Fluorescence of 6-Methoxyquinoline, Quinine, and Quinidine in Aqueous Media

STEPHEN G. SCHULMAN[x], ROSE MARIE THREATTE, ANTHONY C. CAPOMACCHIA, and W. LARRY PAUL

Abstract □ The absorption and fluorescence spectra of 6-methoxyquinoline, quinine, and quinidine were studied as a continuous function of pH. 6-Methoxyquinoline is a stronger base in the lowest excited singlet state, as reflected by the pH dependence of its fluorescence. The latter is used to calculate the rates and equilibria of proton exchange in the excited state. Quinine and quinidine, however, do not protonate during the lifetimes of their excited states, an observation attributed to the lower basicity of their aromatic heterocyclic nitrogen than that in 6-methoxyquinoline. The differences in basic properties between the alkaloids and 6-methoxyquinoline are employed to infer the ketonic character of the quinolinemethanol group of the alkaloids and the existence of an intramolecular hydrogen bond in quinine but not in quinidine.

Keyphrases □ 6-Methoxyquinoline nucleus—absorption and fluorescence spectra, pH dependence □ Quinine—absorption and fluorescence spectra, pH dependence □ Quinidine—absorption and fluorescence spectra, pH dependence □ Fluorescence spectra—6-methoxyquinoline nucleus, quinine, and quinidine, pH dependence

The cinchona alkaloids quinine and quinidine are derived from the 6-methoxyquinoline nucleus and are well known for their pharmaceutical applications (1), quinine as an antimalarial and quinidine as an antipyretic and depressant of cardiac fibrillation. These drugs are generally regarded as diastereomers of one another (1), and their pharmacological actions are thought to differ as a result of their geometric differences. The fluorescent properties of the doubly charged cation derived from quinine are well known, this species being the most widely employed as a standard for the determination of absolute fluorescence quantum yields (2–4). However, the fluorescences of other prototropic species derived from quinine as well as those of all species derived from quinidine have been less extensively investigated. At biological pH, the singly charged species derived from both quinine and quinidine are predominant in the ground electronic state. However, the species that fluoresces at biological pH may depend upon the following: the dissociation constant in the lowest excited singlet state (if prototropic equilibrium is attained during the lifetime of the lowest excited singlet state), the dissociation constant in the ground state (if proton exchange is too slow to occur during the lifetime of the excited state), and the rate constants for protonation and dissociation in the excited state (if proton exchange is comparable in rate with the fluorescence of the acid and conjugate base) (5).

Because of an interest in employing the fluorescences of quinine and quinidine as probes of the interactions of these drugs with biological macromolecules and cellular components, the present study of the pH dependences of their fluorescence spectra as well as that of the parent aromatic nucleus, 6-methoxyquinoline, was undertaken.

EXPERIMENTAL

6-Methoxyquinoline[1] was precipitated as the perchlorate salt from absolute ethanol solution and recrystallized three times from 90% ethanol. Quinine bisulfate[2] and quinidine sulfate[2] were triply recrystallized from 90% ethanol. Analytical reagent grade sulfuric acid[3] and sodium hydroxide[3] were used without further purification. Distilled, deionized water was employed to prepare solutions for spectroscopic measurement.

Absorption spectra were taken on a spectrophotometer[4]. Fluorescence spectra were taken on a fluorescence spectrophotometer[5] whose monochromators were calibrated against the xenon line emission spectrum and whose output was corrected for instrumental response by means of a rhodamine-B quantum counter. The pH measurements were made on a digital pH meter[6] employing a silver–silver chloride–glass combination pH electrode[7]. Fluorescence lifetimes were measured on a nanosecond decay time fluorometer[8], employing an 18-w pulsed deuterium lamp (pulse time 1.6 nsec) and a dual-beam oscilloscope[9] with two IAI plug-in dual-channel amplifiers. Lifetimes of excited states as short as 1.6 nsec could be measured with this apparatus. Absolute quantum yields of fluorescence were evaluated by the relative method using the quantum yield of the doubly charged quinine cation (0.55) as a standard (2).

