# Coenzyme Models. 30. On the Unusual Spectroscopic Behaviors of Amphiphilic Flavins and 5-Deazaflavins

Seiji SHINKAI,* Akiko HARADA, Osamu MANABE, and Fumio YONEDA†

*Department of Industrial Chemistry, Faculty of Engineering, Nagasaki University, Nagasaki 852*

*†Faculty of Pharmaceutical Sciences, Kumamoto University, Kumamoto 862*

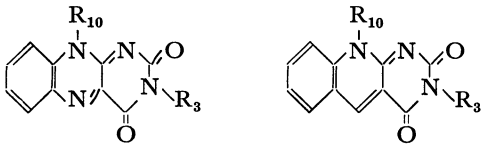
(Received September 16, 1981)

At room temperature in aqueous solution, isoalloxazines and 5-deazaisoalloxazines with 10- dodecyl substituent gave a well-resolved, three-banded S1 peak which has been believed to appear only in nonpolar solvents or in enzymic hydrophobic pockets where isoalloxazines and 5-deazaisoalloxazines become free of hydrogen-bonding effects. On the other hand, 3-methyl-10-ethylisoalloxazine and 5-deazaisoalloxazines with 10- ethyl or 10- octyl group did not give such a fine-structured S1 peak. We have found that (i) the fine structure disappears on the addition of organic solvents (ethanol>40 vol% or pyridine>20 vol%), (ii) the fine structure also disappears on the addition of surfactants (CTAB, Brij-35, and SDS) above the critical micelle concentrations, (iii) 10-dodecylisoalloxazine with hydrophilic 3-(thiazolinio)propyl group at 3-position has not the fine structure, and (iv) the fluorescent intensity of 3-methyl-10-dodecyl-5-deazaisoalloxazine is much weaker than that of 3-methyl-10-ethyl-5-deazaisoalloxazine in aqueous solution but these intensities become comparable above 40 vol% ethanol. These findings are consistently rationalized in terms of an aggregation-deaggregation equilibrium of amphiphilic flavin analogues, supporting that the fine structure in aqueous solution is due to the "stacking" association of (5-deaza)isoalloxazine nuclei, like that of dye molecules. The aggregation-deaggregation phenomenon is sensitively reflected by the reactivity: the reaction of 3-methyl-10-ethyl-5-deazaisoalloxazine and cyanide ion gave $k_2=1.27\ M^{-1}\ s^{-1}$ in aqueous solution at 30 °C, whereas aggregated 3-methyl-10-dodecyl-5-deazaisoalloxazine did not react with cyanide ion. These findings have significant implications on the chemistry of flavoproteins, because the absorption spectra of enzyme-bound flavins and 5-deazaflavins have frequently been cited to discuss the flavin reactivities.

The light absorption spectra of a flavin (vitamin $B_2$) family have characteristic dependence upon the medium polarity and have been used as a useful probe in enzymic and membrane biology. Flavins usually have two characteristic absorption maxima at the ultraviolet region (around 330 nm: peak S2) and the visible region (440 nm: peak S1). The absorption spectra can be classified into three categories on the basis of solvent effects on the S1 peak: a simple gaussian-type peak in aqueous solution (type A), two-to-three shoulders in dipolar solvents (DMF, acetonitrile, tetrahydrofuran, *etc.*) (type B), and a three-banded fine structure in nonpolar solvents (benzene, 3-methylpentane, *etc.*) (type C).[1–5] It is also known that the absorption maximum of S2 shifts to shorter wavelengths in nonpolar solvents.[1,2] Thus, the S2 band is frequently used as an indicator of solvent polarity.

We previously found that the S1 peak of 3-methyl-10-dodecylisoalloxazine (amphiphilic flavin analogue) gives a well-resolved, three-banded fine structure even in aqueous solution at room temperature.[6] Since the fine structure of S1 has been believed to appear only in nonpolar solvents or in enzymic hydrophobic pockets where isoalloxazine becomes free of hydrogen-bonding effects,[1–5,7,8] the novel finding in the nonenzymatic aqueous solution helps understanding the origin of the fine structure in enzymatic systems. Based on the spectral examination, we proposed that the fine structure is attributable to "stacking" association of the isoalloxazine nuclei which is induced by hydrophobic aggregation of 10-dodecyl groups.[6,9]

As an extention of the study, we examined the spectral characteristics of following isoalloxazines (**1**) and 5-deazaisoalloxazines (**2**) (see Table 1 for their 3- and 10- substituents) in aqueous solution. The results support again the importance of the aggregation in the absorption and the fluorescent spectrum.



