Excited-State Prototropic Reactivity in Salicylamide and Salicylanilide

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Abstract: Steady-state fluorescence measurements have suggested the existence in both salicylamide and salicylanilide of "open" and "closed-ring" ground-state conformers which give rise to different emissions upon excitation. Excitation of the "closed-ring" conformer leads to intramolecular proton transfer across the preexisting H bond to give the excited zwitterion. Excitation of the "open-ring" conformer gives rise to both emission from an uncharged excited state and emission from an excited phenolate species, the relative intensities depending on the pH. Laser/streak camera measurements have yielded the phenolate and zwitterion lifetimes and indicate that the protolytic reactions are extremely fast.

Introduction

The multiple fluorescences of methyl salicylate have been extensively studied since Weller first discussed them in 1956.[1] The fluorescence spectrum is characterized by two bands—the longer wavelength band having an unusually large Stokes shift. Furthermore, the intensity ratio of the two bands is both solvent and excitation wavelength dependent.[2,3]

The commonly accepted explanation for these phenomena is that developed from the work of Klöpffer and Naundorf[3] and, later, Sandros,[2] who postulated the existence of cis and trans conformers in a ground-state equilibrium. The "open-ring" trans form is stabilized in appropriate cases by intermolecular H bonding with the solvent. Excitation of the intramolecularly H-bonded cis conformer leads to a rapid and virtually complete proton transfer across the H bond, and the resulting resonance-stabilized zwitterion is responsible for the long-wavelength emission. Excitation of the trans conformer, in which intramolecular proton transfer is impossible, gives rise to the short-wavelength emission. Recent work performed in our laboratory[4] has suggested the existence of a third ground-state conformer which, when excited, contributes to the short-wavelength emission.

Although methyl salicylate has been thoroughly studied, it is only recently that detailed investigations of other salicylic acid derivatives have begun.[5-9] One of the more interesting derivatives is salicylamide. In a recent paper[6] we have discussed kinetic evidence which suggests that the 420-nm fluorescence band of salicylamide consists of two overlapping components. We interpreted these results in terms of a dual, excited-state proton-transfer mechanism in which the phenolic proton can undergo either intramolecular transfer, forming an excited zwitterion, or intermolecular transfer to the solvent, resulting in the excited-state phenolate anion (Figure 1). Recently, Schulman and Underberg[7] have reported results which substantiate our findings.

Another salicylic acid derivative, closely related to salicylamide, is salicylanilide. While there have been some investigations of this molecule,[5,8] these have been confined to studies of absorption spectra and fluorescence in nonaqueous solvents. This is probably due to a combination of its very low quantum yield and its low solubility in water.

In this paper we extend our investigations of salicylamide to include comprehensive steady-state measurements. We report the results of both kinetic and steady-state measurements on salicylanilide and compare them with those for salicylamide. These studies have revealed certain similarities to the photochemistry of methyl salicylate and help in establishing general principles of the photochemistry of salicylic acid derivatives.

Experimental Section

Salicylamide was obtained from BDH and its purity checked both spectroscopically and by melting point determination. Salicylanilide (Purum grade) was supplied by Fluka. It was purified by vacuum sublimation and the purity was then checked both spectroscopically and by melting point determination. D_2O (99.75%) was supplied by the Australian Atomic Energy Commission. Methanol was redistilled, and spectroscopic grade cyclohexane was chromatographically purified by passing it through a column of 60–120 mesh silica gel (BDH) prior to use. These procedures rendered solvents free of fluorescent impurities. All aqueous solutions were made by using triply-distilled water. Adjustments of pH were made with either sodium hydroxide or hydrochloric acid. Measurements of pH were made by using a glass electrode/pH meter combination, and estimates of pD were obtained from pD ≈ (meter reading + 0.4).[10]

Fluorescence emission and excitation spectra were recorded on a Perkin-Elmer MPF-44A spectrofluorimeter. Excitation spectra were run on dilute solutions (maximum absorbance ≤0.11) to avoid distortions of the spectrum which arise out of the optical geometry of the instrument. All excitation and emission spectra reported here are uncorrected. Because of the low quantum yield and solubility of salicylanilide in water and D_2O, saturated solutions (ca. 7×10^{-5} M) were used for all measurements other than the fluorescence excitation spectra.

Fluorescence lifetime measurements in nonaqueous solvents were made by using 10^{-3} M solutions. The laser/streak camera/OMA apparatus used for the fluorescence decay measurements was similar to that previously described by Fleming et al.[11] A single pulse selected from the output of a mode-locked Nd^{3+}–phosphate glass laser was amplified, and the fourth harmonic was generated in two frequency-doubling stages. The resulting pulse ($\lambda \approx 264$ nm, $t_p \approx 6$ ps, $E \approx 0.1$ mJ) was focused into the solution contained in a 1-cm quartz cuvette. Previous work has established that distortion of fluorescence decay curves by rotational relaxation is not a problem with these systems.[6] The input optics of our streak camera are of heavy flint glass, having a transmission cut-off at ca. 400 nm. Fluorescence decay data were analyzed by nonlinear least-squares curve fitting, using a NOVA 2/10 computer.

Results and Discussion

A. Salicylamide. The fluorescence spectra, as a function of excitation wavelength, for salicylamide (1×10^{-4} M) in aqueous solution at pH values of 3.0 and 0.3 are shown in Figures 2 and 3, respectively. From the equilibrium constants for ground-state protolytic reactions of salicylamide,[8] we can be sure that all ground-state salicylamide in solution will exist in the neutral molecule form at the above pH values. An examination of Figures

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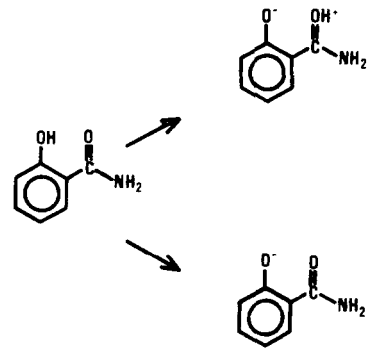

Figure 1. Excited-state reaction scheme for aqueous salicylamide.

