Flavin Activation by Intramolecular Acid Catalysis at N(1) Position†

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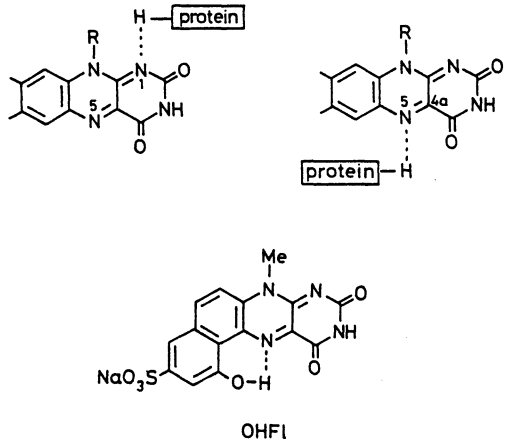
In order to assess the effect of intramolecular acid catalysis on the specific flavin reactivities, 3-methyl-10-(2-hydroxyphenyl)isoalloxazine (**1**(2OH)) and 3-methyl-10-(2-hydroxy-1-naphthyl)isoalloxazine (**2**(2OH)) were synthesized and the redox properties were compared with those of reference flavins such as 3-methyl-10-(2-methoxyphenyl)isoalloxazine (**1**(2OMe)) and 3-methyl-10-(2-methoxy-1-naphthyl)isoalloxazine (**2**(2OMe)). The pK_a values for **1**(2OH) and **2**(2OH) were determined to be 7.7 and 7.0, respectively, which were lower than that of 3-methyl-10-(4-hydroxyphenyl)isoalloxazine (**1**(4OH): pK_a 8.6). It is thus unlikely that 2′-OH and N(1) form a hydrogen bond at the initial state of the reaction (i.e., at the oxidized state). The X-ray crystallographic studies indicated that the phenyl ring makes an angle of 79.7° with the isoalloxazine ring and the 2′-OH group forms a hydrogen bond with methanol included in the crystal lattice. In acetonitrile at 30 °C 1-benzyl-1,4-dihydronicotinamide was not oxidized by **1**(2OMe), **1**(4OH), and **2**(2OMe). On the other hand, the oxidation took place with **1**(2OH) and **2**(2OH) which have an acidic OH group at the 2′-position of the 10-aryl substituent. The presence of the intramolecular acid catalysis suggests that 2′-OH and N(1) can interact at least at the transition state or at the final state of the reaction (i.e., at the reduced state). In general, the oxidized flavin adopts a "planar" structure which is sterically tense while the reduced flavin adopts a "bent" structure which is sterically relaxed. As the structure of the transition state is more or less similar to the reduced form, the hydrogen-bonding interaction could increase on going from the tense initial state to the relaxed transition state. This is a novel example for acid catalysis in flavin-mediated reactions.

Flavin coenzymes act as versatile redox catalysts in many biological systems, and it is now known that more than 100 proteins called flavoproteins require flavin coenzymes as their prosthetic groups.[1-3] In 1980, Massey and Hemmerich[4] proposed a stimulative hypothesis that a large number of flavoproteins may be classified into two groups from a viewpoint of their regiospecific reactivities: the first group is characterized by red semiquinone, high sulfite affinity, and bent structure of the reduced form, whereas the second group is characterized by blue semiquinone, low sulfite affinity, and almost planar structure of the reduced form. They explained that the most essential difference between these two groups is caused by regiospecific hydrogen bonding between flavin coenzymes and apoproteins:[4] that is, the first group has a hydrogen bond with N(1) leading to activation of the N(5) position while the second group has a hydrogen bond with the N(5) leading to activation of the C(4a) position. The situation reminds us of the role of 3-OH in pyridoxal coenzymes.

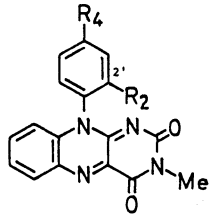
In order to obtain an insight into the latent capability of the hydrogen bond, we previously synthesized a flavin with hydrogen-bonded N(5) (OHFl: sodium 1-hydroxy-7-methyl-9,10-dioxo-7,9,10,11-tetrahydronaphtho-[1,2-g]pteridine-3-sulfonate).[5,6] We found that OHFl is "regioselectively" activated toward reactions involving 4a intermediates, for example, oxidation of thiols.[5,6] On the basis of several lines of evidence, the specific reactivity was ascribed to activation of the C(4a) position through hydrogen bonding with N(5).[5,6] Thus, the half of the hypothesis has been proved with OHFl, but the another half of the hypothesis (i.e., activation of the N(5) position through hydrogen bonding with N(1)) has been left equivocal. Based on our experience, it is extremely difficult to design a flavin derivative which involves hydrogen-bonded N(1).[7] Unlike OHFl, it is almost impossible to attach a fused aromatic ring to N(10) because of steric crowding. Also, $CH_2CH_2NH_3^+$ and CH_2COOH groups on N(10) were ineffective.[7] These examples of past failure suggest that the proximity effect plays a crucial role in the intramolecular acid catalysis.[5-7] Here, we wish to report the redox properties of 3-methyl-10-(2-hydrophenyl)isoalloxazine (**1**(2OH)) and 3-methyl-10-(2-hydroxy-1-naphthyl)isoalloxazine (**2**(2OH)). Examination of space-filling molecular models suggests that although it seems difficult for 2′-OH and N(1) to form a direct hydrogen bond at the oxidized state, the interaction is latently



OHFl

† Coenzyme Models 50.

possible at the sterically-relaxed reduced state. We have found that the acid catalysis arising from the hydrogen-bonding interaction (probably at the transition state) is observable in acetonitrile.



