Fluorescence Spectroscopic Study on Tautomeric Equilibria of 2(1*H*)-Pyridones

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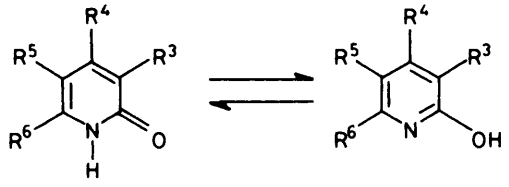
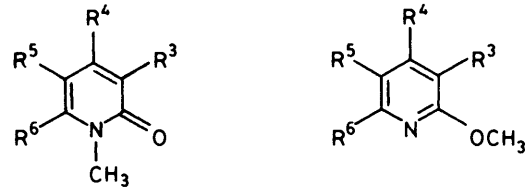
The minor tautomers of 2(1*H*)-pyridones (**1a**—**h**), the pyridinol forms, have been directly observed in fluorescence spectra. The ratios of the pyridinol form obtained from the measurements correspond to the tautomeric equilibria and the substituent effects of methyl groups. Thus, the usefulness of fluorescence measurements for the study of the tautomeric properties of 2(1*H*)-pyridones in solution has been substantiated.

2(1*H*)-Pyridone is a fundamental, tautomeric heteroaromatic system, and many theoretical and experimental studies of the tautomeric equilibria have been reported.[1] It has well been established that there is no significant difference in the fundamental stability of the unimolecular state of the tautomers[1,2] and that the tautomeric equilibria are strongly affected by self-association and solvation.[3] As a result, most 2(1*H*)-pyridones, except for some 6-polar-substituted derivatives,[4] exist almost exclusively in the pyridone form in solution due to the greater association ability of the pyridone form.

In the u.v. spectrum of 2(1*H*)-pyridone (**1a**), it is difficult to show the existence of the pyridinol form. This occurs in a variety of 2(1*H*)-pyridones and the apparent observation of the pyridinol form was restricted only in highly dilute non-polar solution.[2,3] In this study, we describe the direct observation of the rare tautomer of 2(1*H*)-pyridone and several methyl-substituted derivatives (**1b**—**j**) by the fluorescence measurements. We also discuss substituent effects on the tautomeric equilibria.



(1)



(2) (3)

a; $R^3 = R^4 = R^5 = R^6 = H$
b; $R^4 = R^5 = R^6 = H$, $R^3 = CH_3$
c; $R^3 = R^5 = R^6 = H$, $R^4 = CH_3$
d; $R^3 = R^4 = R^6 = H$, $R^5 = CH_3$
e; $R^3 = R^4 = R^5 = H$, $R^6 = CH_3$
f; $R^3 = R^4 = H$, $R^5 = R^6 = CH_3$
g; $R^3 = H$, $R^4 = R^5 = R^6 = CH_3$
h; $R^3 = H$, $R^4 = CH_3$, $R^5 - R^6 = —(CH_2)_3—$
i; $R^3 = R^4 = R^5 = H$, $R^6 = Cl$
j; $R^3 = R^4 = R^5 = H$, $R^6 = OCH_3$

Results and Discussion

(1). *Fluorescence Spectra of* 2(1H)-*Pyridone* (**1a**) *and Tautomeric Features.*—Some fluorescence studies on 2(1*H*)-pyridone (**1a**) have already been reported, but little attention has been paid to the tautomeric properties and the excitation wavelength used has always been the u.v. absorption maximum (*ca.* 300 nm) of the pyridone form.[5] The fluorescence intensities are largely dependent on the absorbance at the excitation wavelength. Therefore, it was expected that excitation near the u.v. absorption maximum of the pyridinol form (*ca.* 270 nm) is favourable for the observation of the fluorescence of the pyridinol form. Thus, the fluorescence spectra in cyclohexane, acetonitrile, and ethanol at the excitation wavelength, 270 nm, were measured.† It was found that the spectra for 4×10^{-5} mol dm^{-3} solutions in cyclohexane and acetonitrile exhibited two fluorescence emission bands at *ca.* 310 and 370 nm, as shown in Figure 1. On the basis of the reported spectra,[5] the fluorescence band at the longer wavelength is characteristic of that due to the pyridone form. The band at shorter wavelength was assigned to that due to the pyridonol form based on the fluorescence excitation spectra monitored at the wavelength of the emission maximum shown in Figure 1, and from the fluorescence emission spectra of the methylated tautomeric models, 1-methyl-2(1*H*)-pyridone (**2a**) and 2-methoxypyridine (**3a**).

