

PROTOLYTIC REACTIONS OF ACRIDINE IN THE TRIPLET STATE

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Summary

The triplet-triplet (T-T) absorption spectrum of the acridinium ion ($\lambda_{\max} = 490$ nm) was measured in acidic aqueous solution at pH 1.0 by means of the triplet energy transfer from the disodium salt of naphthalene disulphonic acid to the acridinium ion. The triplet acridinium ion decays by a first order process with a rate constant of $1.5 \times 10^8 \text{ s}^{-1}$ at a pH below about 3.8. The pK_a of acridine in the triplet state was determined to be 5.6 at 23 °C from the measurement of T-T absorption spectra of both the acridine molecule and the acridinium ion. The rate constants for protonation and deprotonation in the triplet state were determined to be $(3.4 - 3.8) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and $(8.5 - 9.5) \times 10^4 \text{ s}^{-1}$ respectively.

1. Introduction

The pK_a of acridine in the triplet state has been reported to be 5.6 by Jackson and Porter [1]. Since an acidic solution of acridine does not show any transient absorption and an alkaline solution shows a strong triplet-triplet (T-T) absorption ($\lambda_{\max} = 443$ nm) with an ordinary flash photolysis, they determined the pK_a from the pH dependence of the relative concentration of the acridine molecule (A) in the triplet state, assuming that the acridinium ion (AH⁺) has no observable triplet absorption spectrum. However, there are three possible reasons why the T-T absorption of AH⁺ has not been observed: (1) the triplet yield is not low and the molar extinction coefficient of the T-T absorption is small; (2) the triplet yield is low and the molar extinction coefficient is not small; (3) the triplet yield is low and the molar extinction coefficient is small.

If the fact that an acidic solution of acridine does not give a T-T absorption is caused by the low triplet yield of AH⁺, then the pK_a determined from the T-T absorption of A alone may reflect the pK_a (5.5) of the ground state [1]. Therefore the T-T absorption spectrum of AH⁺ should be measured in order to determine the true pK_a of the triplet state.

In this work the acid-base equilibrium as well as protolytic reactions in the triplet state were investigated by taking account of the T-T absorption spectrum of the acridinium ion.

2. Experimental

Acridine (CP grade, Tokyo Kasei) was recrystallized from a water-ethanol mixture after pretreatment with activated charcoal in ethanol. The disodium salt of 1,5-naphthalene disulphonic acid (EP grade, Tokyo Kasei) was recrystallized twice from a water-ethanol mixture. Proflavine sulphate (Tokyo Kasei) was treated with a 10 N aqueous ammonia solution. The precipitate of proflavine base was recrystallized three times from ethanol.

Absorption spectra were recorded on an Hitachi EPS-3T spectrophotometer. The flash energy was usually about 130 J and the full width at half-maximum (FWHM) was about 10 μ s. A Toshiba UV-D25 bandpass filter and a solution filter (an aqueous solution of 0.27 g of K_2CrO_4 and 1 g of Na_2CO_3 in 1 dm^3) were used for the selective excitation of 1,5-naphthalene disulphonate unless otherwise stated. The transmittance is shown in Fig. 1(d). The rate of the protonation reaction was measured using a frequency-doubled Q-switched ruby laser of 15 ns FWHM.

All sample solutions were prepared using a $CH_3COOH-CH_3COONa$ buffer solution. The pH of the solution was measured with a Toa HM-18B pH meter when necessary. The solutions were degassed by freeze-pump-thaw cycles. All measurements were made at 23 $^{\circ}C$.

3. Results and discussion

The absorption spectra of A, AH^+ and 1,5-naphthalene disulphonate (D) are shown in Fig. 1. When the concentration of D is above 1 mM and that of acridine is below 30 μ M, the use of the filter system whose transmittance is shown in Fig. 1(d) allows D to be excited selectively. It is known that D is a water-soluble triplet sensitizer [2].