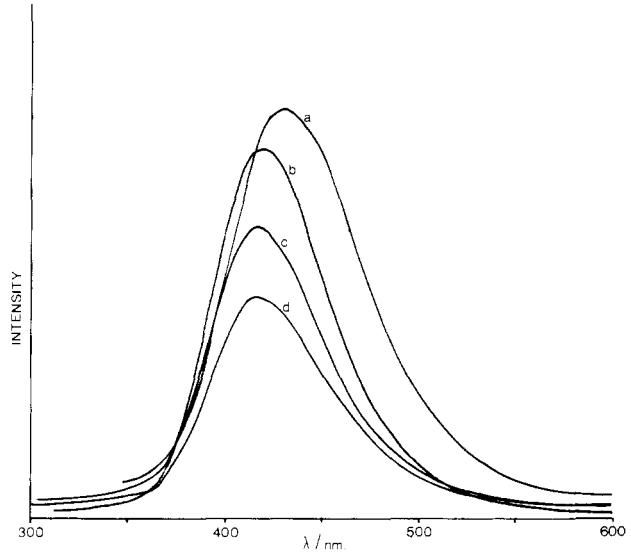

Figure 2. Fluorescence of aqueous salicylamide (1×10^{-4} M) at pH 3. Excitation wavelengths (nm): (a) 330; (b) 300; (c) 265; (d) 230. (Raman scatter peaks have been removed.) Intensities have been adjusted for the sake of clarity.

2 and 3 reveals some interesting differences. Firstly, we note the appearance, at the lower pH, of a new, short-wavelength emission at ca. 355 nm. This band is either very weak or nonexistent at pH 3. Furthermore, if the pH is gradually reduced from a value of 3.0 to 0.3, one observes a corresponding increase in the intensity of the 355-nm emission relative to that of the long-wavelength band. Figure 3 further illustrates that the intensity ratio of short- to long-wavelength emission, in addition to being pH dependent, is excitation wavelength dependent. Shorter wavelength excitation increases the intensity of the 355-nm band relative to that of the longer wavelength emission.

Another obvious difference between Figures 2 and 3 relates to the long-wavelength emission maximum. At a pH of 3, this maximum shifts to shorter wavelengths as the excitation wavelength is decreased, while for a pH of 0.3, no such shift is observed. The long-wavelength emission maximum is at 434 nm for pH 0.3, whereas at pH 3.0, it shifts from 432 nm (λ_{ex} = 330 nm) to 420 nm (λ_{ex} = 300 nm) and finally to 417 nm (λ_{ex} = 265 nm and 230 nm). In a separate experiment on an aqueous solution of salicylamide at a pH of 11, where virtually all ground-state salicylamide exists as the phenolate anion, the emission consisted of a single band, maximal at 417 nm, and exhibited no shift with excitation wavelength. These results indicate the existence of more than one ground-state conformer of neutral salicylamide, these conformers having different absorption spectra.

Our results can most plausibly be explained by postulating the existence of three distinct *excited-state* species. The first of these is neutral salicylamide (I), which is responsible for the emission band at ca. 355 nm. Emitting further to the red is the phenolate anion (II), resulting from excited-state proton transfer to the solvent, and the zwitterion (III) formed by intramolecular proton

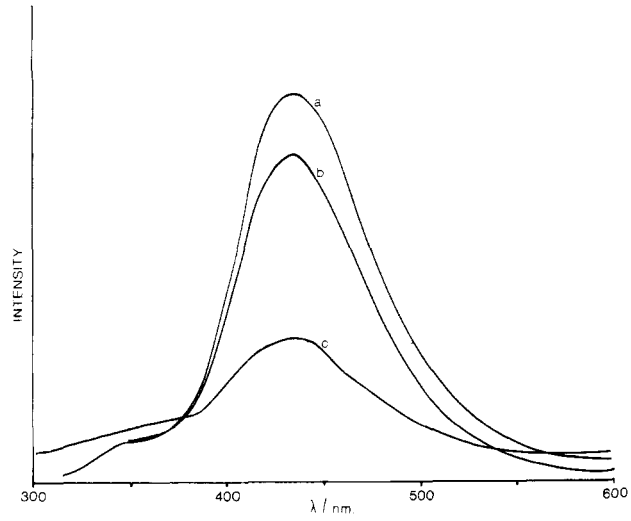

Figure 3. Fluorescence of aqueous salicylamide (1×10^{-4} M) at pH 0.3. Excitation wavelengths (nm): (a) 330; (b) 300; (c) 265.

Table I

wavelength band, nm	fraction of short-lived component
400	0.34 ± 0.09
>495	0.80 ± 0.02

transfer. The excited phenolate anion is responsible for an emission band having its maximum at 417 nm, while the zwitterion fluorescence is maximal at 434 nm.

A considerable body of evidence favors these assignments. First, they can be rationalized on the basis of the expected Stokes shifts of fluorescence. Both the zwitterion and the phenolate anion are capable of considerable resonance stabilization by delocalization of their formal charges. It is therefore expected that their fluorescences will exhibit unusually large Stokes shifts. The neutral molecule, on the other hand, can undergo no stabilization, and its fluorescence should exhibit a more normal Stokes shift. Effects such as these are commonly observed in molecules such as 2-naphthol[12] and methyl salicylate,[13] which undergo excited-state proton-transfer reactions.

Further justification of the assignment of an emission maximum of 417 nm to the phenolate anion is obtained from the emission spectrum for salicylamide in aqueous solution at a pH of 11. At this pH salicylamide exists solely as the phenolate anion in both the ground- and first excited-singlet states.[8] As mentioned earlier, the spectrum consists of a single band having its maximum at 417 nm. It must be stressed, however, that at pH values below 6, the phenolate anion is created after excitation of a neutral molecule ground state rather than by direct excitation of a ground-state phenolate anion.

