November, 1979] BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 52 (11), 3163—3170 (1979) 3163

Film Dichroism. II. Linearly-polarized Absorption Spectra of Acridine Dyes in the Stretched Poly(vinyl alcohol) Films[1)]

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(Received January 5, 1979)

The linear dichroic absorption spectra of the protonated and quaternized acridine dyes were measured in the UV and visible regions by the stretched film technique. The dyes examined were acridine, 10-methylacridine, Proflavine, Trypaflavine, Acridine Yellow, 10-Methylacridine Yellow, Acridine Orange, and 10-Methylacridine Orange, all of which belong to the C_{2v} point symmetry group. Poly(vinyl alcohol) was used as a film matrix. The absorption spectrum of each dye was resolved into the long-axis and short-axis polarized components. Two orthogonally-polarized electronic transitions (the ^1L_a and ^1L_b bands) of 3,6-disubstituted acridine dyes were reduced to overlap in the visible region of the spectra. The orientation factors K_y and K_z of each dye were evaluated at a constant degree of stretching and were related to the geometry of the dye molecule.

Studies of the interaction between acridine dyes and natural or synthetic polymers have been carried out by various optical methods.[2–8)] However, the optical properties of the dyes *per se* were treated qualitatively in those studies. To obtain useful and unambiguous information on the optical properties of those polymer-dye complexes, it is imperative to accumulate the detailed data on the polarization direction of the electronic bands of individual dyes.[9–11)]

One promising technique is the measurement of the linear dichroism of dyes oriented in a film by mechanical stretching. The importance of this method has been recognized in the assignment of the polarization directions of absorption bands.[12–18)] Tanizaki[12)] derived a theoretical expression which relates the dichroic ratio to the stretch ratio of a matrix film, and applied it to the absorption band of naphthols[13)] and acridine[14)] to determine the relative directions of their transition moments. Thulstrup *et al.*[15)] reported a method (the reduction procedure) for the quantitative evaluation of "reduced spectra" from the dichroic absorption spectra of planar molecules belonging to the C_{2v} or D_{2h} point symmetry group.

The first paper of this series has established the groundwork for the measurement and analysis of the dichroic spectra.[19)] The purposes of this paper are, therefore, (1) to determine the relative direction of the transition moment of eight acridine dyes,[20,21)] (2) to divide the isotropic spectrum of each dye into the long-axis and short-axis polarized components (the reduced spectra), (3) to evaluate the orientation factors K_y and K_z for each dye, and (4) to relate the molecular shape of the dye with the orientation factors. The major findings are as follows: (1) Isotropic spectra of acridine and 10-methylacridine contain a short-axis polarized (^1L_a) and two long-axis polarized transitions (^1B_b and ^1L_b). (2) The isotropic spectrum of each 3,6-disubstituted acridine dye consists of two orthogonally-polarized transitions (^1B_a and ^1B_b) in the UV region, a dominant long-axis polarized transition (^1L_b) and a weak short-axis polarized transition (^1L_a) in the visible region. (3) The values of K_y and K_z obtained for acridine and 10-methylacridine change remarkably upon substitution of the amino or dimethylamino groups at the 3,6-positions of the acridine nucleus. (4) Both Acridine Yellow and 10-Methylacridine Yellow behave like a rod-shaped molecule as regards their orientation property.

Experimental

Materials. Acridine dyes were all in the monocationic form, the anion being chloride (for details, see Ref. 20): Acridine (Acr) and 10-methylacridine (MeAcr), 3,6-diaminoacridine or Proflavine (PF) and 3,6-diamino-10-methylacridine or Trypaflavine (TF), 3,6-diamino-2,7-dimethylacridine or Acridine Yellow (AY) and 3,6-diamino-2,7-dimethyl-10-methylacridine or 10-Methylacridine Yellow (MeAY), 3,6-bis(dimethylamino)acridine or Acridine Orange (AO) and 3,6-bis(dimethylamino)-10-methylacridine or 10-Methylacridine Orange (MeAO). All these dyes were purified by recrystallization from ethanol and their purity was confirmed by the method of thin layer chromatography. The powdered poly(vinyl alcohol) (PVA) samples were obtained from Tokyo Kasei Co., Ltd., and Kuraray Co. (Kuraray Poval 117-H). Their nominal degrees of polymerization were 1750 and 1680, respectively.

Preparation of Sample Films. A stock aqueous PVA solution (*ca.* 9.1 wt %) was prepared as described elsewhere.[19)] To the stock PVA solution (5×10^{-2} dm^3), an aqueous dye solution (*ca.* 1×10^{-3} mol dm^{-3}) was added dropwise. The mixture was then stirred for 20—30 min to make a dye-PVA solution (the final dye concentration was about 1×10^{-5} mol dm^{-3}). (Each solution spectrum was measured at this stage to compare with the corresponding film spectrum.) To prepare a nonoriented sample film, the dye-PVA solution was spread onto a glass plate and kept for 7—10 days in an open room to ensure dryness. The glass plate was adjustable horizontally by screws and equipped with four removable sides (10×10 cm) which allowed the dried film to be detached easily from the glass surface. The reference film was prepared from an aqueous PVA solution which contained no dye under the same conditions.