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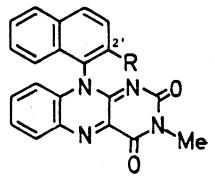


2

1(H): R_2=R_4=H
1(2OH): R_2=OH, R_4=H
1(2OMe): R_2=OMe, R_4=H
1(4OH): R_2=H, R_4=OH
1(4OMe): R_2=H, R_4=OMe

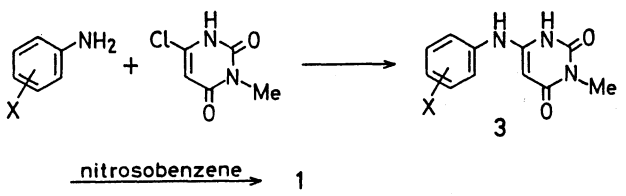
2(H): R=H
2(2OH): R=OH
2(2OMe): R=OMe

Experimental

Materials. Preparations of 3-methyl-10-phenylisoalloxazine (**1**(H)), 3-methyl-10-(1-naphthyl)isoalloxazine (**2**(H)), and 3-methyl-10-(2-methoxy-1-naphthyl)isoalloxazine (**2**(2OMe)) were described previously.[8]

3-Methyl-10-(2-hydroxy-1-naphthyl)isoalloxazine (2(2OH)). **2**(2OMe) (0.80 g; 2.08 mmol) was treated with 47% HBr (20 ml) at 130 °C. The progress of the reaction was followed by a TLC method. After 3 h the reaction mixture was diluted with water (100 ml) and neutralized by $NaHCO_3$. The precipitate was collected by filtration, washed with water, and then dried in vacuo. The yellow solid was recrystallized from acetic acid; mp >300 °C, yield 51%. Found: C, 63.90; H, 4.18; N, 12.99%. Calcd for $C_{21}H_{14}N_4O_3 \cdot CH_3CO_2H$: C, 64.18; H, 4.22; N, 13.02%. The elemental analysis indicates that the crystals contain one mol of acetic acid per one mol of **2**(2OH). We thus repeated recrystallization from ethanol; mp >300 °C, yield 49%. Found: C, 66.06; H, 4.76; N, 13.45%. Calcd for $C_{21}H_{14}N_4O_3 \cdot C_2H_5OH$: C, 66.33; H, 4.84; N, 13.46%. The result suggests that one mol of ethanol is included in the crystals. Toda et al.[9] demonstrated that molecules having crossed planes and the hydroxyl group(s) at the suitable position(s) easily form host-guest complexes in the solid state. Probably, **2**(2OH) satisfies this structural prerequisite to act as host molecules. Since the inclusion of acetic acid is very unfavorable for the titration experiments, we used the sample containing ethanol all through the present study.

Compounds **1** were synthesized according to the following scheme. The synthetic method was originally exploited by Yoneda et al.[10]



Scheme 1.

3-Methyl-6-(2-hydroxyanilino)uracil (3(2OH)). 3-Methyl-6-chlorouracil (1.00 g; 6.23 mmol) and *o*-aminophenol (2.71 g; 24.8 mmol) were stirred at 180—190 °C under a nitrogen stream. After 10 min the precipitate was formed from the melted reaction mixture. The product was well washed with 4 M§HCl; mp 273—275 °C, yield 68%; IR(KBr) ν_{OH}3400 cm^{-1} $\nu_{C=O}$ 1700, 1740 cm^{-1}. Found: C, 56.36; H, 4.76; N, 17.95%. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02%.

3-Methyl-6-(2-methoxyanilino)uracil (3(2OMe)). 3-Methyl-6-chlorouracil (1.00 g; 6.23 mmol) and *o*-methoxyaniline (3.06 g; 24.8 mmol) were stirred at 150—160 °C under a nitrogen stream. After 20 min the precipitate was formed from the melted reaction mixture. The product was well dispersed in ethanol and the precipitate was recovered by filtration. This operation was repeated once again; mp 261—263 °C, yield 85%; IR(KBr) ν_{NH} 3240 cm^{-1}, $\nu_{C=O}$ 1700, 1715 cm^{-1}. Found: C, 58.30; H, 5.18; N, 17.26%. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00%.

3(4OH) and **3**(4OMe) were prepared from *p*-aminophenol and *p*-methoxyaniline in manners similar to those described for **3**(2OH) and **3**(2OMe), respectively. We thus omit the detailed description of the experimental methods and recorded their analytical data.

3-Methyl-6-(4-hydroxyanilino)uracil (3(4OH)). Mp >300 °C, yield 82%; IR(KBr) ν_{OH} 3300 cm^{-1}, $\nu_{C=O}$ 1700, 1720 cm^{-1}. Found: C, 56.22; H, 4.77; N, 17.94%. Calcd for $C_{11}H_{11}N_3O_3$: C, 56.65; H, 4.75; N, 18.02%.

3-Methyl-6-(4-methoxyanilino)uracil (3(4OMe)). Mp 290—292 °C, yield 88%; IR(KBr) ν_{NH} 3250 cm^{-1}, $\nu_{C=O}$ 1720 cm^{-1}. Found: C, 58.26; H, 5.26; N, 17.17%. Calcd for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 17.00%.

3-Methyl-10-(2-hydroxyphenyl)isoalloxazine (1(2OH)). **3**(2OH) (1.90 g; 8.15 mmol) and nitrosobenzene (2.62 g; 24.5 mmol) were refluxed in 40 ml of acetic acid-acetic anhydride (1 : 4 v/v) under a nitrogen stream. The progress of the reaction was followed by a TLC method. After 30 min the reaction mixture was cooled and concentrated in vacuo. The residual oil was subjected to preparative TLC (silica gel, chloroform-methanol 30 : 1 v/v). There were two yellow spots on the TLC plate, which were identified to be **1**(2OH) (R_f=0.5) and 3-methyl-10-(2-acetoxyphenyl)isoalloxazine (R_f=0.7). We thus collected two isoalloxazines together and the mixture was refluxed for 1 h in 16 ml of 4 M HCl to hydrolyze the ester. The solution was neutralized by $NaHCO_3$ and the precipitate was recrystallized from acetic acid; mp >300 °C, yield 12%; IR(KBr) ν_{OH} 3350 cm^{-1}, $\nu_{C=O}$ 1660, 1710 cm^{-1}. ^1H NMR (Me_2SO-d_6): δ=3.27 (3H, NCH_3), 6.88 (1H, 9-H), 7.11 (1H, 5′-H), 7.16 (1H, 3′-H), 7.28 (1H, 6′-H), 7.49 (1H, 7-H), 7.65 (1H, 4′-H), 7.81 (1H, 8-H), 8.25 (1H, 6-H), 9.96 (1H, OH). Found: C, 63.90; H, 3.83; N, 17.38%. Calcd for $C_{17}H_{12}N_4O_3$: C, 63.75; H, 3.78; N, 17.49%.

