

Dehydrolongicamphenilone (29). Hydroxy mesylate (**28**) (452 mg, 1.5 mmol) in 15 ml of dry *tert*-butyl alcohol was mixed with a slurry of potassium *tert*-butoxide (420 mg, 3.75 mmol) in 15 ml of *tert*-butyl alcohol and warmed at 35° under nitrogen for 1 hr. The reaction mixture was worked up²⁰ and an orange oil was obtained in quantitative yield (306 mg). The material was homogeneous on glc (20% SE-30, 190°) and possessed appropriate spectral properties: ir (film) 3030 (olefinic C-H), 1745 (C=O), and 725 cm⁻¹ (cis olefinic C-H bend); nmr (CCl₄) δ 4.93 (m, 2 H), 1.05 (s, 3 H), 1.00 (s, 3 H), and 0.95 (s, 3 H).

Anal. Calcd for C₁₄H₂₀O: C, 82.30; H, 9.87. Found: C, 82.39; H, 9.78.

Alternatively, **29** could be prepared by stirring the mesylate (119 mg, 0.4 mmol) with a suspension of 60% sodium hydride (80 mg, 2 mmol washed free of mineral oil with hexane) in 4 ml of dry tetrahydrofuran for 1 hr at room temperature. The solvent was evaporated and the residue taken up in water and extracted with ether. Usual work-up²⁰ gave 67 mg (83%) of oily **29**.

(±)-**Longicamphenilone (30).** Trisphenylphosphinerhodium chloride (75 mg) was dissolved in 20 ml of 3:1 benzene-methanol in a three-necked flask fitted with a stirring bar and rubber septum. The catalyst was equilibrated with hydrogen at atmospheric pressure for 45 min. To this solution was added, *via* the septum, keto olefin **29** (115 mg, 0.565 mmol) in 4 ml of benzene-methanol, and the orange solution was stirred in a hydrogen atmosphere for 4.5 days. After 2.5 days the solvent was evaporated, and the residue was taken up in ether and percolated through basic alumina to give, after evaporation, longicamphenilone (**30**) (101 mg, 87%)

as a light red-brown oil. An analytical sample was obtained by preparative glc: ir (film) 1750 cm⁻¹ (C=O); nmr (CCl₄) δ 1.01 (s, 3 H, endo bridge methyl) and 0.91 (s, 6 H, exo and angular methyls).

Anal. Calcd for C₁₄H₂₂O: C, 81.50; H, 10.75. Found: C, 81.29; H, 10.83.

(±)-**Longifolene (1).** To longicamphenilone (**30**) (87 mg, 0.422 mmol) in 10 ml of anhydrous ether was added 10 ml of 1.3 *M* methylolithium, and the resulting solution heated for 3 hr at 48°. Shorter reaction times gave incomplete reaction. The excess methylolithium was destroyed by dropwise addition of water, and the reaction was worked up²⁰ to give 89 mg of carbinol as an oily solid: ir (film) 3470 cm⁻¹ (OH).

The crude carbinol (89 mg, 0.401 mmol) in 7 ml of pyridine was treated with thionyl chloride (251 mg, 2.11 mmol) for 10 min at 0°. The reaction mixture was worked up²⁰ to give 79 mg (85%) of a colorless oil whose spectral properties were identical with those of (+)-longifolene. An analytical sample was obtained by preparative glc: ir (film) 3080, 1655, and 868 cm⁻¹; nmr (CCl₄) exocyclic methylene protons δ 4.72 (s, 1 H) and 4.46 (s, 1 H), endo methyl 0.99 (s, 3 H), exo methyl 0.95 (s, 3 H), and angular methyl 0.90 (s, 3 H).

Anal. Calcd for C₁₃H₂₄: C, 88.16; H, 11.84. Found: C, 88.09; H, 11.70.

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A Direct Determination of the Spatial Geometry of Molecules in Solution. I. Conformation of Chloroquine, an Antimalarial¹

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Abstract: A three-dimensional structural determination is reported for the important antimalarial chloroquine (CQ) in acetone solution. The spatial structure was deduced from the results of detailed proton magnetic resonance studies employing a paramagnetic ion probe, tris(2,2,6,6-tetramethylheptane-3,5-dionato)praseodymium(III), (Pr(tmh)₃). Pseudocontact shifts induced by Pr(tmh)₃ were measured at 220 MHz for all of the CQ protons up to a 1:1 CQ:Pr(tmh)₃ concentration ratio at 20 and 48°. Both the shift data and independent optical measurements were consistent with the formation of a 1:1 complex between CQ and Pr(tmh)₃ with equilibrium constants of 63 and 13 M⁻¹ at 20 and 48°, respectively. An extensive analysis of the pseudocontact shift data was then carried out to obtain a set of coordinates for a time-averaged CQ structure which gives the best overall least-squares fit between calculated and observed chemical shifts for all protons in the molecule. A stereoscopic representation of the time-averaged CQ structure was constructed from the final sets of coordinates at each temperature. The three-dimensional structure of CQ, as found in the CQ·Pr(tmh)₃ complex in solution, is quite compact with the side chain curled over the plane of the quinoline ring. The indication of the compactness is given by the rather short distance of 7.4 Å between the N-1 and N-3 positions at 20°. The structure is opened up somewhat at the higher temperature but the essential features are not altered drastically. The solution structure differs markedly from the structure reported for diprotonated CQ in the crystalline state. Possible reasons for these differences are discussed as are the implications of the solution structure for the binding interaction of CQ with deoxyribonucleic acid. Finally, the present work illustrates the feasibility and merits of paramagnetic ion probes and shift agents in structure studies of biomolecules.

Recent detailed pmr studies show that a wide variety of aliphatic and aromatic compounds interact weakly with tris-β-diketone complexes of the lanthanide ions to produce dramatic chemical-shift changes in the

proton resonances.³ These chemical-shift variations have been used to resolve severe overlap of proton resonances,^{4a} simplify complex spectra,^{4b} and in some cases assign proton resonances from the relative magnitude

(1) Work supported in part by the U. S. Atomic Energy Commission.

(2) (a) Argonne National Laboratory; (b) Walter Reed Army Institute of Research; this paper is contribution no. 1025 from the Army Malaria Research Program.

(3) J. K. M. Sanders and D. H. Williams, *J. Amer. Chem. Soc.*, **93**, 641 (1971).

(4) (a) C. C. Hinckley, *ibid.*, **91**, 5160 (1969); (b) P. V. Demarco, T. K. Elzey, R. B. Lewis, and E. Wenkert, *ibid.*, **92**, 5734 (1970).

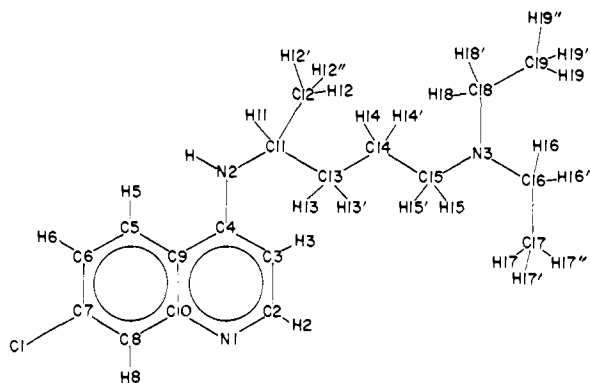


Figure 1. Chemical structure of chloroquine. The atomic numbering scheme follows crystallographic convention (H. S. Preston and J. M. Stewart, private communication; *Chem. Commun.*, 1142 (1970)).

of the chemical shift variation.⁵ Another potentially important and exciting application of the technique is its use to determine molecular conformations in solution.