The absence of bands due to the pyridinol form in ethanol solution and the decrease in the relative intensity of the same band *versus* that of the pyridone form in more concentrated cyclohexane and acetonitrile solutions (in Figure 1) were observed. These are indicative of tautomeric features (*i.e.* the greater stabilisation of the pyridone form either by hydrogen-bonding association in hydroxylic solvents or by self-association in non-polar solvent). However, these decreases in the intensities include the effect due to the reabsorption of the emission of the pyridinol form by the pyridone form, since the maximum of the emission wavelength (*ca.* 310 nm) of the pyridinol form is very close to that of the absorption wavelength of the pyridone form (*ca.* 300 nm). So, the concentration dependences of the fluorescence intensities of the pyridinol form in the range of $1 \times 10^{-5} - 1 \times 10^{-4}$ mol dm^{-3} in cyclohexane and acetonitrile were examined with corrections for the inner filter effect for exciting radiation and emission, and are shown in Figure 2.

It can be seen from Figure 2 that the fluorescence intensities with the corrections in cyclohexane show a non-linear relationship, while those in acetonitrile increase almost linearly as the concentration increases. The results correspond to the characteristics of the tautomeric equilibria of 2(1*H*)-pyridone (**1a**) in solution, considering that, in non-polar solvents, the

† A preliminary account has appeared in reference 6.

