Tautomerism of Singly Protonated Chloroquine and Quinacrine

LEONARD S. ROSENBERG and STEPHEN G. SCHULMAN ×

Received February 17, 1978, from the *College of Pharmacy, University of Florida, Gainesville, FL 32610.* Accepted for publication April 26, 1978.

Abstract □ Differential absorptiometric pH titrations of the antimalarials chloroquine and quinacrine, employing the respective uncharged species at pH 12.5 in the reference cell, show that protolytic dissociation from the heterocyclic ring nitrogen atoms occurs over pH 6–12. This finding indicates that the singly charged cations of the drugs exist measurably in two tautomeric forms. The tautomeric equilibrium constants were calculated from the titration data. The existence of these tautomeric equilibria may have significance in the pharmacodynamics of chloroquine and quinacrine.

Keyphrases □ Chloroquine—protolytic dissociation, spectrophotometric evaluation □ Quinacrine—protolytic dissociation, spectrophotometric evaluation □ Spectrophotometry—evaluation, protolytic dissociation of chloroquine and quinacrine □ Dissociation, protolytic—chloroquine and quinacrine, spectrophotometric evaluation □ Antimalarials—chloroquine and quinacrine, protolytic dissociation, spectrophotometric evaluation

The well-known antimalarials chloroquine (1) and quinacrine (2, 3) exist as singly and doubly charged cations at physiological pH. The doubly charged cations form strong complexes with native DNA (4, 5). It was implied (6–8) that their biological activities are derived from this interaction. Although no direct evidence has been presented to demonstrate the binding of the singly charged cations to the nucleic acids, the possible biological significance of the singly charged cations cannot be dismissed. Richard and Garrett (9), for example, showed that the efficacy of quinacrine against *Escherichia coli* increases with increasing pH and is minimal at low pH where the dication is predominant. This result could indicate either a transport problem involving the dication or the activity of the monocation. In either case, the importance of the monocation for biological effect is apparent.

The dissociation constants related to the dication $\rightleftharpoons$ monocation equilibria (pK_1) and the monocation $\rightleftharpoons$ neutral molecule equilibria (pK_2) of chloroquine (1) and quinacrine (3) were reported, on the basis of spectroscopic evidence, to correspond to dissociation from the heterocyclic nitrogen atom and the aliphatic ammonium group of the side chain, respectively, as shown for chloroquine in Scheme I. However, the proximities of pK_1 and pK_2 for both drugs (for chloroquine, $pK_1 - pK_2 = 2.0$; for quinacrine, $pK_1 - pK_2 = 2.5$) suggested that some dissociation from the alkylamino groups probably would occur in the doubly charged cations. This dissociation would give rise to tautomeric monocations. To examine this possibility more closely, the present spectrophotometric reinvestigation of the protolytic dissociations of chloroquine and quinacrine was undertaken.

EXPERIMENTAL

Chloroquine phosphate[1] and quinacrine hydrochloride[1] were used without further purification. Buffer solutions were prepared by titrating 0.005 *M* dibasic sodium phosphate or boric acid with 0.1 *M* NaOH. Solutions of pH ≥ 11 were prepared by dilution of 1 *M* NaOH with distilled deionized water.

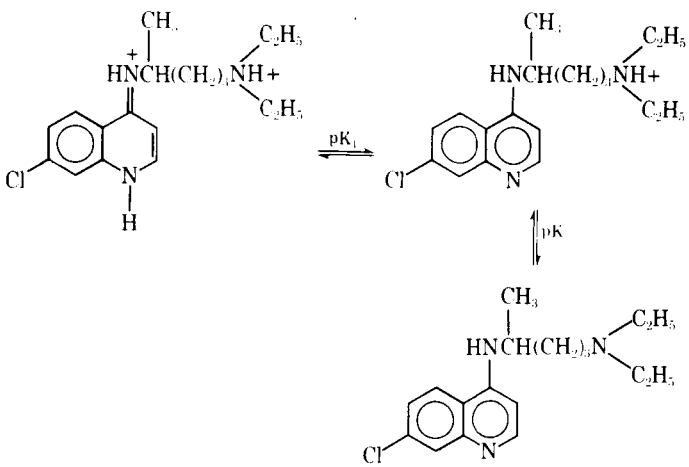
Absorption spectra were taken in 1-cm matched silica cells, thermostated at 30°, on a spectrophotometer[2]. A series of 10-ml volumetric flasks, each containing a buffer solution of a different pH, was injected, each with 100 μl of a stock solution of chloroquine or quinacrine. The final concentrations of chloroquine and quinacrine in each flask were 1.61×10^{-5} and 1.93×10^{-5} *M*, respectively.