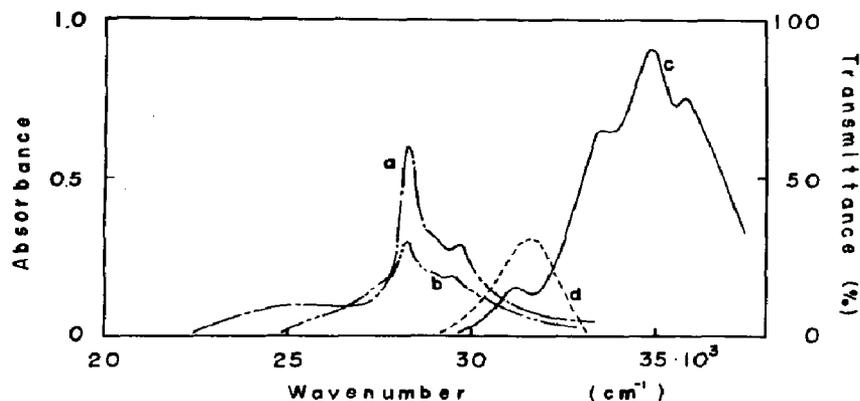


Fig. 1. Absorption spectra of (curve a) AH^+ (30 μ M) at pH 1.51, (curve b) A (30 μ M) at pH 12.1 and (curve c) D (10^{-4} M) in aqueous solution. Curve d, the transmittance of the filter system used.

Flashing of the basic solution ($\text{pH} > 8$) containing D (1 mM) and A ($30 \mu\text{M}$) gives the transient absorption spectrum shown in Fig. 2 (solid curve), which is attributed to the T-T absorption spectrum of A because of its close resemblance to the spectrum reported previously [3]. The apparent first order decay constant k_{dt} depends on both $[A]$ and $[^3A]$:

$$k_{dt} = k_0 + k_1[A] + k_{TT}[^3A]$$

The values $k_0 = (2 - 3) \times 10^3 \text{ s}^{-1}$, $k_1 = (4.5 - 6.0) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{TT} = (2 - 3) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ were obtained.

Flashing of the acidic solution ($\text{pH} < 3$) containing D (1 mM) and AH^+ ($30 \mu\text{M}$) gives the transient absorption spectrum shown in Fig. 2 (broken curve). The decay is first order and the rate constant is $1.5 \times 10^3 \text{ s}^{-1}$ irrespective of the concentration of AH^+ . The decay rate increases with the addition of methylene blue as a triplet energy acceptor. The rate constant of the triplet energy transfer from AH^+ to methylene blue was determined to be $3.5 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$. The lowest triplet level of methylene blue (11650 cm^{-1} [4]) is lower than that of AH^+ (16100 cm^{-1}) which was determined by measuring the singlet-triplet absorption spectrum [5]. Therefore the spectrum given by the broken curve in Fig. 2 is attributed to the T-T absorption spectrum of AH^+ .

The T-T absorption spectra immediately after flashing of the solution containing D (1 mM) and acridine ($30 \mu\text{M}$) at various pH values are shown in Fig. 3. An isosbestic point is observed at about 470 nm. It should be noted