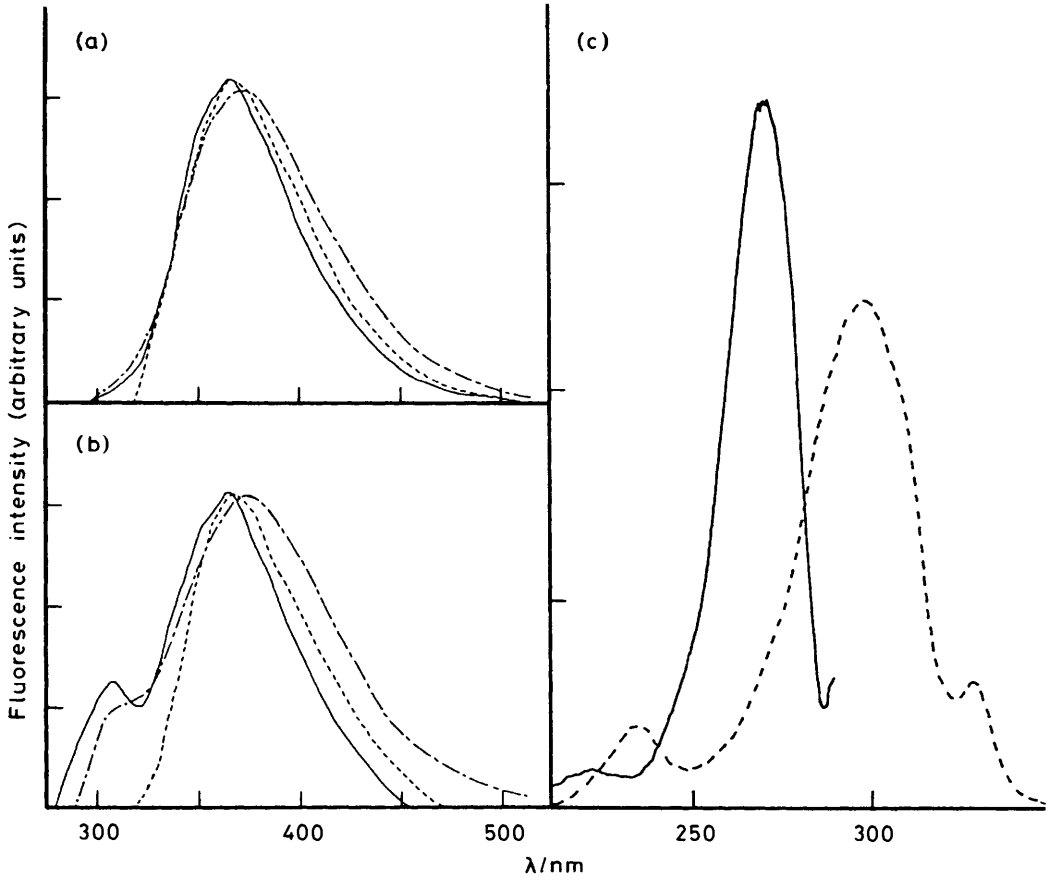


Figure 1. Fluorescence spectra (a) and (b) with excitation wavelength 270 mm and excitation spectra (c) for 2(1*H*)-pyridone (**1a**): (a) 4×10^{-4} mol dm^{-3}; (b) 4×10^{-5} mol dm^{-3}; (c) 1×10^{-5} mol dm^{-3} in cyclohexane. For (a) and (b), ——: in cyclohexane, — · — · —: in acetonitrile, - - - - -: in ethanol. For (c), ——: with emission at 303 nm, - - - - -: with emission at 367 nm

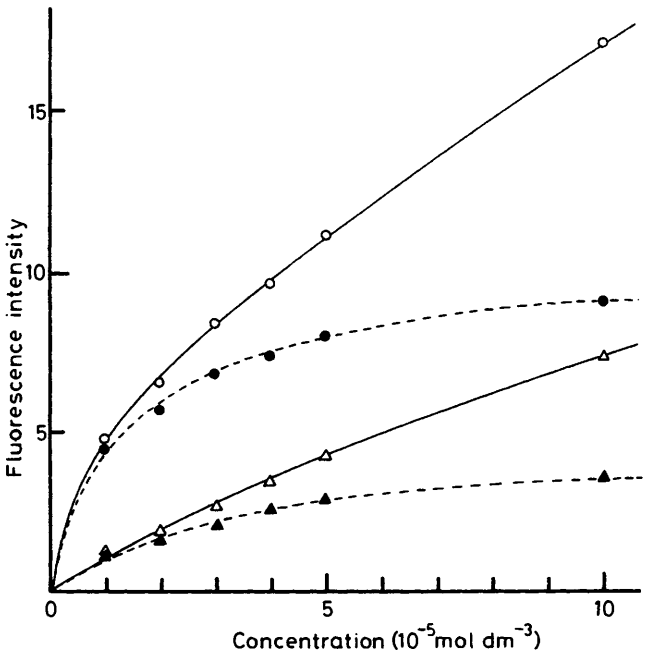


Figure 2. Concentration dependencies of the fluorescence intensity for pyridonol form of (**1a**). ●: Observed (in cyclohexane), ○: corrected (in cyclohexane), ▲: observed (in acetonitrile), △: corrected (in acetonitrile)

molar fraction of the self-associated pyridone form increases as the concentration increases.

(2). *Fluorescence Spectra of Substituted* 2(1H)-*Pyridones* (**1b**—**j**).—The fluorescence spectra of various substituted 2(1*H*)-pyridones (**1b**—**j**) and the corresponding methylated models of each tautomer (**2b**—**j**) and (**3b**—**j**) were also measured and the fluorescence spectral features observed are summarised in Table 1, together with those of the absorption spectra and their quantum yields.

As can be seen from Table 1, two fluorescence emission bands were observed in highly dilute cyclohexane and acetonitrile solutions in all cases. The fluorescence bands were assigned to the emissions due to the pyridinol and the pyridone forms in a similar manner to those for (**1a**). It can also be seen that both the u.v. absorption and fluorescence band maxima of the pyridone form of 2(1*H*)-pyridones (**1**) in cyclohexane tend to shift to shorter wavelengths relative to those in acetonitrile. This spectral feature in the u.v. spectra has been attributed to self-association in non-polar solvents.[7] So, we believe that a greater part of the self-associated pyridone form in the ground state is retained in the excited state.