3-Methyl-10-(4-hydroxyphenyl)isoalloxazine (1(4OH)). This compound was synthesized in a manner similar to that described for **1**(2OH). Mp >300 °C, yield 14%; IR (KBr) ν_{OH} 3370 cm^{-1}, $\nu_{C=O}$ 1660, 1720 cm^{-1}. Found: C, 63.59; H, 3.74; N, 17.30%. Calcd for $C_{17}H_{12}N_4O_3$: C, 63.75; H, 3.78; N, 17.49%.

3-Methyl-10-(4-methoxyphenyl)isoalloxazine (1(2OH)). This compound was synthesized from **3**(2OMe) (1.50 g; 6.07 mmol) and nitrosobenzene (1.95 g; 18.2 mmol) in 40 ml of acetic acid-acetic anhydride (1 : 1 v/v) at the reflux temperature under a nitrogen stream. After 30 min the reaction

§ 1 M=1 mol dm^{-3}.

mixture was cooled and concentrated in vacuo. The residual solid was recrystallized from acetic acid in the presence of activated charcoal: mp >300 °C, yield 47%; IR(KBr) $\nu_{C=O}$ 1660, 1710 cm^{-1}, ν_{C-O-C} 1270 cm^{-1}. Found: C, 64.52; H, 4.21; N, 16.67%. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.66; H, 4.22; N, 16.76%.

3-Methyl-10-(4-methoxyphenyl)isoalloxazine (1(4OMe)). This compound was synthesized in a manner similar to that described for **1**(2OMe). Mp >300 °C, yield 45%: IR(KBr) $\nu_{C=O}$ 1655, 1710 cm^{-1}, ν_{C-O-C} 1270 cm^{-1}. Found: C, 64.66; H, 4.19; N, 16.64%. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.66; H, 4.22; N, 16.76%.

Kinetic Measurements. The aqueous reaction with 1-benzyl-1,4-dihydronicotinamide was carried out aerobically at 30 °C and μ=0.1 with KCl. Under the aerobic conditions flavins are recycled automatically, so that the decrease in the absorption band of 1-benzyl-1,4-dihydronicotinamide (360 nm) satisfies the first-order equation. The medium pH was maintained by 0.01 M phosphate (pH<7.0), 0.01 M *N*-ethylmorpholine (7.0<pH<8.0), 0.01 M borate (8.0<pH<9.5), and 0.01 M carbonate (pH>9.5).

The reaction with 1-benzyl-1,4-dihydronicotinamide in acetonitrile was carried out anaerobically at 30 °C. The decrease in the absorption band of flavins (440 nm) satisfied the first-order equation in the presence of excess 1-benzyl-1,4-dihydronicotinamide (2.09×10^{-3} M; [flavin]=5.00×10^{-5} M). Since the pseudo-first-order rate constants (k_1') were first-order in 1-benzyl-1,4-dihydronicotinamide, the results were discussed with the second-order rate constants ($k_2=k_1'$/[1-benzyl-1,4-dihydronicotinamide]).

X-Ray Crystal Structure Analysis. Crystal data. $C_{17}H_{12}N_4O_3$, CH_3OH, *F.W.* 352.3, monoclinic, space group *C*2/*c*, *a*=23.853(3), *b*=10.342(1), *c*=13.894(1) Å, β=100.16(1)°, *V*=373.9(5) Å^3, D_c=1.387 g cm^{-3} for *Z*=8.

Orange colored, prismatic crystals were obtained by the recrystallization from a methanol-benzene mixed solvent. The crystal used had approximate dimensions of 0.45×0.23×0.18 mm.

Integrated intensities were collected on a Rigaku rotating anode, four-circle diffractometer using nickel-filtered Cu $K\alpha$ radiation(λ=1.5418 Å) by the θ-2θ scan technique up to 2θ=120°. The 2θ scan rate was 8° min^{-1}, and the scan width $\Delta2\theta=(2.4+0.3\tan\theta)°$. Backgrounds were counted for 4 s at both ends of a scan. Three standard reflections measured after every 100 reflections to monitor the stability and orientation of the crystal showed no significant decay through the experiment. Of the 2885 reflections measured, number of reflections observed was 2114 ($|F_o|>2\sigma(F_o)$, where σ is the standard deviation obtained from the counting statistics). Usual Lorentz and polarization corrections were applied but absorption correction was ignored [μ(Cu $K\alpha$)=8.50 cm^{-1}].

The structure was solved by the direct method (MULTAN 78).[11] All the non-hydrogen atoms could be located on successive Fourier syntheses. The hydrogen atoms except those of methanol, the crystalline solvent, were located on the calculated positions, which were confirmed on the difference Fourier map. The structure was refined by the block-diagonal least-squares procedure (HBLS V)[12] with anisotropic thermal parameters for nonhydrogen atoms and isotropic ones for hydrogen atoms. During the course of the refinement, the only remaining peak on the difference Fourier map was found to be assigned as the alternative position of the disordered methanol oxygen atom, O(5), which was included in the refinement with 20% occupancy. The final *R* value was 0.056. The weighting schemes used at the final cycle of the refinement were $w=(\sigma^2+0.01886|F_o|+0.00254|F_o|^2)^{-1}$ for $|F_o|>0$ and $w=0$ for $|F_o|=0$. The final atomic parameters are given in Table 1.[§§] Atomic scattering factors were taken from those of International Tables for X-Ray Crystallography, Vol. IV.[13] Computations were done on an ACOS S850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University.

