Prototropic Equilibria in the Lowest Excited Singlet State of *o*-Phenanthroline[1]

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Abstract: Variations of fluorescence quantum yields with pH and pH-dependent shifting of the long-wavelength excitation maxima and fluorescence maxima have been employed to calculate the successive dissociation constants of protonated species derived from *o*-phenanthroline in the ^1L_b state. The dissociation of the doubly protonated species achieves equilibrium in the ^1L_b state and is about three pK units more acidic than in the ground state. Proton exchange between the singly protonated species and the solvent in the ^1L_b state is too slow to approach equilibrium within the lifetime of the ^1L_b state. However, the singly protonated species is shown to be a weaker acid in the ^1L_b state than in the ground state. Agreement of the experimental results with those predicted by theory and past experience are discussed in terms of the transition polarization and interactions of the ground and excited states of the species studied with the solvent. The significance of a standard state for excited-state pK_a measurements is also discussed.

The title compound, *o*-phenanthroline (1,10-diazaphenanthrene), is well known for its chelating properties and for the highly colored complexes it forms with iron(II) and iron(III). The electronic absorption spectrum of *o*-phenanthroline bears a marked resemblance to that of its hydrocarbon analog, phenanthrene, but does not exhibit the clear vibrational structure observed in the phenanthrene spectrum.[2] Because *o*-phenanthroline contains two nitrogen atoms which are capable of acting as proton acceptors, its electronic absorption spectrum shows a pH dependence in the positions of the absorption bands and to a lesser extent in the intensities of these bands. However, the significance of the pH-dependent shifts of the phenanthroline absorption spectra is difficult to evaluate for all transitions due to strong overlap between successive bands.

The pH dependence of the phosphorescence spectrum of *o*-phenanthroline has been investigated[2] and has indicated that in the lowest excited triplet state, the pK_a for the protonation of the first nitrogen atom is slightly more basic than that for the ground state, a fact consistent with the usual behavior of ring heteroatoms in their lowest excited triplet states.[3] The pK_a for the second protonation (protonation of the singly protonated species) in the lowest excited triplet state, however, is 2.5 units more acidic than the corresponding ground-state pK_a even though this process also consists of protonation of a ring heteroatom. This might be rationalized in view of the normally weak intramolecular charge transfer to the ring heteroatom upon excitation to the lowest triplet state, the positive charge already residing in the singly protonated *o*-phenanthroline molecule, and the proximity of the first proton to the site of the second protonation.

It has been observed that, as a rule, heteroatoms in carbocyclic rings become considerably more basic in their lowest excited singlet states than in their respective ground states.[5] In the present study, in order to test the applicability of the latter rule to *o*-phenanthroline, the dissociation constants (pK_a*) of the two prototropic equilibria of this compound (Figure 1) in its lowest excited singlet state were determined by fluorimetric titration and by the application of the Förster cycle[4] to the pH dependence of the fluorescence emission and fluorescence excitation spectra of the neutral, singly protonated, and doubly protonated forms of *o*-phenanthroline.

Experimental Section

o-Phenanthroline monohydrate was purchased from Aldrich Chemical Co. and was used without further purification. Perchloric acid and sodium hydroxide were purchased from Mallinckrodt Chemical Works, St. Louis, Mo. Perchloric acid solutions were standardized against sodium carbonate and were used for measurements in the acidity range $H_0 = -7.0$ to pH 4.0. Sodium hydroxide solutions were prepared from the carbonate-free aqueous solution, purged with and stored under nitrogen, and standardized against potassium acid phthalate. The solutions were used for measurements in the pH range 10.0–13.0. Beckman buffer solutions were employed in the pH range 4.00–7.00.

Fluorescence emission and excitation spectra were taken on an Aminco spectrophotofluorometer, and absorption spectra were taken on a Cary 15 spectrophotometer. Fluorimetric titrations were performed on a series of solutions each 1.00×10^{-4} M in *o*-phenanthroline and containing varying amounts of perchloric acid, sodium hydroxide, or buffer solution. A complete fluorescence emission and excitation spectrum was taken on each of the latter solutions. Fluorescence intensities used in the fluorimetric titrations were measured at the emission maxima at 363, 420, and 412 mμ of the neutral, singly protonated, and doubly protonated species, respectively. For each species, relative fluorescence intensities I/I_{max} were corrected for overlap by emission from the conjugate acid or base by subtracting the relative fluorescence intensity of the conjugate species (p) at the bottom of the fluorimetric titration curve. The resulting corrected relative fluorescence intensity $(I/I_{max}) - p$ was divided by $1 - p$ to give the relative quantum yield (ϕ/ϕ_0) of the species in question.