Measurements and Analyses of Dichroic Spectra. Dichroic spectra were measured on a Hitachi EPS-3T double beam recording spectrophotometer equipped with a mechanical stretcher that could stretch both sample and reference films simultaneously inside the cell compartment.[19)] In each optical path, a schlieren-free grade Glan-Taylor calcite polarizer (Karl Lambrecht Corp., Chicago) was mounted in front of the stretcher. The stretch ratio, S, of the film was defined as the axial ratio, a/b, (a and b are the semi-major and -minor axes) of an ellipse deformed from a circle of radius r which was initially drawn on the film.[12,19)] Uniform stretching of the film was tested at each S by comparing an imaginary volume of $4\pi ab^2/3$ with the initial volume of $4\pi r^3/3$. These two volumes agreed within an experimental uncertainty of 4%.

The absorbances of dye molecules in the film, with polarizers arranged parallel, $A_{\parallel}$ and perpendicular, $A_{\perp}$ to the direction of stretch, should satisfy the relation

$$k \times A_0 = \frac{A_{\parallel} + 2A_{\perp}}{3} \tag{1}$$

where k is the normalization factor, which depends on S, and A_0 is the absorbance of the nonstretched film. The dichroic ratio, R_d, or the reduced dichroism, $\Delta A/A$, of partially oriented rod-shaped molecules can be related to the transition moment angle, θ, which is defined as the angle between the direction of the transition moment and the orientation axis of a molecule, as[19,22)]

$$R_d = \frac{A_{\parallel}}{A_{\perp}} = \frac{2+2(3\cos^2\theta-1)\Phi}{2-(3\cos^2\theta-1)\Phi}, \tag{2}$$

or

$$\frac{\Delta A}{A} = \frac{3(A_{\parallel}-A_{\perp})}{A_{\parallel}+2A_{\perp}} = \frac{3}{2}(3\cos^2\theta-1)\Phi, \tag{3}$$

where Φ is called the *orientation function* which represents the degree of orientation of an assembly of like molecules. An orientation function was derived for the rod-shaped molecule as[19)]

$$\Phi(S) = \frac{3}{2}\,\frac{S^2-S^2(S^2-1)^{-1/2}\tan^{-1}(S^2-1)^{1/2}}{S^2-1} - \frac{1}{2}. \tag{4}$$

By using Eqs. 2 and 4 or Eqs. 3 and 4, the transition moment angle can be determined according to the method of Tanizaki.[12)]

Since the acridine dyes studied in the present work belong to the C_{2v} point symmetry group, the reduction procedure[15)] can be employed for evaluating the A_y-spectrum (the short-axis polarized component of isotropic spectrum) and the A_z-spectrum (the long-axis polarized component) from the observed dichroic spectra. In the spectral region between 220 and 550 nm, the out-of-plane polarized component may be ignored (*i.e.*, $A_x=0$); therefore, if the molecules in the film are oriented uniaxially, the formulas necessary for the procedure are as follows:[15)]

$$A_{\perp} - d_{\perp}A_{\parallel} = \frac{K_z-K_y}{2K_z}A_y$$

and (5)

$$A_{\parallel} - d_{\parallel}A_{\perp} = \frac{K_z-K_y}{1-K_y}A_z,$$

where $d_{\perp}$ and $d_{\parallel}$ are the reduction factors. The constants K_y and K_z are termed the *orientation factors* and are given by[15)]

$$K_y = \frac{d_{\parallel}}{d_{\parallel}+2} \quad \text{and} \quad K_z = \frac{1}{2d_{\perp}+1} \tag{6}$$

Results and Discussion

Isotropic, Dichroic, and R_d-Spectra. Figures 1 to 4 show the *dichroic spectra, i.e.*, the $A_{\parallel}$-spectrum (dotted curve) and the $A_{\perp}$-spectrum (dashed curve), of eight acridine dyes at a particular stretch ratio, together with the wavelength dependence of the dichroic ratio R_d (filled circles), which is termed the R_d-spectrum. An error bar on the dichroic ratio indicates the experimental uncertainty. The dichroic ratio can be converted into the reduced dichroism,[19)] which should be referred to the left ordinate of each figure. Two kinds of isotropic spectra are also shown in each figure: one, $k \times A_0$ (open circles), was obtained from the left hand side of Eq. 1 and the other, A (solid curve), was calculated from the right hand side of the same equation. An excellent agreement between these two isotropic spectra suggests that the dye molecules are indeed oriented uniaxially in the stretched film. The observed isotropic spectra of all the acridine dyes in the PVA film were similar to the spectra of the dyes in aqueous PVA solution. The apparent band positions of each dye are shown in Table 1.