The spectrum of each solution was scanned against the appropriate buffer blank for the direct titrations and against a blank containing chloroquine or quinacrine at pH 12.5 for the difference titrations. The difference titrations were carried out, with the scale expansion capability of the spectrophotometer's recorder, at 0–0.25 absorbance unit full-scale deflection. The direct titrations were carried out at 0–1.0 absorbance unit full-scale deflection.

RESULTS AND DISCUSSION

The absorption spectrum of chloroquine [$pK_1 = 8.1$ and $pK_2 = 10.1$ (1)] shifted to shorter wavelengths and demonstrated hypochromism with increasing pH in the pH 6–9.5 region. At pH > 9.5, any further changes in the spectrum of chloroquine were essentially imperceptible, in agreement with previous observations (1, 4). The absorption spectrum of quinacrine [$pK_1 = 7.7$ and $pK_2 = 10.2$ (3)] demonstrated hypsochromism and hypochromism with increasing pH from pH 6 to 12. However, changes in the spectrum of quinacrine were quite pronounced from pH 6 to 9 and considerably less so from pH 9 to 12. The pH dependence of the difference spectra, employing chloroquine and quinacrine at pH 12.5 (the neutral species) in the reference cell (Figs. 1 and 2), clearly shows that the absorption spectra moved to shorter wavelengths and lower intensities with increasing pH over pH 6–12.

Since the alkylamino nitrogen atoms are well removed from the aromatic portions of the drugs, the spectroscopic changes observed must be due to ionization of the heterocyclic nitrogen atoms over the pH 6–12 range. This hypothesis indicates that in the region from pH 9 or 10 to 12, where the monocation is virtually the only acidic species present, some dissociation must occur from the heterocyclic nitrogen atoms. Hence, tautomers of the monocations protonated at the ring nitrogen atoms must exist, to a measurable extent, in equilibrium with the tautomers of the

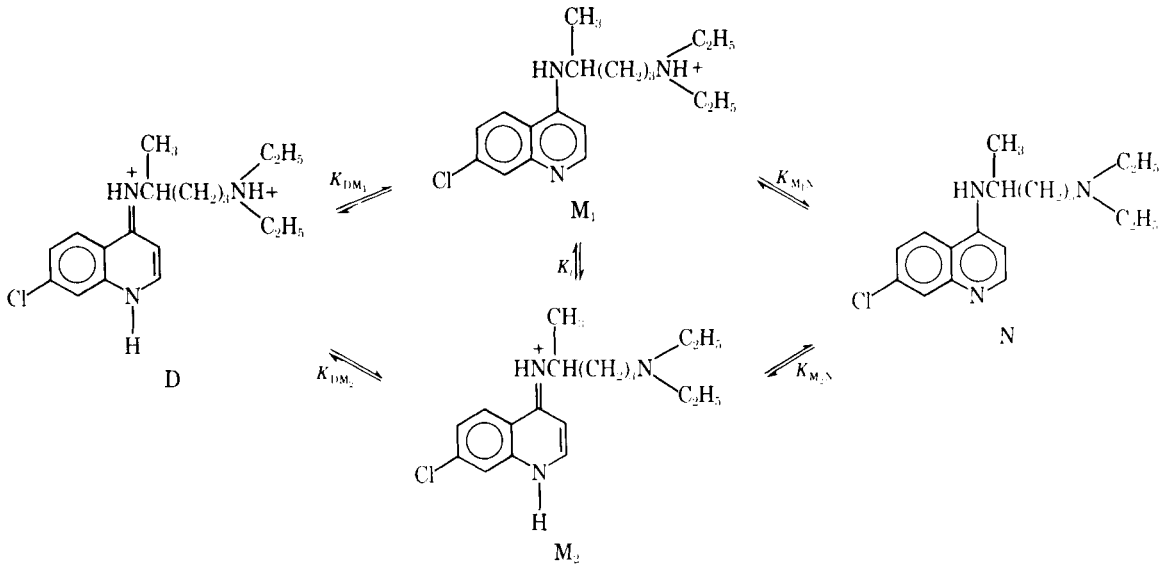


Scheme I

[1] Gift of Dr. S. Archer, Winthrop Research Institute, Rensselaer, N.Y.