Table 1. Final Atomic Parameters with Estimated Standard Deviations in Parentheses

Nonhydrogen atoms with equivalent isotropic temperature factors.[27]

Atom	*x*	*y*	*z*	B_{eq}/Å^2
C(1)	0.18654(15)	−0.0146(4)	0.0355(3)	5.27
C(2)	0.16813(17)	−0.1074(5)	−0.0318(4)	7.09
C(3)	0.20136(18)	−0.1410(5)	−0.0996(4)	6.95
C(4)	0.25281(15)	−0.0821(4)	−0.1036(3)	5.20
C(5)	0.27215(11)	0.0138(3)	−0.0342(2)	3.47
C(6)	0.23899(12)	0.0474(3)	0.0362(3)	3.64
C(7)	0.30620(11)	0.1907(3)	0.10837(19)	3.25
C(8)	0.34319(11)	0.1652(3)	0.03753(19)	3.11
C(9)	0.41213(12)	0.3052(3)	0.1110(3)	4.28
C(10)	0.32745(12)	0.2836(3)	0.1876(3)	3.92
C(11)	0.40526(16)	0.4235(5)	0.2627(3)	6.78
C(12)	0.35763(11)	0.0485(3)	−0.10735(19)	3.18
C(13)	0.34356(13)	0.1033(4)	−0.1984(3)	4.09
C(14)	0.37549(13)	0.0739(4)	−0.2698(3)	4.38
C(15)	0.42118(13)	−0.0102(4)	−0.2473(3)	4.13
C(16)	0.43502(13)	−0.0657(3)	−0.1562(3)	4.16
C(17)	0.40277(12)	−0.0367(3)	−0.0841(2)	3.53
C(18)	0.50905(10)	−0.3136(3)	0.11610(16)	7.90
N(1)	0.25703(9)	0.1377(3)	0.10721(15)	3.59
N(2)	0.32338(10)	0.0769(3)	−0.03314(18)	3.14
N(3)	0.38039(9)	0.3354(3)	0.18365(17)	4.45
N(4)	0.39222(10)	0.2207(3)	0.03773(16)	3.63
O(1)	0.30078(10)	0.3112(3)	0.25191(19)	5.42
O(2)	0.45882(10)	0.3564(3)	0.11613(15)	6.19
O(3)	0.41308(12)	−0.0874(4)	0.0071(3)	5.04
O(4)	0.47930(18)	−0.2888(6)	0.0222(4)	5.81
O(5)	0.5111(4)	−0.2108(8)	0.0609(7)	3.3

Hydrogen atoms with isotropic temperature factors

Atom	*x*	*y*	*z*	B_{eq}/Å^2
H(1)	0.1644(13)	0.004(4)	0.086(3)	3.0(7)
H(2)	0.1327(17)	−0.149(5)	−0.038(3)	6.0(11)
H(3)	0.1841(18)	−0.203(4)	−0.155(3)	5.7(11)
H(4)	0.2781(16)	−0.102(4)	−0.149(3)	4.8(9)
H(5)	0.3789(14)	0.496(4)	0.251(3)	4.4(9)
H(6)	0.493(3)	0.450(6)	0.258(4)	8.4(14)
H(7)	0.401(3)	0.379(6)	0.321(4)	9.3(15)
H(8)	0.3062(14)	0.166(4)	−0.212(3)	3.4(8)
H(9)	0.3663(13)	0.117(4)	−0.340(3)	2.7(7)
H(10)	0.4434(13)	−0.032(3)	−0.297(3)	2.9(7)
H(11)	0.4684(11)	−0.122(3)	−0.134(2)	1.5(6)
H(12)	0.4473(16)	−0.154(4)	0.020(3)	5.1(10)

Results and Discussion

Crystal Structure of 1(2OH). The molecular struc-

§§ Tables of anisotropic thermal parameters of nonhydrogen atoms, coordinates of hydrogen atoms, and observed and calculated structure factors are kept at the Chemical Society of Japan, Document No. 8803.

ture is shown in Fig. 1.[14] The atom numbers in Fig. 1 are used only for the crystal structure. Selected bond lengths and bond angles are given in Table 2. The remarkable feature of the structure is that three six-membered hetero rings in the isoalloxazine ring is not coplanar: the dihedral angle between the rings I and II is 2.2(1)° and that between II and III also 2.2(1)°. The ring IV attached to the N(2) atom of the ring II makes an angle of 79.7° with the ring II. The *o*-hydroxyl group of the ring IV is, therefore, located too far from the N(4) atom of the ring III to form the hydrogen bond [O(3)···N(4)=3.265(3) and H(12)···N(4)=4.11(5) Å].

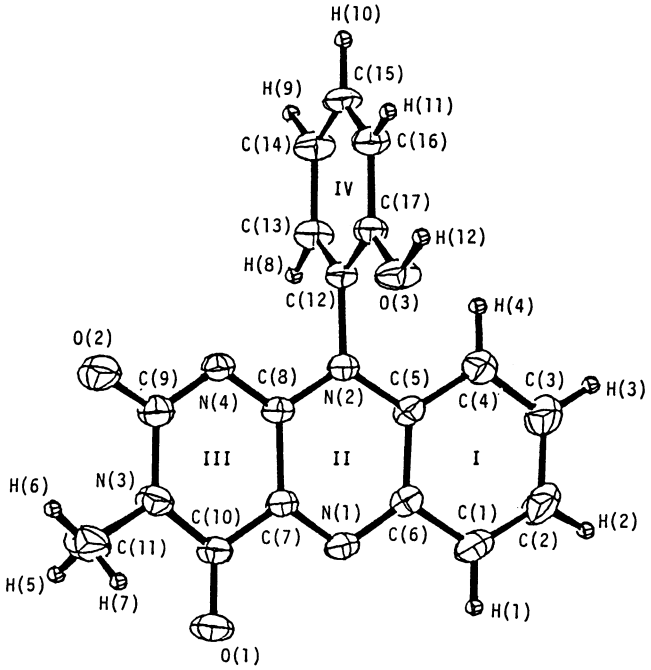


Fig. 1. A perspective view[14] of **1**(2OH). Nonhydrogen atoms are expressed as thermal ellipsoids with 20% probability level, and hydrogen atoms as spheres with B=1.0 Å^2.

