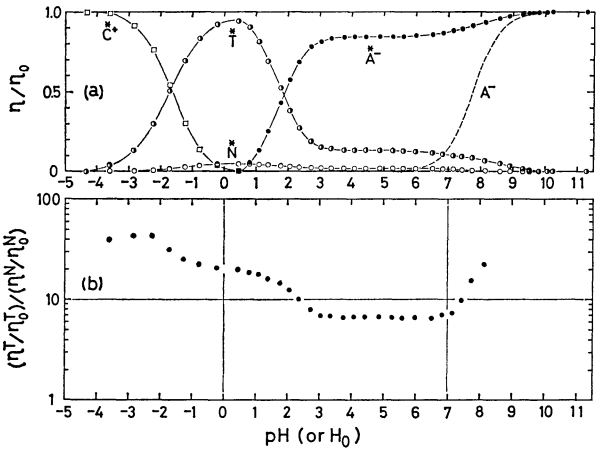


Fig. 4. Relative fluorescence efficiency of the excited species of 7-hydroxycoumarin (**1**) in aqueous solution (6.1×10^{-5} M); $\lambda_{ex}\approx337$ nm; 20 °C. (a) Dependence of the relative efficiency η/η_0 on pH: (○) $\overset{*}{N}$, (●) $\overset{*}{A}^-$, (◑) $\overset{*}{T}$, (□) $\overset{*}{C}^+$. The ground-state equilibration between N and A^- is also indicated by a dashed curve. (b) Dependence of $\overset{*}{K}_t=(\eta^T/\eta_0{}^T)/(\eta^N/\eta_0{}^N)$ on pH.

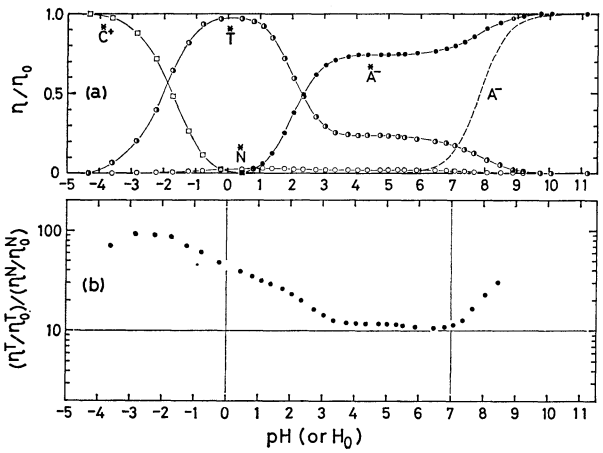


Fig. 5. Relative fluorescence efficiency of the excited species of 7-hydroxy-4-methylcoumarin (**2**) in aqueous solution (6.3×10^{-5} M); $\lambda_{ex}\approx334$ nm; 20 °C. (a) Dependence of the relative efficiency η/η_0 on pH: (○) $\overset{*}{N}$, (●) $\overset{*}{A}^-$, (◑) $\overset{*}{T}$, (□) $\overset{*}{C}^+$. The ground-state equilibration between N and A^- is also indicated by a dashed curve. (b) Dependence of $\overset{*}{K}_t=(\eta^T/\eta_0{}^T)/(\eta^N/\eta_0{}^N)$ on pH.

which are the base and acid common to both the neutral molecule and the tautomer. Because of the low concentration of the substrate and the hydroxide ion, and the weak contribution of water as a proton-donating acid, it is inferred, by considering the mechanism of general acid-base catalytic reactions,[17] that, typically, two reaction pathways, *i.e.*, $\overset{*}{N}\rightleftharpoons\overset{*}{A}^-+H^+\rightleftharpoons\overset{*}{T}$ and $\overset{*}{N}+H^+\rightleftharpoons\overset{*}{C}^+\rightleftharpoons\overset{*}{T}+H^+$, are effective for the tautomerization. In the former reaction process, water participates in practice as a proton-accepting base. In addition to the two above-mentioned processes, we have now proposed a direct nondissociative tautomerization process such as $\overset{*}{N}\rightleftharpoons\overset{*}{T}$. Here, the k_t's represent the rate constants for such a nondissociative intramolecular hopping of a hydrogen ion. The mechanism and structural requirements for this type of proton transfer have been studied by Bensaude *et al.*[17] Although their investigation was performed on phenomena in the ground state of molecules, the situation is very similar to our case; the importance of such terms as $\vec{k}_t$ and $\overleftarrow{k}_t$ can be clearly seen by using a rate-equation analysis as will be shown below.