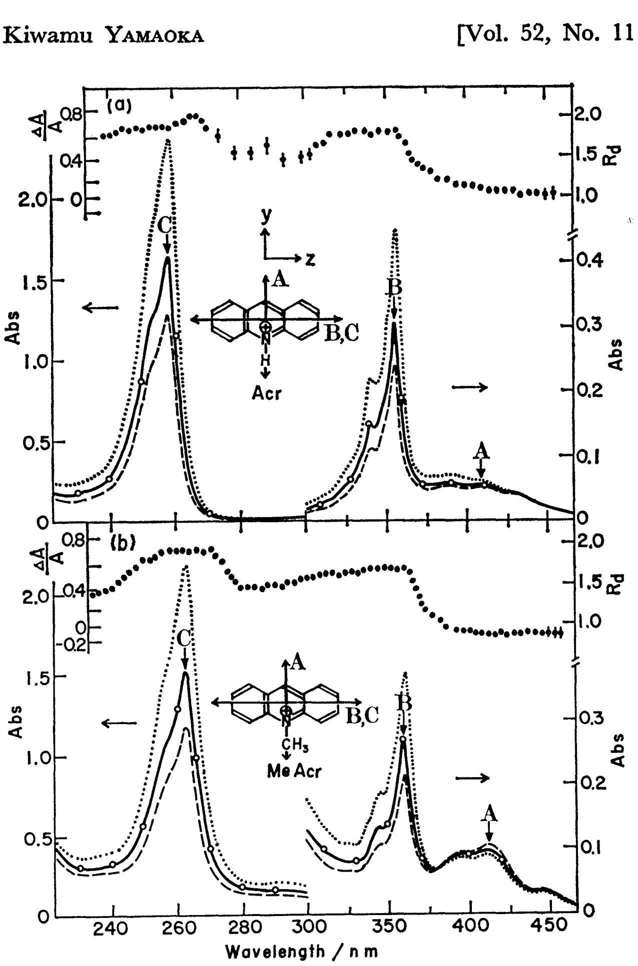

Fig. 1. Isotropic, dichroic, and R_d-spectra of Acr and MeAcr in the stretched PVA film. Symbols are: the isotropic spectrum (——), the parallel (······) and perpendicular (----) polarized spectra, the R_d-spectrum (••••) and the normalized isotropic absorbance (○). Letters **A** to **F** indicate the apparent peak or shoulder positions in each isotropic spectrum and the polarization directions in the acridine nucleus. Acr at $S=4.3$ and $\bar{k}=0.62$. MeAcr at $S=4.3$ and $\bar{k}=0.65$. Factor $\bar{k}$ is an average of the values of k at about 30 wavelengths.

The dye AO has drawn special attention because of its strong ability of dimerization in aqueous solution. The "monomer" spectrum, which was unmasked by the monomer-dimer analysis, gave a value of 1.5—1.6 for the ratio of the peak intensity (492 nm) to the shoulder intensity (≈470 nm).[23,24)] The spectrum of AO in an aqueous PVA solution (2.5×10^{-5} mol dm^{-3} in 9.1 wt %) also gave nearly the same value, which indicates AO is probably in the monomeric form.

Table 1. The apparent band positions (**A**—**F**) in the isotropic spectra and the transition moment angles (θ_A—θ_F) at the corresponding band positions of eight acridine dyes

Dyes	Positions^a)/nm			Angles/deg		
	A	**B**	**C**	θ_A	θ_B	θ_C
Acr	408 (403)	356 (354)	258	54±1	41±1	40±1
MeAcr	412 (416)	360 (358)	263	58±1	40±1	38±1
	D	**E**	**F**	θ_D	θ_E	θ_F
PF	465 (452)	305	263 (261)	8±2	—	22±2
TF	470	305	265	8±2	—	24±2
AY	467 (453)	314	266 (265)	—	—	—
MeAY	471 (461)	317	270 (267)	8±2	—	23±2
AO	502 (496)	330	272 (271)	12±2	70±2	23±2
MeAO	506 (500)	335	274 (272)	10±2	—	27±2

a) The values in the parentheses are the corresponding positions in the aqueous PVA solution.

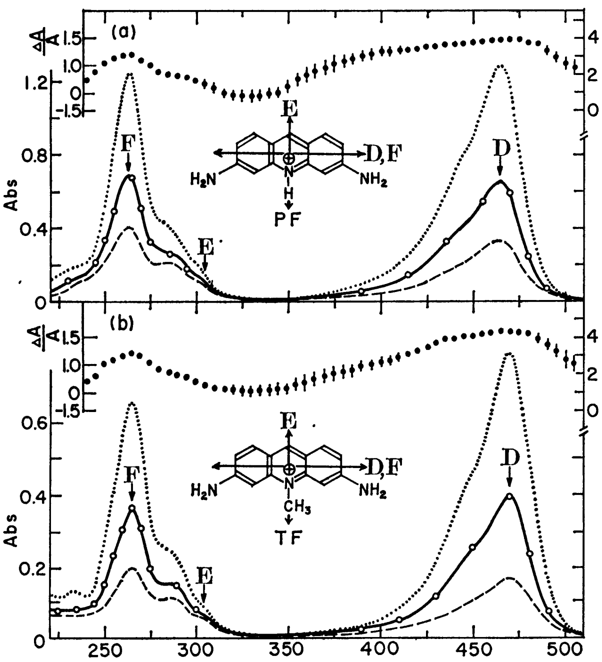

Fig. 2. Isotropic, dichroic, and R_d-spectra of PF and TF in the stretched PVA film. Symbols are the same as in Fig. 1. PF at $S=3.7$ and $\bar{k}=0.65$. TF at $S=3.7$ and $\bar{k}=0.65$.

In the isotropic spectrum of AO in the PVA film (Fig. 4a), the ratio was as high as 2.0. This value compares favorably with the values of 2.1 in chloroform (1—10×10^{-6} mol dm^{-3}) and 1.9 in ethanol (2×10^{-5} mol dm^{-3}). These data suggest that AO is also in the monomeric form in the PVA film. Results similar to the above were observed for such associative dyes as PF,[25,26] TF,[26] and MeAO. Therefore, the isotropic spectra in Figs. 1 to 4 were concluded to be essentially the monomer spectra of the respective dyes.