[2] Model 25, Beckman Instruments, Fullerton, Calif.

0022-3549/78/1200-1770$01.00/0



Scheme II

monocations protonated at the alkylamino nitrogen atoms, as shown for chloroquine in Scheme II where D is dication, M_1 and M_2 are monocations, and N is the neutral species.

The tautomeric ratio K_t of the equilibrium concentration ($[M_1]$) of cation M_1 to that ($[M_2]$) of M_2 may be evaluated as follows. According to Robinson and Biggs (10), for a doubly charged diprotic acid with overlapping protolytic equilibria:

$$(a - \epsilon_D C_B l)[H^+]^2 + (a - \epsilon_M C_B l)K_1[H^+] + (a - \epsilon_N C_B l)K_1K_2 = 0 \quad \text{(Eq. 1)}$$

where a is the absorbance at the analytical wavelength, C_B is the formal concentration of drug, l is the optical depth of the sample, K_1 and K_2 are the prototropic equilibrium constants of the drug, and ϵ_D, ϵ_M, and ϵ_N are the molar absorptivities of the dication, monocation, and neutral species, respectively.

From absorbance measurements at $[H^+] \gg K_1$ and $[H^+] \ll K_2$, ϵ_D and ϵ_N can easily be evaluated. However, since the successive equilibria overlap, the monocation cannot be isolated, and ϵ_M may be evaluated along with K_1 and K_2 from the simultaneous solution of Eq. 1 for at least three different values of $[H^+]$ and a. If the dissociating groups of the dication D are dissimilar, ϵ_M is not a true molar absorptivity, because [M] is not the concentration of a single species but rather a sum of the concentrations ($[M_1] + [M_2]$) of two tautomeric monocations. Since the absorbance of all monocationic species is equal to the sum of the absorbances of the tautomers, *i.e.*:

$$\epsilon_M[M]l = \epsilon_M[M_1]l + \epsilon_M[M_2]l = \epsilon_{M_1}[M_1]l + \epsilon_{M_2}[M_2]l \quad \text{(Eq. 2)}$$

the tautomeric ratio K_t is given by:

$$K_t = \frac{[M_1]}{[M_2]} = \frac{\epsilon_{M_2} - \epsilon_M}{\epsilon_M - \epsilon_{M_1}} \quad \text{(Eq. 3)}$$

Because the monocation M_2 differs from the dication D only in the absence of a proton on the aliphatic amino group, M_2 and D are essentially identical electronically in their aromatic (chromophoric) systems. Hence, it is reasonable to write $\epsilon_D = \epsilon_{M_2}$. Similarly, the monocation M_1 and the neutral molecule N have virtually identical chromophores and $\epsilon_N = \epsilon_{M_1}$. Substituting ϵ_D for ϵ_{M_2} and ϵ_N for ϵ_{M_1} in Eq. 3 yields:

$$K_t = \frac{\epsilon_D - \epsilon_M}{\epsilon_M - \epsilon_N} \quad \text{(Eq. 4)}$$

The preceding approach may be applied directly to differential titrations, such as those carried out here, without evaluation of the molar absorptivities at the analytical wavelength. In Eq. 1, let $\epsilon_D C_B l = a_D$, $\epsilon_M C_B l = a_M$, and $\epsilon_N C_B l = a_N$ so that:

$$(a - a_D)[H^+]^2 + (a - a_M)K_1[H^+] + (a - a_N)K_1K_2 = 0 \quad \text{(Eq. 5)}$$