**1** **2**

Table 1. Abbreviations of isoalloxazines and 5-deazaisoalloxazines

| Abbreviation | $R_3$ | $R_{10}$ |
|---|---|---|
| **1**MeEt | $CH_3$- | $CH_3CH_2$- |
| **1**MeDod | $CH_3$- | $CH_3(CH_2)_{11}$- |
| **1**HDod | H- | $CH_3(CH_2)_{11}$- |
| **1**ThDod | $Br^-S^+N(CH_2)_3$- | $CH_3(CH_2)_{11}$- |
| **2**MeEt | $CH_3$- | $CH_3CH_2$- |
| **2**MeOct | $CH_3$- | $CH_3(CH_2)_7$- |
| **2**MeDod | $CH_3$- | $CH_3(CH_2)_{11}$- |

## Experimental

Preparations of isoalloxazine derivatives (**1**MeEt, **1**MeDod, **1**HDod, and **1**ThDod) were described previously.[9–11] The preparation method of **2**MeEt was also reported.[12] Preparations of **2**MeOct and **2**MeDod will be described soon in a separate paper.

The absorption spectra of isoalloxazines and 5-deazaisoalloxazines were taken at 30±0.1 °C on a Hitachi 200 spectrophotometer equipped with a thermostated cell-holder. The concentration was usually $5.00\times10^{-5}$ M.†† The fluorescent spectra were taken at 30±0.1 °C on a Hitachi 650-10S spectrophotometer equipped with a thermostated cell-holder. The concentration was usually $5.00\times10^{-7}$ M. The excitation wavelengths for isoalloxazines and 5-deazaisoalloxazines were 380 and 330 nm, respectively, and the slit widths of excitation and emission were 3 and

†† 1 M=1 mol $dm^{-3}$.

15 nm, respectively.

The reaction of cyanide ion and 5-deazaisoalloxazines was monitored spectrophotometrically at 30±0.1 °C by following the disappearance of 5-deazaisoalloxazines at their $\lambda_{max}$. The typical conditions were: $[CN^-]=1.00\times10^{-3}$ M and [5-deazaisoalloxazine]$=5.00\times10^{-5}$ M in aqueous 2 vol% DMF solution. The first-order behavior was observed for at least up to three half-lives. The pseudo-first-order rate constants thus obtained were proportional to the cyanide ion concentration. Hence, the reaction is first-order in cyanide ion and 5-deazaisoalloxazine.

## Results and Discussion

*Absorption Spectra in Various Solvents.* The typical absorption maxima of isoalloxazines and 5-deazaisoalloxazines are summarized in Tables 2 and 3, respectively. Table 2 shows that **1**MeEt is subject to typical solvent effects:[1-5] S1 is a gaussian-type peak in aqueous solution (type A), has shoulders in polar solvents (type B), and has a three-banded fine structure in nonpolar solvents (type C). The $\lambda_{max}$ of S2 shifted to shorter wavelengths with lowering the solvent polarity, as reported by Koziol and others.[1-5] On the other hand, S1 of **1**MeDod and **1**HDod was a well-resolved fine structure even in aqueous solution (*i.e.*, type C: Fig. 1). The shape of the S1 peak was almost unaffected at the concentration range $(0.10—2.40)\times10^{-4}$ M. Although these fine-structured peaks in aqueous solution are similar to that of **1**MeEt in benzene, the blue shift of S2 was not observed for aqueous **1**MeDod and **1**HDod (*e.g.*, 340 and 343 nm, respectively; compare with the blue shift of **1**MeEt, 341 (water)→328(benzene)). The result suggests that the fine structure observed in the aqueous system cannot be rationalized in terms of the simple solvent effect.