pK_a* values were estimated from the intercept of the plot of H_0 or pH *vs.* log [PhH^{+}*]/[PhH$_2^{2+}$*] or log [Ph*]/[PhH^{+}*], where [Ph*], [PhH^{+}*], and [PhH$_2^{+}$*] are the equilibrium concentrations of the neutral, single protonated, and doubly protonated species, respectively, in the lowest excited singlet state. The ratio [PhH^{+}*]/[PhH$_2^{2+}$*] for each value of H_0 in the fluorimetric titration is given by $[1 - (\phi/\phi_0)]/(\phi/\phi_0)$ measured at 412 mμ in concentrated perchloric acid. That of [Ph*]/[PhH^{+}*] for each value of pH is given

(1) Research was carried out as a part of a study on the phosphorimetric analysis of drugs in blood and urine, supported by U. S. Public Health Service Grant No. GM-11373-07.

(2) J. S. Brinen, D. D. Rosebrook, and R. C. Hirt, *J. Phys. Chem.*, 67, 2651 (1963).

(3) G. Jackson and G. Porter, *Proc. Roy. Soc., Ser. A*, **260**, 13 (1961).

(4) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).

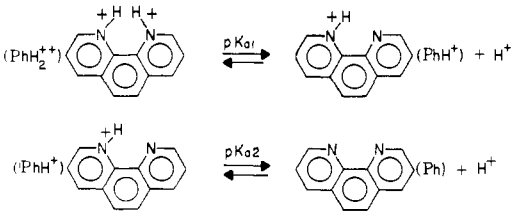

Figure 1. Prototropic equilibria of *o*-phenanthroline. Ph is the neutral species, PhH^+ is the singly protonated species, and PhH_2^{++} is the doubly protonated species.

by $[1 - (\phi/\phi_0)]/(\phi/\phi_0)$ measured at 420 mμ or by $(\phi/\phi_0)/[1 - (\phi/\phi_0)]$ measured at 363 mμ, in aqueous solutions.

In the Förster-cycle calculations, the pK_s^* values were estimated from the relationships

$$pK_{s_1}^* = pK_{a_1} - 2.10 \times 10^{-3}(\bar{\nu}_{PhH_2^{++}} - \bar{\nu}_{PhH^+})$$

and

$$pK_{s_2}^* = pK_{a_2} - 2.10 \times 10^{-3}(\bar{\nu}_{PhH^+} - \bar{\nu}_{Ph})$$

where $pK_{s_1}^*$ and $pK_{s_2}^*$ are the dissociation constants of the doubly and singly protonated species in the lowest singlet state, respectively, and pK_{a_1} and pK_{a_2} are the corresponding dissociation constants in the ground state. $\bar{\nu}_{PhH_2^{2+}}$, $\bar{\nu}_{PhH^+}$, and $\bar{\nu}_{Ph}$ are the wave numbers of the ground singlet–lowest excited singlet transition (in reciprocal centimeters) of the doubly protonated, singly protonated, and neutral species, respectively. For purposes of comparison, the $\bar{\nu}$ values were evaluated from the fluorescence maxima, the long-wavelength excitation maxima (which coincided with the apparent long-wavelength absorption maxima of the *o*-phenanthroline absorption spectra), and the averages of the corresponding fluorescence and excitation maxima (to locate the 0–0 bands), respectively. Because the response of the RCA 1P21 phototube employed here, with respect to frequency, is relatively flat in the region of the spectrum studied, the excitation maxima were taken to reasonably represent the true absorption maxima and so fluorescence and emission spectra were not corrected for the wavelength dependences of the phototube or the monochromators. However, the monochromators were calibrated against the mercury emission lines from a Pen-Ray low-pressure mercury lamp. For the Förster-cycle calculations, spectra representative of the doubly protonated, singly protonated, and neutral species were taken at $H_0 = -7.0$, pH 2.00, and pH 12.00, respectively.