Acridines: Both the dichroic and R_d-spectra of Acr and MeAcr are shown in Fig. 1. In order to ascertain that Acr is fully protonated, an appropriate amount of HCl (0.1 mol dm^{-3}) was added to the aqueous dye–PVA solution prior to casting. The isotropic spectrum of Acr in the film resembles the solution spectrum of the acridinium ion. The $A_{\parallel}$-spectrum of Acr is always more intense than the $A_{\perp}$-spectrum, except for the long-wavelength region. The R_d-spectrum of Acr is nearly flat in 460—400 nm ($R_d=1.0$—1.1) and again in 360—320 nm ($R_d\approx1.7$), sharply changing at about 370 nm. Another plateau region is observed in 260—240 nm ($R_d\approx1.8$). These dichroic features are in excellent agreement with a previous report.[27] The isotropic spectrum of MeAcr shifts toward the red, as compared with the spectrum of Acr, because of the substitution of a methyl group on the ring nitrogen. The R_d-spectrum is constant over 460—390 nm ($R_d\approx0.9$), 360—330 nm ($R_d\approx1.8$), and 270—250 nm ($R_d\approx1.9$).

3,6-Diaminoacridines: Figure 2 shows the dichroic and R_d-spectra of PF and TF, which are 3,6-diamino derivatives of Acr and MeAcr, respectively. The isotropic spectra of PF and TF differ from those of Acr and MeAcr in that PF and TF show a deceptively simple absorption peak in the visible region and two conspicuous shoulders in 280—320 nm. In spite of the simple isotropic absorption in the visible region, the R_d-spectra of PF and TF are not flat: the largest values occur near 465 nm and 470 nm, respectively. They descend rather sharply on the long-wavelength side and slowly but irregularly on the short-wavelength side. These descending trends are well beyond the experimental uncertainty.

3,6-Diamino-2,7-dimethylacridines: Figure 3 shows the dichroic data of AY and MeAY, which are 3,6-diamino-2,7-dimethyl derivatives of Acr and MeAcr, respectively. In the isotropic spectrum of AY, there are at least three humps at *ca.* 445, 428, and 400 nm, and a shoulder at *ca.* 255 nm. The profile of the isotropic spectrum of MeAY resembles that of AY in the visible and UV regions. A new feature was discovered for MeAY in the 340—310 nm region, where the $A_{\perp}$-spectrum is more intense that the $A_{\parallel}$-spectrum (inset of Fig. 3). Such a reverse trend is always found for more heavily substituted dyes, such as AO and MeAO. The R_d-spectra of AY and MeAY give the highest value near the main peak in the visible region and show the lowest value in the 340—310 nm region.

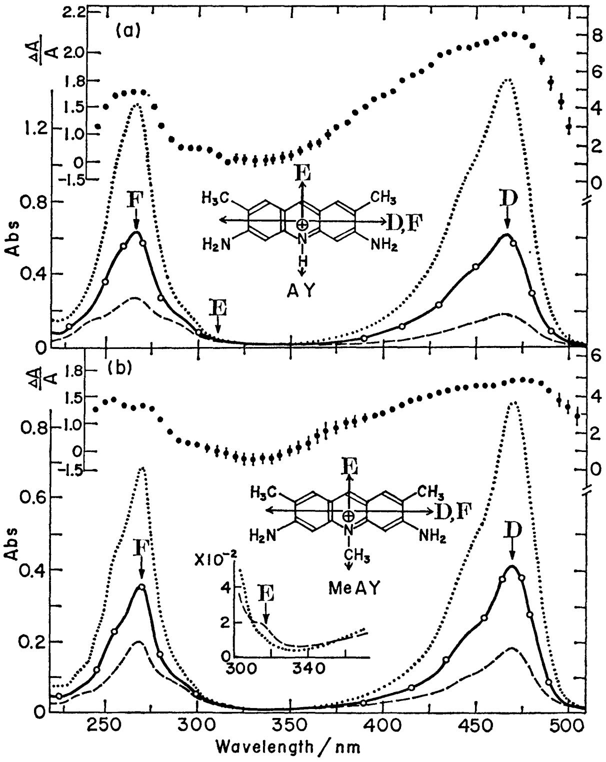

Fig. 3. Isotropic, dichroic, and R_d-spectra of AY and MeAY in the stretched PVA film. Symbols are the same as in Fig. 1. AY at $S=4.3$ and $\bar{k}=0.57$. MeAY at $S=4.3$ and $\bar{k}=0.62$.

Especially, the R_d-value becomes smaller than unity for MeAY, indicating that a weak absorption band exists in 340—310 nm. On the analogy of the R_d-spectrum of MeAY in 340—310 nm, all AY, TF, and PF should also have such a weak band in the same region.

3,6-Bis(dimethylamino)acridines: The dichroic and R_d-spectra of AO, which is the 3,6-bis(dimethylamino) derivative of Acr, are shown in Fig. 4a. The $A_{\parallel}$-spectrum is more intense than the $A_{\perp}$-spectrum in the entire spectral region, except for the 360—320 nm region, where the behavior of the $A_{\parallel}$- and $A_{\perp}$-spectra is reversed. Such a reverse trend has also been reported.[28,29] The R_d-spectrum of AO descends on the long-wavelength side of the 502 nm principal band, while it decreases irregularly on the short-wavelength side and becomes smallest at *ca.* 330 nm. Figure 4b shows the dichroic data of MeAO. The isotropic spectrum of MeAO is bathochromic relative to AO; the spectral features are almost the same. The reverse trend of the dichroic spectra was also observed in the 350—320 nm region. The changes in the R_d-spectrum on both sides of the 506 nm principal band become more pronounced than those of AO. The change of the R_d-spectrum of MeAO in the visible region can be explained reasonably well by introducing the short-axis polarized transition (the ^1L_a band) with a vibrational structure which is overlapped by the 506 nm band (the ^1L_b band). Similar interpretations can be put on the changes of the R_d-spectra of all 3,6-disubstituted dyes: AO, AY, MeAY, PF, and TF.