The possibility of phototautomerisation which is known to occur in several tautomeric systems needs to be considered. For the 2(1*H*)-pyridones investigated here, however, phototautomerisation from the pyridone to the pyridinol form seems highly unlikely, since the energy differences in excitation between the two tautomers are far greater than those in the ground state.[3] Although the reverse phototautomerisation is possible from the energetic point of view, it is considered to be

Table 1. Fluorescence and absorption characteristics of 2(1*H*)-pyridones (1) and the methylated tautomeric models (2) and (3) in cyclohexane and acetonitrile[a]

		2(1*H*)-Pyridones			1-Methyl-2(1*H*)-pyridones			2-Methoxypyridines		
Substituents	Solvents[b]	$\lambda^{ab}_{max.}$/nm	$\lambda_{em}(\lambda_{ex})$[c]/nm	Φ_F[d]($\times 10^2$)	$\lambda^{ab}_{max.}$/nm	λ_{em}[e]/nm	Φ_F($\times 10^2$)	$\lambda^{ab}_{max.}$/nm	λ_{em}[e]/nm	Φ_F($\times 10^2$)
a	C	299	303(268), 367(300)	0.80	309	384	0.20	273	303	1.5
	A	303	313(272), 375(307)		306	379		271	312	
b	C	297	308(269), 371(296)	1.2	304	384	0.25	272	308	2.1
	A	301	313(272), 381(302)		302	379		272	313	
c	C	294	302(269), 367(294)	0.59	304	384	0.10	270	302	1.5
	A	299	308(267), 377(301)		306	375		269	308	
d	C	307	309(278), 381(306)	0.73	316	394	1.0	280	311	3.6
	A	312	314(278), 386(312)		320	387		279	315	
e	C	302	308(270), 396(302)	0.38	310	388	0.02	274	306	2.6
	A	307	315(274), 380(308)		315	380		273	312	
f	C	309	313(278), 381(311)	0.26	319	392	0.03	280	312	2.6
	A	320	314(279), 388(322)		324	390		279	315	
g	C	304	313(274), 379(307)	0.07	314	416[f]	0.00	277	310	1.2
	A	316	315(275), 390(314)		317	385		276	315	
h	C	310	315(283), 387(311)	5.5	325	393	46	284	316	6.1
	A	321	316(284), 388(318)		322	385		282	316	
i	C	278	309(276)	0.07[g]	311	—		279	312	0.07
j	C	279	315(279)	2.6[g]	314	—		280	316	2.6

[a] Measured at ambient temperature. [b] C, cyclohexane; A, acetonitrile. [c] Excitation maxima. [d] Fluorescence quantum yields for the pyridone form. [e] Excited at the wavelength of the $\lambda^{ab}_{max.}$. [f] Containing some uncertainty due to the weakness of the fluorescence. [g] For the pyridinol form.