Results and Discussion

o-Phenanthroline was found to exhibit a weak, structured fluorescence in the ultraviolet in basic and neutral solutions. In the region near pH 5, the ultraviolet fluorescence diminished in intensity concurrently with the appearance and increase in intensity, with decreasing pH, of a weak, unstructured blue fluorescence. In the same pH region, the excitation spectrum shifted to lower frequency as the pH decreased. An isoemissive point (Figure 2) was observed between the ultraviolet and blue emission spectra, indicating that in the region near pH 5 only two prototropic species are in equilibrium and that their quantum yields of fluorescence at high and low pH, respectively (*i.e.*, in the absence of pH dependence of the quantum yields), are identical.[5] The blue fluorescence rose to a maximum at pH ~3.5, with disappearance of the ultraviolet fluorescence, and remained fairly constant in intensity until a Hammett acidity of ~−2.0 was reached. The excitation spectrum of the blue fluorescence shifted to still lower frequencies in the Hammett acidity region 0

(5) S. G. Schulman and J. D. Winefordner, *Talanta*, 17, 607 (1970).

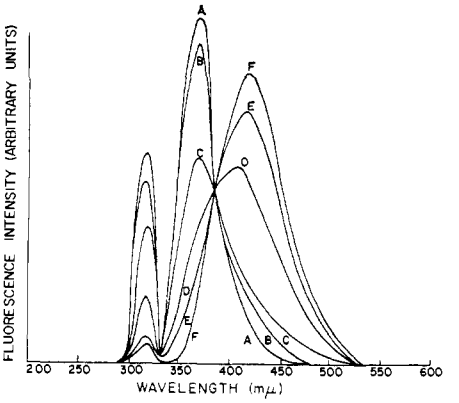

Figure 2. Fluorescence spectra of *o*-phenanthroline in the pH region 2.97–13.00, showing an isoemissive point at λ 388 mμ; excitation wavelength 318 mμ: (A) pH 13.00, (B) pH 6.86, (C) pH 5.70, (D) pH 4.70, (E) pH 4.48, (F) pH 2.97.

to −3.0 and became narrower in bandwidth. At Hammett acidities below −2.0, the blue fluorescence shifted slightly toward the red and increased sharply in intensity, reaching a maximum at $H_0 \sim -7.0$.

The ground-state pK_a values of the singly protonated and doubly protonated forms of *o*-phenanthroline have been determined[6] and are 4.85 and −1.4, respectively. Because both the excitation and emission spectra of *o*-phenanthroline change in the region near pH 5 and show an isoemissive point, the ultraviolet excitation and emission spectra obtained in basic and neutral solutions are assigned to the neutral *o*-phenanthroline species. Correspondingly, the excitation and blue emission bands obtained in dilute acidic media are presumed to be due to the singly protonated species. The excitation spectra appearing at $H_0 < -2$ are assigned to the doubly protonated *o*-phenanthroline. The excitation and emission maxima of the three prototropic forms of *o*-phenanthroline are listed in Table I.

Table I. Long-Wavelength Excitation Maxima and Fluorescence Maxima of *o*-Phenanthroline[a] in Aqueous and Perchloric Acid Media

	Maxima, cm^{-1}	
	Excitation	Fluorescence
Ph	3.13×10^4	2.76×10^4
PhH^+	2.89×10^4	2.39×10^4
PhH_2^{2+}	2.73×10^4	2.43×10^4

[a] Ph = neutral species, PhH^+ = singly protonated species, PhH_2^{2+} = doubly protonated species.

Graphical representation of the fluorimetric titration data adapted to the Henderson–Hasselbach equation is presented in Figure 3.

Perkampus, *et al.*,[6,7] have investigated the pH dependence of the absorption spectra of three isomeric phenanthrolines, including *o*-phenanthroline. They have also studied the polarizations of the fluorescence

(6) H. H. Perkampus and H. Köhler, *Z. Elektrochem.*, 64, 365 (1960).
(7) (a) H. H. Perkampus, J. V. Knop, A. Knop, and G. Kossebeer, *Z. Naturforsch. A*, 22, 1419 (1967); (b) H. H. Perkampus, A. Knop, and J. V. Knop, *ibid.*, *A*, 23, 840 (1968).