In order to study the reaction kinetics between the ionic species formed in aqueous solutions with various hydrogen-ion concentrations, it was essential to control precisely the pH value over very wide ranges. Each spectrum measured with a given pH value was separated into the respective emission bands, $\overset{*}{N}$, $\overset{*}{A}^-$, $\overset{*}{C}^+$, and $\overset{*}{T}$; then their emission intensity was represented by the relative emission efficiency, η/η_0, in Figs. 4 and 5 rather than by the emission intensity itself, where η is the quantum efficiency of light emission under a given condition and where η_0 is the efficiency when all the molecules are hypothetically of the same species. This relative efficiency is obtainable directly from the ratio of the actual emission intensity, I, and the maximum emission intensity, I_0, the latter being the fluorescence intensity when all the excited molecules are in the single corresponding species. Assuming that the natural lifetime, τ, of the excited molecule and the maximum efficiency, η_0, are independent of the pH value of the solution, the following equation is satisfied under steady-state excitation over the whole pH range:

$$\frac{\eta^N}{\eta_0^N}+\frac{\eta^A}{\eta_0^A}+\frac{\eta^T}{\eta_0^T}+\frac{\eta^C}{\eta_0^C}=1, \qquad (1)$$

where the superscript of η indicates the respective molecular species. Equation 1 is indispensable for the determination of η^N/η_0^N and η^T/η_0^T, because the $\overset{*}{N}$ and $\overset{*}{T}$ bands do not have a pH range in which only the emission from either $\overset{*}{N}$ or $\overset{*}{T}$ is observed, and therefore I_0^N and I_0^T cannot be known directly. The absolute values of η^N/η_0^N and η^T/η_0^T must, then, be consistently determined so as to satisfy Eq. 1 over the whole pH range. The relative emission efficiencies, η/η_0, for the emissimon bands, $\overset{*}{N}$, $\overset{*}{A}^-$, $\overset{*}{T}$, and $\overset{*}{C}^+$, of **1** and **2** are plotted in Figs. 4(a) and 5(a). Excitation was effected at the isosbestic points of **1** and **2**, which are indicated in Figs. 1 and 2 by small arrows. Consequently, the generation rate of the excited molecule was always the same, regardless of species being excited by light absorption, and of course only the neutral molecule could be photo-excited when pH$\leqslant$6. From an inspection of Figs. 4(a) and 5(a), it may be noticed that between pH$\approx$3 and pH$\approx$6 each η/η_0 value is constant. Further, the $\overset{*}{A}^-$ band vanishes if pH$\leqslant$0; instead, the $\overset{*}{C}^+$ band begins to increase, along with the decrease in the pH value. The contribution of the $\overset{*}{T}$ emission to the relative efficiency is larger than that of the $\overset{*}{N}$ emission in the whole pH range. This was first

found by us because we plotted the data not in terms of the emission intensity, but in terms of the relative efficiency. It is noted that the large contribution to the relative efficiency does not always mean a high emission intensity because of the difference in the quantum efficiency of each molecular species.

After all, the fate of the photo-excited 7-hydroxycoumarins can be thoroughly described by the generalized scheme of Fig. 3. In the case of pH$\leqslant$6, where our main concern resides, illuminating light is absorbed solely by neutral molecules, so the generation rates of the molecular species, $\overset{*}{\mathrm{N}}$, $\overset{*}{\mathrm{A}}^-$, $\overset{*}{\mathrm{T}}$, and $\overset{*}{\mathrm{C}}^+$, in the singlet excited-state are expressed as:

$$\frac{\mathrm{d}[\overset{*}{\mathrm{N}}]}{\mathrm{d}t} = G - (n_1+\vec{k}_1+\overleftarrow{k}_3[\mathrm{H}^+]+\vec{k}_t)[\overset{*}{\mathrm{N}}] + \overleftarrow{k}_1[\mathrm{H}^+][\overset{*}{\mathrm{A}}^-] + \vec{k}_3[\overset{*}{\mathrm{C}}^+] + \overleftarrow{k}_t[\overset{*}{\mathrm{T}}], \quad (2)$$

$$\frac{\mathrm{d}[\overset{*}{\mathrm{A}}^-]}{\mathrm{d}t} = -(n_2+\overleftarrow{k}_1[\mathrm{H}^+]+\overleftarrow{k}_2[\mathrm{H}^+])[\overset{*}{\mathrm{A}}^-]+\vec{k}_1[\overset{*}{\mathrm{N}}]+\vec{k}_2[\overset{*}{\mathrm{T}}], \quad (3)$$

$$\frac{\mathrm{d}[\overset{*}{\mathrm{T}}]}{\mathrm{d}t} = -(n_3+\vec{k}_2+\overleftarrow{k}_4[\mathrm{H}^+]+\overleftarrow{k}_t)[\overset{*}{\mathrm{T}}] + \overleftarrow{k}_2[\mathrm{H}^+][\overset{*}{\mathrm{A}}^-] + \vec{k}_4[\overset{*}{\mathrm{C}}^+] + \vec{k}_t[\overset{*}{\mathrm{N}}], \quad (4)$$