Table 2. Absorption maxima(nm) of isoalloxazines in various media (30 °C)[a]

| Medium | 1MeEt S2 | 1MeEt S1 | 1MeDod S2 | 1MeDod S1 | 1HDod S2 | 1HDod S1 | 1ThDod S2 | 1ThDod S1 |
|---|---|---|---|---|---|---|---|---|
| Water | 341, | 433 | 340, | 427<br>447<br>480 | 343, | 423<br>441<br>472 | 345, | 437 |
| CTAB(10 mM) | 340, | 433 | 338, | 440(S) | 339, | 438(S) | 337, | 438(S) |
| SDS(10 mM) | | | 341, | 440(S) | 343, | 440(S) | | |
| Brij-35(10 mM) | | | 340, | 445(S) | 346, | 440(S) | | |
| Ethanol | 332, | 435(S) | 332, | 435(S) | 332, | 435(S) | | |
| Pyridine | 327, | 438(S) | 328, | 438(S) | 327, | 438(S) | | |
| Benzene | 328, | 418<br>440<br>466 | 329, | 420<br>440<br>467 | 327, | 417<br>438<br>464 | | |

a) (S) indicases that S1 has shoulders. Three values given for S1 indicate each absorption maximum for the fine structure split.

Table 3. Absorption maxima(nm) of 5-deazaisoalloxazines in various media (30 °C)[a]

| Medium | 2MeEt S2 | 2MeEt S1 | 2MeOct S2 | 2MeOct S1 | 2MeDod S2 | 2MeDod S1 |
|---|---|---|---|---|---|---|
| Water | 323, | 392 | 325, | 400 | 325, | 386<br>403<br>428 |
| CTAB(10 mM) | 322, | 393 | 323, | 399 | 322, | 399(S) |
| SDS(10 mM) | 323, | 392 | 323, | 397 | 324, | 397(S) |
| Brij-35(10 mM) | 323, | 391 | 323, | 398 | 322, | 400(S) |
| Ethanol | 318, | 398(S) | 320, | 398(S) | 320, | 398(S) |
| DMF | 318, | 400(S) | | | 318, | 401(S) |
| $CH_3CN$ | 316, | 397(S) | | | 316, | 398(S) |
| *o*-Dichlorobenzene | 321(S), | 404(S)<br>426 | | | 322(S), | 405(S)<br>427 |
| Benzene | 318(S), | 405(S)<br>427 | | | 318(S), | 405(S)<br>428 |

a) (S) indicates that the peak has shoulders. Two-to-three values given for S1 indicate each absorption maximum for the fine structure split.

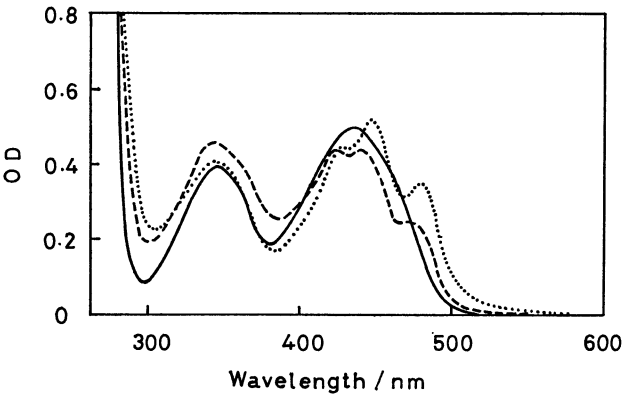



Fig. 1. Absorption spectra of **1**HDod(- - -), **1**MeDod (·····), and **1**ThDod(——) in water. [isoalloxazine]$=5.00\times10^{-5}$ M.