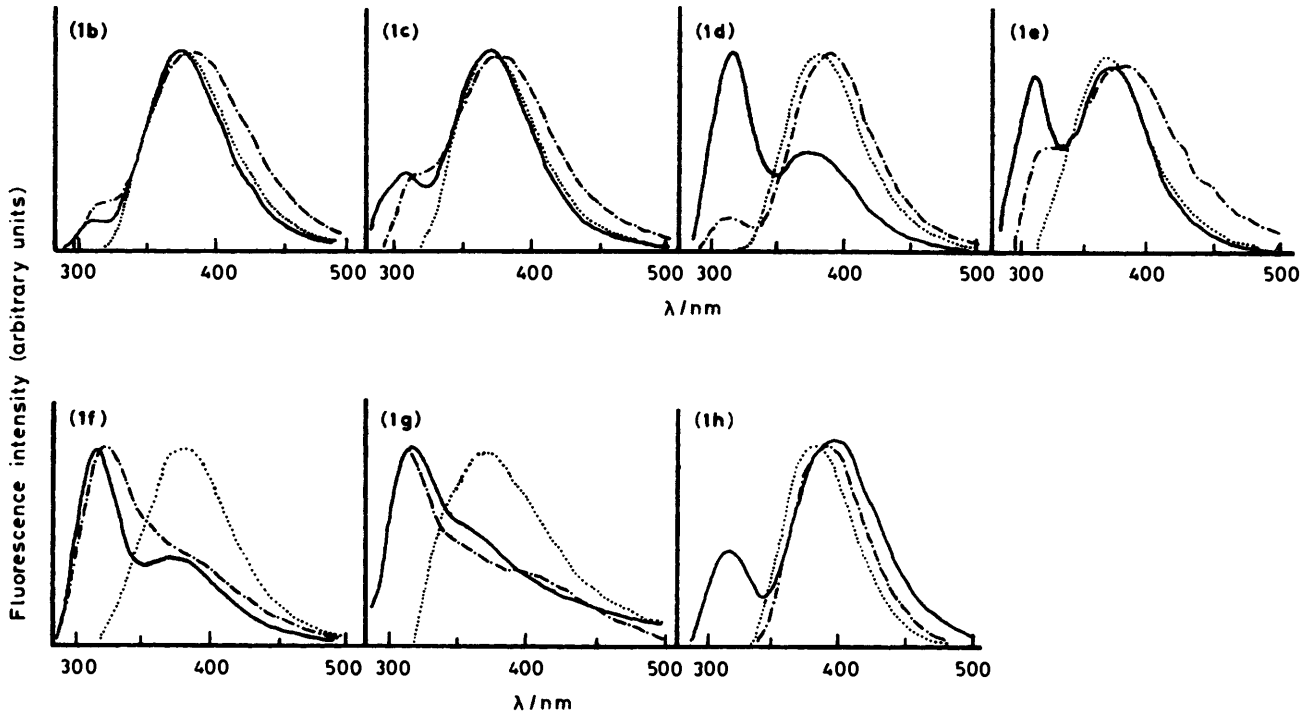


Figure 3. Fluorescence spectra of 2(1*H*)-pyridones (**1b**—**h**) (4 × 10^{-5} mol dm^{-3}) with excitation wavelength 270 nm. ——: in cyclohexane, — · — · —: in acetonitrile, · · · · ·: in ethanol

negligible in the present system in view of the similar fluorescence spectral features (*i.e.* the emission wavelength and the fluorescence efficiency) of (**1i** and **j**) as the corresponding *O*-methyl derivatives (**3i** and **j**).

(3). *Elucidation of the Substituent Effects for the Tautomeric Equilibria of* 2(1H)-*Pyridones by Fluorescence Spectra.*—We have recently reported the theoretical evaluation of substituent effects for the tautomeric equilibria of 2(1*H*)-pyridones based on MO calculations (MINDO/3).[8] Therein, the 6-methyl substituent, particularly the 5,6-dimethyl substituent, has been shown to make the tautomeric equilibria labile to phase, solvent, and concentration changes.[8,9] These effects were investigated by means of fluorescence spectra.

For the comparisons of the relative intensities of the two bands, the fluorescence spectra of (**1b**—**h**) at the excitation wavelength of 270 nm at 4 × 10^{-5} mol dm^{-3} in cyclohexane, acetonitrile, and ethanol are shown in Figure 3.

Table 2. Contents of the pyridinol form for 2(1*H*)-pyridones in cyclohexane and acetonitrile[a]

	Cyclohexane solution		Acetonitrile solution	
Compound	I_f^{OH}[b] (I_f^{OMe})[c]	% OH form	I_f^{OH}[b] (I_f^{OMe})[c]	% OH form
(**1a**)	3.76 (36.9)	4.08 (5.47)[d]	1.05 (34.5)	1.22
(**1b**)	2.77 (62.3)	1.78	1.00 (63.1)	0.63
(**1c**)	2.07 (22.8)	3.63	0.63 (24.6)	1.02
(**1d**)	8.87 (105.9)	3.35	2.97 (112.5)	1.06
(**1e**)	2.81 (68.5)	1.64	1.27 (72.7)	0.70
(**1f**)	6.03 (78.5)	3.07	3.60 (83.1)	1.73
(**1g**)	2.75 (31.6)	3.48	1.64 (38.8)	1.69
(**1h**)	27.2 (278.3)	3.91	15.9 (307.3)	2.07

[a] Measured in 1×10^{-5} mol dm^{-3} at 20 °C. [b] At an emission maximum excited at the wavelength of the excitation maximum. [c] Measured in 4×10^{-6} M at an emission maximum excited at the wavelength of the absorption maximum. [d] Calculated using known equilibrium constants.[1a]

It is immediately seen that the relative intensities of the pyridinol form *versus* the pyridone form vary according to the substituents. However, elucidation of the effect of substituents on the tautomeric properties of 2(1*H*)-pyridones cannot be made directly from the fluorescence spectra, since the quantum yields vary according to the substituents (Table 1).