In a previous paper[6] we presented evidence concerning the fluorescence decay of the long-wavelength emission ($\lambda_{em} \geq 400$ nm) of aqueous salicylamide at a pH of 5.6. At this pH virtually all ground-state salicylamide exists as the neutral molecule.[8] It was found that the fluorescence decay could be well fitted by a double-exponential decay function where τ_1 and τ_2 are fluorescence

$$I(t) = A[f \exp(-t/\tau_1) + (1-f) \exp(-t/\tau_2)] + B \quad (1)$$

lifetimes, f is the fractional contribution to the zero-time intensity by the component having lifetime τ_1, and B is a baseline parameter. A fluorescence decay curve was recorded for each of three different wavelength sections of the emission, using suitable filters. Bands at 400 and 440 nm were isolated by interference filters having a band-pass of 35 and 20 nm, respectively, and the emission above 495 nm was isolated with a Schott GG-495 sharp cut filter.

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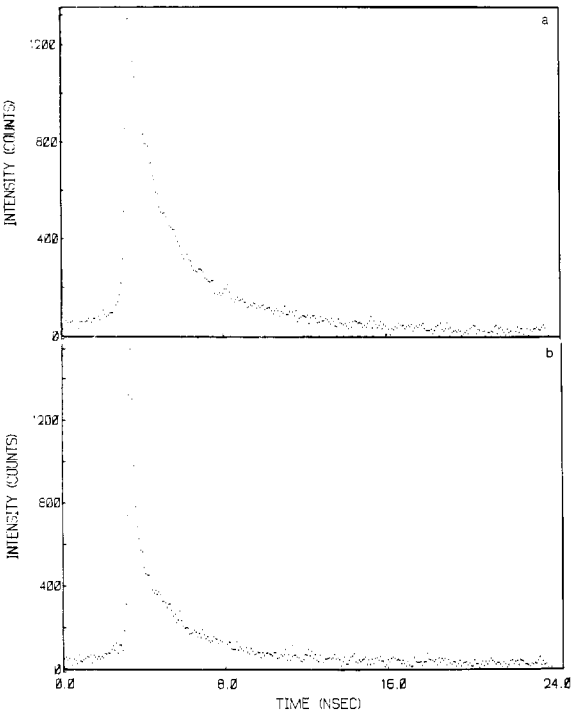

Figure 4. Fluorescence decay curves for aqueous salicylamide (1×10^{-3} M): (a) λ_{em} = 400–430 nm; (b) $\lambda_{em} \geq$ 495 nm.

The data are fitted by the same two lifetimes in all three cases, but with an increase in the fraction of the short-lived component as the emission wavelength increases. The lifetimes of the two components were found to be 1.87 ± 0.20 and 0.11 ± 0.01 ns. The decay curves for the 400 and >495 nm emissions are shown in Figure 4. Table I summarizes the results. Similar results were obtained for acidified solutions. In the earlier work it was assumed that what was observed was the decay of two distinct species with the growth times being negligible. A more complete discussion of the kinetics expected in these systems is given in a later section.

The fluorescence decay curve for an aqueous salicylamide solution at a pH of 9.1, where almost all ground-state salicylamide exists as the phenolate anion, is a single exponential with a lifetime of 1.96 ± 0.12 ns. The coincidence between this lifetime and that of the long-lived component of the pH 5.6 decay curves led us to suggest that the phenolate anion is a major contributor to the long-wavelength emission band of aqueous salicylamide. Furthermore, the trend in the value of the fraction of short-lived component with emission wavelength indicates that the zwitterion fluorescence lies to the red of that of the phenolate anion. This compelling evidence is the final justification of our original assignment of the fluorescence bands.

For aqueous salicylamide at a pH of 5.6, we did not observe any dependence of the emission spectrum upon excitation wavelength.[6] This is hardly surprising, considering the kinetic results suggest that more than 95% of the total emission intensity is due to the excited phenolate anion. Because the fluorescence lifetime of the zwitterion is considerably less than that of the phenolate anion, its contribution of less than 5% to the emission shows up clearly in the measured fluorescence decay. Obviously kinetic measurements can be a highly sensitive method of detecting minor contributions to a multicomponent fluorescence, especially in cases where the fluorescence lifetimes of the species are widely separated. Conversely, had the kinetic measurements been taken under conditions where there were equal contributions to the total emission intensity by both zwitterion and phenolate anion, detection of the two-component decay would have been rendered more difficult. This difficulty would arise because these conditions, and the relative values of the fluorescence lifetimes, require that the parameter f in eq 1 assume a value of ca. 0.94. This means that only 6% of the "zero-time" intensity would arise from the excited phenolate anion. It appears, therefore, that the combination of steady-state and kinetic measurements is a far more powerful tool, in many photochemical investigations, than either technique alone.

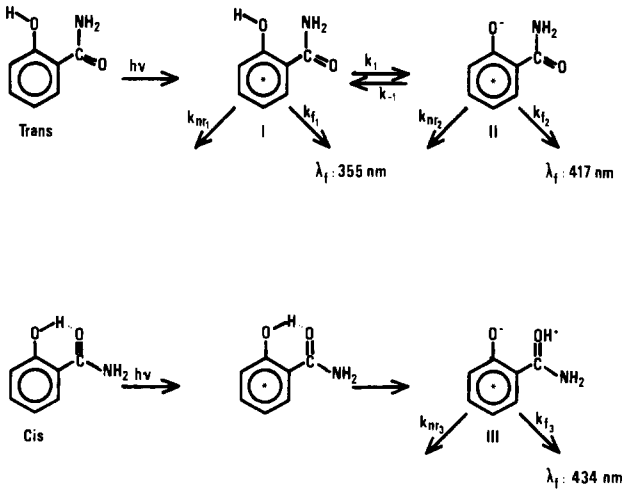

Figure 5. Proposed photochemical scheme for aqueous salicylamide.