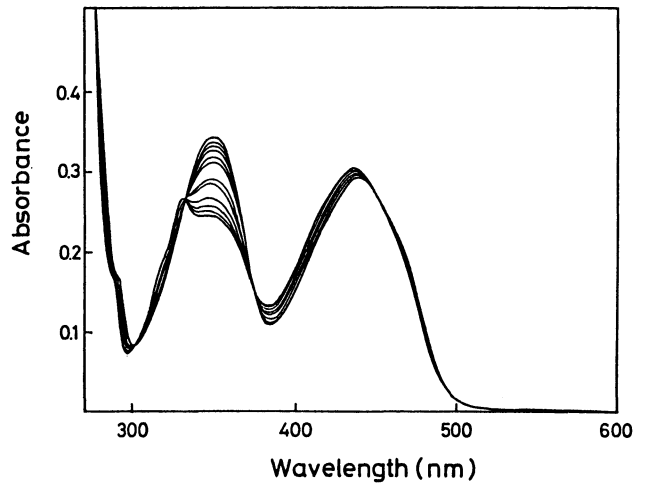


Fig. 2. pH Dependent spectral change in **2**(2OH) (4.00×10^{-5} M).

Instead, the hydroxyl group is bound by the hydrogen bond with the methanol molecule, the solvent of crystallization. However, the methanol showed a positional disorder: the major component, C(18)H_3O(4)H with 80% occupancy and the minor, C(18)H_3O(5)H with 20% occupancy [O(3)···O(4)=2.599(4), O(3)-H(12)=1.06(5), H(12)···O(4)=1.59(4) Å, and O(3)-H(12)-O(4)=158(4)°, and O(3)···O(5)=2.652(10), H(12)···O(5)=1.64(4) Å, and O(3)-H(12)-O(5)=159(4)°].

Phototitration of 1(2OH), 1(4OH), and 2(2OH). The pK_a values of the 2′-OH in three isoalloxazines were estimated by phototitration. As shown in Fig. 2, the absorption spectra of **2**(2OH) changed as a function of pH. This change is attributed to the dissociation of the 2′-OH because this group is the sole dissociable group in **2**(2OH) at pH 4—10. In Fig. 3 we

Table 2. Bond Lengths (l/Å) and Bond Angles (ϕ/°) with Estimated Standard Deviations in Parentheses

Bond lengths			
C(1)-C(2)	1.358(6)	C(1)-C(6)	1.405(5)
C(2)-C(3)	1.379(4)	C(3)-C(4)	1.380(6)
C(4)-C(5)	1.403(5)	C(5)-C(6)	1.406(4)
C(7)-C(8)	1.457(4)	C(7)-C(10)	1.482(4)
N(1)-C(6)	1.371(4)	N(1)-C(7)	1.292(4)
N(2)-C(5)	1.383(4)	N(2)-C(8)	1.363(4)
N(2)-C(12)	1.454(4)	N(3)-C(9)	1.400(5)
N(3)-C(10)	1.382(5)	N(3)-C(11)	1.469(6)
N(4)-C(8)	1.302(4)	N(4)-C(9)	1.362(4)
O(1)-C(10)	1.218(4)	O(2)-C(9)	1.224(5)
C(12)-C(13)	1.373(5)	C(12)-C(17)	1.385(4)
C(13)-C(14)	1.387(7)	C(14)-C(15)	1.386(5)
C(15)-C(16)	1.376(5)	C(16)-C(17)	1.399(5)
O(3)-C(17)	1.354(4)		
C(18)-O(4)[a]	1.395(7)	C(18)-O(5)[b]	1.32(2)
Bond angles			
C(2)-C(1)-C(6)	120.4(4)	C(1)-C(2)-C(3)	119.6(4)
C(2)-C(3)-C(4)	122.5(4)	C(3)-C(4)-C(5)	118.2(4)
C(4)-C(5)-C(6)	119.7(3)	C(3)-C(5)-N(2)	121.6(3)
C(6)-C(5)-N(2)	118.7(3)	C(1)-C(6)-C(5)	119.5(3)
C(1)-C(6)-N(1)	118.8(3)	C(5)-C(6)-N(1)	121.7(3)
C(8)-C(7)-C(10)	116.8(3)	C(8)-C(7)-N(1)	124.8(3)
C(10)-C(7)-N(1)	118.4(3)	C(7)-C(8)-N(2)	115.7(3)
C(7)-C(8)-N(4)	124.7(3)	N(2)-C(8)-N(4)	119.6(3)
N(3)-C(9)-N(4)	121.4(3)	N(3)-C(9)-O(2)	117.6(3)
N(4)-C(9)-O(2)	121.0(3)	C(7)-C(10)-N(3)	114.6(3)
C(7)-C(10)-O(1)	122.8(3)	N(3)-C(10)-O(1)	122.6(3)
C(6)-N(1)-C(7)	117.8(3)	C(5)-N(2)-C(8)	121.2(3)
C(5)-N(2)-C(12)	120.2(3)	C(8)-N(2)-C(12)	118.7(3)
C(9)-N(3)-C(10)	123.8(3)	C(9)-N(3)-C(11)	117.5(3)
C(10)-N(3)-C(11)	118.6(3)	C(8)-N(4)-C(9)	118.6(3)
C(13)-C(12)-C(17)	122.1(3)	C(13)-C(12)-N(2)	119.6(3)
C(17)-C(12)-N(2)	118.3(3)	C(12)-C(13)-C(14)	119.5(3)
C(13)-C(14)-C(15)	119.0(3)	C(14)-C(15)-C(16)	121.4(3)
C(15)-C(16)-C(17)	119.7(3)	C(12)-C(17)-C(16)	118.2(3)
C(12)-C(17)-O(3)	118.0(3)	C(16)-C(17)-O(3)	123.7(3)
C(17)-O(3)-H(12)	115(3)		
Hydrogen bonds			
O(3)···O(4)[a]	2.599(4)	O(3)···O(5)[b]	2.652(10)
O(3)-O(12)	1.06(5)		
H(12)···O(4)[a]	1.59(4)	H(12)···O(5)[b]	1.64(4)
O(3)-H(12)···O(4)	158(4)	O(3)-H(12)···O(5)[b]	159(4)