$$\frac{\mathrm{d}[\overset{*}{\mathrm{C}}^+]}{\mathrm{d}t} = -(n_4+\vec{k}_3+\vec{k}_4)[\overset{*}{\mathrm{C}}^+]+\overleftarrow{k}_3[\mathrm{H}^+][\overset{*}{\mathrm{N}}]+\overleftarrow{k}_4[\mathrm{H}^+][\overset{*}{\mathrm{T}}], \quad (5)$$

where G is the conversion rate of the neutral molecule to its excited state by the light absorption, and where n_1—n_4, the rates of the deactivation of the excited molecule, are the reciprocals of the lifetimes of $\overset{*}{\mathrm{N}}$, $\overset{*}{\mathrm{A}}^-$, $\overset{*}{\mathrm{T}}$, and $\overset{*}{\mathrm{C}}^+$ respectively. If the steady-state condition is satisfied, we can get the densities of the excited molecules by letting the left-hand side of Eqs. 2—5 be zero, and the relative emission efficiencies are given as:

$$\frac{\eta^{\mathrm{N}}}{\eta_0^{\mathrm{N}}} = \frac{n_1[\overset{*}{\mathrm{N}}]}{G}, \quad \frac{\eta^{\mathrm{A}}}{\eta_0^{\mathrm{A}}} = \frac{n_2[\overset{*}{\mathrm{A}}^-]}{G}, \quad \frac{\eta^{\mathrm{T}}}{\eta_0^{\mathrm{T}}} = \frac{n_3[\overset{*}{\mathrm{T}}]}{G}, \quad \frac{\eta^{\mathrm{C}}}{\eta_0^{\mathrm{C}}} = \frac{n_4[\overset{*}{\mathrm{C}}^+]}{G}. \quad (6)$$

The dependence of η/η_0 on pH predicted by Eq. 6 can be shown to explain extremely well the results in Figs. 4(a) and 5(a). In the relatively high pH region, *i.e.*, pH 4—6, all terms containing $[\mathrm{H}^+]$ become negligible compared with those that do not. This situation can readily be understood by the following consideration. The values of n_1—n_4 are of the order of $10^8\ \mathrm{s}^{-1}$, judging from the fluorescence lifetimes of the excited molecules, which will be discussed later. When the hydrogen-ion concentration $[\mathrm{H}^+]$ is as low as 10^{-4} mol dm^{-3} or less, the terms of $\overleftarrow{k}_{1-4}[\mathrm{H}^+]$ become negligibly small compared with n_{1-4}, because the values of $\overleftarrow{k}_{1-4}$ never exceed the order of the rate constants estimated from diffusion-controlled processes, which are about 10^9—$10^{10}\ \mathrm{mol}^{-1}\ \mathrm{dm}^3\ \mathrm{s}^{-1}$.[18] The appearance of a flat region in the η/η_0 *vs.* pH curves around pH 5 can be consistently explained by these facts. Now, formally letting $[\mathrm{H}^+]\to 0$ in Eqs. 2—5, the limits of the relative efficiencies in the higher pH region are given as:

$$\frac{\eta^{\mathrm{N}}}{\eta_0^{\mathrm{N}}} \to \frac{n_1(n_3+\overleftarrow{k}_t+\vec{k}_2)}{(n_1+\vec{k}_1)(n_3+\overleftarrow{k}_t+\vec{k}_2)+(n_3+\vec{k}_2)\vec{k}_t}, \quad (7)$$

$$\frac{\eta^{\mathrm{A}}}{\eta_0^{\mathrm{A}}} \to \frac{\vec{k}_1(n_3+\overleftarrow{k}_t+\vec{k}_2)+\vec{k}_2\vec{k}_t}{(n_1+\vec{k}_1)(n_3+\overleftarrow{k}_t+\vec{k}_2)+(n_3+\vec{k}_2)\vec{k}_t}, \quad (8)$$

$$\frac{\eta^{\mathrm{T}}}{\eta_0^{\mathrm{T}}} \to \frac{n_3\vec{k}_t}{(n_1+\vec{k}_1)(n_3+\overleftarrow{k}_t+\vec{k}_2)+(n_3+\vec{k}_2)\vec{k}_t}, \quad (9)$$

$$\frac{\eta^{\mathrm{C}}}{\eta_0^{\mathrm{C}}} \to 0. \quad (10)$$

The experimental values obtained from Figs. 4(a) and 5(a), which correspond to Eqs. 7—9 were, at pH 3.5—5.5, $\eta^{\mathrm{N}}/\eta_0^{\mathrm{N}}$:0.020, $\eta^{\mathrm{A}}/\eta_0^{\mathrm{A}}$:0.847, $\eta^{\mathrm{T}}/\eta_0^{\mathrm{T}}$:0.136 for **1**, and $\eta^{\mathrm{N}}/\eta_0^{\mathrm{N}}$:0.021, $\eta^{\mathrm{A}}/\eta_0^{\mathrm{A}}$:0.742, $\eta^{\mathrm{T}}/\eta_0^{\mathrm{T}}$:0.240 for **2**.