The solutions used for determining the spectra were prepared by diluting sulfuric acid or sodium hydroxide in a 10-ml volumetric flask and then delivering 0.10 ml of about 1×10^{-3} *M* aqueous stock solution of 6-methoxyquinoline, quinine, or quinidine, with a micropipet, to the 10-ml flask. Each solution was prepared immediately prior to taking the spectra to minimize decomposition errors and errors due to pH changes of the poorly buffered solutions in the middle pH range.

Dilute sulfuric acid and sodium hydroxide solutions were employed in preference to fairly concentrated buffer solutions, because buffer ions are known to react with excited aromatic acids and bases during the lifetime of the lowest excited singlet state (6). This reaction does not, of course, affect the thermodynamics of proton exchange in the excited state but does often affect the mechanism and, therefore, the kinetics of excited-state proton transfer. It was deemed desirable, at least initially, to avoid this added complication.

RESULTS

The absorption and fluorescence spectral maxima of the various species derived from 6-methoxyquinoline, quinine, and quinidine are presented in Table I. The ground-state dissociation constants for the prototropic equilibria between the singly charged cation and neutral molecule derived from 6-methoxyquinoline and between the doubly and singly charged cations derived from

[1] K and K Laboratories, Plainview, N.Y.
[2] Merck and Co., Rahway, N.J.
[3] Mallinckrodt Chemical Works, St. Louis, Mo.
[4] Beckman DB-GT.
[5] Perkin-Elmer MPF-2A.
[6] Orion model 801.
[7] Corning.
[8] TRW.
[9] Tektronix 556.

Table I—Absorption (λ^1L_a and λ^1L_b) and Corrected Fluorescence (λ_f) Maxima of the Various Prototropic Species Derived from 6-Methoxyquinoline, Quinine, and Quinidine

Compound	$\lambda_{^1L_a}$, nm	$\epsilon_{^1L_a}$, nm	$\lambda_{^1L_b}$, nm	$\epsilon_{^1L_b}$, nm	λ_f, nm
6-Methoxyquinoline:					
Cation (pH 2.0)	314	4.10×10^3	338	3.83×10^3	438
Neutral molecule (pH 13.2)	272	2.80×10^3	325	3.65×10^3	362
Quinine:					
Dication (pH 2.0)	317	4.33×10^3	347	5.40×10^3	448
Monocation (pH 7.0)	281	3.35×10^3	331	4.93×10^3	382
Neutral molecule (pH 11.0)	281	3.68×10^3	330	4.83×10^3	380
Quinidine:					
Dication (pH 2.0)	317	4.30×10^3	347	5.39×10^3	447
Monocation (pH 7.0)	281	3.40×10^3	331	5.00×10^3	380
Neutral molecule (pH 11.0)	281	3.61×10^3	331	4.89×10^3	380

quinine and quinidine correspond to dissociation from the nitrogen atom of the quinoline ring and, therefore, affect the absorption spectra. These dissociation constants were determined spectrophotometrically (Table II) under the experimental conditions employed here and were found to be in good agreement with those previously reported (7). The equilibria between the singly charged cations and the neutral molecules derived from quinidine and quinine correspond to dissociation from the quinuclidine nitrogen atoms, which are three atoms removed from the aromatic ring and sterically prohibited from interaction with the aromatic ring. Therefore, they do not affect the electronic structure of the aromatic system or the electronic absorption spectra to an extent that permits the determination of pKa. Consequently, these dissociations were studied potentiometrically. The equilibrium constants derived from the potentiometric measurements (Table II) were also in good agreement with the literature values (7).

In each case, fluorescence was excited at an isosbestic point in the absorption spectrum of each compound to eliminate the effect of absorption on the fluorometric titrations and to make the latter correspond only to variations of fluorescence efficiency with pH.

The fluorometric titrations of the cation and neutral molecule derived from 6-methoxyquinoline are represented in Fig. 1. At pH < 4, only the blue-green emission of the cation is observed. From pH 4 to 6, the fluorescence of the cation falls to 0.41 of its maximum intensity (at pH 4) and the violet fluorescence of the neutral species appears and rises to 0.85 of its maximum value (at pH 12). From pH 6 to 10, the fluorescences of the cation and neutral molecule remain constant. However, in the interval from pH 10 to 13 the cation emission is completely quenched and the fluorescence of the neutral species rises to a maximum at pH 12 and then falls sharply with further increase in pH.