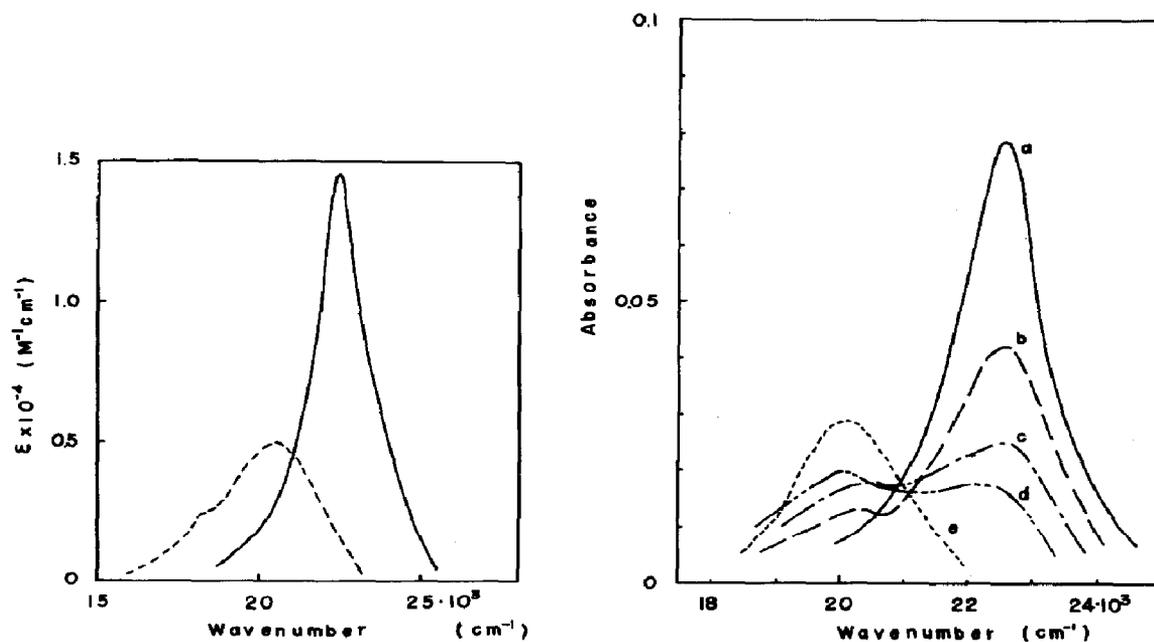


Fig. 2. The T-T absorption spectra of (—) A and (---) AH^+ .

Fig. 3. The T-T absorption spectra at various pH values: curve a, 10.86; curve b, 5.85; curve c, 5.00; curve d, 4.70; curve e, 1.00.

that the total concentration of the two kinds of triplet acridine ${}^3\text{A}$ and ${}^3\text{AH}^+$ immediately after flashing is constant and is equal to the triplet concentration of D produced by one flash. The spectrum shape at any pH value does not change throughout its decay. Therefore the acid–base equilibrium is established immediately after flashing in the time scale of the microsecond flash method.

In order to determine the pKa of acridine in the triplet state, the results shown in Fig. 3 were analysed using the following equation:

$$\frac{C_a}{C_b} = \frac{\epsilon_b(\lambda_1) \epsilon_b(\lambda_2)/\epsilon_b(\lambda_1) - A(\lambda_2)/A(\lambda_1)}{\epsilon_a(\lambda_1) A(\lambda_2)/A(\lambda_1) - \epsilon_a(\lambda_2)/\epsilon_a(\lambda_1)} \quad (1)$$

where $\epsilon_a(\lambda)$ and $\epsilon_b(\lambda)$ are the molar extinction coefficients of ${}^3\text{AH}^+$ and ${}^3\text{A}$ at wavelength λ respectively and $A(\lambda)$ is the absorbance at λ . Since ${}^3\text{AH}^+$ and ${}^3\text{A}$ have their absorption maxima at 490 and 443 nm respectively, we chose $\lambda_1 = 490$ nm and $\lambda_2 = 443$ nm. From Fig. 2 $\epsilon_a(\lambda_2)/\epsilon_a(\lambda_1)$ and $\epsilon_b(\lambda_2)/\epsilon_b(\lambda_1)$ were determined to be 0.21 and 8.4₃ respectively. The values $\epsilon_a(490) = 5.5 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ and $\epsilon_b(443) = 1.4_5 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ were determined using a triplet energy transfer method [6]. D was used as an energy donor. Proflavine (P^+) was used as an energy-accepting standard, because the use of the filter system whose transmittance is shown in Fig. 1(d) prevented the direct excitation of P^+ . We determined the molar extinction coefficient of the T–T absorption of P^+ ($\epsilon_p = 5.7 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ at 549 nm) in an aqueous solution of pH 4.1 using a light saturation method [7]. The absorbance of triplet proflavine (${}^3\text{P}^+$) immediately after flashing of the solution containing D (1 mM) and P^+ (30 μM) is 0.027 at 549 nm. The corresponding absorbance of ${}^3\text{A}$ is 0.069 at 443 nm. The same concentration of ${}^3\text{A}$ as that of ${}^3\text{P}^+$ will be produced under the condition of complete energy transfer when the quantity of photons absorbed by D is kept constant. From the known value of ϵ_p we obtain the triplet concentration and consequently the ϵ_b value just mentioned. The same method can be used to obtain ϵ_a . The value of $\epsilon_b(443)$ obtained is smaller than that, $\epsilon_b(\lambda_{\text{max}}) = 1.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$, reported by Kellmann [7]. A plot of $C_b/(C_a + C_b)$ versus pH is shown in Fig. 4, from which the pKa of acridine in the triplet state is determined to be 5.6 using no assumptions. This value is very close to that of the ground state and agrees with that of Jackson and Porter [1].