It is convenient here to define $\overset{*}{K}_t$ by:

$$\overset{*}{K}_t = \frac{\eta^{\mathrm{T}}/\eta_0^{\mathrm{T}}}{\eta^{\mathrm{N}}/\eta_0^{\mathrm{N}}} = \frac{n_3[\overset{*}{\mathrm{T}}]}{n_1[\overset{*}{\mathrm{N}}]}, \quad (11)$$

which is proportional to the ratio of the concentration of $\overset{*}{\mathrm{T}}$ to the concentration of $\overset{*}{\mathrm{N}}$. Figures 4(b) and 5(b) show the dependence of $\overset{*}{K}_t$ on the pH value. The theoretical expression obtained from Eq. 11 can elucidate fairly well the behavior of $\overset{*}{K}_t$ over a wide pH range. If the hydrogen-ion concentration is so small that the terms containing $[\mathrm{H}^+]$ are negligible in the expression, as before, then Eqs. 7 and 9 give:

$$\overset{*}{K}_t \to \frac{n_3}{n_1}\frac{\vec{k}_t}{n_3+\overleftarrow{k}_t+\vec{k}_2}. \quad (12)$$

The corresponding values of **1** and **2** were, at pH 3.5—5.5, 6 7, and 11.7 respectively.

In the excited state, the protonated carbonyl group of the cation shown in Fig. 3 is a very strong acid comparable with the oxonium ion, $\mathrm{H_3O^+}$ ($\mathrm{p}K_{\mathrm{H_3O^+}} \approx -1.7$), while, in spite of the drastic decrease in the pK of the hydroxyl substituent of the neutral molecule, $\overset{*}{\mathrm{N}}$, with excitation, $\mathrm{p}\overset{*}{K}_{\mathrm{N}}$ remains positive, as will be mentioned later; therefore, the $\mathrm{p}\overset{*}{K}_{\mathrm{C}^+} < \mathrm{p}\overset{*}{K}_{\mathrm{N}}$ relation is satisfied. Combining this inference with the fact that the rate constants for the reaction between charged species with an attractive force, such as $\overleftarrow{k}_1$ and $\overleftarrow{k}_2$, are larger than those without an attractive force, such as $\overleftarrow{k}_3$ and $\overleftarrow{k}_4$,[10] it is naturally assumed that $\overleftarrow{k}_1$ and $\overleftarrow{k}_2$ are much larger than $\overleftarrow{k}_3$ and $\overleftarrow{k}_4$. In the case of Compounds **1** and **2**, as the pH was decreased toward zero, there occurred a pH region around pH 0 where the terms of $\overleftarrow{k}_1[\mathrm{H}^+]$ and $\overleftarrow{k}_2[\mathrm{H}^+]$ become very large, but the terms of $\overleftarrow{k}_3[\mathrm{H}^+]$ and $\overleftarrow{k}_4[\mathrm{H}^+]$ are still negligible compared with the other rate constants. The limiting value of $\overset{*}{K}_t$ calculated from Eq. 11 is written:

$$\overset{*}{K}_t \approx \frac{n_3}{n_1}\frac{\vec{k}_t+\vec{k}_1\overleftarrow{k}_2/(\overleftarrow{k}_1+\overleftarrow{k}_2)}{n_3+\overleftarrow{k}_t+\vec{k}_2\overleftarrow{k}_1/(\overleftarrow{k}_1+\overleftarrow{k}_2)}. \quad (13)$$

The experimental values of $\overset{*}{K}_t$ around pH 0 were ≈20 for **1** and ≈40 for **2**.

When the solution becomes most acidic, such as when $H_0 \leqslant -3$, the effective concentration of the hydrogen-ion [H^+] is formally taken to be more than 10^3 mol dm^{-3},[8] so the terms of $\overleftarrow{k}_{1-4}[H^+]$ dominate all the other rate constants. Therefore, within such a limit, we have:

$$\overset{*}{K}_t \to \frac{n_3}{n_1} \frac{\overleftarrow{k}_3}{n_4+\overrightarrow{k}_3} \frac{\overrightarrow{k}_4}{\overleftarrow{k}_4}. \quad (14)$$

The experimental values of **1** and **2** corresponding to this condition were, for $H_0 \leqslant -3$, ≈43, and ≈92 respectively.