a) Occupancy 0.8. b) Occupancy 0.2.

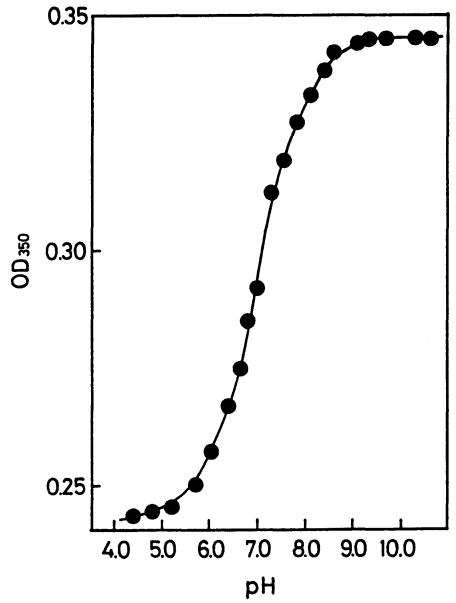


Fig. 3. Plot of OD_{350} vs. pH for **2**(2OH).

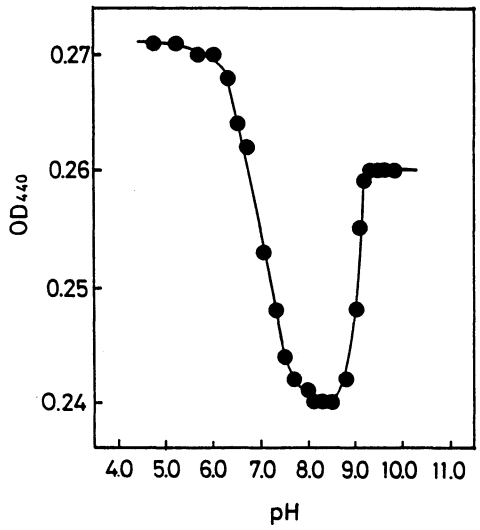
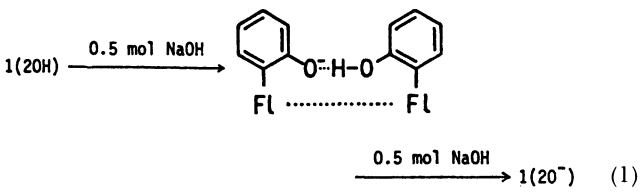


Fig. 4. Plot of OD_{440} vs. pH for **1**(2OH) (4.00×10^{-5} M).

plotted OD_{350} against medium pH. The analysis of this plot by a titration equation afforded pK_a=7.0 for **2**(2OH). Similarly, the pK_a of the 2′-OH in **1**(4OH) was estimated to be 8.6. On the other hand, **1**(2OH) showed a spectral change different from others: with increasing medium pH the absorbance at 320—470 nm region first decreases with isosbestic points (314 and 478 nm) and then increases with different isosbestic points (275 and 490 nm). The plot of OD_{440} vs. pH gave a minimum absorbance at pH 8.3 (Fig. 4). We carefully examined the correlation between the spectral change and the consumed NaOH. It was found that 0.5 mol of NaOH is consumed per one mol of **1**(2OH) at pH 5.0—8.3 and another 0.5 mol of NaOH is consumed at pH 8.3—9.3. The finding supports that the biphasic spectral change is caused by the specific interaction between neutral **1**(2OH) and dissociated **1**($2O^-$). It is known that flavins easily form stacked aggregates in aqueous solution.[15,16] This is due to the poor solubility of flavins in water and also due to the large dipole moment across flavin molecules.[17] If this is the case in **1**(2OH), the flavin-flavin interaction would become maximum at the half-titrated pH, for example, owing to intermolecular hydrogen bonding between neutral **1**(2OH) and dissociated **1**($2O^-$) (Eq. 1). Anyway, we estimated the pK_a of **1**(2OH) to be the half-titrated pH 7.7.



(1)

It is not clear yet why such a biphasic spectral

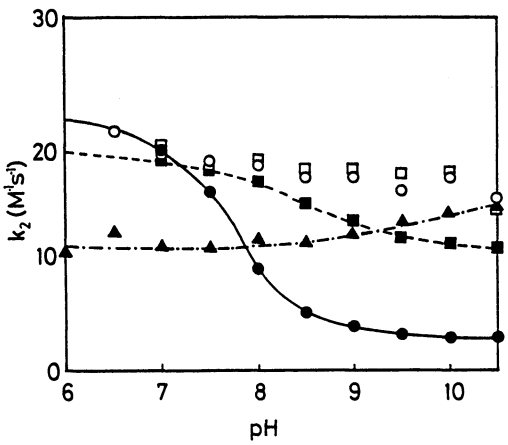


Fig. 5. pH Dependence of the k_2 for the reaction of **1**(2.75×10^{-5} M) and BNAH (7.91×10^{-5} M): ▲ **1**(H), ● **1**(2OH), ○ **1**(2OMe), ■ **1**(4OH), □ **1**(4OMe).

change is observed for **1**(2OH) but not for **2**(2OH). We have found that the rotation of the aryl group at the N(10) position of flavins is considerably restricted because of the steric effect and the rotation of the naphthyl group is much more difficult than the phenyl group.[8,18] Probably, the two isoalloxazines in **2**(2OH) cannot come closer enough to interact electronically because of steric hindrance arising from the 10-naphthyl group.