Such a possibility arises if the observed paramagnetic-induced pmr chemical shifts are the result of a pseudocontact process. In this instance the time-averaged pseudocontact shift induced in nuclei of a molecule by the paramagnetic species is given by⁶⁻⁸

$$\Delta\nu_{\text{obsd}} - \Delta\nu_0 = \Delta\nu_i = \rho\beta \left\{ \frac{3 \cos^2 \phi_i - 1}{r_i^3} \right\} \quad (1)$$

where i refers to the i th nucleus, $\Delta\nu_{\text{obsd}}$ and $\Delta\nu_0$ are the chemical shifts of the protons in the molecule with respect to an internal reference for the solution with and without the paramagnetic substance, respectively, ρ is the probability that the molecule and paramagnetic substance are complexed, ϕ_i is the angle between the molecular axis and the i th nucleus, r_i is the interaction distance between the paramagnetic center and the i th nucleus, and β is given as

$$\beta = \frac{\nu_0 B^2 S(S+1)}{27kT} (g_{\parallel} + 2g_{\perp})(g_{\parallel} - g_{\perp}) \quad (2)$$

The parameters in eq 2 have their usual meaning: ν_0 , operating frequency; k , Boltzmann's constant; S , sum of effective free spins; B , Bohr magneton; T , absolute temperature; g_{\parallel} and g_{\perp} are the anisotropic g values parallel and perpendicular to the molecular axis, respectively. If the parametric constants in these equations can be determined, then ϕ_i and r_i can also be established for each chemically shifted nucleus using an iterative approach. Finally, with appropriate bond distance and bond angle information for the remaining atoms in the molecule it is then possible to obtain an average molecular conformation for all nuclei in the molecule which is complexed to the shift reagent.

The approach outlined above is of immediate importance in determining the conformation of drug molecules because of the limitations of present methods

(5) (a) C. C. Hinckley, *J. Org. Chem.*, **35**, 2834 (1970); (b) C. C. Hinckley, M. R. Klotz, and F. Patil, *J. Amer. Chem. Soc.*, **93**, 2417 (1971).

(6) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).

(7) D. R. Eaton, *J. Amer. Chem. Soc.*, **87**, 14 (1965).

(8) J. P. Jesson, *J. Chem. Phys.*, **47**, 582 (1967).

for establishing molecular conformations in solution directly. Common pmr methods rely on empirical correlations of coupling constants and vicinal angles, various chemical-shift correlations, and on the solvent and temperature dependences of chemical shifts and coupling constants.⁹ The derivation of molecular conformations by these techniques is often ambiguous and yields nonunique structures. On this basis, studies in our laboratory requiring a knowledge of the conformation of chloroquine, an antimalarial drug, prompted an investigation of the drug's structure using the shift reagent tris(2,2,6,6-tetramethylheptane-3,5-dionato)praseodymium(III), Pr(tmh)₃. It has been shown in studies using molecules with known conformation in solution that Pr(tmh)₃ gives only pseudocontact shifts.¹⁰

Experimental Section

Preparation of Chloroquine. Chloroquine was prepared from commercially available *rac*-chloroquine diphosphate (Hoffman-LaRoche, Inc.). Chloroquine diphosphate (25 g) was dissolved in 500 ml of distilled water. The free base of chloroquine was separated as a yellow oil from this solution by adding 200 ml of 10 *N* sodium hydroxide. The oil was then extracted into 3 vol of methylene chloride. After drying 1 hr over anhydrous sodium sulfate the methylene chloride was removed by vacuum distillation leaving a white crystalline solid.

Pmr Solutions. Each sample was prepared by first weighing 32 mg ($\sim 10^{-4}$ mol) of chloroquine into an imperial 5-in. Wilmad nmr spinning tube. The samples were then diluted to a given fixed volume (0.5 ml) with each of two stock solutions A and B. Stock solution A was composed of deuterioacetone with 2% tetramethylsilane (TMS), and stock solution B was composed of 0.20 *M* tris(2,2,6,6-tetramethylheptane-3,5-dionato)praseodymium(III) (Pr(tmh)₃) in deuterioacetone with 2% TMS; the volumes of stock solutions A and B were varied to obtain a range of Pr(tmh)₃ concentrations between 0.01 and 0.20 *M* while the concentration of chloroquine (0.20 *M*) remained constant.

Spectrophotometry Solutions. All samples were prepared by combining different volumes of two stock solutions. Stock solution A consisted of 5.0×10^{-4} *M* chloroquine in deuterioacetone and stock solution B consisted of 5.0×10^{-4} *M* chloroquine and 5.0×10^{-4} *M* Pr(tmh)₃ in deuterioacetone. The volumes of the two stock solutions were varied to obtain a range of Pr(tmh)₃ concentrations varying from 0.01×10^{-4} *M* to 5.0×10^{-4} *M* while the concentration of chloroquine remained constant.

Instrumentation. The proton magnetic resonance spectra were measured with a Varian HR-220 pmr spectrometer equipped with a variable temperature probe and controller. Spectra were obtained in the continuous wave (CW) mode, and the sweep was calibrated by superimposing audio side bands of the internal tetramethylsilane reference signal at 1000.0-Hz intervals; line positions were then measured by interpolation. Signal positions relative to internal tetramethylsilane were measured with a precision of ± 0.01 ppm.

Signal intensities were obtained by signal area integration using both electronic and planimetric methods. Probe temperatures in the pmr experiments were determined using standard methanol and ethylene glycol temperature calibration samples. An equilibration time of 1 hr was allowed for each sample. Double resonance measurements were made with the field-sweep method.

Epr spectra were measured with a Varian spectrometer using E-9 accessories and a 12-in. electromagnet. Spectra were obtained at 5- and 100-mW power levels and at ambient temperature, 20°. A sample 0.2 *M* in both CQ and Pr(tmh)₃ was contained in a flat-topped quartz tube.

Visible spectra were measured on a Gilford recording Model 2400S spectrophotometer with variable temperature accessories. All measurements were made in 1.00-cm quartz cuvettes against a solvent reference.

Computation. The IBM 360-75 computer of the Applied Mathematics Division of Argonne National Laboratory was used for all

(9) T. A. Victor, F. E. Hruska, C. L. Bell, and S. S. Danyluk, *Tetrahedron Lett.*, **53**, 4721 (1969).

(10) J. Briggs, F. A. Hart, and G. P. Moss, *Chem. Commun.*, 1506 (1970).

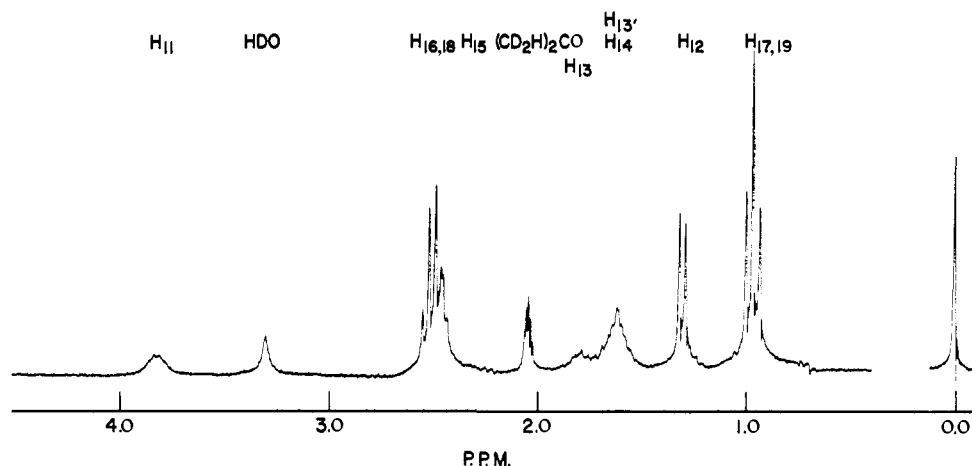


Figure 2. Pmr spectrum at 220 MHz of 0.20 *M* chloroquine in deuterioacetone at 20°. The assigned signals represent the protons of the aliphatic portion of the molecule.

calculations. The basic iterative computer programs NMREN and NMRIT as well as the CalComp plot program used in this study are available at Argonne National Laboratory. Problems solved by least-squares fitting and parameter estimation utilized the non-linear parameter estimation program and can be obtained directly from the IBM Program Library (No. 360D-13,6,003). Computer programs for evaluating atomic cartesian coordinates from bond angles, bond distances, and dihedral angles were obtained from the Quantum Chemistry Program Exchange Library at Bloomington, Ind.; these programs were appropriately modified for the present study. Three-dimensional molecular structure plots of chloroquine were computed by the Oak Ridge thermal ellipsoid plot program (ORNL-3794).