The estimation of the content of the pyridinol form by means of the fluorescence intensities of the methylated tautomeric models was considered, since the u.v. spectra and the quantum efficiencies (Table 1) of 6-polar-substituted 2(1*H*)-pyridones (**1i** and **j**) (which almost exclusively exist as the pyridinol form in cyclohexane)[4,10] have been shown to be nearly identical with those of the corresponding methylated models (**3i** and **j**). Similar relations also hold in acetonitrile solution, although the quantum efficiencies and the molar extinction coefficients for (**1i** and **j**) are rather greater than those for (**3i** and **j**) and this overestimates the content of the pyridinol forms by *ca.* 30% in calculations using the fluorescence intensities of (**3i** and **j**), respectively.

So, assuming that the u.v. spectrum and fluorescence intensity of the other pyridinol forms are the same as those of the methylated models, the content of the pyridinol form can be simply calculated from comparison of the fluorescence intensities. The result thus obtained at 1×10^{-5} mol dm^{-3} in cyclohexane and acetonitrile are shown in Table 2.

Table 2 shows that some of the characteristics of the tautomeric equilibria (*i.e.*, the preference of the pyridinol form in non-polar dilute solution) was well demonstrated in comparison of the content of the pyridinol form in cyclohexane and in acetonitrile. Also, the relatively greater content of the pyridinol form for the 5,6-dimethylated derivatives (**1f** and **g**) and the pyrindine derivative (**1h**) in acetonitrile solutions seem to show the greater stability of the pyridinol form in these compounds. That is, in acetonitrile solution it is considered that the self-associated dimers are dissociated to a considerable extent and that the stabilising effect for the pyridone form is not so strong and specific as the effect in hydroxylic solvents. So, the results in acetonitrile solutions are considered to represent to some extent the characteristics of the substituent effects for tautomeric equilibria in the unimolecular state. On the other hand, the rather smaller content of the pyridinol form for the 6-methylated derivatives (**1e**—**g**) in cyclohexane shows the greater self-association ability of the pyridone form. All these results are consistent with the previous conclusions derived from theoretical calculations and i.r. spectral measurements.[8,9]

(4). *Attempt to detect the Minor Tautomer in 6-Polar-substituted* 2(1H)-*Pyridones.*—In the light of the fluorescence spectral observation of the minor tautomer (pyridinol form) for 2(1*H*)-pyridones (**1a**—**h**), one may also expect that the minor tautomer (pyridone form) of the 6-polar-substituted 2(1*H*)-pyridones (**1i** and **j**)[4,10] would be detected in fluorescence spectra with excitation near the u.v. absorption maxima of the pyridone form. In fact, such minor tautomers were not detected. The failure seems to be due to the very low fluorescence efficiencies of the pyridone forms, since the fluorescence of the corresponding 1-methyl-6-polar-substituted 2(1*H*)-pyridones (**2i** and **j**) was not detected (Table 1). The detailed nature of the 6-substituent effect on the fluorescence efficiency will be published elsewhere.

Experimental

All m.p.s and b.p.s are uncorrected. The instruments used in this study were as follows: i.r. spectra (KBr disks), JASCO A-102; ^{1}H n.m.r. spectra, Hitachi R-24B ($CDCl_3$; tetramethylsilane as internal standard); mass spectra, JEOL JMS-D300; u.v. spectra, Hitachi 323; fluorescence spectra, Hitachi 650-60.

Materials—2(1*H*)-Pyridones (**1a**—**g**, **i**, and **j**) and the 1-methylated compounds (**2a**—**g**, **i**, and **j**) were described in our previous papers.[9,11] 1,5,6,7-Tetrahydro-4-methyl-1-pyrindin-2-one (**1h**) was prepared according to the literature.[12]

1,5,6,7-Tetrahydro-1,4-dimethyl-1-pyrindin-2-one (**2h**) was prepared by the methylation of (**1h**) by methyl iodide in the presence of potassium carbonate in acetone and purified by sublimation, m.p. 100—103 °C (Found: C, 73.3; H, 8.1; N, 8.55. $C_{10}H_{13}NO$ requires C, 73.6; H, 8.0; N, 8.6%); $\nu_{max.}$ 1 660 cm^{-1}; δ 2.05 (3 H, s), 1.93—3.07 (6 H, m), 3.4 (3 H, s), and 6.17 (1 H, s); *m/z* 163 (M^+, 100%), 162 (93), 135 (30), and 134 (49).