It now appears that the shift in the emission spectrum with excitation wavelength at pH 3 (Figure 2) can be explained by a competition between the phenolate anion and the zwitterion emissions. Shorter wavelength excitation favors phenolate emission, indicating that the ground-state precursor of the excited phenolate anion absorbs at shorter wavelengths than its excited zwitterion counterpart.

Previously, we noted that as the pH is lowered (i) the intensity ratio of short- to long-wavelength emission increases and (ii) the magnitude of the excitation wavelength dependent shift of the long-wavelength emission maximum decreases until at a pH of 0.3 only zwitterion emission (and hence no shift) is observed. These two features together indicate that as the pH is lowered, the neutral molecule fluorescence intensifies while that of the phenolate anion diminishes. This suggests that the excited-state neutral molecule and phenolate anion have a common ground-state precursor.

The probable ground-state precursor of the excited zwitterion is a "closed-ring", intramolecularly H-bonded salicylamide conformer. An "open-ring" ground-state conformer could be expected to give rise to both the excited-state neutral molecule and phenolate anion. These ground-state conformers, together with the proposed photochemical scheme for salicylamide, are shown in Figure 5. For the sake of consistency, we will follow Sandros' assignments for methyl salicylate and call the "open-ring" form the trans conformer and the intramolecularly H-bonded form the cis conformer.

This model predicts expressions 2 and 3 for the fluorescence

$$\phi_f(S^*) = \frac{k_{f_1}(k_{-1}[H^+] + k_{f_2} + k_{nr_2})}{(k_1 + k_{f_1} + k_{nr_1})(k_{f_2} + k_{nr_2}) + (k_{f_1} + k_{nr_1})k_{-1}[H^+]} \quad (2)$$

$$\phi_f(P^*) = \frac{k_1 k_{f_2}}{k_1(k_{f_2} + k_{nr_2}) + (k_{f_1} + k_{nr_1})(k_{-1}[H^+] + k_{f_2} + k_{nr_2})} \quad (3)$$

quantum yields for excited neutral salicylamide (S*) and phenolate anion (P*). Therefore, at high pH values ([H+] → 0), we see that

$$\phi_f(S^*) \rightarrow \frac{k_{f_1}}{k_1 + k_{f_1} + k_{nr_1}} \quad (4)$$

and

$$\phi_f(P^*) \rightarrow \frac{k_1 k_{f_2}}{(k_1 + k_{f_1} + k_{nr_1})(k_{f_2} + k_{nr_2})} \quad (5)$$

These equations also show that as the pH is lowered, the

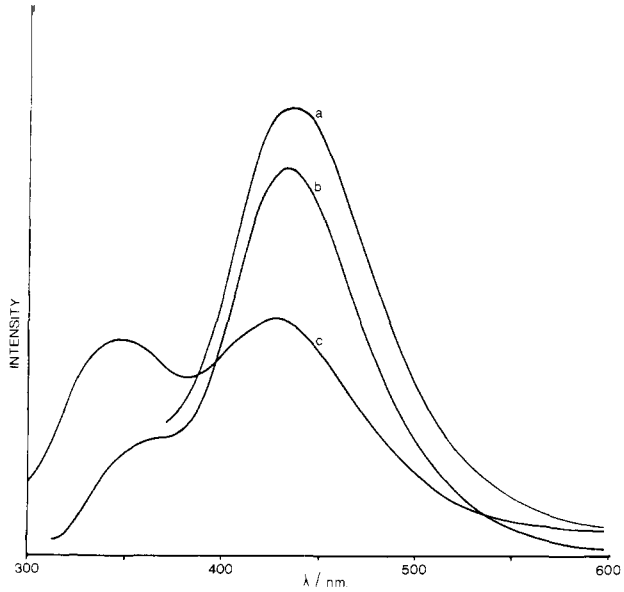

Figure 6. Fluorescence of salicylamide in D_2O (1.7×10^{-4} M). Excitation wavelengths (nm): (a) 330; (b) 300; (c) 265.

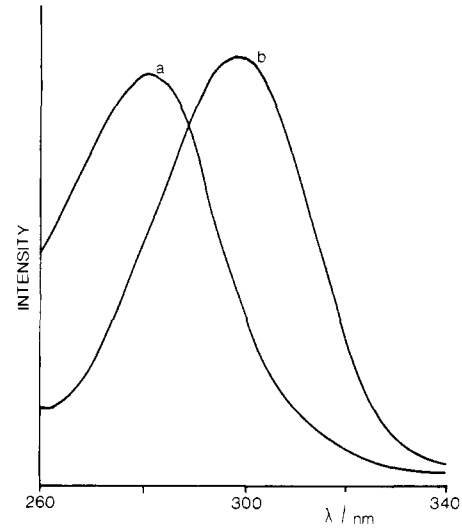

Figure 7. Fluorescence excitation spectra for salicylamide in D_2O. Emission wavelengths (nm): (a) 350; (b) 430.

fluorescence quantum yield of the neutral molecule increases, while that of the phenolate anion decreases. The model thus qualitatively predicts the observed pH dependence of the neutral molecule and phenolate anion emission intensities.

In simpler terms, the relative excited-state populations, and hence the steady-state emission intensities of the two species, are governed by the rates of interconversion between these species. The forward rate (having rate constant k_1) is first order with respect to neutral excited salicylamide, and at sufficiently high H^+ concentrations (pH $\leq$3), the back-reaction is pseudo first order with respect to phenolate anion. The pseudo-first-order rate constant will be $k_{-1}[H^+]$. As the hydrogen ion concentration increases, this rate constant will increase relative to the radiative and nonradiative deactivation rates for phenolate anion, resulting in a decrease in the phenolate quantum yield. The increased rate of the back-reaction should also lead to a more neutral molecule and consequently to an increase in its emission intensity.