Results

1. Spectral Analysis. An illustration of the chemical structure of CQ and the numbering scheme used in this work is given in Figure 1. Partial assignments of the proton spectrum for CQ diphosphate have been reported in D_2O at 60 MHz¹¹ and in trifluoroacetic acid at 100 MHz,¹² but signal overlap of some of the methylenes limited a complete assignment. Because a complete and detailed assignment is essential for the present work a reinvestigation of the CQ (basic form) spectrum was made at 220 MHz in deuterioacetone. The latter solvent was used because both CQ and the praseodymium chelate were soluble in it, and it did not cause a decomposition of the latter.

Figure 2 shows the upfield portion of the spectrum for a 0.20 *M* solution of CQ in $(CD_3)_2CO$ at 20°. At 220 MHz the side-chain proton signals are sufficiently well resolved to permit a straightforward assignment of the ethyl group protons and the C-12 methyl group protons. The locations of H-2-N, H-11, and C-12 methyl protons were further confirmed by double resonance measurements. The remaining three sets of methylene protons at C-13, C-14, and C-15 give rise to a more complicated pattern and although H-15, H-15' can be assigned to the multiplet (partially overlapped) at 2.48 ppm on the basis of a lower chemical shielding (due to the adjacent N) and H-14, H-14' in turn can be assigned by decoupling from H-15, H-15' the derivation of quantitative parameters requires additional measurements.

(11) H. Sternglanz, K. L. Yelding, and K. M. Pruitt, *Mol. Pharm.*, **5**, 376 (1969).

(12) T. A. Victor, F. E. Hahn, and E. A. Hansen, *Progr. Mol. Subcell. Biol.*, **2**, 91 (1970).

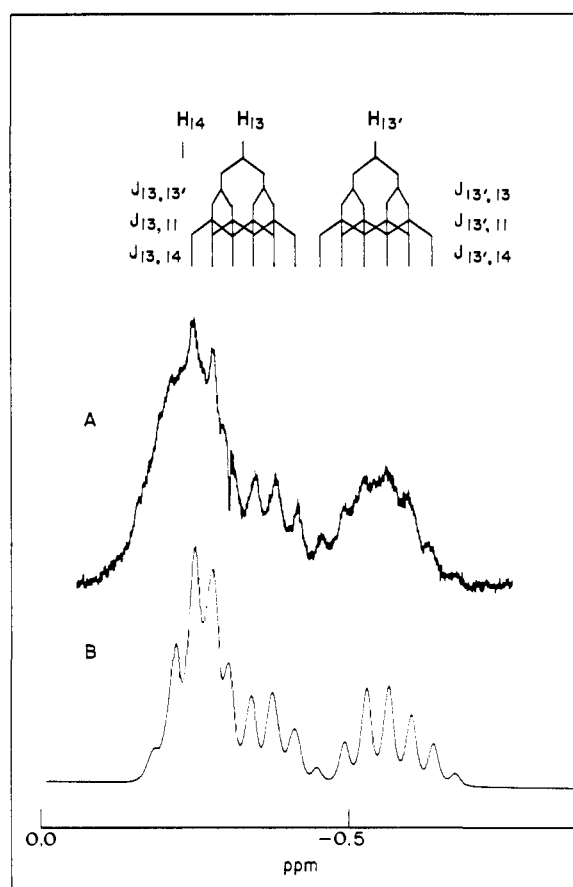


Figure 3. Pmr spectrum at 220 MHz of 0.20 *M* chloroquine and 0.20 *M* $Pr(tmh)_3$ in deuterioacetone at 20°. The C-13 and C-14 proton signals are shown in the spectrum with an analysis of the spin-spin coupling.

The paramagnetic ion chelate, $Pr(tmh)_3$, was added to the CQ solution to produce a somewhat better separation of the H-13, H-13' and H-14, H-14' patterns. Specifically, addition of $Pr(tmh)_3$ to equal molar concentrations of CQ shifts the multiplet at 1.79 ppm (integrated area for one proton) upfield to -0.34 ppm, relative to internal TMS, and the multiplet at 1.61 ppm to two sets of signals centered at -0.23 and -0.55 ppm, each with an integrated area of two protons and one proton, respectively. If the reasonable assumption

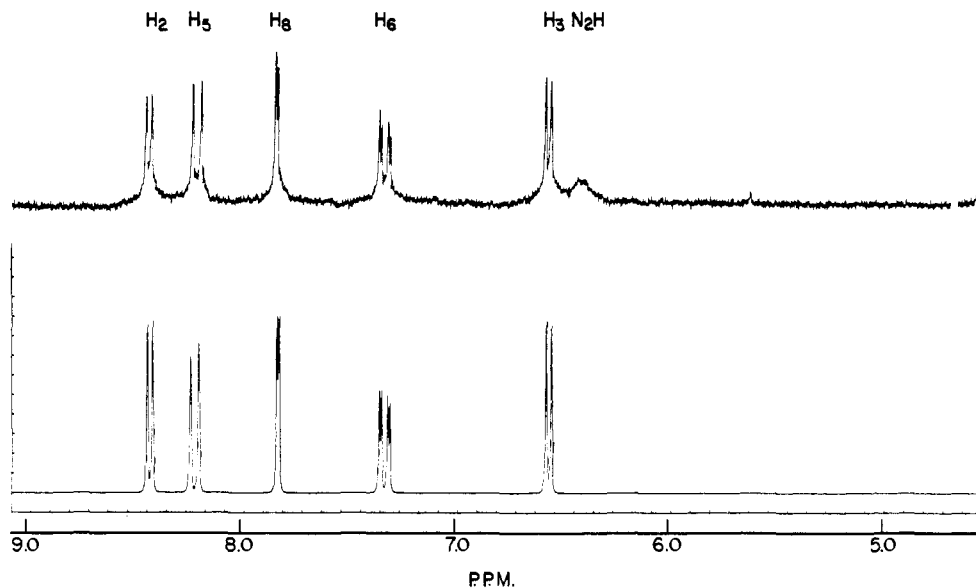


Figure 4. PMR spectrum at 220 MHz of 0.20 *M* chloroquine in deuterioacetone at 20°. The aromatic region of the spectrum is shown above with the calculated spectrum below for comparison.

Table I. Proton Chemical Shifts^a for Chloroquine^b in Deuterioacetone

		Proton type (aromatic)					Proton type (aliphatic)								
		H-2	H-3	H-5	H-6	H-8	H-2-N	H-11	H-12	H-13	H-13'	H-14(14')	H-15(15')	H-16= H-18	H-17= H-19
Chemical shifts	20°	8.46	6.58	8.24	7.35	7.85	6.41	3.82	1.30	1.70	1.79	1.61	2.46	2.50	0.96
	48°	8.43	6.55	8.18	7.29	7.83	6.30	3.82	1.31	1.70	1.79	1.62	2.47	2.50	0.97

^a Chemical shifts in ppm relative to internal TMS are accurate to ± 0.01 ppm. ^b 0.20 *M* chloroquine in deuterioacetone at 20 and 48°.