In contrast to the rather complex fluorometric titration behavior of 6-methoxyquinoline, the dependences of the relative quantum yields of fluorescence of the various species derived from quinine (Fig. 2) and quinidine upon pH are fairly straightforward. Below pH 2.5, only the fluorescences of the doubly protonated cations are apparent. These emissions fall in intensity from pH 2.5 to 6.5, vanishing completely at pH 6.5. In this interval, the fluorescences of the singly protonated cations appear and rise with increasing pH to a maximum at pH ~ 6.5. The inflection points of the fluorometric titrations of quinine and quinidine in the 2.5–6.5 pH region coincide with the ground-state pKa values for the dissociations occurring in this pH region. In the interval from pH 7 to 11, the fluorescences of the singly charged cations derived from quinine and quinidine are quenched. The inflection points in these regions of the fluorometric titration curves coincide with the ground-state pKa values for conversion of the singly protonated species to the neutral molecules. At pH 11 the maximum of the fluorescence of quinine has shifted 2 nm to a shorter wavelength relative to that at pH 7 and its quantum yield is 0.018 of the fluorescence efficiency at pH 7. The fluorescence maximum of quinidine does not change on going from pH 7 to 11, but the quantum yield of fluorescence decreases by a factor of 0.032. At pH higher than 11, the fluorescences of quinine and quinidine are quenched more gradually with increasing pH. However, quenching is not complete at pH 14.0.

The fluorescence efficiencies and lifetimes of the lowest excited singlet states of the various prototropic species derived from 6-methoxyquinoline, quinine, and quinidine were measured at pH values where the fluorescences of each species were maximal and isolated (pH 2.0 and 12.2 for 6-methoxyquinoline and pH 2.0, 7.0, and 11.0 for quinine and quinidine) (Table III). Because of the quenching of neutral 6-methoxyquinoline fluorescence by hydroxide ion at high pH, it is believed that the fluorometric titration curve of the latter emission does not correspond to only an acid–base reaction and, therefore, that the maximum emission intensity (at pH 12) from which ϕ_0 and τ_0 (Table III) were measured does not give their true values for the neutral molecule. The fluorometric titration of the 6-methoxyquinolinium cation does, however, appear to be well behaved. Consequently, the theoretical fluorometric titration curve of the neutral molecule (as if only proton exchange occurred) was constructed by plotting $1 - (\phi'/\phi_0')$ against pH, where ϕ'/ϕ_0' is the relative quantum yield of fluorescence of the 6-methoxyquinoline cation. The true values of ϕ_0 and τ_0 for the neutral species were calculated from the measured values and the ratio of the relative fluorescence intensities of the theoretical fluorometric titration to that of the actual titration at

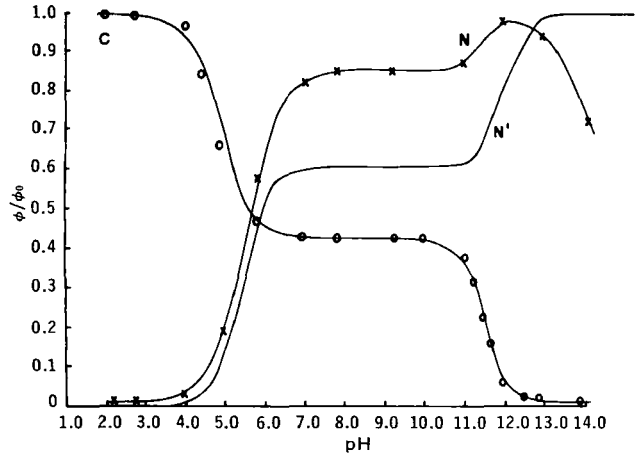


Figure 1—*The pH dependences of the relative quantum yields of fluorescence of the neutral molecule (N) and cation (C) derived from 6-methoxyquinoline. The curve represented by N is the experimental fluorometric titration curve of the neutral molecule. That represented by N′ is the theoretical fluorometric titration of the neutral molecule (in the absence of OH^- quenching at high pH).*