A Förster cycle calculation of the excited-state pK_a for the loss of the salicylamide phenolic proton gives a value of ca. 1.1. While this value is consistent with our observations, we are unable to positively assert that equilibrium is established in the excited state.

B. Isotope Effect. A kinetic isotope effect on the rate of proton-transfer reactions has been widely observed. In an attempt to decrease the rate of deprotonation, and consequently increase the neutral molecule emission intensity, the steady-state experiments reported above were repeated in D_2O. The fluorescence spectra for salicylamide in D_2O (pD 0.5) at various excitation wavelengths are shown in Figure 6. In changing from H_2O to D_2O, we observed a large increase in the intensity of the 355-nm emission band relative to that of the long-wavelength band. Emission spectra at various excitation wavelengths recorded at pDs in the range from 5.7 to 0.5 showed features identical with those recorded in water, other than the 355-nm band always being relatively more intense. The effect of deuteration will be complex depending on the degree to which the various rate constants are affected. It is also possible that there is some effect of deuteration on the nonradiative rate of the neutral form. The zwitterion concentration remains constant on deuteration. The intramolecular proton transfer is expected to be so rapid that even when slowed by deuteration, the zwitterion yield is unaffected. The increased intensity of the neutral molecule emission shown in Figure 6 makes it possible to record the excitation spectra of the neutral and zwitterion species. These spectra are shown in Figure 7. As expected, the ground-state precursor (trans conformer) of excited neutral salicylamide absorbs at shorter wavelengths than the cis ground-state conformer, which leads to excited-state zwitterion. The excitation spectra are maximal at 281 and 298 nm. It would be desirable to check the postulate that excitation of the trans conformer results in both neutral molecule and phenolate anion emissions by a comparison of the excitation spectra of these species. Unfortunately, the zwitterion emission considerably overlaps that of the phenolate anion, making it impossible to find a wavelength at which phenolate is the sole emitter. The excitation spectra for 400- and 470-nm emission in D_2O at a pD of 2.6 have been measured. That of the 400-nm emission lies at shorter wavelength (λ_{max} = 294 nm) than that of the 470-nm emission (λ_{max} = 296 nm). The phenolate anion makes a greater contribution than the zwitterion to the 400-nm emission intensity and vice versa at 470 nm. The observation is consistent with the phenolate anion excitation spectrum lying at shorter wavelengths than that of the zwitterion. It appears that both excited neutral salicylamide and phenolate anion have ground-state precursors which absorb to the blue of that of the excited zwitterion. While the excitation spectra do not unambiguously show both the excited neutral molecule and the phenolate anion to have the same ground-state precursor, they are still entirely consistent with the proposed model.

It is interesting to consider the parallels between this work and that of Klöpffer and Naundorf,[3] who observed that the dual emission of methyl salicylate arose out of two distinct ground-state conformers. In both their study and ours, the conformer giving rise to the excited zwitterion had its absorption at longer wavelengths than that giving rise to the neutral molecule emission.

C. Salicylanilide. The study of aqueous solutions of salicylanilide is complicated by the extremely low solubility. It is desirable to work with a saturated solution, and under these circumstances care must be taken to ensure complete dissolution. We have observed the 350-nm emission, which we attribute to the neutral salicylanilide molecule, to be less intense in solutions that have been aged. Furthermore, the emission bands appear to be broadened in nonaged solutions. We believe that this may be due to the slow breakup of micellar aggregates which form in the first stage of dissolution. The greater intensity of the 350-nm band is compatible with an inhibition of intramolecular hydrogen bond formation in the micellar situation. We have adopted a practice of gently warming our solutions in a water bath at 40 °C for approximately 1 h. This produces no apparent decomposition of the solution (as determined spectroscopically) and gives a sufficiently concentrated solution for spectra of average quality to be obtained. Results obtained by using this method are identical with those produced from a solution that has aged for 1 week.

The fluorescence spectra, as a function of excitation wavelength, for aqueous salicylanilide at pH values between 3 and 0.3 have been measured. At these values of pH all ground-state salicylanilide will exist as the neutral molecule.[8] Figures 8 and 9 illustrate the results for pH values of 3.0 and 0.3, respectively. In all of the spectra we observe a weak emission band in the vicinity of

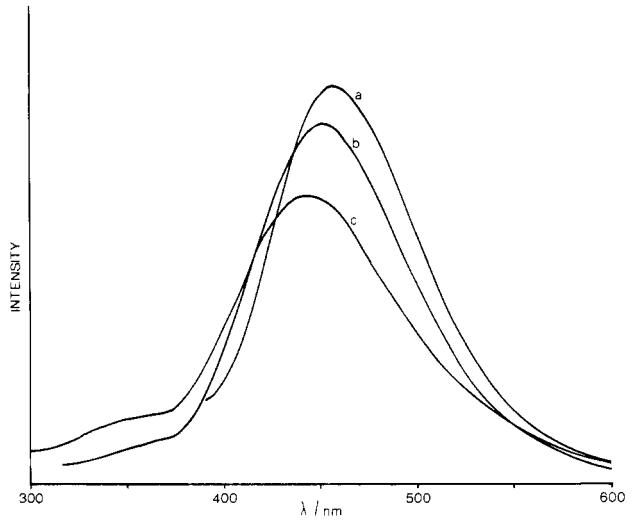

Figure 8. Fluorescence of aqueous salicylanilide (7×10^{-5} M) at pH 3. Excitation wavelengths (nm): (a) 330; (b) 300; (c) 265.