Table II. Proton Coupling Constants^a for Chloroquine^b in Deuterioacetone at 20°^c

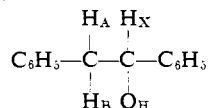
	Proton type (aromatic)					Proton type (aliphatic)						
	H-2	H-3	H-5	H-6	H-8	H-2-N	H-11	H-12	H-13	H-13'	H-14	H-16=H-18 H-17=H-19
Coupling constants (± 0.05 Hz)	$J_{2,3} = 5.40$	$J_{3,2} = 5.40$	$J_{3,6} = 8.58,$ $J_{3,8} = 0.62$	$J_{5,6} = 0.62,$ $J_{6,8} = 2.30$	$J_{3,8} = 0.62,$ $J_{6,8} = 2.30$	$J_{H-2-N,11} = 7.0$ ± 0.2	$J_{11,13} = 8.0^d$ ± 0.1 $J_{11,13'} = 8.0$ $\pm 0.2^d$	$J_{11,12} = 6.0$ ± 0.2	$J_{13,13'} = 16.2^d$ $J_{13,14} = 8.0$ $\pm 0.1^d$	$J_{13',14} = 8.0$ $\pm 0.2^d$	$J_{14,15} = 6.0$ $\pm 0.1^d$	$J_{16,17} = J_{18,19} = 6.9 \pm 0.1$

^a Proton coupling constants measured in Hz. ^b 0.20 *M* chloroquine in deuterioacetone. ^c Proton coupling constants at 48° were identical, within experimental error, to those at 20°. ^d Derived from coupling constants in the CQ·Pr(tmh)₃ complex at 1:1. (There was no change of the other coupling constants for this spectrum compared to the CQ spectrum.)

is made that H-13 and H-13' are nonequivalent because C-13 is adjacent to an asymmetric center,¹³ then the multiplet patterns at -0.34 and -0.55 ppm can be assigned to these protons.^{14a} A consideration of

(13) K. Mislow and M. Raban, *Top. Stereochem.*, 1, 1 (1967); 2, 199 (1968).

(14) (a) A report outlining the use of a lanthanide shift reagent to induce shift nonequivalence for protons bonded to carbon atoms adjacent to asymmetric centers has been published very recently.^{14b} A shift nonequivalence was induced in



various coupling possibilities with protons at the C-11 and C-14 positions leads to the coupling scheme in Figure 3 as being the most likely for H-13, H-13'. Final pmr parameters were derived by computer simulation of the relevant regions of the side-chain spectrum (Figure 3).

The multiplet patterns observed for the aromatic ring protons at 220 MHz, Figure 4, are very similar to those reported for CQ diphosphate at 100 MHz.¹² Assignment of the signals was made from a considera-

by adding Eu(dpm)₃; $J_{AB} = 13.5$ Hz; $J_{AX} = 6.8$ Hz; $J_{BX} = 5.8$ Hz. (b) G. P. Schiemenz and H. Rast, *Tetrahedron Lett.*, 48, 4685 (1971).

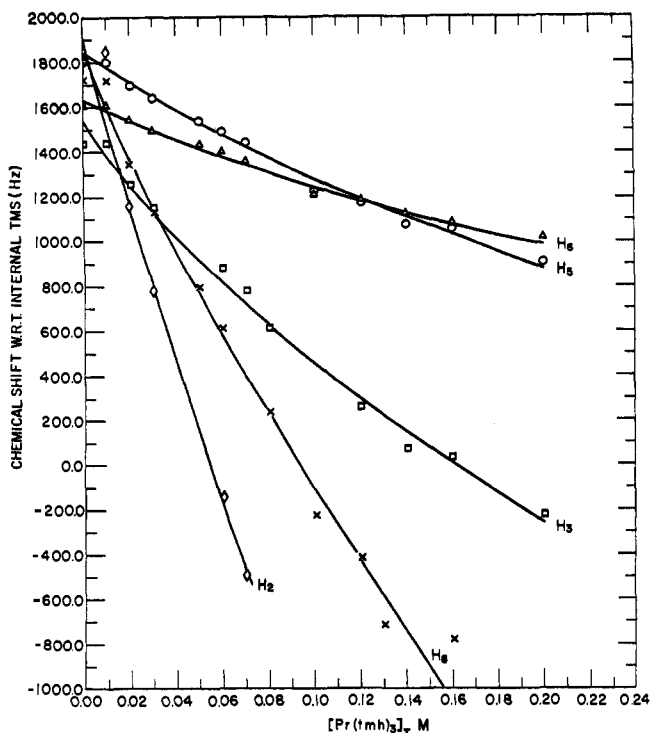


Figure 5. The chemical-shift dependence of the aliphatic proton signals of chloroquine ($[CQ]_T = 0.2 M$) on the concentration of $Pr(tmh)_3$ in deuterioacetone at 20° . Chemical shifts in Figures 5–8 are given in hertz relative to internal TMS; +ve shifts are to low field of TMS.

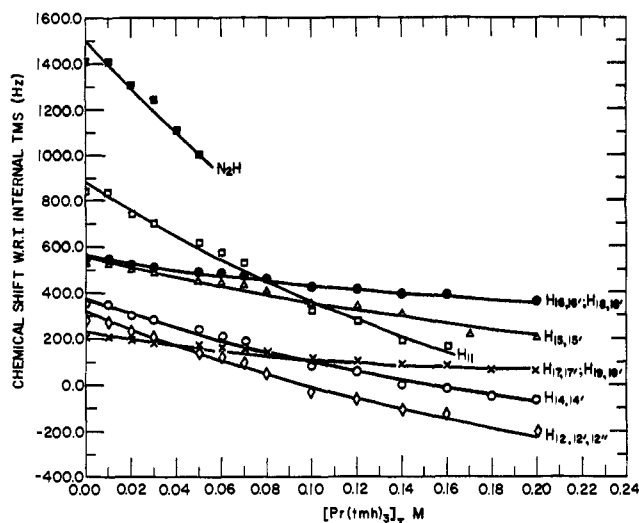


Figure 6. The chemical-shift dependence of the aromatic proton signals of chloroquine ($[CQ]_T = 0.2 M$) on the concentration of $Pr(tmh)_3$ in deuterioacetone at 20° .

tion of observed splitting patterns combined with double resonance measurements. Chemical shifts and coupling constants were refined further by iterative calculation of the spectrum; the resulting calculated spectrum is shown in the lower portion of Figure 4.

A summary of all the chemical shifts and coupling constants for CQ derived from the analyses is given in Tables I and II.

2. Determination of the Chloroquine Conformation.

(i) **Stoichiometry of the Complex.** Following the proton spectral assignment a detailed study was made of the

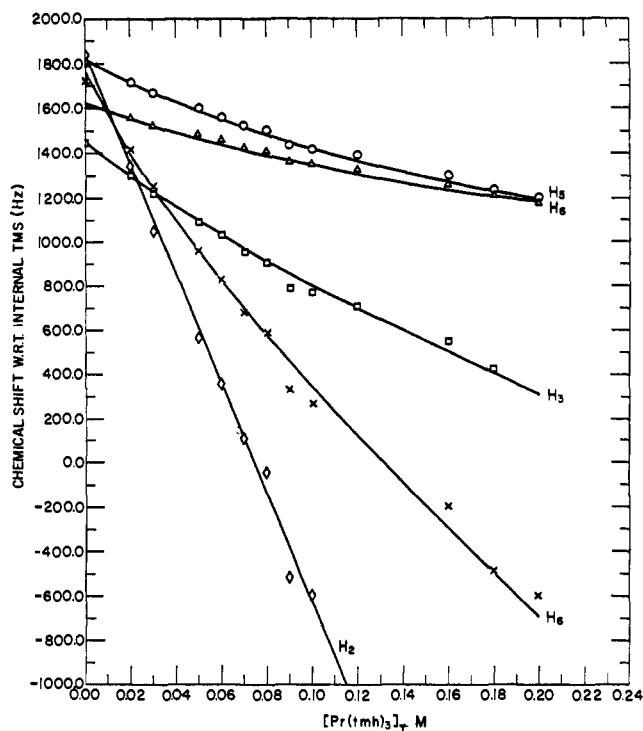


Figure 7. The chemical-shift dependence of the aliphatic proton signals of chloroquine ($[CQ]_T = 0.2 M$) on the concentration of $Pr(tmh)_3$ in deuterioacetone at 48° .