If the reaction pathway represented by $\overrightarrow{k}_t$ and $\overleftarrow{k}_t$ does not exist, the limiting value of $\overset{*}{K}_t$ in the high pH region apparently becomes zero, as $\overrightarrow{k}_t \to 0$ in Eq. 12. This contradicts the experimental fact that there is in reality no pH region having a zero value of $\overset{*}{K}_t$ as may be seen in Figs. 4(b) and 5(b). Therefore, we must admit the existence of a tautomerization mechanism unrelated to the general acid-base catalyzed scheme. Further, it may be noted that, by comparing Eq. 12 with Eq. 13, the limiting value of $\overset{*}{K}_t$ in the lower pH region can be predicted theoretically to be larger than that in the higher pH region, as is again clearly shown in the results (Figs. 4(b) and 5(b)) between pH 0 and 6. Corresponding to this change in $\overset{*}{K}_t$, η^T/η^T_0 as a function pH has its maximum at about pH 0 and a flat region between pH 3 and 6. Around pH 0, the contribution from η^A/η^A_0, like as that from η^C/η^C_0, becomes nearly zero; on the contrary, the contribution from η^T/η^T_0 becomes nearly unity, although that from η^N/η^N_0 still small. Toward the highest-concentration region of the hydrogen-ion, η^C/η^C_0 increases and reaches unity at the end, since the terms of $\overleftarrow{k}_3[H^+]$ and $\overleftarrow{k}_4[H^+]$ overcome all other terms. Parallel with the change in η^C/η^C_0, $\overset{*}{K}_t$ increases when pH (or H_0) decreases from 0 to −3. This increase is rather accidental, as there is no relation in magnitude between the values of Eqs. 13 and 14. In contrast to these results, the rise in $\overset{*}{K}_t$ and the approach of η^A/η^A_0 to unity toward a pH higher than 6 is caused by another situation, because the amount of the anionic molecule in the ground state becomes large at pH>6; therefore, the assumption used above—that only the neutral molecule is excited from the ground state—must be changed. Although this modification is simple and straightforward, we would not here be concerned with this matter further.

Mechanism for Tautomerization. In earlier studies[4-7] only the dissociative processes such as $\overset{*}{N} \rightleftarrows \overset{*}{A}^- + H^+ \rightleftarrows \overset{*}{T}$ were emphasized for the tautomeric interconversion of the excited molecule; however, this led to erroneous conclusions about the reaction mechanism in the case of 7-hydroxycoumarins. From a careful consideration of $\overset{*}{K}_t$ as a function of the pH, we have come to the conclusion that the direct conversion between the neutral molecule and the tautomeric molecule which proceeds without generating dissociated ionic species as an intermediate, *i.e.*, $\overset{*}{N} \rightleftarrows \overset{*}{T}$, is most essential in the tautomerization reaction. In view of the results of the investigation of Bensaude *et al.*,[17] the most probable model for the origin of the nondissociative process represented by the $\overrightarrow{k}_t$ and $\overleftarrow{k}_t$ terms is a concerted bifunctional proton-transfer through water molecules, as is described schematically in Fig. 6. The existence of such a tautomerization process is strongly supported by the following discussion. In general, substituents to aromatic and heteroaromatic compounds can be classified in two types of groups.[18] One is composed of substituents that become more acidic in the excited state than in the ground state, such as hydroxyl and amino groups. The other is composed of substituents that become more basic in the excited state, such as carbonyl and carboxyl groups. In the case of 7-hydroxycoumarins, this tendency makes the transfer of a proton from the hydroxyl group to the carbonyl group very easy in their electronic excited-state. Because of the ability of water to form chains of hydrogen bonds and the Grotthus-type migration of the proton (or proton-hole) along the chain,[19] the proton transfer between the two active sites in a molecule can be promoted cooperatively and the interconversion of the neutral form to the tautomeric form can occur without liberating any proton or ionic species to an outside region of the molecule which is hydrated by participating water molecules. Because this type of proton jump in the molecule is considered to proceed at an extremely high velocity in comparison with other reaction rates, no intermediate species can appear in the rate-equation formulation; instead, the rate constants which represent the direct conversion between the neutral molecule and the tautomeric molecule, *i.e.*, $\overrightarrow{k}_t$ and $\overleftarrow{k}_t$, appear.