The pK_a values determined above are generally lower than those for analogous compounds (e.g., 9.71 for *o*-aminophenol and 10.30 for *p*-aminophenol).[19] Furthermore, **1**(2OH) has the pK_a lower than **1**(4OH). When the OH group forms the stable intramolecular hydrogen bond, the pK_a should be raised. Hence, these pK_a data suggest that **1**(2OH) and **2**(2OH) do not form the stable hydrogen bond at least at the initial state (i.e., oxidized state). The conclusion obtained in solution is in line with that obtained from the X-ray crys-

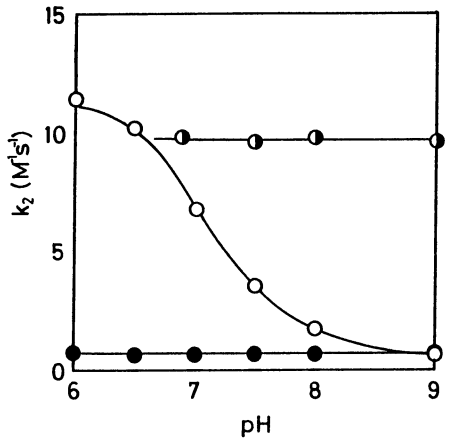
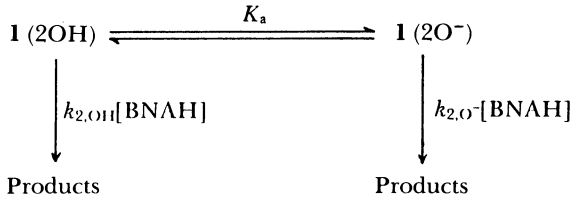


Fig. 6. pH Dependence of the k_2 for the reaction of **2**(2.75×10^{-5} M) and BNAH (7.91×10^{-5} M): ○ **2**(H), ○ **2**(2OH), ● **2**(2OMe).

tallographic analysis.

pH Dependence for the Reaction with 1-Benzyl-1,4-dihydronicotinamide. The hydrogen-transfer reaction from 1-benzyl-1,4-dihydronicotinamide (BNAH) to five flavins was carried out at pH 7.0—10.4. As a prelude to kinetic studies, we confirmed that this reaction is not catalyzed by buffers (10^{-3}—10^{-1} M) in water. In Figs. 5 and 6 the second-order rate constants (k_2) are plotted against medium pH. It is seen from these figures that the k_2 values for **1**(H), **1**(2OMe), **1**(4OMe), **2**(H), and **2**(2OMe) which have no dissociable group at this pH range are almost constant. On the other hand, **1**(2OH), **1**(4OH), and **2**(2OH) gave the sigmoid curves which could be expressed by Scheme 2 (**1**(2OH) is taken as an example). Thus, the k_2 is expressed by Eq. 2 including the true second-order rate constants for each species, $k_{2,OH}$ and k_{2,O^-}.



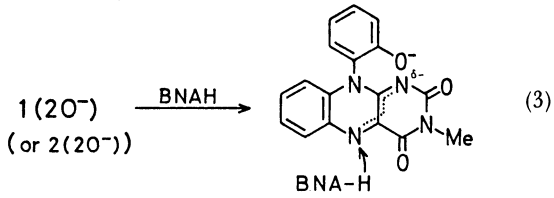
Scheme 2.

$$k_2 = \frac{k_{2,OH}[H^+] + k_{2,O^-}K_a}{[H^+] + K_a} \quad (2)$$

The $k_{2,OH}$ and k_{2,O^-} determined by computer-assisted curve-fitting with Eq. 2 are summarized in Table 3. Examination of Table 3 reveals that (i) in **1**(2OH) and **2**(2OH) the $k_{2,OH}$ values are greater by 8.1—27 fold than the k_{2,O^-} values but in **1**(4OH) the $k_{2,OH}$ is greater only by 1.8 fold than the k_{2,O^-} and (ii) the $k_{2,OH}$ for **2**(2OH) is greater by 2 fold than that for the 2′-methoxy counterpart but the $k_{2,OH}$ values for **1**(2OH) and **1**(4OH) are almost equal to those for the 2′-methoxy counterparts (less than 1.3). As described above, the aryl group at the N(10) position cannot enjoy the coplanarity with the isoalloxazine ring.[8,18] Therefore, the resonance effect of the 10-substituent should be relatively small. In fact, the dissociation of the 2′-OH group in **1**(4OH) decreased the rate constant only by a factor of 1.8. In contrast, the large rate decrease was observed for **1**(2OH) and **2**(2OH). The difference observed between the 2′-substituent and the 4′-substituent would be explained by the spatial electrostatic effect but not by the resonance effect: that is, reduction of these flavins develops an anionic charge on the N(1) position which is very close to the 2′-oxide anion (Eq. 3). Therefore, the progress of this reaction would induce an energetically-unfavorable electrostatic repulsion. Probably, this is why the large diffence was observed for **1**(2OH) and **2**(2OH).