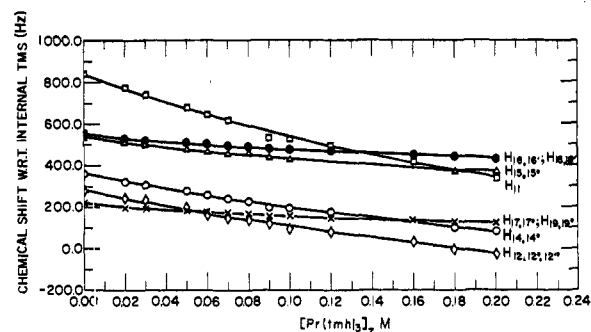


Figure 8. The chemical-shift dependence of the aromatic proton signals of chloroquine ($[CQ]_T = 0.2 M$) on the concentration of $Pr(tmh)_3$ in deuterioacetone at 48° .

influence of the paramagnetic ion chelate, $Pr(tmh)_3$, upon the CQ pmr spectrum. Spectra were measured for a fixed CQ concentration ($0.20 M$) and varying concentrations of $Pr(tmh)_3$ up to $0.20 M$. A summary of the results at 20 and 48° is given in Figures 5–8.

The data show that in every instance addition of the paramagnetic ion shifts the proton signal to higher field, with the largest perturbation occurring for protons in the quinoline ring. Assuming that $Pr(tmh)_3$ gives rise to pseudocontact shifts only, it follows that the observed shift change will be inversely proportional to the cube of the interaction distance for each $Pr(III)$ -proton pair. On this basis the data in Figures 5–8 show that $Pr(tmh)_3$ interacts preferentially at the N-1 position of CQ in the concentration range covered.

Before analyzing the pmr shift data an independent determination of the equilibrium constant for the CQ- $Pr(tmh)_3$ interaction was made using spectrophotometric techniques. Because there are potentially three sites

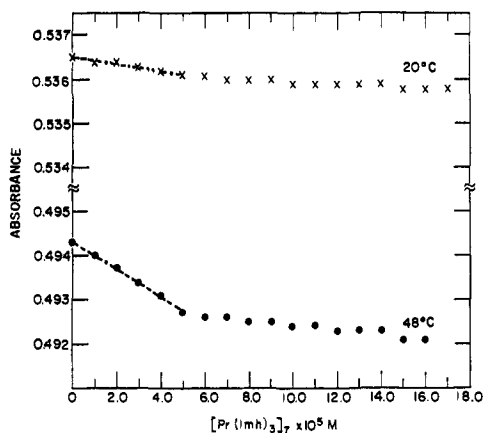
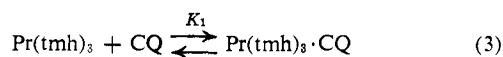


Figure 9. The absorbance change of the chloroquine absorbance at 332 $m\mu$ with the addition of $\text{Pr}(\text{tmh})_3$, $[\text{CQ}] = 5.0 \times 10^{-4} M$.

for complexation of $\text{Pr}(\text{tmh})_3$, *i.e.*, N-1, N-2, and N-3, a check was made to establish whether K_1 , 1:1, was sufficiently greater than K_2 , 1:2, and K_3 , 1:3, such that any contributions to the shift changes from 1:2 and 1:3 complexes would be minimal up to equimolar CQ- $\text{Pr}(\text{tmh})_3$ concentrations. The optical results are shown in Figure 9 at 332 $m\mu$.¹⁵ Although the absorbance changes are not large, there is a very definite break at CQ· $\text{Pr}(\text{tmh})_3$ of 1:1 which suggests that K_1 is at least a factor of 10 larger than K_2 or K_3 . Since there is not sufficient absorbance change to warrant a rigorous determination of K_2 and K_3 it was assumed that only a 1:1 complex was formed up to equal mole ratios of CQ to $\text{Pr}(\text{tmh})_3$. Therefore, the absorbance change could be analyzed according to the following equilibrium



The equilibrium constant was obtained by a nonlinear least-squares fit of the spectrophotometric data up to CQ· $\text{Pr}(\text{tmh})_3$ of 1:1 to the equation

$$A = \epsilon b[\text{CQ}] \quad (4)$$

where ϵ is the extinction coefficient of CQ, b is the optical path length, and A is the absorbance at the corresponding CQ concentration, $[\text{CQ}]$. The latter was determined from a knowledge of the total CQ and $\text{Pr}(\text{tmh})_3$ concentrations and the following expression for the equilibrium constant (eq 5). The results of

$$K_1 = \frac{[\text{Pr}(\text{tmh})_3 \cdot \text{CQ}]}{[\text{Pr}(\text{tmh})_3][\text{CQ}]} M^{-1} \quad (5)$$

the analysis at 20 and 48° are shown by the solid lines in Figure 9 and the parameters are given in Table III. Because of the relatively small absorbance changes with complexation a further determination of the equilibrium constants was made using the pmr shift data. The shifts in Figures 5-8 were analyzed in the following manner. The pseudocontact chemical-shift change for a given proton upon complexation of CQ to $\text{Pr}(\text{tmh})_3$ can be evaluated according to

$$\Delta\nu_i = -\rho\beta\xi_i \quad (6)$$

(15) $\text{Pr}(\text{tmh})_3$ does not absorb at 332 $m\mu$ nor does its spectrum change in the concentration range covered suggesting no dimerization of this compound.

Table III. Equilibrium Data for the Formation of $[\text{Pr}(\text{tmh})_3 \cdot \text{CQ}]$

Temp, °C ± 1	Equilibrium constants $K, M^{-1} \pm 0.5$		Extinction coeff of CQ $\epsilon, M^{-1} \text{ cm}^{-1} \pm 0.005 \times 10^3 M^{-1} \text{ cm}^{-1}$ at 332 $m\mu$
	Nmr	Spectrophotometric	
20	6.410×10	6.317×10	9.886×10^3
48	1.393×10	1.346×10	1.073×10^4

where ρ , the probability that CQ is complexed, is given by

$$\rho = \frac{[\text{Pr}(\text{tmh})_3 \cdot \text{CQ}]}{[\text{CQ}]_T - [\text{Pr}(\text{tmh})_3 \cdot \text{CQ}]} \quad (7)$$

and can be evaluated in turn from K_1 , $[\text{Pr}(\text{tmh})_3]_T$ and $[\text{CQ}]_T$, where T is total; β was defined previously and ξ_i is taken as

$$\xi_i = \frac{3 \cos^2 \phi_i - 1}{r_i^3} \quad (8)$$

Equation 6, in effect, has two adjustable parameters, K_1 and $\beta\xi_i$, with the latter being unique for each magnetically nonequivalent proton. A nonlinear least-squares procedure was used to fit the pmr shift data with these adjustable parameters. The final K_1 values showed a moderate scatter for the various protons; however, the average of these K_1 values agrees remarkably well with the spectrophotometric K_1 values, Table III. This result substantiated the assumption that only a 1:1 complex is being formed. Furthermore, analysis of the shift data assuming a mole ratio of 1:2 for $\text{Pr}(\text{tmh})_3 \cdot \text{CQ}$ or $\text{CQ} \cdot \text{Pr}(\text{tmh})_3$ gave larger standard deviations in the nonlinear least-squares fit and K values which differ markedly from the spectrophotometric K_1 values. Therefore, the pmr data were re-analyzed using the spectrophotometric K_1 values and assuming formation of a 1:1 complex. An indication of the excellent agreement between the observed and calculated $\delta-[\text{Pr}(\text{tmh})_3]$ curves is provided in Figures 5-8 where the calculated curves are shown as solid lines.

(ii) **Orientation of $\text{Pr}(\text{tmh})_3$ with Respect to the Quinoline Ring Protons.** A determination of the CQ structure essentially involves a fixing of the $\text{Pr}(\text{tmh})_3$ location relative to the quinoline ring and side-chain protons by a correlation of the observed pseudocontact shift data with $\beta\xi_i$, adjusted for the fraction of CQ molecules complexed, ρ . Since ρ can be evaluated from K_1 , the next step therefore requires a knowledge of β and the coordinates of the $\text{Pr}(\text{tmh})_3$ interaction center relative to the CQ molecule in order to evaluate ξ_i . The problem can be considered in two parts, the first being an evaluation of β and the location of $\text{Pr}(\text{III})$ with respect to the quinoline ring CQ, and the second a determination of the side-chain structure. A direct calculation of β from eq 2 was not feasible in this work and an indirect approach utilizing the quinoline ring proton shift data was, therefore, followed. The choice of the quinoline protons for this purpose was governed by several factors among which the most important are the location of the complexing site, *i.e.*, at the N-1 position on the quinoline ring, and the rigid planar structure of the quinoline ring (Figure 11). Distances between atoms in the latter are fixed, and it can be

reasonably assumed that ring proton locations in solution are the same as in the crystalline state.