excitation and phosphorescence excitation spectra, in alcohol–ether solvent, and performed SCF–CI molecular orbital calculations on ten isomeric phenanthrolines, including *o*-phenanthroline. Their studies indicate that for *o*-phenanthroline the same ordering of (absorptive) transitions is preserved for the three prototropic species; that the longest wavelength absorption in each species is π–π^*; that the lowest excited singlet state in both the absorption and fluorescence spectra of the neutral species is ^1L_b and is polarized along the twofold axis of symmetry; and that the lowest excited triplet state, from which phosphorescence originates in the neutral species, is ^3L_a and is polarized in the molecular plane along the axis perpendicular to the twofold axis. The latter result is consistent with the triplet-state pK_a values of *o*-phenanthroline.[2] In studies of two other isomeric diazaphenanthrenes, Lippert,[8] in an investigation of 9,10-diazaphenanthrene, and Ballard and Edwards,[9] in a study of 3,4-benzocinnoline (5,6-diazaphenanthrene), found the lowest excited singlet state to be n–π^*. Ballard and Edwards also found the pK_s^* of the lowest excited singlet state of 3,4-benzocinnoline to be more acidic than the ground state, a result which is consistent with an n–π^* state.

In the present study, we draw upon the results of Perkampus and Köhler to infer that the three long-wavelength excitation maxima arise from the $^1A \rightarrow {}^1L_b$ transitions in the neutral, singly protonated, and doubly protonated species, respectively. Furthermore, both the excitation and fluorescence maxima of the neutral *o*-phenanthroline shift to longer wavelength upon addition of a single proton. Substitution of this result into the Förster cycle indicates that the *o*-phenanthroline becomes more basic in the lowest excited singlet state, with respect to the first protonation. This result is inconsistent with an n–π^* lowest excited singlet state for either the neutral or singly protonated species and is typical for protonation of a nitrogen atom in a heterocyclic ring when the lowest excited singlet state is π–π^*.

Consequently, it is concluded that the fluorescences of the neutral (in agreement with Perkampus and Köhler) and singly protonated species arise from π–π^* lowest excited singlet states and not from n–π^* transitions buried under the long-wavelength ends of the lowest π–π^* transitions. Because the fluorescence spectra of the latter species show reasonably good mirror-image relationships with their respective excitation spectra, both emissions apparently arise from ^1L_b excited states. In the doubly protonated species, there are no lone electron pairs, so the emission must originate from a π–π^* state. The mirror-image relationship between the excitation and emission spectra is not particularly good, the excitation band half-width being about half that of the emission band. However, the proximity of the two bands infers that they both arise from the ^1L_b state.

Provided that the errors in the Förster-cycle calculation, due to the nonequivalence of the vibrational substructures of the ground and excited states, are not large by comparison to $pK_a - pK_s^*$, the Förster cycle can be used to obtain qualitative pK_s^* values which can yield valuable information regarding the redistribution of electronic charge upon excitation. The ultimate test of the legitimacy of these calculations, however, is how well pK_s^* values calculated from the Förster cycle agree with those determined by fluorimetric titration when equilibrium is established within the lifetime of the lowest excited singlet state. Unfortunately, for those conjugate pairs in which neither member is fluorescent or in which equilibrium is not established during the lifetime of the lowest singlet state, the Förster cycle is the only means of assessing pK_s^*.

Application of the Förster cycle to the data of Table I along with the ground-state values of pK_{a_1} and pK_{a_2} (−1.4 and 4.85, respectively) enabled calculation of $pK_{s_1}^*$ and $pK_{s_2}^*$. The latter dissociation constants calculated from the shifts in excitation maxima, fluorescence maxima, and 0–0 bands of the ground state to lowest excited singlet transitions (averages of the excitation and fluorescence maxima), respectively, are given in Table II.

Table II. pK_s^* Values of the Prototropic Equilibria of *o*-Phenanthroline (Figure 1) in the Lowest Excited Singlet State[a]

	pK_s^*(e)	pK_s^*(f)	pK_s^*(0-0)
$PhH_2^{2+*} \overset{pK_{s_1}^*}{\rightleftharpoons} PhH^{+*} + H^+$	2.0	−2.2	−0.1
$PhH^{+*} \overset{pK_{s_2}^*}{\rightleftharpoons} Ph^* + H^+$	9.9	12.7	11.4

[a] Calculated from shifts in excitation maxima (pK_s^*(e)), emission maxima (pK_s^*(f)), and the 0–0 bands of the ground state to lowest excited singlet transitions (pK_s^*(0–0)), respectively. Ground-state values, $pK_{a_1} = -1.4$ and $pK_{a_2} = 4.85$.