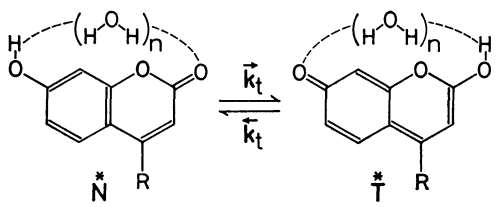


Fig. 6. Concerted mechanism for the tautomerization of 7-hydroxycoumarins in the excited state.

Such a mechanism of tautomerization is also expected for lower alcohols, but the effect will be weaker in that case because the hydrogen bond is much weaker than that of water and the motion of the proton is highly restricted. It has been pointed out by several authors[1-5,7] that the addition of a small amount of water to alcoholic solutions of 7-hydroxycoumarins induced a very large effect on the fluorescent behavior of the excited molecule. As it has become clear that the tautomerization of 7-hydroxycoumarins proceeds through the proton transfer between two sites in the molecule and is accelerated by the participation of water molecules, the reported increase in the ratio of the emission intensity of $\overset{*}{T}$ to that of $\overset{*}{N}$ with an

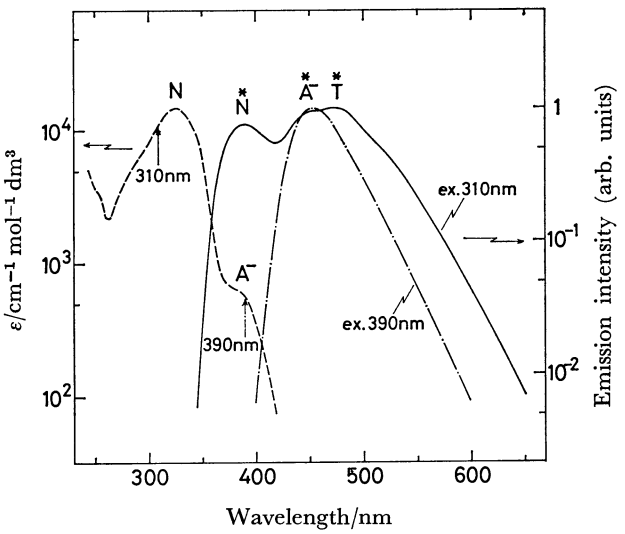
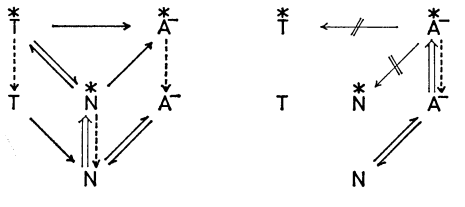


Fig. 7. Absorption and fluorescence spectra of 7-hydroxycoumarin (**1**) in ethanol–water solution (90:10 vol%).
The fluorescence spectra are obtained by using the excitation wavelength at 310 nm (——) and 390 nm (—·—).

increased water content[4,7] is considered to arise from the increase in the value of $\vec{k}_t$, not from the increased rate of the $\overset{*}{N} \rightleftharpoons \overset{*}{A}^- + H \rightleftharpoons \overset{*}{T}$ reaction. This prediction is distinctly proved by the result shown in Fig. 7. From the absorption spectrum of 7-hydroxycoumarin (**1**) it was found, in neutral solutions of ethanol–water mixtures, that both the neutral and the anionic molecule existed in the ground state, as may be seen from Fig. 7. By choosing an appropriate excitation wavelength, it was possible to excite separately either the neutral or the anionic molecule. If the neutral molecule alone was excited by using the wavelength of 310 nm, three of the fluorescence bands, *i.e.*, $\overset{*}{N}$, $\overset{*}{A}^-$, and $\overset{*}{T}$, were obtained simultaneously. On the other hand, only the fluorescence band of $\overset{*}{A}^-$ was detectable if the anionic molecule was excited by using the wavelength of 390 nm. The results are summarized in the following scheme:



As may easily be recognized from this scheme, the reaction pathway $\overset{*}{N} \rightarrow \overset{*}{A}^- + H^+ \rightarrow \overset{*}{T}$ cannot exist in the ethanol–water solution because the $\overset{*}{A}^- + H^+ \rightarrow \overset{*}{T}$ reaction is forbidden in reality and the $\overset{*}{N} \rightarrow \overset{*}{T}$ pathway alone can generate the tautomeric form of the molecule. The same conclusion was obtained for 7-hydroxy-4-methylcoumarin (**2**).