Interestingly, the fine structure of **1**MeDod and **1**HDod has disappeared completely in aqueous solutions containing surfactants above the critical micelle concentrations(cmc), and the spectra were classified as type B (Table 2). Similarly, the fine structure disappeared on the addition of organic solvents (*vide infra*). The fine structure also disappeared by introducing a hydrophilic 3-(thiazolinio)propyl group at 3-position of 10-dodecylisoalloxazine (Fig. 1). These results, together with our previous findings,[6,9] strongly suggest that the fine structure of aqueous **1**MeDod and **1**HDod stems from the association of the isoalloxazine nuclei which is induced by hydrophobic interaction between 10- dodecyl groups. The disappearance of the fine structure in the micellar system is accounted for by the loss of the interaction between the isoalloxazine nuclei in the micellar phase (*i.e.*, dilution effect). The phenomenon is similar to that of the absorption spectrum of dye molecules: "stacking" association of dye molecules induces the split of the absorption bands in aqueous solution, but it disappears in micellar solutions.[13-16] The disappearance of the fine structure by adding organic solvents is accounted for by deaggregation due to the enhanced solubility.

It occurred to us that if the fine structure is induced by the aggregation, the S1 peak of **1**ThDod may split above its cmc. The cmc of cationic $C_{12}$-surfactants is about 10 mM,[17] so that we measured the spectrum of **1**ThDod at 0.05—30 mM by using 1 cm—0.1 mm width cuvettes. As shown in Fig. 2, the shape of the S1 peak was almost identical below 10 mM while clear shoulders were observed above 20 mM. Probably, the cmc of **1**ThDod is at this concentration. The result supports that the split of the S1 peak is significantly associated with the aggregation phenomenon.

The solvent effect on the absorption spectrum of 5-deazaisoalloxazine has not been examined systematically. Table 3 and Fig. 3 show that in general, the solvent effects on S1 of **2**MeEt are quite similar

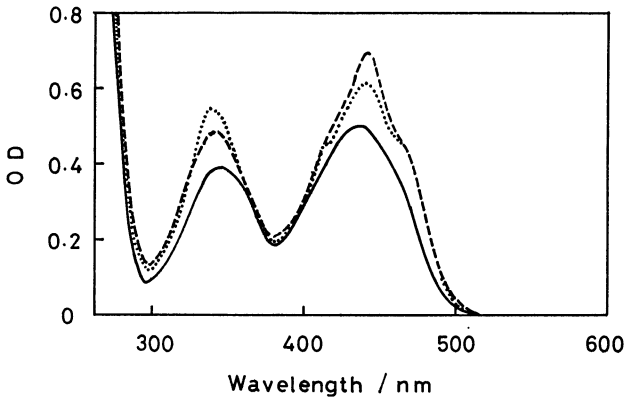



Fig. 2. Absorption spectra of **1**ThDod in water at different concentrations.
——: 10 mM, - - -: 20 mM, ·····: 30 mM. The Y-axis is normalized to $5\times10^{-5}$ M in 1 cm cell.

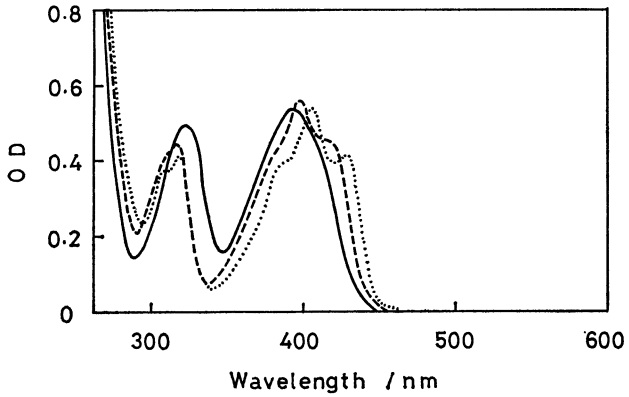



Fig. 3. Absorption spectra of **2**MeEt in water (——), acetonitrile (- - -), and benzene (·····).
[**2**MeEt]$=5.00\times10^{-5}$ M.

to those on **1**MeEt. The following points are noticeable differences: (i) the blue shift of S2 in nonpolar solvents is smaller than that of S2 of isoalloxazines (*e.g.*, 323 nm in water→318 nm in benzene) and (ii) the S2 peak in benzene and *o*-dichlorobenzene(*o*-DCB) has a shoulder at 310 nm (Fig. 3). A similar shoulder on S2 has been observed for tetra-*O*-butyrylriboflavin in carbon tetrachloride.[4] As expected, **2**MeDod (amphiphilic 5-deazaisoalloxazine) gave a three-banded S1 peak. Being different from that of amphiphilic isoalloxazines, the $\varepsilon_{max}$ of S2 (12300) is greater than any of S1 (9000, 10400, and 6040). Since the fine structure disappeared in surfactant micellar solutions, the association of 5-deazaisoalloxazine nuclei is responsible for the appearance of the fine structure.