The data of Table II are obviously not in good agreement, showing the corresponding pK_s^* values calculated from excitation and emission spectra differing by about 3–4 units. The values calculated from the 0–0 bands[10] are merely averages of the former values. However, while the values of $pK_{s_1}^*$ are not even in agreement as to the change in direction of the acidity of the doubly protonated species upon excitation to the lowest excited singlet state, the values of $pK_{s_2}^*$ do indicate that upon excitation the singly protonated species becomes more basic in the excited state. The fluorimetric titration data contained in Figure 3a yield a $pK_{s_2}^*$ value of 4.84, in excellent agreement with the ground-state pK_{a_2} of 4.85. Because the Förster-cycle data for the latter equilibrium indicate that the singly protonated species should become more basic in the lowest excited singlet state, it is concluded that the radiative lifetimes of the neutral and singly protonated species must be too short for proton exchange to occur appreciably within these lifetimes at pH ~5 (*i.e.*, the reaction is diffusion limited). Consequently, prototropic equilibrium is not established within the lifetime of the lowest excited singlet states of these molecules, with the result that the fluorescence intensities measured at pH ~5 reflect the relative ground-state concentrations of Ph and PhH^+. Hence, the ground-state pK_{a_2} is determined by fluorimetric titration in this instance.

The fluorimetric titration represented in Figure 3b yields a value of $pK_{s_1}^*$ of −4.3, a value about three

(8) E. Lippert in "Luminescence of Organic and Inorganic Materials," H. P. Kallmann and G. M. Spruch, Ed., Wiley, New York, N. Y., 1962.
(9) R. E. Ballard and J. W. Edwards, *Spectrochim. Acta*, **20**, 1275 (1964).
(10) W. Bartok, P. J. Lucchesi, and N. S. Snider, *J. Amer. Chem. Soc.*, **84**, 1842 (1962).

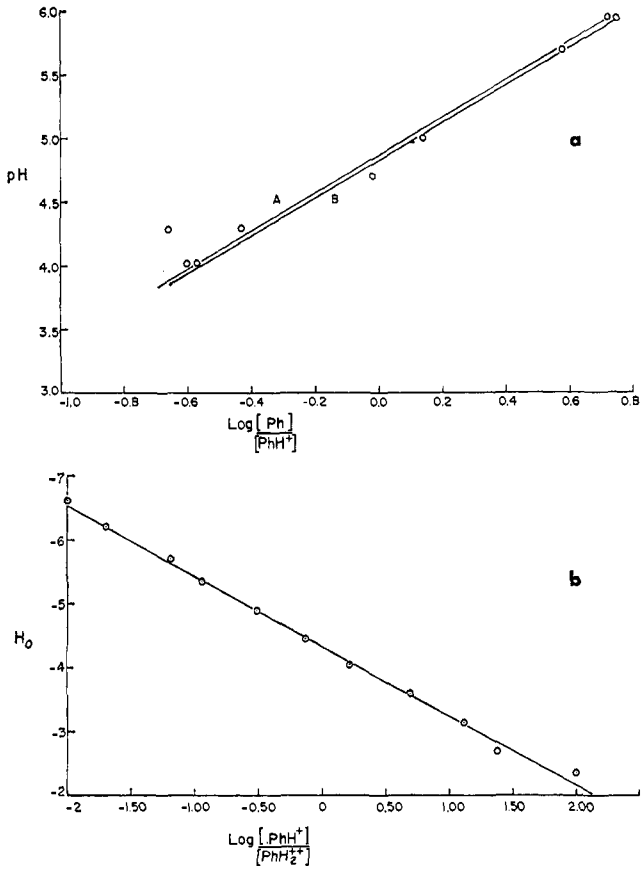

Figure 3. (a) Plot of pH *vs.* log [Ph]/[PhH$^+$]: (A) calculated from fluorescence spectrum of neutral species (λ_f 363 mμ), (B) calculated from fluorescence spectrum of singly protonated species (λ_f 420 mμ). (b) Plot of H_0 *vs.* log [PhH^{+*}]/[PhH$_2^{2+*}$], pK^* = −4.3.