If the reference cell in the spectrophotometer contains the analyte of concentration C_B at a pH high enough that it is all in the N form, each absorbance measurement will give the difference between the total absorbance of the solution in the sample cell and a_N:

$$([a - a_N] - [a_D - a_N])[H^+]^2 + ([a - a_N] - [a_M - a_N])K_1[H^+] + ([a - a_N] - [a_N - a_N])K_1K_2 = 0 \quad \text{(Eq. 6)}$$

If $a - a_N = \Delta a$, $a_D - a_N = \Delta a_D$, and $a_M - a_N = \Delta a_M$:

$$(\Delta a - \Delta a_D)[H^+]^2 + (\Delta a - \Delta a_M)K_1[H^+] + \Delta a K_1K_2 = 0 \quad \text{(Eq. 7)}$$

Equation 7 can be solved simultaneously in the titration of the analyte for K_1, K_2, and a_M. Multiplying the numerator and denominator of Eq.

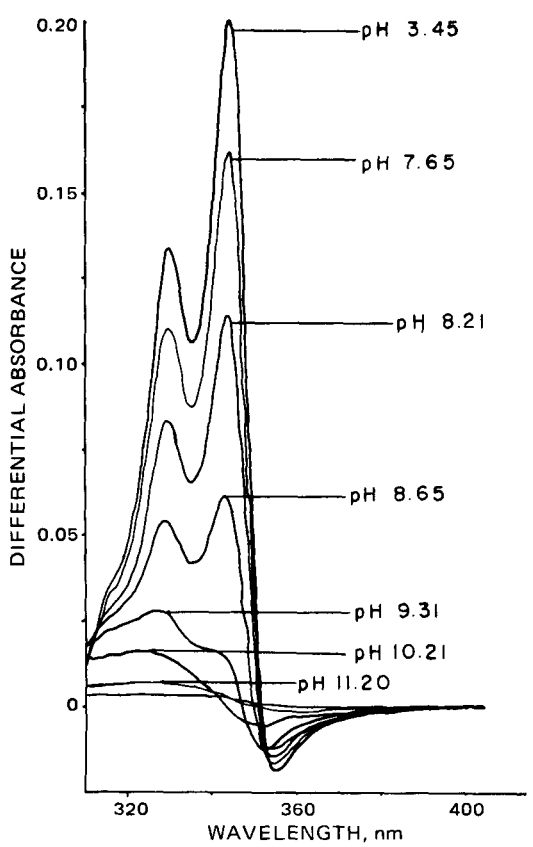


Figure 1—*The pH dependence of the differential absorption spectrum of 1.61 × 10⁻⁵ M chloroquine at 30°. The reference cell of the spectrophotometer contained 1.61 × 10⁻⁵ M chloroquine at a fixed pH of 12.5. Recorder scale expansion was 0–0.25 absorbance unit full-scale deflection.*

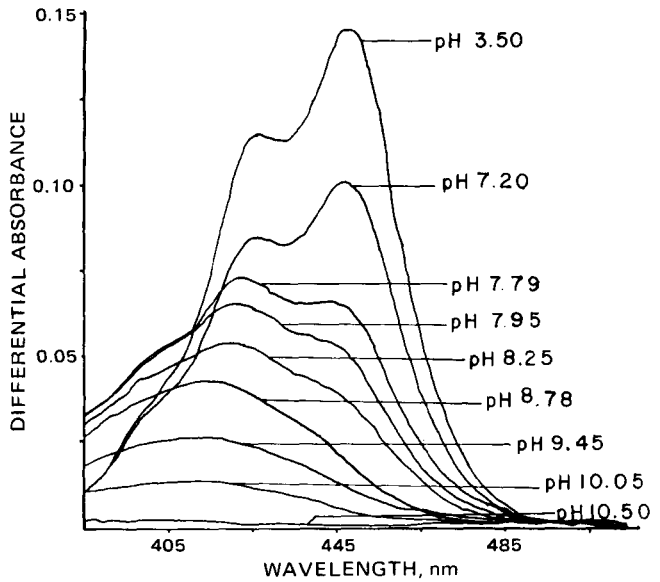


Figure 2—*The pH dependence of the differential absorption spectrum of 1.93 × 10^{-5} M quinacrine at 30°. The reference cell of the spectrophotometer contained 1.93 × 10^{-5} M quinacrine at a fixed pH of 12.5. Recorder scale expansion was 0–0.25 absorbance unit full-scale deflection.*