The triplet yield of AH^+ is so low that ${}^3\text{A}$ alone is produced by flashing. When the solution of pH 5.5 with no sensitizer, when $[\text{A}] = [\text{AH}^+]$, is irradiated by a very short-lived light pulse such as a laser flash, the T–T absorption observed at the end of the pulse is entirely due to ${}^3\text{A}$, *i.e.* the acid–base equilibrium in the triplet state is not established. Because the pKa in the triplet state is 5.6, the concentration ratio of ${}^3\text{AH}^+$ to ${}^3\text{A}$ at equilibrium is 1.259 at pH 5.5. Therefore, 55.7% of the ${}^3\text{A}$ produced by flashing the solution of pH 5.5 is protonated towards the equilibrium. Since $\epsilon_b(443)$ is greater than $\epsilon_a(443)$, it is expected that the protonation of ${}^3\text{A}$ occurs directly at 443 nm when the solution at about pH 5.5 is irradiated by a laser flash.

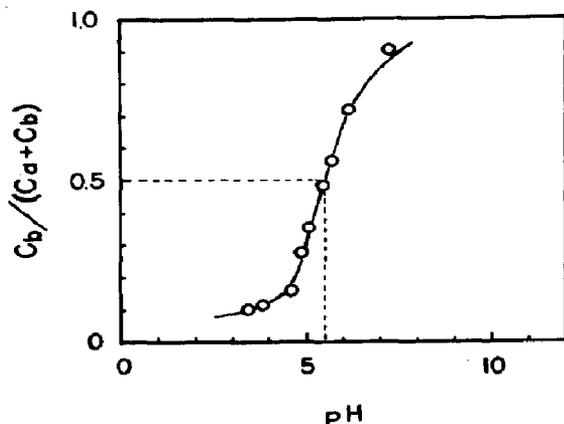
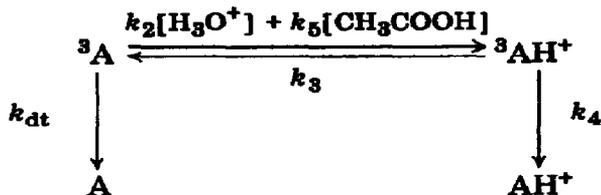


Fig. 4. A plot of $C_b/(C_a + C_b)$ vs. pH.

As well as the protonation of 3A by hydrogen ions, we must consider the protonation due to the acid catalytic reaction of the acetic acid included in the buffer solution. It is necessary to study concentration effects of both the hydrogen ion and the acetic acid on the decay rate of 3A . Therefore the elementary processes related with the proton transfer in the triplet state of acridine are those involved in the following scheme:



The differential equations to be solved are

$$-\frac{d[{}^3A]}{dt} = (k_{dt} + k_2[H_3O^+] + k_5[CH_3COOH])[{}^3A] - k_3[{}^3AH^+] \quad (2)$$

and

$$-\frac{d[{}^3AH^+]}{dt} = (k_3 + k_4)[{}^3AH^+] - (k_2[H_3O^+] + k_5[CH_3COOH])[{}^3A] \quad (3)$$

Since the triplet yield of AH^+ is negligible, the initial conditions are given by

$$[{}^3A] = [{}^3A]_0$$

and

$$[{}^3AH^+] = 0$$

In the early stages of the decay of 3A , eqns. (2) and (3) can be given, to a good approximation, as

$$-\frac{d[{}^3\text{A}]}{dt} = (k_{\text{dt}} + k_2[\text{H}_3\text{O}^+] + k_5[\text{CH}_3\text{COOH}])({}^3\text{A}) \quad (4)$$

and

$$\frac{d[{}^3\text{AH}^+]}{dt} = (k_2[\text{H}_3\text{O}^+] + k_5[\text{CH}_3\text{COOH}])({}^3\text{A}) \quad (5)$$