Table II—Dissociation Constants for the Ground-State Prototropic Equilibria between the Cation and Neutral Molecule Derived from 6-Methoxyquinoline and the Dications and Monocations Derived from Quinine and Quinidine (pKa_1) and between the Monocations and Neutral Molecules Derived from Quinine and Quinidine (pKa_2)

	pKa_1	pKa_2
6-Methoxyquinoline	5.13	
Quinine	4.34	8.43
Quinidine	4.34	8.79

Table III—Quantum Yields of Fluorescence (ϕ_0) and Lifetimes of the Fluorescing States (τ_0) of the Various Species Derived from 6-Methoxyquinoline, Quinine, and Quinidine

Compound	ϕ_0	τ_0, sec $\times 10^{-9}$
6-Methoxyquinoline:		
Cation (pH 2.0)	0.51	23.7[a]
Neutral molecule (pH 12.2)	0.16 (0.23)[b]	7.6 (10.8)[b]
Quinine:		
Dication (pH 2.0)	0.55	23.6[7]
Monocation (pH 7.0)	0.50	13.4
Neutral molecule (pH 11.0)	0.009	—[d]
Quinidine:		
Dication (pH 2.0)	0.50	24.6
Monocation (pH 7.0)	0.43	19.9
Neutral molecule (pH 11.0)	0.014	—[d]

[a] τ_0 = 22.8 nsec was reported (4) for 6-methoxyquinolinium ion. [b] Values in parentheses are corrected for OH^- quenching (see text). [c] τ_0 = 19.0 nsec was reported (4) for quinine dication. [d] The fluorescences of quinine and quinidine were too weak at pH 11.0 for their lifetimes to be determined with the apparatus.

pH 12.2. The corrected values of ϕ_0 and τ_0 are also given in Table III and were employed in all calculations.

DISCUSSION

The shifts in the long wavelength absorption and fluorescence maxima of 6-methoxyquinoline indicate that this compound is about seven orders of magnitude more basic in the lowest excited singlet state than in the ground state (6). This means that, since pKa ~5, if equilibrium is complete during the lifetime of the excited state, only the fluorescence of the cation should be observed in the 7–10 pH interval even though the neutral molecule is the only species excited in this interval. Alternatively, if proton exchange is too slow to occur at all during the lifetime of the excited state, the fluorometric titration characteristics should be identical with the ground-state titration characteristics and only the fluorescence of the neutral molecule should be observed at pH > 7. The observation of fluorescence from both the cation and the neutral molecule derived from 6-methoxyquinoline over the long pH interval from 3 to 13 is indicative of partial establishment of equilibrium in the lowest excited singlet state (8). Thus, in the 7–10 pH interval where only the neutral molecule is excited and only the cation is thermodynamically stable, a steady state is established in which a fraction (0.41) of the excited neutral molecules is converted to the cation while the remaining fraction (0.59) fluoresces from the neutral form before equilibrium in the excited state is fully established. At pH > 12, the excited cation

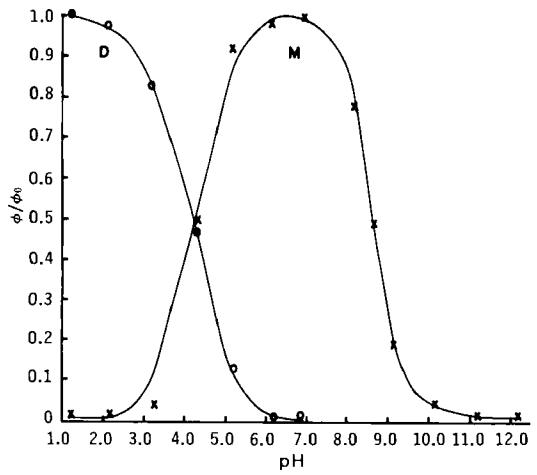
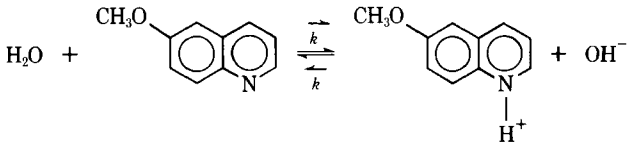


Figure 2—*The pH dependences of the relative quantum yields of fluorescence of the dication (D) and the monocation (M) derived from quinine. The fluorescence of the neutral molecule is almost identical in position with that of the monocation but much weaker in intensity.*

becomes thermodynamically unstable, so virtually all of the neutral molecules excited fluoresce as such.