4 by $C_B l$ yields:

$$K_t = \frac{a_D - a_M}{a_M - a_N} \qquad \text{(Eq. 8)}$$

Moreover:

$$K_t = \frac{(a_D - a_N) - (a_M - a_N)}{(a_M - a_N) - (a_N - a_N)} \qquad \text{(Eq. 9)}$$

or:

$$K_t = \frac{\Delta a_D - \Delta a_M}{\Delta a_M} \qquad \text{(Eq. 10)}$$

Consequently, the value of Δa_M determined by the solution of Eq. 7 and that of Δa_D taken from the low pH limit of the differential titration can be used to calculate K_t directly.

Values of pK_1, pK_2, and Δa_M for chloroquine and quinacrine, calculated from the differential titrations, are presented in Table I. The analytical wavelengths of 330 nm for chloroquine and 445 nm for quinacrine were chosen for these calculations. The values of K_t resulting from these calculations are listed in Table II.

Table I—Spectroscopic and Thermodynamic Parameters of the Absorptiometric pH Titrations at 30° of Chloroquine and Quinacrine Calculated from Eq. 7 and the Data Represented in Figs. 1 and 2

	Δa_D	Δa_M	pK_1	pK_2
Chloroquine (λ = 330 nm)	0.128	0.013	8.35 ± 0.05 (8.08)[a]	10.40 ± 0.05 (10.1)[a]
Quinacrine (λ = 445 nm)	0.146	0.0158	7.72 ± 0.03 (7.69)[b]	10.39 ± 0.03 (10.18)[b]

[a] Reference 1. [b] Reference 3.

Table II—Tautomerization Constant K_t and Microequilibrium Constants for the Prototropic Equilibria of Chloroquine and Quinacrine (Scheme II) Calculated from the Data of Table I

	K_t	pK_{DM_1}	pK_{DM_2}	pK_{M_1N}	pK_{M_2N}
Chloroquine	8.9 ± 1.0	8.40	9.32	10.35	9.43
Quinacrine	8.2 ± 0.5	7.77	8.69	10.34	9.42

The tautomeric ratio K_t and the macroequilibrium constants K_1 and K_2 can be used to calculate the microequilibrium constants for the prototropic equilibria of chloroquine and quinacrine represented in Scheme II (11). The microequilibrium constants are presented in Table II.

The fact that two tautomers of chloroquine and quinacrine occur, each as substantial fractions of the population of singly charged molecules, may be of great significance in the pharmacodynamics of these drugs. For example, it is possible that one tautomer could be responsible for biological response and the other for the transport or metabolic disposition of either drug. This area is worthy of further consideration.

REFERENCES

(1) J. L. Irvin and E. M. Irvin, *J. Am. Chem. Soc.*, **69**, 1091 (1947).
(2) S. R. Christophers, *Ann. Trop. Med. Parasitol.*, **31**, 43 (1937).
(3) J. L. Irvin and E. M. Irvin, *J. Am. Chem. Soc.*, **72**, 2745 (1950).
(4) S. N. Cohen and K. L. Yielding, *J. Biol. Chem.*, **240**, 3123 (1965).
(5) D. S. Drummond, V. F. W. Simpson-Gildmeister, and A. R. Peacocke, *Biopolymers*, **3**, 135 (1965).
(6) S. N. Cohen and K. L. Yielding, *Proc. Natl. Acad. Sci. USA*, **54**, 521 (1965).
(7) J. Ciak and F. E. Hahn, *Science*, **156**, 655 (1967).
(8) M. L. Seligman and H. G. Mandel, *J. Gen. Microbiol.*, **68**, 135 (1971).
(9) A. J. Richard and E. R. Garrett, *J. Pharm. Sci.*, **63**, 894 (1974).
(10) R. A. Robinson and A. I. Biggs, *Aust. J. Chem.*, **10**, 128 (1957).
(11) R. J. Sturgeon and S. G. Schulman, *J. Pharm. Sci.*, **66**, 958 (1977).