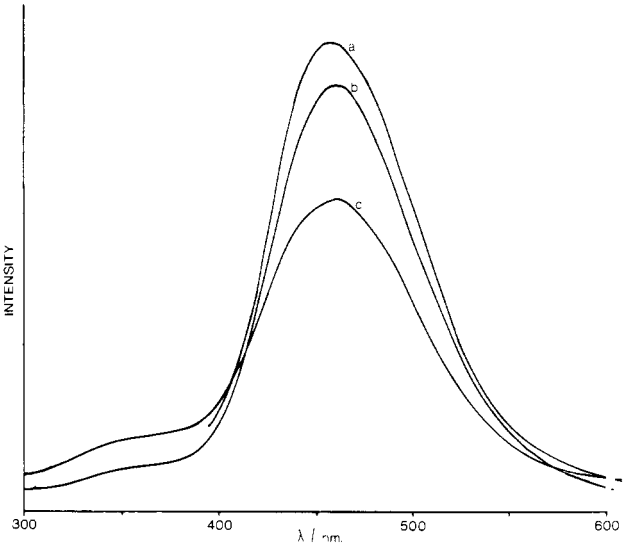

Figure 9. Fluorescence of aqueous salicylanilide (7×10^{-5} M) at pH 0.3. Excitation wavelengths (nm): (a) 330; (b) 300; (c) 265.

350 nm. The intensity of this band relative to that of the long-wavelength emission is both pH and excitation wavelength dependent. It becomes relatively more intense as the pH is lowered and also as the excitation wavelength is decreased. The long-wavelength emission band exhibits an excitation wavelength dependent shift. The magnitude of this shift decreases as the pH is lowered until at a pH of 0.3 no shift is observed. In this case the long-wavelength emission band is maximal at 457 nm. An aqueous salicylanilide solution at a pH of 12, where all ground-state molecules exist as the phenolate anion,[8] showed a single fluorescence band with its maximum at 423 nm.

Aqueous salicylanilide shows behavior almost identical with that of aqueous salicylamide. Therefore we believe that the explanations proposed for the latter apply equally to the former. For salicylanilide we assign the band at ca. 350 nm to the neutral molecule, a band at 423 nm to the excited phenolate anion, and a band at 457 nm to the excited state zwitterion. The latter two bands overlap considerably to form what we term the long-wavelength emission band.

There are two noticeable differences between the emission spectra of salicylamide and those of salicylanilide: (i) At a pH of 3, the short wavelength emission band of salicylanilide is observed, whereas for salicylamide it is not. (ii) The increase in the relative intensity of the short-wavelength emission as the pH is lowered is much less pronounced for salicylanilide than it is for salicylamide. These differences can be explained by a lower rate

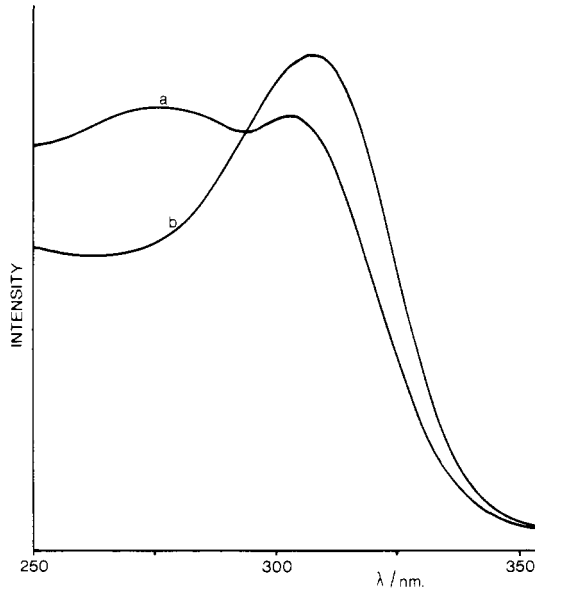

Figure 10. Fluorescence excitation spectra for aqueous salicylanilide at pH 3. Emission wavelengths (nm): (a) 425; (b) 490.

of deprotonation (k_1) in salicylanilide than in salicylamide.

In aqueous salicylanilide, the neutral molecule (350-nm) emission is too weak to permit recording of its excitation spectrum. The excitation spectra for the 425- and 490-nm emissions can, however, be obtained. Although both the zwitterion and the phenolate anion contribute to the emission at these wavelengths, the 490-nm emission has a considerably higher contribution from the zwitterion than does the 425-nm emission. Conversely the 425-nm emission is mainly due to the phenolate anion but with a minor contribution from the zwitterion. The excitation spectra are shown in Figure 10.

On the basis of the proposed model it appears that the 300-nm shoulder in the absorption spectrum[8] can be identified with the "closed-ring" cis conformer, while the band in the 270-nm region is more characteristic of the "open-ring" trans conformer. These conclusions are consistent with those of Klöpffer, who commented on the decrease in the long-wavelength absorption in changing the solvent from hexane to ethanol.[5] It is a consistent feature of salicylic acid derivatives that the zwitterion ground-state precursor absorbs at longer wavelengths than the precursor of either the uncharged or phenolate excited species.

D. Lifetime Measurements. The very low quantum yield of aqueous salicylanilide has prevented us from unambiguously observing multicomponent fluorescence decays similar to those reported earlier for salicylamide.[6] The steady-state experiments undoubtedly point to their existence, but our streak camera is insufficiently sensitive to produce data of the required signal-to-noise ratio for their clear observation. Nevertheless, we have measured the fluorescence decay of aqueous salicylanilide at pH values of 12 and 2. The fluorescence decay at a pH of 12 is solely that of the phenolate anion which exhibits a single exponential decay with a lifetime of 150 ± 15 ps. It should be remembered that in this case the phenolate anion is the sole species in both ground and excited states and hence a single exponential fluorescence decay is expected. At the lower pH, however, the excited phenolate anion arises from excitation of a neutral molecule ground state and hence its fluorescence decay is expected to be nonexponential (see below). The measured fluorescence decay at a pH of 2 should be a superposition of the excited zwitterion and the phenolate anion (arising from proton transfer to the solvent) fluorescence decays. Although in this case our data are quite noisy ($S/N \approx 10$ at maximum), we have been able to extract an upper limit estimate for the zwitterion lifetime of 40 ps. It is interesting that both this and the phenolate anion decay times are considerably shorter than the corresponding decay times in the salicylamide case; however, the reason for this large difference is not apparent.