Table 3. pK_a of the 2′-OH Group and the Second-Order Rate Constants (k_2) in an Aqueous System (30 °C)

Flavin	pK_a	$k_2/M^{-1}s^{-1}$ Neutral ($k_{2,OH}$)	$k_2/M^{-1}s^{-1}$ Dissociated (k_{2,O^-})
1(H)	—	11.6	—
1(2OH)	7.7	22.0	2.70
1(2OMe)	—	16.6	—
1(4OH)	8.6	18.0	9.94
1(4OMe)	—	16.8	—
2(H)	—	9.71	—
2(2OH)	7.0	13.3	0.50
2(2OMe)	—	0.66	—



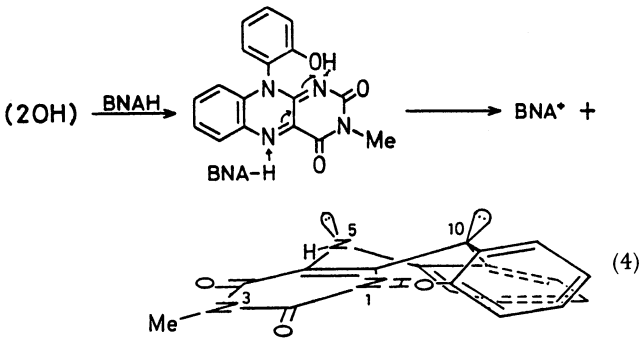
(3)

More important is the finding (ii). The rate difference between **2**(2OH) and **2**(2OMe) may be rationalized in terms of hydrogen bonding. On the other hand, the absence of the rate difference between **1**(2OH) and **1**(2OMe) may be due to the aggregate formation discovered through the photititration. Subsequently, we carried out the reaction in acetonitrile where the effect of hydrogen bonding would be more strengthened.

Reaction with BNAH in Acetonitrile. It is known that the electrostatic and the hydrogen-bonding effect appear more clearly in organic solvents (especially, in aprotic solvents). For example, the influence of hydrogen bonding with trichloroacetic acid or trifluoroacetic acid on the absorption spectra of flavins was studied in detail in CCl_4.[20] Similarly, Sigman et al.[21,22] demonstrated that an anionic charge placed in the vicinity of N(1) in 1,4-dihydronicotinamide effectively

enhances the reducing ability in acetonitrile but not in an aqueous system. Yagi et al.[20] also found that the photoreduction of riboflavin tetrabutyrate by *N*-benzyl-*N*,*N*′-dimethylethylenediamine in CCl_4 is weakly catalyzed (1.2—1.4 fold) by strong proton acids such as trichloroacetic acid and trifluoroacetic acid. This suggests that the reduction of flavins may be general-acid-catalyzed in aprotic solvents such as CCl_4 although the position of the acid catalysis cannot be specified. We thus chose acetonitrile to obtain a further insight into the hydrogen bonding effect.

In the anaerobic reaction with BNAH (2.09×10^{-3} M) we found that only **1**(2OH) and **2**(2OH) (5.00×10^{-5} M) can oxidize BNAH with $k_2=2.06\times10^{-2}$ $M^{-1}s^{-1}$ and 7.31×10^{-2} $M^{-1}s^{-1}$, respecticely. The reaction with other six flavins was not detected at all ($k_2<10^{-5}$ $M^{-1}s^{-1}$). It is particularly worthy mentioning that **1**(2OH) is reactive but **1**(4OH) is totally unreactive in acetonitrile. The essential difference in the reactivity indicates that the reaction with **1**(2OH) (and also with **2**(2OH)) is acid catalyzed intramolecularly. It is known that oxidized flavins employ the planar structure with sp^2-hybridized N(5) and N(10) while reduced flavins employ the bent structure folded through sp^3-hybridized N(5) and N(10).[23-25] Thus, the steric crowding in oxidized flavins is significantly relaxed in folded reduced flavins.[8,18] Examination of Corey-Pauling-Koltun models suggests that it is not impossible to form a hydrogen bond in reduced **1**(2OH) (and also **2**(2OH)) (Eq. 4).



(4)

Presumably, intramolecular general-acid catalysis by the 2′-OH begins when the anionic charge is partially developed on N(1) and the isoalloxazine ring is partially folded. This implies that the transition state of this reaction lies more or less close to the final state.

We also found that the reaction with BNAH is general-acid-catalyzed by added acidic species in acetonitrile. As shown in Fig. 7, the k_2 increased linearly with increasing *p*-cyanophenol concentration: k_{ga} and 1.8×10^{-2} $M^{-2}s^{-1}$ for **2**(2OMe). If the acidity difference between 2′-OH and *p*-cyanophenol (pK_a 7.95)[19] is neglected, the effective concentration (k_2/k_{ga}) of the 2′-OH group in general-acid catalysis amounts to 0.71 M for **1**(2OH) and 4.1 M for **2**(2OH). These values are relatively small and are classified as a less effective contribution of general-acid catalysis.[26] It is concluded, therefore, that flavin activation through hydrogen bonding with N(1) does exist but is not so effective in these model compounds, **1**(2OH) and **2**(OH).

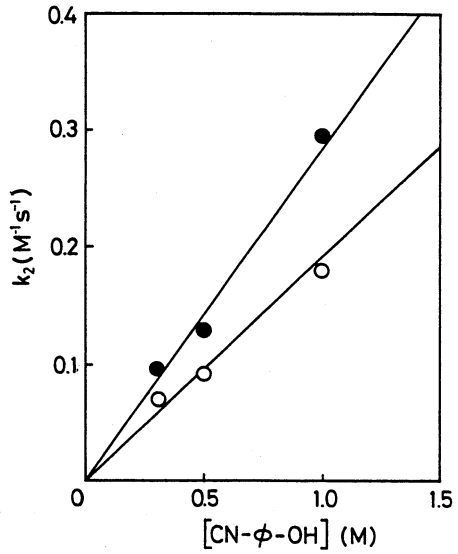


Fig. 7. General-acid catalysis by *p*-cyanophenol for the reaction of **1**(2OMe) (●) or **2**(2OMe) (○) (5.00×10^{-5} M) with BNAH (2.09×10^{-3} M) in acetonitrile.

We are currently devoting our research efforts on the development of more effective model systmes to prove the flavin activation through hydrogen bonding with N(1).

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