Determination of Transition Moment Angles. If a planar molecule has at least one C_2 symmetry axis in the molecular plane, the observed isotropic spectrum can be divided into two components (*i.e.*, the divided spectra)[13] which are polarized parallel and perpendicular to the symmetry axis, respectively. In order to obtain the divided spectra of each acridine dye, the transition moment angle must be evaluated correctly.[13,30] For this purpose, the dichroic spectra were analyzed, in the first place, by Tanizaki's method.[12] As examples, the dependences of R_d (and $\Delta A/A$) on S are shown for Acr and AY in Fig. 5. The transition moment angles, θ, relative to the orientation axis are given in Table 1, together with those of the remaining acridine dyes.

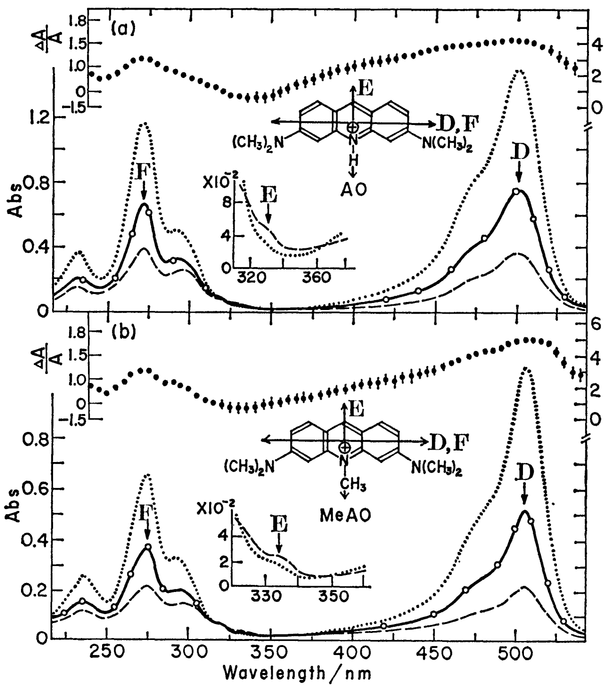

Fig. 4. Isotropic, dichroic, and R_d-spectra of AO and MeAO in the stretched PVA film. Symbols are the same as in Fig. 1. AO at $S=4.3$ and $\bar{k}=0.55$. MeAO at $S=4.3$ and $\bar{k}=0.60$.

The sums of the transition moment angles of θ_A and θ_C, and θ_A and θ_B, for Acr are 94° and 95°, respectively. These values are in good agreement with the results obtained by Yoshino *et al.*,[27] and assure the equal precision of the experimental procedures of the present work. From the symmetry consideration, the transition moments of the **A** and **C** bands of Acr or MeAcr (each band can be regarded as a single transition from the flatness of the R_d-spectrum) should be orthogonal *i.e.*, $\theta_A+\theta_C=90°$. However, the values are 94° for Acr and 96° for MeAcr, always larger than 90°. The deviation from the orthogonality is more conspicuous for MeAcr than Acr; this implies that those transition moment angles contain some kind of systematic error which is associated with the molecular shape, *i.e.*, the shape of Acr or MeAcr can not be generally represented by a rod, but is more likely to be planar. The most unrealistic example is AY, for which the dependence of R_d (or $\Delta A/A$) on S at position **D** exceeds the theoretically permissible curve

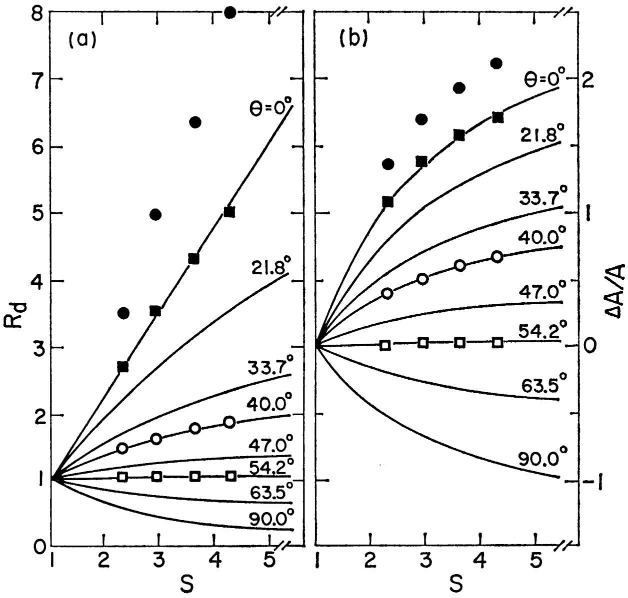

Fig. 5. Dependence of (a) the observed R_d and (b) $\Delta A/A$ on S for Acr and AY. Symbols (□ and ○) denote the values of Acr at wavelength positions **A** and **C**; and (■ and ●) denote the values of AY at **F** and **D**, respectively. Solid curves are theoretical and calculated with the angle θ specified for respective curves by the use of Eqs. 2, 3, and 4.