Because the excited-state proton exchanges and the fluorescences of the two species derived from 6-methoxyquinoline are temporally competitive, measurement of the lifetimes of the excited singlet states (the lifetimes of the isolated species in the absence of reaction with H^+ or OH^-) permits evaluation of the kinetics of proton exchange (Scheme I and Eqs. 1 and 2) in the excited state from the fluorometric titration curve according to the steady-state approach developed by Weller (8). For the reaction:



Scheme I

$$\phi/\phi_0 = \frac{1 + \overleftarrow{k}\tau_0'[OH^-]}{1 + \vec{k}\tau_0 + \overleftarrow{k}\tau_0'[OH^-]} \quad \text{(Eq. 1)}$$

and:

$$\phi'/\phi_0' = \frac{\vec{k}\tau_0}{1 + \vec{k}\tau_0 + \overleftarrow{k}\tau_0'[OH^-]} \quad \text{(Eq. 2)}$$

where ϕ/ϕ_0 is the relative quantum yield of fluorescence of the neutral molecule at any point in the fluorometric titration (ϕ/ϕ_0 is unity at pH > 13 and zero at pH < 3 for 6-methoxyquinoline), ϕ'/ϕ_0' is the relative quantum yield of fluorescence of the cation at any point in the titration (for the 6-methoxyquinolinium cation, ϕ'/ϕ_0' = 1 when pH < 3 and ϕ'/ϕ_0' = 0 when pH > 13), $\vec{k}$ is the pseudo-first-order rate constant for protonation of the neutral molecule in the excited state by the solvent, $\overleftarrow{k}$ is the bimolecular rate constant for deprotonation of the cation by the hydroxide ion, and τ_0 and τ_0' are the lifetimes of the lowest excited singlet states when ϕ/ϕ_0 = 1 and when ϕ'/ϕ_0' = 1, respectively. The equilibrium between the neutral molecule and the cation, in the excited state, is written as a reaction of the cation with hydroxide ion rather than of the neutral molecule with hydrogen ion because in the basic region, where the reaction occurs, the hydrogen ion is in too low a concentration to be responsible for the observation of a diffusion-controlled reaction during the short lifetimes of the excited states concerned here. This, of course, does not affect the thermodynamic aspect of the reaction (pKa*) but is of mechanistic consequence.

Although Eqs. 1 and 2 indicate that ϕ/ϕ_0 and ϕ'/ϕ_0' are continuously dependent upon $[OH^-]$, both relative quantum yields are constant in the 7–10 pH interval. This is a consequence of the relative magnitudes of the terms 1 and $\overleftarrow{k}\tau_0'[OH^-]$ in Eqs. 1 and 2. In order for the relative quantum yields of fluorescence to vary with $[OH^-]$ measurably, $\overleftarrow{k}\tau_0'[OH^-]$ must be greater than or comparable in magnitude to 1. Now, τ_0 and τ_0' are generally 10^{-9}–10^{-7} sec, while $\vec{k}$ depends on the proton affinity of the excited conjugate base and $\overleftarrow{k}$ is usually 10^{11}–10^9 M^{-1} sec^{-1} for diffusion-limited protonation of bases. Consequently, at very low values of $[OH^-]$ (*i.e.*, in the mid pH range), $\overleftarrow{k}\tau_0'[OH^-]$ is usually much smaller than 1. As a result, when $[OH^-]$ is very low, Eqs. 1 and 2 reduce to:

$$(\phi/\phi_0)_{const} = \frac{1}{1 + \vec{k}\tau_0} \quad \text{(Eq. 3)}$$

and:

$$(\phi'/\phi_0')_{const} = \frac{\vec{k}\tau_0}{1 + \vec{k}\tau_0} \quad \text{(Eq. 4)}$$

which are, of course, independent of pH (*i.e.*, the appearance of cation fluorescence is governed exclusively by the forward reaction) and from which $\vec{k}$ may be immediately calculated from the value of τ_0 given for 6-methoxyquinoline (neutral) in Table I and the value of $(\phi/\phi_0)_{const}$ or $(\phi'/\phi_0')_{const}$ (0.59 and 0.41, respectively). The value of $\vec{k}$ for 6-methoxyquinoline was found to be 6.4 × 10^7 sec^{-1}. This value was then employed in Eq. 2 along with the