E. Salicylanilide in Methanol. The absorption spectrum for salicylanilide in methanol is similar to that reported in ethanol.[5] An absorption maximum is observed at 267 nm with a shoulder at 302 nm, the latter thought to be characteristic of the intramolecularly H-bonded ground state.[5] The fluorescence spectrum exhibits a marked dependence upon the excitation wavelength, and there is no evidence of any 350-nm emission. The excitation wavelength dependence differs markedly from that observed in aqueous solution, and detailed investigations have shown that it is caused by the presence of a minute amount of ground-state phenolate anion. The amount is sufficiently small that it cannot be detected in the absorption spectrum but is nevertheless able to influence the emission spectrum because of the high quantum yield of phenolate compared to zwitterion. When excited at 265 nm, the emission is apparently a single band with maximum at 422 nm. The fluorescence decay of salicylanilide in methanol is well fitted by the sum of two exponentials (eq 1), the two decay times being those of the zwitterion and the phenolate anion.

When the solution is made 10^{-3} M with respect to HCl, the ground-state dissociation is suppressed and the fluorescence now consists of a single band with maximum at 457 nm and exhibits no excitation wavelength dependence. The absorption spectrum is, however, indistinguishable from that in pure methanol. The 457-nm emission is characteristic of the excited zwitterion. Our inability to detect any fluorescence from the neutral molecule, when the absorption spectrum points to the existence of the "open-ring", trans ground-state conformer, suggests that the excited neutral molecule has an exceedingly low quantum yield in methanol. Similar behavior has been reported for salicylanilide in ethanol.[5] The fluorescence decay of the zwitterion is a single exponential with a lifetime of 70 ± 10 ps. No risetime for the fluorescence is observed, indicating a lower limit for the rate of intramolecular proton transfer in methanol of 2×10^{11} s^{-1}.

When the solutions are made 10^{-3} M in NaOH, the phenolate anion becomes the sole ground-state species. The absorption spectrum of this species is radically different from that of the neutral molecule, consisting of a maximum at 338 nm, with another at 270 nm, and a weak shoulder at ca. 290 nm. This agrees with earlier observations.[14] The fluorescence spectrum is a single intense band, maximal at 420 nm, and exhibits no shift with excitation wavelength. The fluorescence decay of the phenolate anion in methanol is a single exponential with a lifetime of 2.32 ± 0.15 ns.

In an earlier paper, we drew attention to the Stokes shift for the long-wavelength band of salicylamide in ethanol.[6] This band lies to the red of that observed in water but to the blue of that observed in cyclohexane. It is now clear that while the emissions in water and cyclohexane are dominated by phenolate and zwitterion, respectively, the long-wavelength emission in ethanol contains appreciable contributions from both species.

F. The Kinetic Model. The suggested kinetic model leads to the coupled differential equations 6 and 7, where [S*] and [P*]

$$\frac{d[S^*]}{dt} = k_{-1}[H^+][P^*] - (k_1 + k_{f_1} + k_{nr_1})[S^*] \quad (6)$$

$$\frac{d[P^*]}{dt} = k_1[S^*] - (k_{-1}[H^+] + k_{f_2} + k_{nr_2})[P^*] \quad (7)$$

represent the concentration of excited-state neutral molecule and phenolate anion, respectively. The constants are defined in Figure 5.

These equations can be solved by standard methods[15] to yield expressions 8 and 9 for the excited-state concentrations of the two

$$[S^*] = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) \quad (8)$$

$$[P^*] = A_3\{\exp(-t/\tau_1) - \exp(-t/\tau_2)\} \quad (9)$$

species. Hence, both of these species should exhibit a nonexponential fluorescence decay. The nonexponentiality arises as a consequence of the excited state interconversion between neutral molecule and phenolate anion.

The lifetimes, τ_1 and τ_2, are given by expressions 10 and 11,

$$\tau_1^{-1} = \tfrac{1}{2}[p - (p^2 - 4q)^{1/2}] \quad (10)$$

$$\tau_2^{-1} = \tfrac{1}{2}[p + (p^2 - 4q)^{1/2}] \quad (11)$$

where $p = k_1 + k_{-1}[H^+] + k_{f_1} + k_{nr_1} + k_{f_2} + k_{nr_2}$ and $q = k_1(k_{f_2} + k_{nr_2}) + k_{-1}[H^+](k_{f_1} + k_{nr_1}) + (k_{f_1} + k_{nr_1})(k_{f_2} + k_{nr_2})$. The preexponential factors can be calculated by making use of the boundary conditions.

The excited-state zwitterion can be treated independently of this model. Provided that the rate of intramolecular proton transfer is fast, it should exhibit a single exponential fluorescence decay.

Equation 9 indicates that the excited phenolate anion fluorescence, where it originates from excitation of a ground-state neutral trans conformer, should display a "grow-in" or risetime. It has previously been mentioned[6] that in our measurements of the salicylamide fluorescence decay ($\lambda_{em} \geq 400$ nm) no "grow-in" was observed. The nonexponential decay was well fitted by the sum of two exponentials, the lifetimes of which represent the *decay* times for excited zwitterion and phenolate anion. We believe that our inability to observe the growth of the phenolate anion fluorescence does not imply any deficiencies in the model but is simply a consequence of the growth being obscured by the superimposed zwitterion decay and the shortness of the growth time.

The rate constant for excited-state protonation of 2-naphthol-6-sulfonate has recently been reported to be (9.0 ± 3) $\times 10^{10}$ M^{-1} s^{-1}.[16] This is consistent with the reaction being diffusion-controlled modified by a charge-charge attraction.[17] On these grounds it is not unreasonable to assign a similar value to k_{-1} in our model. A value of at least 1×10^{10} is reasonable for the value of k_1, considering the reaction is likely to be simply one of translocation across a preformed intermolecular H bond. Estimates of k_{f_2} and k_{nr_2} can be obtained from the measured phenolate anion decay times ($k_{f_2} + k_{nr_2} \approx 5 \times 10^8$ s^{-1}). Computer simulations using these values for the rate constants predict a growth time of less than 100 ps, depending on the value of $k_{f_1} + k_{nr_1}$. On the sweep speed used for the experiments this represents a risetime of less than 5 channels. It is not surprising, therefore, that the zwitterion decay, with its much larger fraction of zero-time intensity, could obscure such a "grow-in".