The procedure followed in evaluating β involved an initial assumption concerning the distance between the interaction center of $\text{Pr}(\text{tmh})_3$ and N-1. A consideration of distances observed in representative transition metal ion complexes¹⁰ suggested a $\text{Pr}(\text{III})$ -N-1 distance of 3 Å as being a reasonable starting point. If the molecular axis is defined along the $\text{Pr}(\text{III})$ -N-1 axis then ϕ_i and r_i values can be calculated readily for each ring proton. The following ratio (eq 9) can then

$$\frac{\beta\xi_i}{\beta\xi_j} = \frac{3 \cos^2 \phi_i - 1}{r_i^3} \bigg/ \frac{3 \cos^2 \phi_j - 1}{r_j^3} \quad (9)$$

be compared for all possible pairwise combinations $i \rightarrow j$ ($i \neq j$) of the aromatic protons for different positions of the $\text{Pr}(\text{III})$ origin, until the set of proton coordinates giving the minimum relative deviations from respective $\beta\xi_i$ ratios was obtained. Least-squares $\beta\xi_i$ values derived in the preceding section were used in this computation and the origin of $\text{Pr}(\text{III})$ was varied about the initial position in 0.1-Å increments along orthogonal x , y , and z axes up to ± 3 Å. The initial position was taken in-plane of the quinoline ring and directed out 3 Å from N-1. The final refined $\text{Pr}(\text{III})$ -N-1 distances were 3.98 Å at 20° and 3.77 Å at 48° with the $\text{Pr}(\text{III})$ located 3.00 and 2.85 Å, respectively, above the plane of the quinoline ring. In the next step β was calculated for each ring proton from the expression

$$\beta = \beta\xi_i(\text{obsd}) \left(\frac{3 \cos^2 \phi_i - 1}{r_i^3} \right)^{-1} \quad (10)$$

and the mean values $\beta_{20^\circ} = 3.50 \pm 0.09 \times 10^5 \text{ sec}^{-1}$ and $\beta_{48^\circ} = 3.49 \pm 0.09 \times 10^5 \text{ sec}^{-1}$, taken as the best values. The maximum difference between the independent β values was less than 3%, and the overall $\Delta\nu_i$ uncertainties between calculated and observed values were $\sim 5\%$.

(iii) Determination of the Side-Chain Conformation.

The procedure for determining the side-chain conformation essentially involved a comparison between observed and calculated $\beta\xi_i$ values for all side-chain protons in all CQ conformations. Because β is constant for the entire molecule, and all the bond angles and bond distances are known, it follows that $\beta\xi_i$ values can be calculated for all the chemically shifted side-chain protons for various x , y , and z proton coordinates. Coordinates for the protons were calculated by a computer program using bond angles,¹⁶ bond distances,¹⁶ and dihedral angles as input parameters. In the actual computational procedure the dihedral angles along the entire chain were varied in a sequential manner through 360° in 5° increments¹⁸ until a conformational model giving the best fit between observed and calculated $\beta\xi_i$ values for the entire set of side-chain protons was obtained.

(16) These parameters were obtained from the crystal-structure data.¹⁷

(17) (a) H. S. Preston and J. M. Stewart, private communication; (b) H. S. Preston and J. M. Stewart, *Chem. Commun.*, 1142 (1970).

(18) Although the number of different conformations at first appears to be impossibly large the restrictions imposed by bonding arrangements reduce the number of allowed conformations to a manageable proportion.

Following this procedure, positions for H-2-N and H-11 could be calculated directly from the $\beta\xi_i$ values. For those cases where rotational motion (and/or torsional oscillation) gives rise to magnetically equivalent signals (C-12, C-14, C-15, C-16 and C-18, and C-17 and C-19 protons) a calculation of coordinates for individual protons in the group is not possible directly from the shift data. However, the observed $\beta\xi_i$ value can be considered to represent an average of $\beta\xi_n$ values for the n protons of the group (*i.e.*, for a methyl group, $n = 3$, and for a methylene group, $n = 2$). The locations of the individual protons were, therefore, determined indirectly by calculating ϕ_i and r_i and ϵ_i independently for each proton and then taking the average ξ_i . The best fit of $\beta\xi_i(\text{obsd})$ and $\beta\xi_n$ (calculated average) was used to fix the location of the individual protons. At 48° it was not possible to obtain $\beta\xi_i$ data for H-2-N or H-13, H-13' because these signals were overlapped with other resonance signals and/or their chemical shifts could not be accurately determined. Therefore, a greater uncertainty exists for their positions. An estimate of the H-2-N location was made by assuming that it had the same orientation with respect to the C-4-C-11 bond as in the crystal. Similarly, the position of C-13 was established from the H-11 calculation and the relative carbon chemical bond configuration. The C-13-C-14 bond location could be determined from the H-14, H-14' data; therefore, only two bonding positions on C-13 were left available for H-13 and H-13' where again the relative bonding angles were taken from the crystallographic results. These assumptions were not required for the H-2-N data at 20° since its resonance shift could be followed over part of the concentration range. An accurate estimate of the individual H-13, H-13' positions was not possible at 20° because of signal overlap and the procedure followed at 48° was again employed.

A complete listing of the final coordinates for all of the atoms in CQ can be obtained from the authors. Stereoscopic projections of the molecular conformation plotted using the ORTEP plot routine are shown in Figure 10 (20°) and Figure 11 (48°).

Discussion

1. Complex Formation and Pseudocontact Shifts for Chloroquine.

The three key requirements for the paramagnetic ion probe approach to be feasible in biomolecular structure work are: (i) formation of a stoichiometrically well-defined complex with the donor molecule, (ii) an accurate determination of the complex equilibrium constant, and (iii) sizable paramagnetic ion-induced pseudocontact shifts for protons in the biomolecule. All three criteria are fulfilled in the CQ-Pr(tmh)₃ system. Formation of a 1:1 complex between CQ and Pr(tmh)₃ is clearly established by both the spectrophotometric and pmr measurements. Moreover, the chemical-shift data point to the N-1 atom of CQ as the point of complexation. The N-1 atom of the quinoline ring is likely to have electron donor characteristics and complexing would be favored at this position with appropriate acceptors.¹⁹ The spectrophotometric and pmr data show that the CQ·Pr(tmh)₃ complex is relatively weak ($K_1 = 63.2 \text{ M}^{-1}$ at 20° and $K_1 =$

(19) (a) C. S. Kerr, J. M. Stewart, H. S. Preston, and H. L. Annon, private communication; (b) A. Albert, *Annu. Rev. Pharm.*, 11, 13 (1971).