observed values of ϕ'/ϕ_0' at several values of [OH^-] in the pH 10–13 region (where the back-reaction as well as the forward-reaction affects the relative fluorescence efficiencies) to calculate the average value of $\bar{k}$ of $2.6 \times 10^{10}\ M^{-1}\ sec^{-1}$. From $\bar{k}$ and $\bar{k}$, one obtains the hydrolysis constant $K_b^* = (\bar{k}/\bar{k}) = 4.2 \times 10^{-3}$. Thus, pKa* = 11.40, in excellent agreement with the value of 11.5 calculated from the Förster cycle (6) employing the absorption and fluorescence maxima of Table I and the pKa value for 6-methoxyquinoline in Table II. It should be borne in mind here that the values of ϕ/ϕ_0 and ϕ'/ϕ_0' used in these calculations were taken only from the fluorometric titration curve of the 6-methoxyquinolinium cation. The fluorometric titration curve of the neutral 6-methoxyquinoline molecule shows not only an acid-base reaction but also some unspecified quenching reaction at high pH. Thus, whereas the cation emission demonstrates a clean titration curve close to the theoretical behavior predicted by Eq. 2, the fluorescence of the neutral molecule is quenched by OH^- before the acid-base titration is complete, giving apparently too short an acid-base titration interval and too high a value of ϕ/ϕ_0 (Fig. 1).

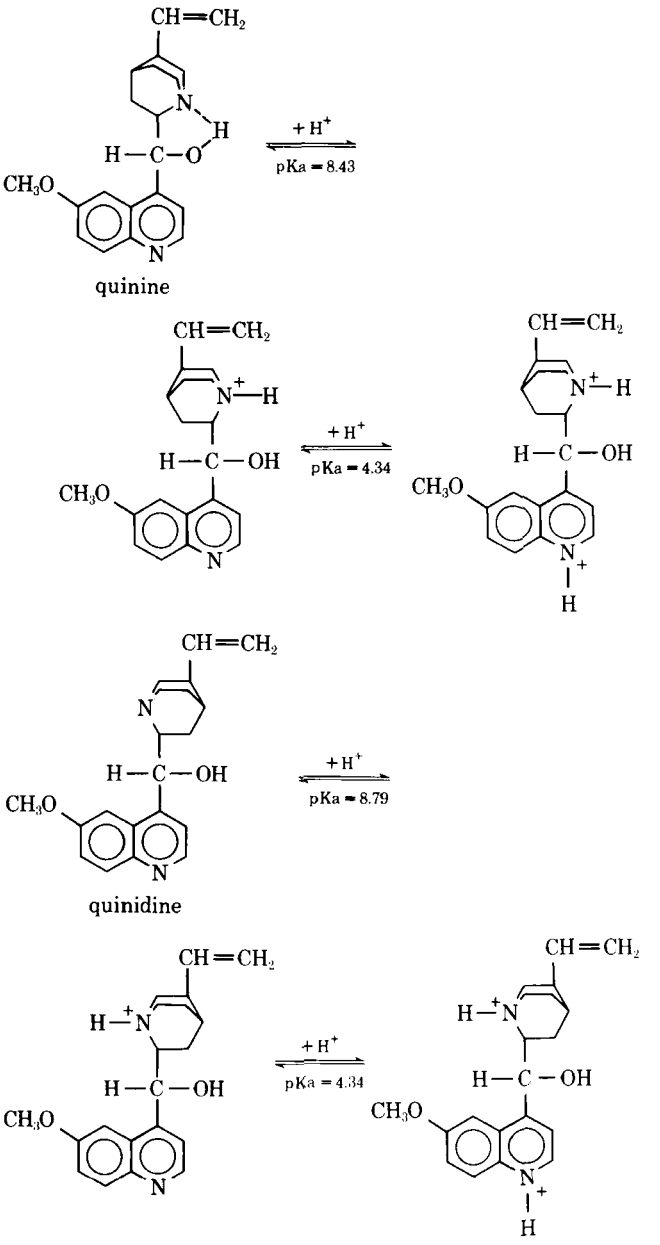
By comparison with 6-methoxyquinoline, quinine and quinidine are unusual in their ground-state chemistry and photochemistry. The ground-state pKa values of these compounds are considerably less basic than would be anticipated. The pKa of quinuclidine is 10.6 (9). However, in quinine and quinidine, the quinuclidine nitrogen atoms are 2.2 and 1.8 orders of magnitude less basic, respectively, suggesting that the quinoline methanol group is electron withdrawing. Moreover, the nitrogen atoms of the quinoline rings of quinine and quinidine are both 0.8 order of magnitude less basic than that of 6-methoxyquinoline, indicating the electron-withdrawing effect of the exocyclic group in the 4-position. The low basicities of the quinuclidine nitrogen atoms of the alkaloids are surprising, because they are three atoms removed from the quinoline ring and would not be expected to be diminished as much as two orders of magnitude in basicity solely by hyperconjugation of the methanolic carbon atom with the quinoline ring. The low basicities of the quinoline nitrogen atoms of the alkaloids are surprising, because even with the quinuclidine nitrogen atoms protonated, it is to be expected that the effect of the substituent in the 4-position of the quinoline ring would be hyperconjugative, similar to a methyl group in the same position, and should therefore be base strengthening [*e.g.*, the pKa of quinoline is 4.94 (10) but that of 4-methylquinoline is 5.34 (11)].