2-Methoxypyridine (**3a**) and 2,6-dimethoxypyridine (**3j**) were obtained from Aldrich Chemical Company, Inc. and purified before use. 6-Chloro-2-methoxypyridine (**3i**) was obtained by the methylation of (**1i**) as same as described above and purified by fractional distillation, b.p. 80—81 °C at 20 Torr (lit.,[10] 184—185 °C). Other 2-methoxypyridines (**3b**—**h**) were prepared from the corresponding 2(1*H*)-pyridones by the method of the literature.[13] 2-Methoxy-3-methyl- (**3b**),[14] 2-methoxy-4-methyl- (**3c**),[15] 2-methoxy-5-methyl- (**3d**),[16] 2-methoxy-6-methyl- (**3e**),[17] and 2-methoxy-5,6-dimethyl-pyridine (**3f**)[4] are described in the literature. 2-Methoxy-4,5,6-trimethylpyridine (**3g**) had b.p. 60—61 °C at 1 Torr; δ 2.03 (3 H, s), 2.17 (3 H, s), 2.37 (3 H, s), 3.83 (3 H, s), and 6.30 (1 H, s); *m/z* 151 (M^+, 60%), 150 (100), 122 (26), 121 (41), 120 (27), and 106 (12). 1,5,6,7-Tetrahydro-2-methoxy-4-methyl-1-pyrindine (**3h**) had b.p. 76—77 °C at 1.5 Torr; δ 1.87—2.37 (2 H, m), 2.17 (3 H, s), 2.57—3.07 (4 H, m), 3.82 (3 H, s), and 6.20 (1 H, s); *m/z* 163 (M^+, 63%), 162 (100), 134

(24), 133 (31), and 132 (19). All compounds were purified by distillation or sublimation before use.

Fluorescence Measurements.—Spectrograde solvents (no fluorescent impurity) were used. Fluorescence measurements were performed at ambient temperature. The excitation was carried out at right angles to the direction in which the emission was monitored. The spectra were corrected for the wavelength sensitivity of the photomultiplier–monochrometer system with Rhodamine B by the automated data correction equipped in a spectrometer and measured as difference spectra from a solvent. For the measurements of the quantum yield (Φ_F), solutions of quinine sulphate in 1.0N-H_2SO_4, optically matched with the solutions of 2(1*H*)-pyridones at the excitation wavelengths, were used as reference ($\Phi = 0.55$).[18]

Correction of Fluorescence Intensity of the Pyridinol Form.—For solutions containing more than two components, the fluorescence emission intensities are largely affected by an inner filter effect such as reabsorption of the emission and absorption of the exciting radiation.[19] The fluorescence intensity was measured as that at the emission maximum. The self-absorption of the emission could be ignored based on the inspection of the u.v. absorption spectra of the methylated tautomeric models. Thus, the corrections needed are the reabsorption by the pyridone form and the absorption of the exciting radiation. As for the reabsorption, the corrected intensity (I'_f) is given by equation (1) where I_f^{obs} is the observed

$$I'_f = \{(a - b)I_f^{obs} + I_R\int_b^a (1 - 10^{-A_{em}x})dx\}/\int_b^a 10^{-A_{em}x}dx \quad (1)$$

fluorescence intensity, I_R is the intensity of the Raman scattering of the solvent at the measured emission wavelength, a and b are the cell parameters and were calculated as 0.75 and 0.25 cm, respectively, by considering the band-path (0.5 cm) for both the exciting radiation and the detection of the emission light, and the cell dimension (1 cm^2), and A_{em} is the absorption coefficient at the measured emission wavelength.

For the absorption of the exciting radiation, since the absorption coefficient of the pyridinol form is considered to be quite small due to its fairly small importance, the corrected fluorescence intensity (I_f) is given by equation (2) where I'_f, a,

$$I_f = I'_f(a - b)A_{ex}\ln 10/(10^{-A_{ex}b} - 10^{-A_{ex}a}) \quad (2)$$

and b are as described above, and A_{ex} is the absorption coefficient at the excitation wavelength.

The validity of these corrections was ascertained for solutions of various concentrations of 1-methyl-2-pyridone (**2a**) and 2-methoxypyridine (**3a**).

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