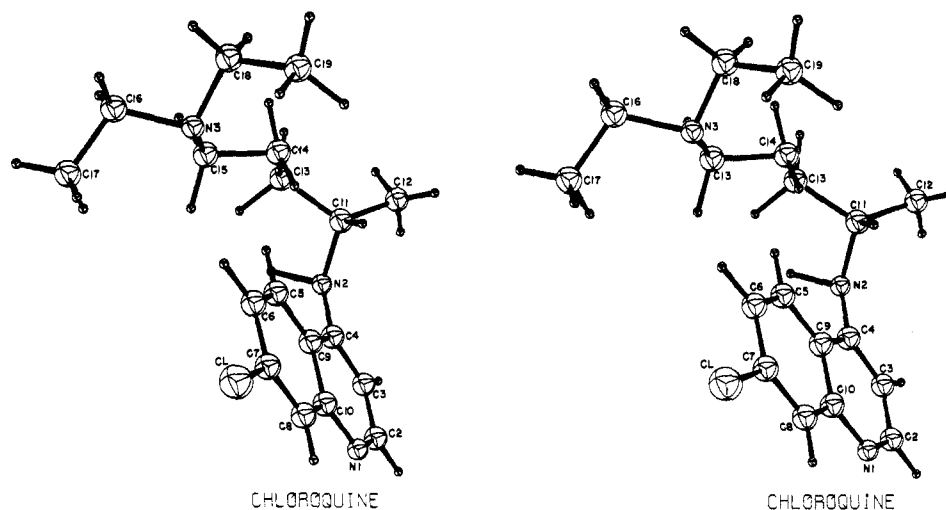


Figure 10. A stereoscopic projection of the molecular conformation of chloroquine in solution at 20°. The numbering scheme corresponds to the crystallographic convention used in the literature.¹⁷

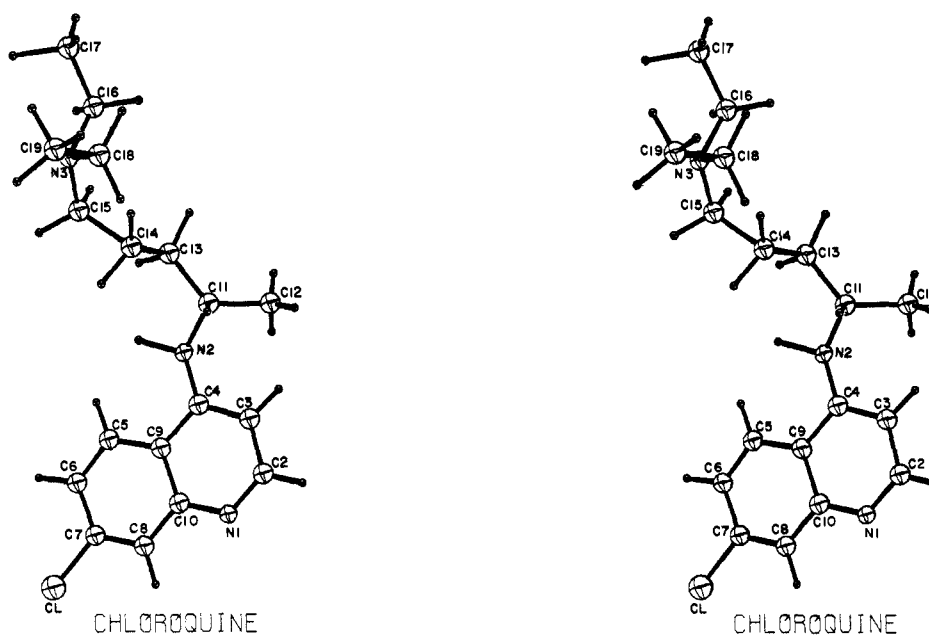


Figure 11. A stereoscopic projection of the molecular conformation of chloroquine in solution at 48°. The numbering scheme corresponds to the crystallographic convention used in the literature.¹⁷

13.5 M^{-1} at 48°), as is generally the case in other systems of this type.²⁰ Although this introduces a complication in that ρ will be <1 below 1:1 proportions of CQ to $\text{Pr}(\text{tmh})_3$ the accuracy of the K_1 determinations permits accurate ρ calculations, and the latter are not likely to be a troublesome source of error in the conformational calculations.

A more important question regarding the accuracy of the conformation derived from shift changes is the extent to which the observed shifts are due to a contact or pseudocontact process. An insight into the process dominating the shift change can be obtained from an analysis of the line-width variation with total concentration of $\text{Pr}(\text{tmh})_3$.

Since a temperature increase from 20 to 48° produced no change in line width for the quinoline protons it can

be reasonably assumed that $\text{Pr}(\text{tmh})_3$ is undergoing rapid exchange between complexed and uncomplexed forms. Accordingly, at equilibrium the exchange process can be described by the equation²¹

$$\frac{1}{T_{2(p)}} = \frac{1}{T_{2(\text{obsd})}} - \frac{1}{T_{2(s)}} = \pi(\Delta\nu_{1/2(\text{obsd})} - \Delta\nu_{1/2(0)}) = \frac{\rho}{T_{2(M)}} \quad (11)$$

where $T_{2(\text{obsd})}$ and $T_{2(s)}$ are the transverse relaxation times of the chloroquine nuclei, respectively, in the presence of and in the absence of $\text{Pr}(\text{tmh})_3$ in solution; $\Delta\nu_{1/2(\text{obsd})}$ and $\Delta\nu_{1/2(0)}$ are the line widths at half-height in hertz of the chloroquine nuclear resonances in the presence and in the absence of $\text{Pr}(\text{tmh})_3$ in solution, respectively; ρ ,

(20) I. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Chem. Commun.*, 1281 (1971).

(21) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, 37, 307 (1962).

the probability factor, has been previously defined; and $1/T_{2(M)}$ is given by^{22, 23}

$$\frac{1}{T_{2(M)}} = \frac{4S(S+1)\gamma_I^2 g^2 \beta^2}{3r_i^6} \tau_c + \frac{2}{3} S(S+1) \left(\frac{A}{\hbar}\right)^2 \tau_e \quad (12)$$

where S is the total effective free spin of the $\text{Pr}(\text{tmh})_3$, $\{\frac{5}{2}\}$; ²⁴ γ_I is the magnetogyric ratio of the proton; g is the average g value for $\text{Pr}(\text{tmh})_3$ and is taken as 1.55; ^{25, 26} r_i is the distance between the paramagnetic interaction center and the proton; A/\hbar is the coupling constant between the effective free electron on the paramagnetic center and the proton; τ_c and τ_e are taken as the tumbling time, τ_R , of $\text{Pr}(\text{tmh})_3 \cdot \text{CQ}$ and as the electron relaxation time, τ_S , respectively.²⁷

In the limit of rapid exchange, the line width can be represented by eq 11 and the contact shift by

$$\Delta\omega = \Delta\omega_{\text{obsd}} - \Delta\omega_0 = \rho\Delta\omega_M \quad (13)$$

$\Delta\omega_M$ has the following form

$$\Delta\omega_M = \frac{-\omega_0 S(S+1)\hbar}{3kT} \left(\frac{A}{\hbar}\right) \frac{\gamma_e}{\gamma_I} \quad (14)$$

where ω_0 is the operating frequency in radians, γ_e is the magnetogyric ratio for the electron, and the other parameters have their previously defined meanings.

Since the largest line-width variation with $[\text{Pr}(\text{tmh})_3]_T$ concentration was observed for the H-2 proton (48°) these data were used in the analysis of the contact-pseudocontact shift contribution.

All of the parameters in eq 12 are known except for r_i , τ_R , A/\hbar , and τ_S . If τ_R is assumed to have the reasonable value of 1.0×10^{-12} sec²⁸ then a nonlinear-least-squares fit to the line-width data for H-2 gives $r_i = 4.1 \pm 0.1 \text{ \AA}$. The final fit to the broadening data is shown in Figure 12. This value for r_i is in excellent agreement with that determined for the Pr-H-2 distance 3.90 Å from the pseudocontact shift analysis. From this analysis it can be concluded that the contact shift cannot contribute more than 5% (~250 Hz) to the total chemical shift at $[\text{Pr}(\text{tmh})_3]_T = 0.20 \text{ M}$. Therefore, the contact coupling constant can be estimated ($A/\hbar < 5 \pm 1 \times 10^4$ rad/sec) from eq 13 and 14. This estimate for A/\hbar can then be substituted into the second term in eq 12 along with an estimate for $\tau_S < 10^{-10}$ sec²⁹ to yield $\Delta\nu_{1/2} < 1 \text{ Hz}$. This small line width caused by a contact interaction is consistent with the initial assumption that the total line width is due principally to a dipole-dipole interaction.