units more acidic than the corresponding ground-state pK_a. The relatively narrow H_0 interval over which the fluorimetric titration occurs and the excellent fit of the data points of the fluorimetric titration curve to the Henderson–Hasselbach equation indicate that prototropic equilibrium between the doubly protonated and singly protonated species in their respective lowest excited singlet states is established, essentially completely, within the lifetimes of the latter electronic states. It is now in order to consider an apparent anomaly. *o*-Phenanthroline, having C_{2v} symmetry and having its $^1L_b \leftrightarrow {}^1A$ transition polarized along the twofold axis, should have π charge either accumulate on or depart from both nitrogen atoms as a result of excitation to the ^1L_b state. From studies of other nitrogen heteroatomics[11] and from molecular orbital calculations, it is known that heterocyclic nitrogen atoms gain charge upon excitation. Consequently, on the basis of intramolecular charge-transfer considerations alone, both ^1L_b-state pK_{s_1}* values should be more basic than their ground-state counterparts. This result is predicted by the Förster-cycle calculations based upon excitation spectra and the 0–0 band approximation. On the other hand, Förster-cycle data employing fluorescence data and fluorimetric titration data for pK_{s_1}* indicate that one nitrogen atom becomes more acidic in the ^1L_b state.

There are two possible explanations for the above apparent anomaly which will still satisfy the charge-transfer properties of the ^1L_b state. First, it is possible that, owing to the geometry of *o*-phenanthroline, intramolecular hydrogen bonding occurs in the singly protonated species in the ^1L_b state, thereby stabilizing this species and making it more difficult to protonate.[12] Second, the energy of relaxation of the solvent cage from the equilibrium ground-state configuration to the equilibrium excited-state configuration might be so much greater for the singly protonated species than for the doubly protonated species that the gain in basicity of the nitrogen atom due to charge transfer to the nitrogen in the ^1L_b state would be overwhelmed by the stabilization of the singly protonated species in the ^1L_b state with respect to its doubly protonated counterpart.

In order to test the above hypotheses, the excitation and fluorescence spectra of Ph, PhH$^+$Cl, and PhH$_2^{2+}$-(ClO$_4$)$_2$ were taken in *n*-pentane. Because this solvent is nonpolar and non-hydrogen bonding, solvent relaxation effects therein of the three species derived from *o*-phenanthroline are expected to be very small compared with those in aqueous and perchloric acid media whereas intramolecular hydrogen bonding should be relatively unaffected. The long wavelength excitation maxima and fluorescence maxima of Ph, PhH$^+$ and PhH$_2^{2+}$ are presented in Table III.

Table III. Long-Wavelength Excitation Maxima and Fluorescence Maxima of *o*-Phenanthroline[a] in *n*-Pentane

	Maxima, cm^{-1}	
	Excitation	Fluorescence
Ph	3.12×10^4	2.79×10^4
PhH$^+$	2.92×10^4	2.61×10^4
PhH$_2^{2+}$	2.78×10^4	2.48×10^4

[a] Ph = neutral species, PhH$^+$ = singly protonated species, PhH$_2^{2+}$ = doubly protonated species.

The data of Table III were then used to calculate hypothetical values of pK_{s_1}* and pK_{s_2}* using the ground-state pK_a values and the Förster cycle. "pK_s*" values were calculated using excitation spectra alone, fluorescence spectra alone, and the average of the two to locate the 0–0 band. These results are shown in Table IV.

Table IV. "pK_s*" Values of the Prototropic Equilibria of *o*-Phenanthroline in the Lowest Excited Singlet State (^1L_b) in *n*-Pentane[a]

	"pK_s*(e)"	"pK_s*(f)"	"pK_s*(0–0)"
PhH$_2^{2+*}$ $\overset{\text{"p}K_{s_1}\text{"}}{\rightleftharpoons}$ PhH^{+*} + H$^+$	1.5	1.3	1.4
PhH$_2^{+*}$ $\overset{\text{"p}K_{s_2}\text{*"}}{\rightleftharpoons}$ Ph* + H$^+$	9.1	8.7	8.9

[a] Calculated from shifts in excitation maxima ("pK*(e)"), emission maxima ("pK*(f)"), and the 0–0 bands of the $^1A \leftrightarrow {}^1L_b$ transitions ("pK_s*(0–0)"), respectively. The ground-state values are pK_{a_1} = −1.4 and pK_{a_2} = 4.85.