Another amphiphilic 5-deazaisoalloxazine, **2**MeOct in water did not give a three-banded S1 peak but a broad absorption band spreading to 500—600 nm, and the $\varepsilon_{max}$ of S1 was smaller than that of S2 (Fig. 4). The longer-wavelength absorption band disappeared in 20 vol% ethanol. One may thus presume that **2**MeOct forms some aggregate in aqueous solution though it is not stable enough to induce a fine structure split. Attempting to stabilize the aggregate, we measured the spectrum of **2**MeOct ($5.00\times10^{-5}$ M) in KCl (0.25—2 mM) containing aqueous solutions and at high

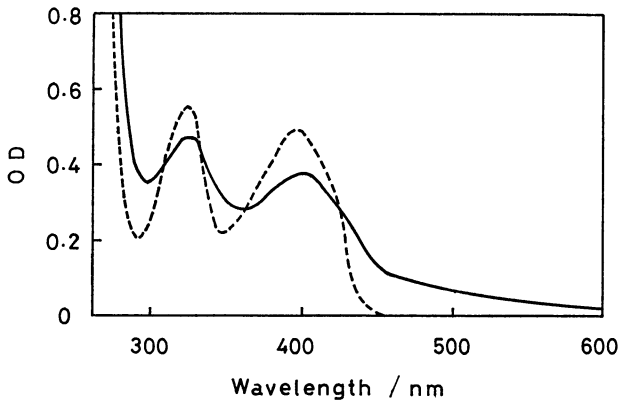



Fig. 4. Absorption spectra of **2**MeOct in water (——) and 20 vol% aqueous ethanol (- - -).
[**2**MeOct]$=5.00\times10^{-5}$ M.

**2**MeOct concentrations ($\approx2.40\times10^{-4}$ M). However, the spectra were apparently identical under these measurement conditions.

*Absorption Spectra in Water–Ethanol and Water–Pyridine Mixed Solvents.* Since the fine structure of amphiphilic isoalloxazines and 5-deazaisoalloxazines stems from the association of the nuclei, addition of organic solvents which causes the deaggregation of these amphiphiles would change the shape of the S1 speak. The spectral results are summarized in Tables 4 and 5. Addition of ethanol or pyridine changed the spectral shape of S1 of **2**MeEt from type A to type B (ethanol) or to type C(pyridine). More peculiar spectral changes were observed for **2**MeDod: a new, broad absorption band appeared at 500—600 nm region below 40 vol% ethanol and 20 vol% pyridine, and both the fine structure and the longer-wavelength absorption band disappeared above these solvent compositions (Fig. 5). We previously reported that a similar longer-wavelength absorption band is observed for **1**MeDod in ethanol (<45 vol%) and pyridine (<20 vol%) solutions.[6,9] In particular, the absorption band in aqueous pyridine was so strong (*e.g.*, $\varepsilon_{550}$ 4420 in

Table 4. Absorption maxima(nm) of 5-deazaisoalloxazines in ethanol–water mixed solvents (30 °C)[a]

| Ethanol (vol%) | **2**MeEt | | **2**MeOct | | **2**MeDod | |
|---|---|---|---|---|---|---|
| | S2 | S1 | S2 | S1 | S2 | S1 |
| 0 | 323, | 392 | 325, | 400 | 325, | 386<br>403<br>428 |
| 20 | 324, | 393 | 325, | 395 | 326, | 386<br>402<br>428 |
| 30 | | | | | 333, | 404(S)<br>428 |
| 40 | 323, | 395 | 324, | 397 | 324, | 397(S) |
| 60 | 322, | 396 | 323, | 397 | 323, | 397(S) |
| 80 | 321, | 397(S) | 322, | 398(S) | 322, | 398(S) |
| 100 | 318, | 398(S) | 320, | 398(S) | 320, | 398(S) |

a) (S) indicates that the peak has shoulders. Two-to-three values given for S1 indicate each absorption maximum for the fine structure split.