The anomalous basicities of the quinuclidine and quinoline nitrogen atoms of quinine and quinidine can both be explained by considering the hyperconjugation of the carbon atom attached to the 4-position of the quinoline ring as a partial oxidation. This would confer some ketonic character upon the C—O bond, which should reduce the basicity of the quinuclidine nitrogen by virtue of the electron-withdrawing influence nearby. Moreover, the polarization of the O—H bond resulting from an increase of the order of the C—O bond would enhance the hydrogen-bonding capability of the O—H proton, which is not normally strongly hydrogen bonding. The presence of a reasonably strong intramolecular hydrogen bond between the O—H proton and the quinuclidine nitrogen in the neutral molecule derived from quinine (but not quinidine) would stabilize the neutral quinine molecule, making it even more difficult to protonate, and thereby account for the substantial difference between the pKa values of the quinuclidine nitrogen atoms of quinine and quinidine (Scheme II).

Protonation of the quinuclidine nitrogen atom of quinine and quinidine introduces a positively polarizing influence on the H—C—OH group and makes it more electron withdrawing than in the uncharged species. This is transmitted with considerable strength to the quinoline nitrogen atom through the aromatic π-system with which the H—C—OH group is hyperconjugated, accounting for the reduction in basicity of singly protonated quinine and quinidine.

That the fluorescences of the doubly charged cations derived from quinine and quinidine are converted, with increasing pH, to those of the singly charged cations with ground-state titration characteristics (Fig. 2) indicates that the protonations of the singly charged cations by water in the excited state are much slower than the rates of fluorescence of the latter species. Since the fluorescence lifetimes of the monocations of quinine and quinidine are greater than those of 6-methoxyquinoline (neutral), the rates of deactivation of the former are lower than those of the latter. Thus, the rates of protonation of the excited quinine and quinidine monocations (which cannot be calculated from their fluorometric titration curves) must be substantially lower than those of the 6-methoxyquinoline molecule. This can be accounted for at least partially by the pKa* values (pKa* = 9.7 for quinine dication $\rightleftarrows$ quinine monocation and pKa* = 9.9 for quinidine dication $\rightleftarrows$ quinidine monocation) estimated from the Förster cycle, employing the corresponding ground-state pKa values and the averages of absorption and emission maxima. The Bronsted relation states that for a single proton transfer, the rate of hydrolysis of a related family of bases decreases with decreasing pKa of the conjugate acid. Thus, quinine and quinidine, which have pKa* values lower than that of 6-methoxyquinoline by 1.9 and 1.7 log units, respectively, would be expected to have hydrolysis rate constants in the excited state between 0 and 1.9 orders of magnitude lower than 6-methoxyquinoline. Whether or not this is sufficient to account completely for the failure of quinine and quinidine to demonstrate measurable excited-state ionization cannot be stated with certainty at present.