($\theta=0°$), as shown in Fig. 5. In fact, the R_d-values of AY are very large in the visible absorption peak, as compared with those of PF and AO. (The dichroic spectra of AY were measured several times to confirm the reproducibility of the R_d-values.)

The above findings suggest that the orientation function given by Eq. 4 does not represent the degree of orientation of AY and Acr correctly. Since this equation was derived from the distribution of unit vectors and contains the variable S only, no consideration has been given to the geometrical shape of a particular guest molecule. For instance, the large R_d-values of AY at two main peaks (467 and 266 nm), as compared with those of MeAY, can be accounted for by assuming that AY is better oriented than MeAY at a given S. Hence, the actual orientation mechanism of dyes in the stretched polymer film seems to be more complex than that described by the orientation function (Eq. 4). The stretch ratio S may need a more critical evaluation: the true stretch ratio for AY could possibly be greater than the macroscopic S. The discrepancy between the true and calculated angles of a transition moment may become pronounced, if any transition moment of the molecule is nearly parallel or perpendicular to the orientation axis, *i.e.*, if the angle θ approaches either 0° or 90°. Because of the reasons cited in this section, the reduction procedure,[15] which does not need to assume the degree of orientation of the guest molecules, was employed to determine the reduced spectra of each dye.

Reduced Spectra of Eight Acridine Dyes. Since each dye belongs to the C_{2v} point symmetry group, its transition moment should be in the molecular plane and along the long (z) or short (y) axis in the visible and UV regions. According to many film dichroism measurements, the molecules embedded in the PVA matrix are generally known to orient predominantly with their long axes aligned to the direction of stretch. This means that an absorption band whose transition moment is polarized along the long axis rather than the short axis should give a larger R_d-value. For example, in the dichroic spectra of Acr and MeAcr (Fig. 1), the change of each R_d-curve indicates the existence of at least one short-axis polarized (denoted as **A**) and two long-axis polarized (denoted as **B** and **C**) transitions. In contrast, the R_d-curves of the 3,6-disubstituted acridine dyes (Figs. 2 to 4) reveal the existence of at least one short-axis polarized (**E**) and two long-axis polarized (**D** and **F**) transitions. On the basis of these apparent spectral features, the reduced spectra of each dye were determined with a HITAC-8700 computer according to the reduction procedure.[15]

The resultant reduced spectra of Acr are shown in the upper half of Fig. 6a. As expected from Fig. 1, the A_z-spectrum (solid curve) gives rise to two strong long-axis polarized transitions at *ca.* 356 and 258 nm (the $^1\mathrm{L_b}$ and $^1\mathrm{B_b}$ bands), while the A_y-spectrum (dotted curve) manifests a short-axis polarized transition at *ca.* 408 nm (the $^1\mathrm{L_a}$ band). These assignments are in excellent agreement with the results of fluorescence polarization[31] and film dichroism.[27] It is interesting to note that the A_y-spectrum is much better resolved with the reduction procedure than the D_y-spectrum,[27] which appears to contain some remaining contributions from the intense D_x-spectrum between 370 and 320 nm. The reduced spectra of MeAcr are shown in the lower half of Fig. 6a. Just as noted for Acr, the A_z-spectrum of MeAcr gives two long-axis polarized transitions at *ca.* 360 and 263 nm (the $^1\mathrm{L_b}$ and $^1\mathrm{B_b}$ bands), whereas the A_y-spectrum shows a short-axis polarized transition at *ca.* 412 nm (the $^1\mathrm{L_a}$ band).[32] The higher-energy transition polarized along the short axis (the $^1\mathrm{B_a}$ band) is rather obscured in the reduced spectra, but can possibly be at 260 nm for Acr and at 280 nm for MeAcr, as was shown for Acr previously,[27] or it may be hidden in the higher-energy region, as in anthracene.[33]

The reduced spectra of 3,6-disubstituted acridine dyes are shown in Figs. 6b to 6d. As already expected from the R_d-spectra in Figs. 2 to 4, there are generally two strong long-axis polarized transitions (the $^1\mathrm{L_b}$ and $^1\mathrm{B_b}$ bands) in the A_z-spectrum and a short-axis polarized transition (the $^1\mathrm{B_a}$ band) in the A_y-spectrum.[34] In addition to these transitions, there is definitely a short-axis polarized, weak transition (probably the $^1\mathrm{L_a}$ band)[34] in the visible region, the presence of which has long been a point of controversy.[35] It should be this transition, hidden in the isotropic spectrum, that is responsible for the decrease in the R_d-values on both sides of the principal peak in the visible region.