If the dependence of the rate constants on the water concentration in alcohol–water solutions is approximately written:

$$\vec{k}_t = \vec{k}_t'[H_2O], \quad \overleftarrow{k}_t = \overleftarrow{k}_t'[H_2O], \qquad \vec{k}_1 = \vec{k}_1'[H_2O], \quad \vec{k}_2 = \vec{k}_2'[H_2O], \tag{15}$$

then, at a low hydrogen-ion concentration (pH 3—6), the ratios of $[\overset{*}{A}^-]$ and $[\overset{*}{T}]$ to $[\overset{*}{N}]$ are given by Eqs. 7—9 as:

$$\frac{n_2[\overset{*}{A}^-]}{n_1[\overset{*}{N}]} \propto \frac{\vec{k}_1'[H_2O](n_3 + \overleftarrow{k}_t'[H_2O] + \vec{k}_2'[H_2O]) + \vec{k}_2'[H_2O]\vec{k}_t'[H_2O]}{n_1(n_3 + \overleftarrow{k}_t'[H_2O] + \vec{k}_2'[H_2O])}, \tag{16}$$

$$\overset{*}{K}_t = \frac{n_3[\overset{*}{T}]}{n_1[\overset{*}{N}]} \propto \frac{n_3\vec{k}_t'[H_2O]}{n_1(n_3 + \overleftarrow{k}_t'[H_2O] + \vec{k}_2'[H_2O])}. \tag{17}$$

In the low-concentration region of the water content where $n_3 \gg \overleftarrow{k}_t'[H_2O]$, $\vec{k}_2'[H_2O]$, $[\overset{*}{T}]/[\overset{*}{N}]$ varies linearly with the water concentration, while $[\overset{*}{A}^-]/[\overset{*}{N}]$ increases superlinearly with the increase in the water component, since the numerator of Eq. 16 has a quadratic term of $[H_2O]$. These predictions explain reasonably well the fluorescence behavior of **1** and **2** in alcohol–water solutions, including the results in Refs. 4 and 7. In this manner, the dependence of η/η_0 and $\overset{*}{K}_t$ on the pH was completely described by the formulation of Eqs. 2—5. Above all, it was impossible to explain the behavior of $\overset{*}{K}_t$ if the terms of $\vec{k}_t$ and $\overleftarrow{k}_t$ were not introduced. Further, the dependence of the relative intensity of $\overset{*}{N}$, $\overset{*}{A}^-$, and $\overset{*}{T}$ in aqueous alcoholic solutions on the water content can be understood in terms of Eqs. 16 and 17 if we admit the assumption of Eq. 15.

Fluorescence Lifetimes and Energy-level Considerations. In order to argue about the rate constants that appear in Eqs. 7—14, it is necessary to know the lifetime, τ, which is the reciprocal of the deactivation rate of the excited molecule, n. Table 1 shows the values of the lifetime measured under various solvent conditions at the wavelength of the emission peak of each molecular species. In the case of the anionic and cationic molecular forms, the lifetime measurement was performed when the luminescence from a single molecular species was observable, and so the measured lifetimes τ_A and τ_C naturally correspond to the true lifetimes of the excited state of the anionic and cationic molecular species. On the other hand, $\bar{\tau}_{TN}$ and $\bar{\tau}_{NT}$ indicate the apparent decay times of the fluorescence from $\overset{*}{T}$ and $\overset{*}{N}$ respectively when they coexist at around pH 0. $\bar{\tau}_{NAT}$ is also the apparent decay time of the excited neutral molecule when it coexists with the anionic and tautomeric forms at around pH 4. In the first-order approximation, they are written:

$$\frac{1}{\bar{\tau}_{NT}} = n_1 + \vec{k}_t, \tag{18}$$

Table 1. Lifetimes of the fluorescent molecular species

Substance	τ_C/ns	τ_A/ns	$\bar{\tau}_{TN}$/ns[a]	$\bar{\tau}_{NT}$/ns[a]	$\bar{\tau}_{NAT}$/ns[a]
1	5.8	5.4	4.6	4.5	1.7
2	5.9	5.5	4.9	3.2	1.6

a) The meanings of $\bar{\tau}_{TN}$, $\bar{\tau}_{NT}$, and $\bar{\tau}_{NAT}$ are described in the text.

Table 2. Separations of the energy levels and changes in the thermodynamical functions

Substance	ΔE_N [a] / kcal mol^{-1}	ΔE_A [a] / kcal mol^{-1}	ΔE_T [b] / kcal mol^{-1}	ΔE_C [b] / kcal mol^{-1}	ΔH_{NA} / kcal mol^{-1}	ΔS_{NA} / cal mol^{-1} K^{-1}
1	80.11	70.60	67.61	74.13	4.29	-21.0_4
2	81.17	71.56	67.93	75.97	4.16	-21.8_1

a) Obtained from the absorption and fluorescence data, and accurate within ± 0.1 kcal mol^{-1}. b) Inferred from the fluorescence data, and accurate within ± 0.5 kcal mol^{-1}.