(11) A. Weller, *Progr. React. Kinet.*, 1, 187 (1961).

(12) S. G. Schulman and H. Gershon, *J. Phys. Chem.*, 72, 3279 (1968).

The data of Table IV are quite different from those in Table II, all "pK_s*" values corresponding to the same equilibrium being in good agreement. Furthermore, the values of "pK_{s_1}*" are all more basic than pK_{a_1}. Because intramolecular hydrogen bonding should be relatively unaffected by the change in solvent and because the "pK_{s_1}*" values indicate that in the weakly relaxing *n*-pentane solvent the intramolecular charge transfer accompanying excitation to the ^1L_b state does indeed increase the basicity of both nitrogen atoms relative to the ground state, the anomalously acidic values of pK_{s1}* obtained from fluorescence measurements in aqueous and perchloric acid media must indeed be due to strong stabilization of PhH^{2+} by solvent relaxation.

Additional evidence for the importance of solvent relaxation in the hydroxylic solvents is to be had from the relative separations of the excitation and emission maxima (the Stokes shifts) of each species in *n*-pentane and in aqueous and perchloric acid media. At room temperature, Stokes shifts are due to vibrational and solvent relaxation in the fluorescing species relative to the corresponding absorbing species. In *n*-pentane, the Stokes shifts for Ph, PhH^+, and PhH_2^{2+} are respectively 3.3×10^3, 3.1×10^3, and 3.0×10^3 cm^{-1}. In the more strongly relaxing aqueous and perchloric acid media, the corresponding Stokes shifts are 3.7×10^3, 5.0×10^3, and 3.0×10^3 cm^{-1}, respectively. The large discrepancy between the Stokes shifts in PhH^+ in *n*-pentane and water must be due to solvent relaxation, because the change in solvent should not exhibit a very marked effect upon the vibrational structure of the ground or excited states of the molecule. The large Stokes shift of PhH^+ in water indicates that this species is stabilized in the excited state much more than in the ground state relative to the species with which it is in prototropic equilibrium. The good agreement obtained in *n*-pentane between all "pK_s*" values corresponding to the same equilibria strongly suggests that the major part of the disagreement between the pK_s* values determined in water and in perchloric acid, by the Förster cycle, is due to the differences in the solvent environment about the molecules from which fluorescence and absorption occur.

The above anomaly was also discussed by Wehry and Rogers[13] for a series of hydroxyaromatics. However, in the latter study, it was suggested that pK_s* determined from the Förster cycle using fluorescence spectra alone were no more valid than those obtained using absorption spectra alone and that the averaging of absorption and emission spectra to obtain the 0–0 band for use in the Förster cycle gave the most meaningful pK_s* value. We disagree with this means of obtaining a pK_s* value. The technique of locating the 0–0 band might be meaningful if solvent relaxation were not involved. However, relaxation of the excited species with the solvent entails the result that the 0–0 band for absorption and that for emission will not coincide. Consequently, *averaging of absorption and emission maxima do not yield a thermodynamically significant value for the transition energy unless solvent relaxation is identical for both members of a conjugate pair.* Because ground-state thermodynamic reaction parameters are defined with respect to the ground-state molecules in the ground-state equilibrium solvent-cage configuration, it would probably be most meaningful to define excited-state thermodynamic parameters with respect to excited species in their excited-state equilibrium solvent-cage configurations. In this scheme, the "best" value of a pK_s* would be obtained by fluorimetric titration under conditions where equilibrium is established in the excited state. If this is not possible, because solvent relaxation discrepancies seem to outweigh vibrational discrepancies, the next best value would be obtained by application of the Förster cycle using fluorescence maxima alone (if both members of the conjugate pair are fluorescent). Conversely, while fluorimetric titration data and Förster cycle calculations employing fluorescence maxima measured in water at room temperature yield thermodynamic information about the excited state, they do not reliably yield information about transition polarizations. The latter information is to be had from Förster-cycle-type calculations using only absorption spectra or emission spectra taken in rigid or nonpolar media where solvent relaxation does not interfere with spectral shifts occurring as a result of the change of the intrinsic electronic dipole moment upon absorption or emission. The results of this study support these conclusions.

(13) E. L. Wehry and L. B. Rogers, *Spectrochim. Acta*, 21, 1976 (1965).