From comparison of the reduced spectra between Acr and PF or between MeAcr and TF, it is clear that the $^1\mathrm{L_a}$ and $^1\mathrm{L_b}$ bands of Acr or MeAcr are shifted, on the substitution of amino groups at 3,6-positions, toward the long wavelength in such a way that the $^1\mathrm{L_b}$ band overtakes the $^1\mathrm{L_a}$ band.[36] The $^1\mathrm{B_a}$ transition is definitely unraveled in the UV regions of PF and

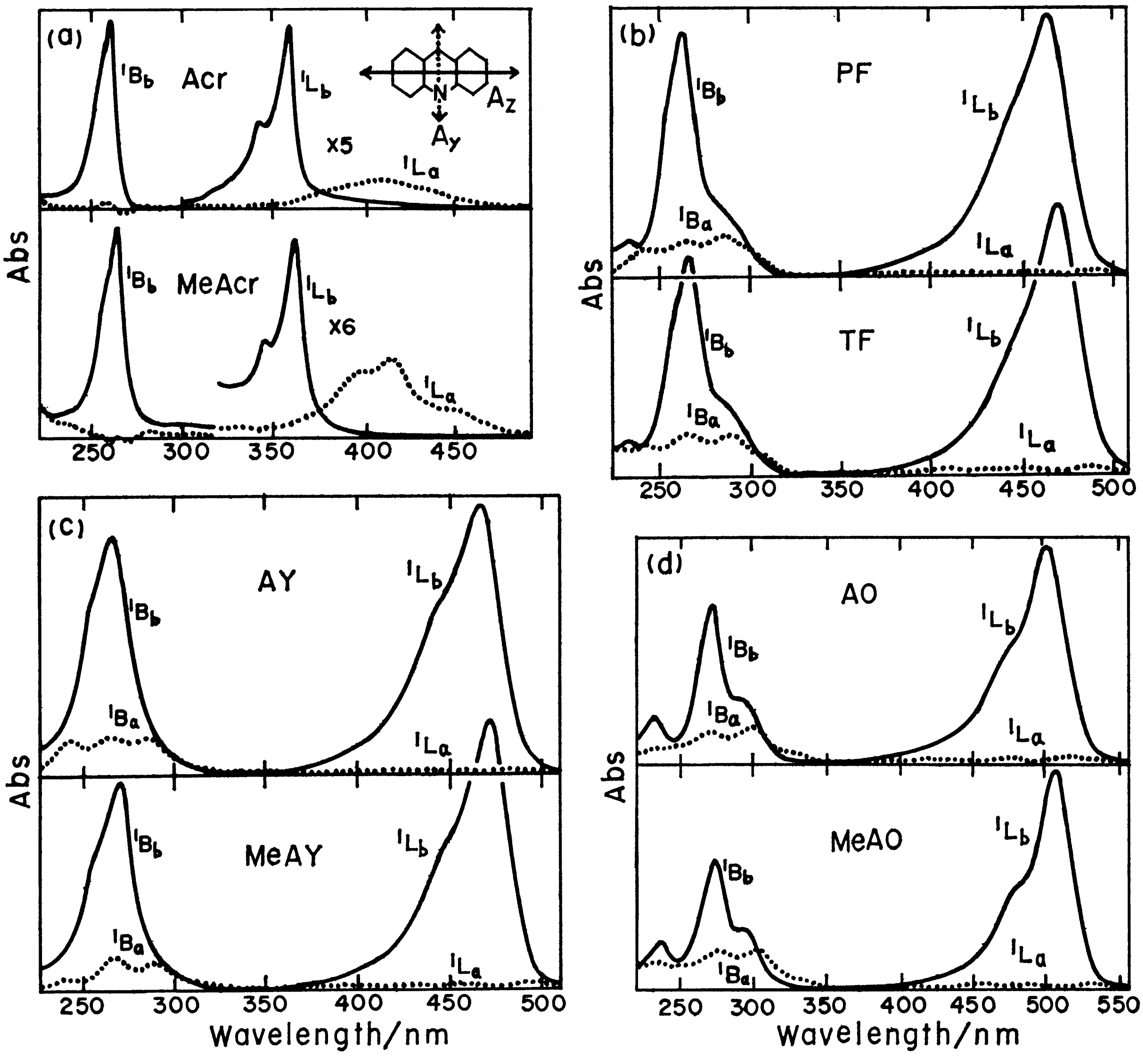

Fig. 6. The reduced spectra (the A_z-spectrum and the A_y-spectrum) of eight acridine dyes at S=4.3. The long-axis (z-axis) and short-axis (y-axis) polarized absorbances (arbitrary units) are shown by the solid and the dotted curves, respectively. (a) Acr and MeAcr (The absorbance between 320 and 480 nm is expanded as indicated.), (b) PF and TF, (c) AY and MeAY, and (d) AO and MeAO.

TF, in contrast to the reduced spectra of Acr and MeAcr. These features of the reduced spectra are also evident from Figs. 6c and 6d for AY and AO relative to Acr and for MeAY and MeAO relative to MeAcr.

The A_y-spectrum of AO shows the ^1L_a band in 550—400 nm (probably the 0-0 transition near 518 nm). This 0-0 transition of the ^1L_a band is mostly responsible for the decrease of the R_d-spectrum of AO (also PF, TF, AY, MeAY, and MeAO) on the long-wavelength side of the visible peak. In 350—310 nm, the A_y-spectra of AO and MeAO are more intense than the corresponding A_z-spectra. This trend is in excellent accord with the result that the R_d-spectra of AO and MeAO become smallest at *ca.* 330 nm. (This statement is also valid for PF, TF, AY, and MeAY.) Since the 330 nm band is expected to be an in-plane transition, it may be assigned to be the 0-0 transition of the ^1B_a band.

A comprehensive assignment of polarization was tentatively carried out for the reduced spectra of 3,6-disubstituted acridine dyes on the basis of the data of Acr and MeAcr. In order to obtain the complete and detailed assignment of all the transition moments of those dyes, each reduced spectrum should be resolved into individual component bands, the isotropic spectrum should then be reconstructed from those decomposed bands, and above all, the observed R_d-spectrum should be reproduced fully by simulation. The details will be reported shortly.