Table 5. Absorption maxima(nm) of 5-deazaisoalloxazines in pyridine-water mixed solvents (30 °C)[a)]

| Pyridine (vol%) | 2MeEt | | 2MeDod | |
|---|---|---|---|---|
| | S2 | S1 | S2 | S1 |
| 0 | 323, | 392 | 325, | 386<br>403<br>428 |
| 5 | | | 328, | 388<br>403<br>429 |
| 7 | | | 328, | 388<br>403<br>429 |
| 10 | 327, | 395 | 329, | 404(S)<br>429 |
| 14 | | | 330, | 405(S)<br>430 |
| 20 | 326, | 396 | 325, | 398 |
| 40 | 323, | 397 | 323, | 399 |
| 60 | 322, | 399 | 322, | 400 |
| 80 | 321, | 400 | 323, | 402 |
| 100 | 319, | 402<br>423 | 320, | 403<br>423 |

a) (S) indicates that the peak has shoulders. Two-to-three values given for S1 indicate each absorption maximum for the fine structure split.

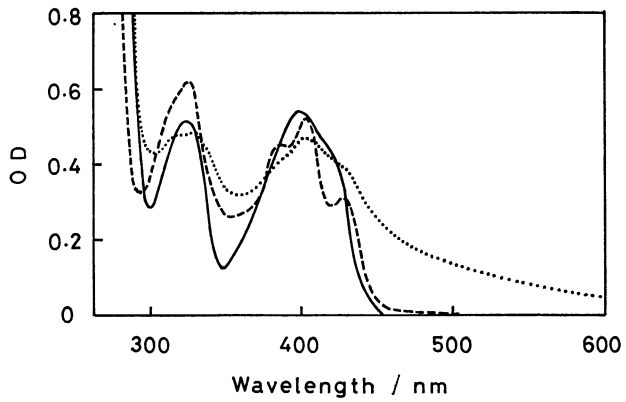



Fig. 5. Absorption spectra of **2**MeDod in water (- - -), 15 vol% pyridine(·····), and 40 vol% pyridine(——).
[**2**MeDod]$=5.00\times10^{-5}$ M.

18 vol% pyridine) that we ascribed the new absorption band to a specific charge transfer interaction between pyridine and isoalloxazine. A similar band was observable in aqueous solutions containing 4-(dimethylamino)pyridine, 3-indoleacetic acid, and *o*-aminobenzoic acid. Since the longer-wavelength absorption band of **2**MeDod in aqueous pyridine was not so strong and was rather comparable with that in aqueous ethanol, this band may not be ascribed to a simple charge transfer interaction. Probably, the electron-acceptability of **2**MeDod may be weaker than that of **1**MeDod.

In Fig. 6, $OD_{500}$ as a measure of the strength of the longer-wavelength absorption band is plotted as a function of solvent compositions. The $OD_{500}$ became largest at 33 vol% ethanol and 13 vol% pyridine and decreased sharply at higher solvent compositions.

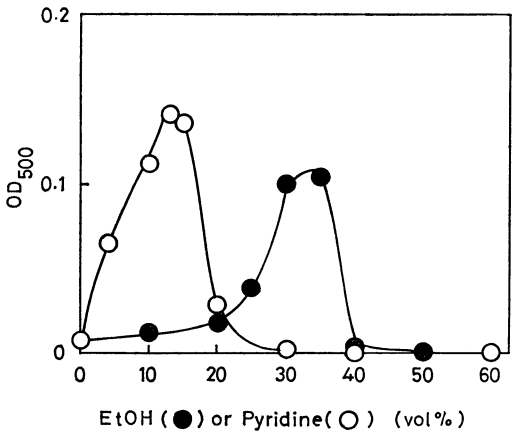



Fig. 6. $OD_{500}$ *vs.* solvent concentration.
[**2**MeDod]$=5.00\times10^{-5}$ M.