$$\frac{1}{\bar{\tau}_{TN}} = n_3 + \overleftarrow{k}_t, \tag{19}$$

$$\frac{1}{\bar{\tau}_{NAT}} = n_1 + \vec{k}_t + \vec{k}_1. \tag{20}$$

By the use of Eqs. 18 and 20 and the values in Table 1, it can readily be known that $\vec{k}_1$ is 3.7×10^8 s^{-1} for **1** and 3.1×10^8 s^{-1} for **2**. The dissociation constant, $p\overset{*}{K}_{NA}$, corresponding formally to the excited-state equilibrium between $\overset{*}{N}$ and $\overset{*}{A}^-$ can be calculated by the method outlined by Weller as:[18]

$$p\overset{*}{K}_{NA} = pK_{NA} - \frac{\Delta E_N - \Delta E_A}{2.303RT}, \tag{21}$$

where ΔE_N and ΔE_A are the energy-level separations between the singlet ground-state and the singlet excited-state of the neutral and anionic species and are obtainable as the mean of the energies at the absorption-band and emission-band maxima.[20] These values are listed in Table 2, together with ΔE_T and ΔE_C, the energy-level separations for the tautomeric and cationic species, which in this case are inferred from only the emission-band maximum assuming that the Stokes shift is the same for all molecular species. Using the values of ΔE_N and ΔE_A in Table 2, and also pK_{NA}, $p\overset{*}{K}_{NA}$ is calculated to be 0.66 for **1** and 0.68 for **2** at 20 °C. Since the equilibrium constant in the excited state is defined by $\overset{*}{K}_{NA}=\vec{k}_1/\overleftarrow{k}_1$, we get $\overleftarrow{k}_1$ of **1** and **2** as 1.7×10^9 s^{-1} mol^{-1} dm^3 and 1.5×10^9 s^{-1} mol^{-1} dm^3 respectively.

ΔH_{NA} and ΔS_{NA} in Table 2 are the enthalpy and entropy changes in the $N \rightleftharpoons A^- + H^+$ reaction; they were obtained by the measurement of the temperature dependence of pK_{NA} with the aid of the thermodynamical relation: $K=\exp[-(\Delta H - T\Delta S)/RT]$. If we can neglect the change in the PV term of the relation, $H=E+PV$, the enthalpy change, ΔH, is equated to the energy change, ΔE. Considering these facts and the values in Table 2, the energy-level scheme of the reacting molecular species of 7-hydroxycoumarins around pH 3—5 can roughly be drawn as Fig. 8. Since $\Delta E_N + \Delta\overset{*}{H}_{NA} = \Delta E_A + \Delta H_{NA}$, the values of $\Delta\overset{*}{H}_{NA}$ for the $\overset{*}{N} \rightleftharpoons \overset{*}{A}^- + H^+$ reaction are -5.22 kcal mol^{-1} for **1** and -5.45 kcal mol^{-1} for **2**. Putting together

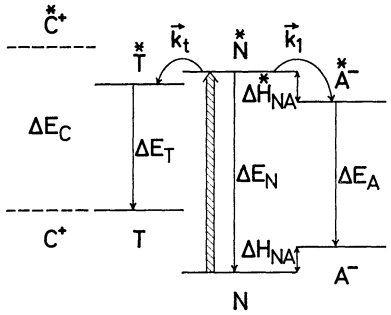


Fig. 8. Energy level diagram of 7-hydroxycoumarins in the weakly acidic solution (pH 3—5). The hatched arrow shows the photo-excitation of N into $\overset{*}{N}$. The levels of C^+ indicated by dashed lines do not take part in the reaction in this pH range.

all the results discussed so far, the interrelation of the energy levels between the neutral and anionic molecules was firmly determined, as is shown in Fig. 8. Although the energy position of the tautomer and the cation relative to that of the neutral molecule could not determined strictly, the energy levels are presumably ordered as $N<A^-\ll C^+$, T in the ground state and as $\overset{*}{A}^-<\overset{*}{T}<\overset{*}{N}<\overset{*}{C}^+$ in the excited state.

Unfortunately, several rate constants defined in Fig. 3 have left unknown because of a lack of information about the reaction process. The main reason for this uncertainty must be sought in the impossibility of transforming the neutral molecule into the tautomeric form in the ground state under any condition of solvents different from the case of the 7-hydroxyquinolines. Therefore, we cannot know about the ground-state equilibration between the neutral and tautomeric molecules, and the direct excitation of the tautomer by light absorption is never possible. In spite of this unfavorable situation, though we are now trying to analyze the decay properties of the excited species over the whole pH range which is controllable. This procedure, which will be described thoroughly in a forthcoming paper, would provide more detailed information about the rate constant and the reaction mechanism.

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