A plot is shown in Fig. 5 of the initial decay rate of ${}^3\text{A}$ versus the acetic acid concentration included in the solution of pH 5.6. It is obvious that ${}^3\text{A}$ is protonated by the acetic acid. From the slope and the intercept we obtain $k_5 = 4.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{dt}} + k_2[\text{H}_3\text{O}^+] = 1.0 \times 10^5 \text{ s}^{-1}$ respectively. Since $[\text{H}_3\text{O}^+] = 2.5 \times 10^{-6} \text{ M}$ and $k_{\text{dt}} = (4 - 5) \times 10^3 \text{ s}^{-1}$ for $[\text{A}] = 1.2 \times 10^{-5} \text{ M}$ and $[{}^3\text{A}] = 5.5 \times 10^{-7} \text{ M}$, we obtain $k_2 = 3.8 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. A plot of the initial decay rate of ${}^3\text{A}$ versus hydrogen ion concentration under the condition that the concentration of acetic acid included in the solution is 1 mM is shown in Fig. 6. From the slope and the intercept we obtain $k_2 = 3.4 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ and $k_{\text{dt}} + k_5[\text{CH}_3\text{COOH}] = 6 \times 10^4 \text{ s}^{-1}$ respectively. Since $[\text{CH}_3\text{COOH}] = 10^{-3} \text{ M}$ and $k_{\text{dt}} = (4 - 5) \times 10^3 \text{ s}^{-1}$, we obtain $k_5 = (5.5 - 5.6) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. Therefore the results shown in Figs. 5 and 6 are consistent with each other. The value for k_2 of $(3.4 - 3.8) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ is the upper limit of the diffusion-controlled rate and indicates that the probability of producing ${}^3\text{AH}^+$ by one collision between ${}^3\text{A}$ and H_3O^+ is very high. In contrast, the value for k_5 of $(4.5 - 5.6) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ indicates that about a thousandth of the collisions between ${}^3\text{A}$ and CH_3COOH produces ${}^3\text{AH}^+$.

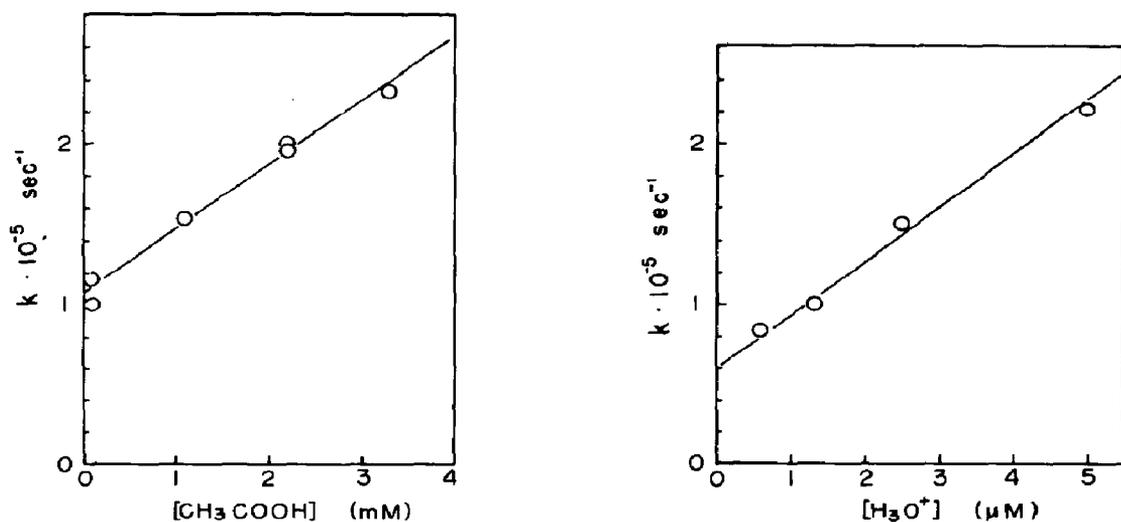


Fig. 5. A plot of the initial decay rate of ${}^3\text{A}$ vs. acetic acid concentration at pH 5.6.

Fig. 6. A plot of the initial decay rate of ${}^3\text{A}$ vs. hydrogen ion concentration for $[\text{CH}_3\text{COOH}] = 1 \text{ mM}$.

The equilibrium constant K_a in the triplet state is expressed by

$$K_a = k_3/k_2$$

Using $K_a = 10^{-5.6} = 2.5 \times 10^{-6}$ M and $k_2 = (3.4 - 3.8) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$, we obtain

$$k_3 = (8.5 - 9.5) \times 10^4 \text{ s}^{-1}$$

The rate constants of the elementary processes pertaining to the triplet state are listed in Table 1.

TABLE 1

The rate constants related to the protolytic reactions of acridine in the triplet state
($k_{dt} = k_0 + k_1[A] + k_{TT}[{}^3A]$)

Rate constant	Value
k_0	$(2 - 3) \times 10^3 \text{ s}^{-1}$
k_1	$(4.5 - 6.0) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
k_{TT}	$(2 - 3) \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$
k_2	$(3.4 - 3.8) \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$
k_3	$(8.5 - 9.5) \times 10^4 \text{ s}^{-1}$
k_4	$1.5 \times 10^3 \text{ s}^{-1}$
k_5	$(4.5 - 5.6) \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$

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