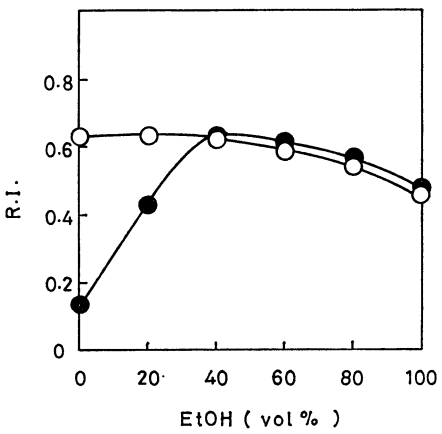


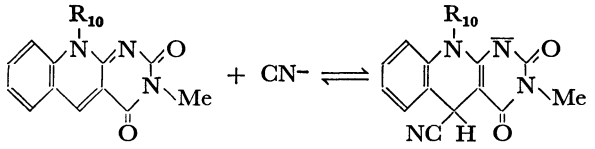

Fig. 7. Relative intensity(R.I.) of emission maxima *vs.* ethanol concentration.
○: **2**MeEt($5.00\times10^{-7}$ M), ●: **2**MeDod($5.00\times10^{-7}$).

These results indicate that there is a critical transition from the aggregated form to the monomeric form at 40 vol% ethanol or 20 vol% pyridine. In **1**MeDod and **2**MeDod as amphiphiles, (5-deaza)isoalloxazines are hydrophilic head groups. The "stacking" association between the head groups induces the fine structure split of S1. On the other hand, addition of organic solvents, more or less, would disturb the orientation of the aggregation form, and probably, the disturbed orientation would be responsible for the longer-wavelength absorption band. At the transition points, both the fine structure and the longer-wavelength absorption disappear because of the deaggregation to the monomeric forms.

*Fluorescent Spectra.* Figure 7 shows the influence of added ethanol on the relative intensity (R.I.) of fluorescent maxima (about 460 nm) of **2**MeEt and **2**MeDod in aqueous solution. The R.I. value of **2**MeEt was affected to a smaller extent by added ethanol, slightly decreasing with increasing ethanol concentration. On the other hand, the R.I. of **2**MeDod in water was very small, increasing rapidly with increasing ethanol concentration. The value at 40 vol% was almost equal to that of **2**MeEt. The results indicate that the fluorescent spectra of **2**MeDod

are also explicable on the basis of the aggregation-deaggregation phenomenon: the weak R.I. of **2**MeDod in water is ascribed to concentration quenching due to the association of 5-deazaisoalloxazine nuclei, and solvent-induced deaggregation leads to the increase in R.I. more than expected from the simple solvent effect. The 40 vol% ethanol composition where the abnormality of **2**MeDod disappears is exactly equal to the transition estimated from the absorption spectra.

*Nucleophilic Reactivity of Cyanide Ion.* It is interesting to assess whether the aggregation-deaggregation equilibrium of **2**MeDod is reflected by its reactivity. To test the possibility, we determined the rate constants for the nucleophilic reaction of cyanide ion toward 5-deazaisoalloxazines[18,19] in water–ethanol mixed solvents.




(1)

The reaction was first-order in cyanide ion and 5-deazaisoalloxazine. In Fig. 8, the second-order rate constants ($k_2$) are plotted as a function of the ethanol concentration. In aqueous solution, the $k_2$ values for **2**MeEt and **2**MeOct were 1.27 and 0.86 $M^{-1}\,s^{-1}$, respectively, whereas that for **2**MeDod was not detected at all under the present experimental conditions ($k_2 < 0.001\ M^{-1}\,s^{-1}$). The $k_2$ for **2**MeEt increased with increasing ethanol concentration. The reaction rate of **2**MeDod in 20 vol% ethanol was still very slow ($k_2 = 0.003\ M^{-1}\,s^{-1}$) but suddenly became fast in 30 vol% ethanol ($k_2 = 1.48\ M^{-1}\,s^{-1}$). **2**MeOct in most cases gave the medium rate constants. The rate constants for three 5-deazaisoalloxazines were almost identical above 40 vol% ethanol. The plots in Fig. 8 are well correlative to those in Figs. 6 and 7, indicating that the reactivity of 5-deazaisoalloxazines is significantly related to the aggregation-deaggregation equilibrium.