Relation between Molecular Shape and Orientation Factors. The orientation factors K_y and K_z were determined from the reduction factors $d_\parallel$ and $d_\perp$ with the aid of Eq. 6. The values of $d_\parallel$, $d_\perp$, K_y, and K_z obtained for each dye at S=4.3 are listed in Table 2. In order to find out the relation between the molecular shape and the orientation factors, the points (K_y,K_z) for all the dyes are plotted in Fig. 7. All these points lie either inside or on the periphery of the "orientation triangle".[18] The accuracy of each point is roughly indicated by its size.

The location of each point in the orientation triangle

Table 2. The average values of the reduction factors ($d_{\parallel}$ and $d_{\perp}$) and the orientation factors (K_y and K_z) at S=4.3 of eight acridine dyes

Dyes	$d_{\parallel}$	$d_{\perp}$	K_y	K_z
Acr	0.95	0.54	0.322	0.481
MeAcr	0.88	0.53	0.305	0.485
PF	0.52	0.21	0.206	0.704
TF	0.47	0.18	0.190	0.735
AY	0.23	0.12	0.099	0.806
MeAY	0.30	0.19	0.130	0.725
AO	0.47	0.24	0.190	0.676
MeAO	0.37	0.18	0.156	0.735

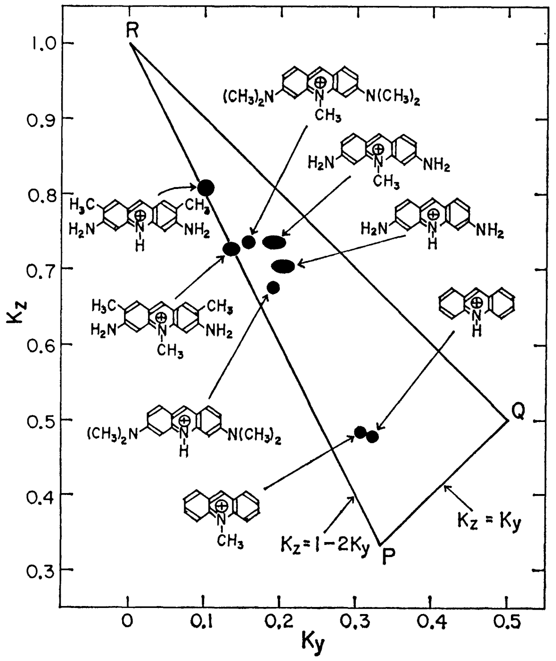

Fig. 7. The orientation factors K_y and K_z of eight acridine dyes at S=4.3 in the coordinates (K_y, K_z). The orientation triangle is denoted by PQR.

is clearly related to the molecular shape of dye. For example, the points for Acr and MeAcr are closest to the apex P (1/3,1/3), indicating that both Acr and MeAcr are the least orientable among those dyes studied here. This is probably due to their molecular shape, which lacks the 3,6-substituents and, as a result, is least prolate. The distance between the apex P and each point increases remarkably on the substitution of the amino or dimethylamino groups at the 3- and 6-positions of acridine nucleus (AO, PF, TF, MeAO, MeAY, and AY in this order). Since these dyes are oriented more favorably than Acr and MeAcr, the increase in the distance is attributable to the lengthening of the molecular shape. The factor K_z is larger for AY than for the quaternized, 10-methyl substituted MeAY, which implies that the quaternization of AY may alter the orientability along the z-axis. However, this tendency is reversed for pairs of PF and TF, and AO and MeAO, which possess the same substituents (either amino or dimethylamino) at 3- and 6-positions but a different one at 10-position (hydrogen or methyl). The reason for this reversal is not yet clear.

Since the points for AY and MeAY are nearly on the line PR (K_z=1−2 K_y), these two dyes can be approximated as a rod-like molecule in their orientation property.[18] It may then be concluded that AY and MeAY are geometrically more symmetric than PF and TF with respect to the z-axis and, accordingly, the orientation axis nearly coinsides with the z-axis. The points for Acr, MeAcr, PF, TF, AO, and MeAO lie not on the line PR but inside the orientation triangle. This result indicates that the orientation axis of each of those six dyes does not coincide with the z-axis but should be somewhere between the y- and z-axes in the molecular plane. Thus the locations of the points in Fig. 7 are related to the molecular shape and, as a consequence, to the orientability of dyes. With the location of those points determined at a single S value, however, the degree of orientation of an assembly of like molecules can not be specified as yet. In this connection, a more comprehensive theory of the film dichroism must be developed under due consideration of the dependence of the degree of orientation on the geometrical shape of the guest molecules.

Conclusion

The film dichroism method has proved to be powerful for obtaining information on the polarization directions and the presence of weak bands for 3,6-disubstituted acridine dyes. From the dependence of R_d on S, the transition moment angle relative to the orientation axis could be determined, only when the R_d-values are near unity. By the reduction procedure, the reduced spectra and the orientation factors for acridine dyes of C_{2v} symmetry could be estimated at a given S without recourse to the orientation function of the guest molecules. The orientation factors of these dyes could be related to their molecular shapes.

The authors thank Mr. Takumi Matsuda for his skilled computer programming, which was used in calculating the reduced spectra.

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