The quenching with increasing pH of the fluorescences of the singly charged cations derived from quinine and quinidine follows the ground-state titration characteristics for conversion of the singly charged cations to the respective neutral molecules. The neutral molecules, therefore, fluoresce much less intensely than



Scheme II

their singly protonated counterparts. In *n*-hexane, however, the neutral molecules and the respective singly charged cations were observed to fluoresce with about the same intensities. This suggests that the diminished quantum yields of fluorescence of the neutral molecules in water are not due to the inherent molecular structures of these species but rather to some interaction with the excited-state solvent cage, presumably hydrogen bonding at the quinuclidine nitrogen atoms. Excited-state hydrogen bonding is known to quench the fluorescences of a wide variety of molecules, presumably by coupling the molecule to the solvent quasilattice. The vibrational modes of the solvent quasilattice provide an efficient means of radiationless deactivation of the lowest excited singlet state. That the dissociations and solvent interactions of the quinuclidine nitrogen atoms have such a dramatic effect upon the fluorescent properties of quinine and quinidine indicates substantial electronic coupling in the lowest excited singlet state between the aromatic and aliphatic portions of the alkaloids. Normally, the solvent interactions and prototropic exchanges involving functional groups not directly bonded to the aromatic fluorophore or sterically free to interact directly with the fluorophore (12, 13) do not have much effect upon the fluorescence arising from the fluorophore.

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[x] To whom inquiries should be directed.

Electron Spin Resonance Studies of Free Radicals in Solution III: pH Dependence of Thiyl Free Radical of Cysteine

JEAN C. KERTESZ, MICHAEL B. WOLF, WALTER WOLF[x], and LI-YA A. CHEN

Abstract □ An electron spin resonance flow technique was utilized to follow the concentration of thiyl free radicals (RS·) generated upon the oxidation of cysteine with ceric ion over the 0.25–7.5 pH range. The thiyl free radical concentration was seen to decrease with increasing pH in a manner dependent on the extent of ionization of cysteine. The formation of thiyl free radicals was favored by the proportion of thiol present in the positively charged form. The diminution of RS· spin concentration at physiological pH was correlated with the increasing prevalence of singly and doubly negative thiolate ions. At acidic pH's below 1.5, thiyl free radical formation was depressed by solutions of high ionic strength. The utility of this technique in studying the potential role of thiyl free radicals in radioprotective processes is indicated.

Keyphrases □ Free radicals in solution—pH dependence of thiyl free radical formation from oxidation of cysteine, electron spin resonance □ Cysteine oxidation—pH dependence of thiyl free radical formation □ Electron spin resonance—monitoring, pH dependence of thiyl free radical formation from oxidation of cysteine

Recent studies in this laboratory identified and characterized thiyl (RS·) and related free radicals in aqueous solution by electron spin resonance (ESR) spectroscopy when they are generated by ceric-ion oxidation of various thiols at low pH (1–6). Since sulfhydryl compounds and thiyl free radicals exhibit roles of considerable importance in the mechanisms of radiation damage and radioprotective processes (7–12), it was of special interest to determine whether the free radicals observed at acidic pH's may also be detected and similarly characterized at physiological pH's (6.8–7.6). Since thiyl free radical signals are observed under steady-state flow conditions, it was necessary to modify the flow method so that as the ESR spectra are recorded the concomitant pH of the reaction mixture can be measured. This paper presents the results of a systematic investigation of the variation of the thiyl free radical spin concentration observed upon the oxidation of cysteine with increasing pH.

EXPERIMENTAL

The ESR continuous-flow system used was described in detail elsewhere (13). A flow rate of 1.0 ml/sec was used uniformly throughout the experiments. ESR spectra were recorded with a ESR spectrometer[1] with associated 4-in. magnet, which operated at an X-band microwave frequency of 9.5 GHz and a modulation frequency of 100 KHz. Each sample was recorded at a modulation amplitude of 1.6 gauss and a microwave power of 200 mw on 50-gauss field scans. There was no evidence of power saturation of the spectra studied in this work. A 1-sec filter was routinely used

[1] Varian model E-3.