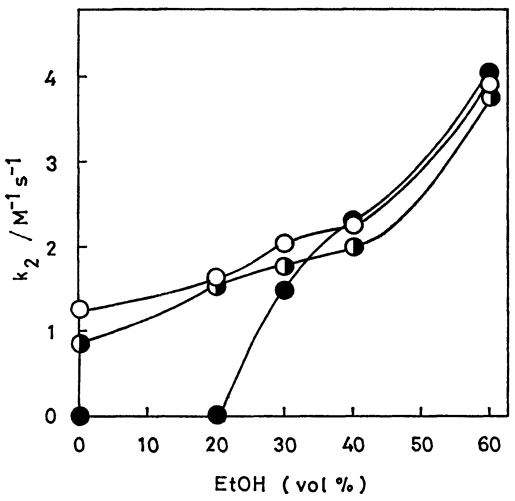



Fig. 8. Second-order rate constants for the reaction of cyanide ion ($1.00 \times 10^{-3}$ M) and 5-deazaisoalloxazines ($5.00 \times 10^{-5}$ M). ○: **2**MeEt, ◑: **2**MeOct, ●: **2**MeDod.

S. S. thanks Ministry of Education of Japan for Grant-in-Aid for Scientific Research (No. 555365).

## References

1) J. Koziol, *Photochem. Photobiol.*, **5**, 41 (1966).
2) J. Koziol, *Photochem. Photobiol.*, **9**, 45 (1969).
3) K. Yagi, J. Okuda, A. A. Dmitrovskii, and R. Honda, *J. Vitaminol.* **7**, 276 (1961).
4) A. Kotaki, M. Naoi, J. Okuda, and K. Yagi, *J. Biochem.*, **61**, 404 (1967).
5) A. J. W. G. Visser and F. Muller, *Helv. Chim. Acta*, **62**, 593 (1979).
6) S. Shinkai, A. Harada, Y. Ishikawa, O. Manabe, and F. Yoneda, *Chem. Lett.*, **1981**, 479.
7) K. Yagi and T. Ozawa, *Biochim. Biophys. Acta*, **56**, 413 (1962).
8) V. Massey and H. Ganther, *Biochemistry*, **4**, 1161 (1965).
9) S. Shinkai, A. Harada, Y. Ishikawa, O. Manabe, and F. Yoneda, *J. Chem. Soc., Perkin Trans. 2*, in press.
10) F. Yoneda, Y. Sakuma, M. Ichiba, and K. Shinomura, *J. Am. Chem. Soc.*, **98**, 830 (1976).
11) S. Shinkai, T. Yamashita, Y. Kusano, and O. Manabe, *J. Am. Chem. Soc.*, **104**, 563 (1982).
12) F. Yoneda, Y. Sakuma, S. Mizumoto, and R. Ito, *J. Chem. Soc., Perkin Trans. 1*, **1976**, 1805.
13) J. T. Martin and H. A. Standing, *J. Textil. Institute*, **1949**, 1671.
14) E. Rabinowitch and L. F. Epstein, *J. Am. Chem. Soc.*, **63**, 69 (1941).
15) O. Manabe, K. Nanzyo, S. Matsubara, M. Nagasaki, and H. Hiyama, *Kagaku To Kogyo*, **35**, 12 (1961).
16) H. Sato, M. Kawasaki, K. Kasatani, N. Nakashima, and K. Yoshihara, *Chem. Lett.*, **1980**, 1529.
17) J. H. Fendler and E. J. Fendler, "Catalysis in Micellar and Macromolecular Systems," Academic Press, New York, N. Y. (1975), Chap. 2.
18) R. L. Chan and T. C. Bruice, *J. Am. Chem. Soc.*, **99**, 6721 (1977).
19) M. S. Jorns and L. B. Hersh, *J. Biol. Chem.*, **251**, 4872 (1976).