Conclusion

The foregoing results reveal a number of unifying features in the photophysics of methyl salicylate, salicylamide, and salicylanilide. In all three cases emission from a neutral or, more precisely, uncharged excited species is observable. This emission is most noticeable in methyl salicylate, where under certain conditions of solvent and excitation wavelength it may dominate the total fluorescence spectrum.[3] By contrast, the uncharged molecule emission in the cases of salicylamide and salicylanilide is a minor feature of the total emission and can only be observed at low pH values, where the excited-state dissociation of the phenolic group is partly suppressed. The greater intensity of the uncharged molecule emission in the case of methyl salicylate is in part due to the fact that only in this case does a ground-state conformer that is stabilized by intramolecular H bonding lead to an uncharged molecule emission.[4] Thus even in hydrocarbon solvents, methyl salicylate shows appreciable uncharged molecule emission.[2,3] It may also be that in methyl salicylate the large influence of solvent on the relative intensity of the long- and short-wavelength bands is due to the availability in the ester group of two oxygen sites for H bonding with hydroxylic solvents. Salicylamide and salicylanilide are much more difficult to study in nonpolar solvents because of the very low solubilities. In all three cases, an emission of unusually large Stokes shift and at-

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tributable to an excited zwitterion is observed. In each case this appears to arise from excitation of a ground-state conformer in which there is intramolecular H bonding between the phenolic proton and the carbonyl oxygen. It is a consistent feature of the three derivatives that the zwitterion ground-state precursor absorbs at longer wavelengths than other ground-state conformers. In the case of salicylamide and salicylanilide, the zwitterion emission is strongly overlapped by emission attributable to excited phenolate. This fact, together with the weakness of the uncharged molecule emission, means that at most pH values the emission appears as a single band and shows marked excitation wavelength dependence. That this single band contains two contributions is, in the case of salicylamide, elegantly demonstrated by kinetic measurements. The very low quantum yield makes this impossible in the case of salicylanilide. Streak camera measurements with a time resolution of 5 ps have shown that proton loss to form the phenolate and proton transfer to form the zwitterion are both very rapid processes. A noteworthy feature of all three molecules is the shortness of the lifetime of the zwitterionic species. Although the zwitterion lifetimes are to some degree solvent dependent, their shortness in comparison to those of the phenolate species in all solvents indicates that intramolecular proton transfer opens up a particularly efficient nonradiative channel in these molecules. The nature of this nonradiative process remains to be elucidated. Temperature dependence measurements could prove informative.

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Electrochemical Production of Chlorophyll a and Pheophytin a Excited States

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Abstract: The reaction of chlorophyll a^+ (Chl a^+) with either Chl a^- or pheophytin a^- (Pheo a^-) in addition to the reaction of Pheo a^+ with Pheo a^- was studied in butyronitrile (BCN), BCN–1% THF, THF, and DMF. The electrochemically produced radical ion pairs Chl a^+–Chl a^- and Pheo a^+–Pheo a^- react in each solvent to produce a 10^{-7}–10^{-6} yield of luminescent states on the basis of the initial number of radical pairs. The Chl a^+–Pheo a^- reaction produces no observable luminescence in any of the solvents examined. The luminescence maximum for the Pheo a^+–Pheo a^- reaction occurs at 730 nm in each solvent and is strongly red-shifted relative to the fluorescence maxima for optically excited Pheo a in these solvents. A similar result is obtained for the Chl a^+–Chl a^- reaction in BCN. However, emission from the Chl a^+–Chl a^- reaction in the other three solvents occurs at 680 nm and corresponds more closely to normal fluorescence from optically excited Chl a. The red-shifted spectra are consistent with the formation of excimers. AC voltammetry of Chl a in BCN provides evidence that Chl a is aggregated in the ground state in this solvent. Chl a reduction shows four waves in BCN and two waves in the other three solvents. Thus, the Chl a^+–Chl a^- reaction in BCN does not form a true excimer, whereas the Pheo a^+–Pheo a^- reactions in each solvent do. The luminescence efficiencies of these charge-transfer neutralization reactions are discussed in terms of the geometric constraints on electron-transfer reactions in photosynthetic reaction centers.

Introduction

The primary processes of both green plant and bacterial photosynthesis involve photoinduced electron transfer from a chlorophyll species to an electron acceptor.[1,2] Evidence based on ESR and ENDOR data suggests that the primary donors in reaction center proteins of photosystem I (PS I) in green plants and of the *Rhodopseudomonas sphaeroides* bacterium consist of a special pair of chlorophyll a (Chl a) and of bacteriochlorophyll a (Bchl a) molecules, respectively.[3,4] In photosystem II (PS II) of green plants and in some strains of photosynthetic bacteria the monomeric or dimeric nature of the primary donor is not yet established. In addition, both optical and ESR evidence indicate that the first electron acceptor in *R. sphaeroides* reaction centers is bacteriopheophytin a.[5,6] However, the nature of the first acceptor in green plant photosystems remains an open question. The two most likely candidates are pheophytin a (Pheo a) and Chl a.[7–12]

For the past several years our research has addressed questions of reaction center structure and function by examining in vitro models possessing two or more chlorophyll-type chromophores for which the geometry of the chromophores relative to one another is constrained by linking the chromophores through several covalent bonding schemes.[13–15] Recently, we have examined photoinduced electron transfer from a dimeric pyrochlorophyll a system to one of two pheophorbide a macrocycles attached to the dimer by coordination bonds.[16] In this model the forward reaction

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