Therefore, it can be concluded that contact interactions are not a significant source of possible error in the

(22) (a) I. Solomon, *Phys. Rev.*, **99**, 559 (1955); (b) I. Solomon and N. Bloembergen, *J. Chem. Phys.*, **25**, 261 (1956).

(23) Equation 12 results from the general expression when $\omega_I \ll \omega_e$ and $\omega_e^2 \tau_c^2$ and $\omega_e^2 \tau_e^2 \ll 1$.

(24) H. J. Emeleus and A. G. Sharpe, *Advan. Inorg. Chem. Radiochem.*, **43** (1970).

(25) S. A. Al'tschuler and B. M. Kozyrev, "Electron Paramagnetic Resonance," Academic Press, New York, N. Y., 1964, p 131.

(26) A. A. Manenkov and R. Orbach, Ed., "Spin-Lattice Relaxation in Ionic Solids," Harper and Row, New York, N. Y., 1966, p 386.

(27) N. S. Angerman and R. B. Jordan, *J. Chem. Phys.*, **48**, 3983 (1968).

(28) J. M. Emsley, J. Feeney, and L. H. Sutcliffe, *Progr. Nucl. Magn. Resonance Spectrosc.*, **3**, 95 (1967).

(29) No epr spectrum was observed for a solution containing 0.2 M CQ and 0.2 M $\text{Pr}(\text{tmh})_3$ at 20°.

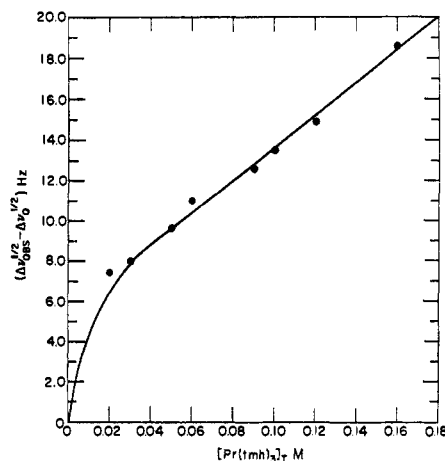


Figure 12. Line-width change of the H-2 proton resonance of chloroquine with varying $[\text{Pr}(\text{tmh})_3]_T$ in deuterioacetone at 48°.

structure calculation for chloroquine. A small contact contribution, if present, is likely reflected in the overall error of ~5% in $\rho\beta\xi_i$. The latter error gives rise to errors of $\sim \pm 2\%$ in r_i and ϕ_i .

Another source of error may arise from the possibility that the spatial arrangement represents an average of two conformationally different optical isomers^{14b} for the $\text{CQ} \cdot \text{Pr}(\text{tmh})_3$ complex. This hypothesis can be eliminated, however, by a consideration of the fact that only one set of signals representing the C-12 methyl protons and the C-11 proton is observed when the concentration of $[\text{Pr}(\text{tmh})_3]_T$ is varied. If the respective protons for each optical isomer were in different magnetic environments in the $\text{Pr}(\text{tmh})_3 \cdot \text{CQ}$ complexes they would experience different chemical shifts; however, this is not the case. Therefore, the *d*-chloroquine· $\text{Pr}(\text{tmh})_3$ and *l*-chloroquine· $\text{Pr}(\text{tmh})_3$ complexes must be mirror images with respect to a mirror plane through asymmetric C-11.

2. Conformation of Chloroquine and the Chloroquine-Praseodymium Complex. (i) **Crystal Conformation of Chloroquine.** An X-ray diffraction study of the crystal structure of CQ phosphate dihydrate has been completed recently by Preston and Stewart,¹⁷ and a stereoscopic representation of the molecular conformation³⁰ is given in Figure 13.

Several features about the CQ conformation in the crystal may be noted. The H-2-N hydrogen and C-11 lie very nearly in the plane of the quinoline ring with the H-2-N proton directed toward H-5. With respect to this orientation the $\text{CH}_3(12)$ methyl lies on one side of the plane of the quinoline ring while the rest of the side chain is extended out on the opposite side and roughly normal to it. This quinoline ring-side chain arrangement may arise from the combined effect of a stacking interaction between adjacent quinoline rings and hydrogen bonding interactions between the phosphate and side-chain nitrogen. The bonds comprising the backbone of the side chain are oriented in an all trans form typical of alkanes, with the C-H bonds (C-13 → C-15) in a staggered form. Overall, the side-chain conformation in CQ has a close similarity to the conformation found in 1,4-diamino alkanes of biological impor-

(30) For purposes of clarity the phosphate and H_2O were not plotted in Figure 13.

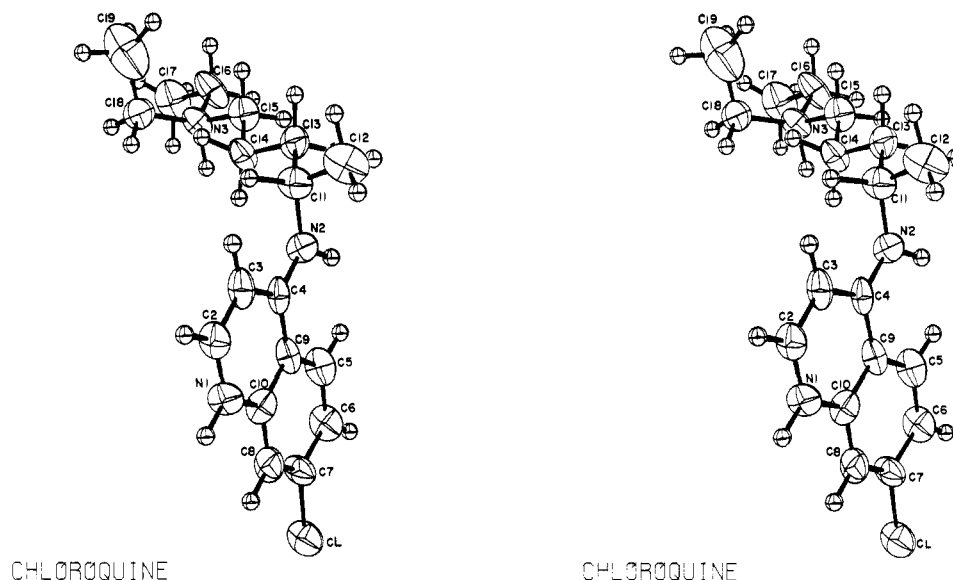


Figure 13. A stereoscopic projection of the molecular conformation of chloroquine diphosphate in the crystalline form. Coordinates were obtained from ref 17a.

tance, *e.g.*, spermine^{31a} and spermidine.^{31b} Finally it is useful to note several intramolecular distances which may have a bearing on the biological activity of CQ. These are the N-1–N-3 and N-2–N-3 distances of 14.1 and 5.5 Å, respectively, and the distance between C-17 and C-19 methyl groups and the center of the quinoline ring, *i.e.*, 8.6 and 8.2 Å, respectively.

(ii) **Solution Conformation of Chloroquine.** The derived pmr parameters (chemical shifts and coupling constants) for CQ provide only a very limited insight into the conformation. No definitive picture of the orientation of the quinoline ring and side chain emerges from the chemical-shift data although the absence of any shift changes with increasing temperature does suggest that the conformation is not altered drastically by thermal effects.

A combination of the splitting patterns and coupling constant magnitudes for protons along the side chain strongly points to the presence of rotational or oscillational averaging about the C–C and C–N bonds. If the side chain retained the conformationally rigid structure found in the crystal then the splitting patterns would be more complex with coupling constants of roughly 2 and 9 Hz for *gauche* and *trans* vicinally coupled protons. The actual vicinal couplings lie in a range, ~6–8 Hz, typical of the single rotationally averaged J_{av} values found in a majority of substituted alkanes (ethanes, propanes) in which rotation about C–C bonds is unhindered.³² Although a uniform rotational averaging along all the C–C bonds would probably give rise to an extended zigzag chain conformation, the chemical shift and coupling constant data are not sufficient to establish this. A recently completed study of the pH dependence of the CQ spectrum³³ in fact raises the interesting possibility that the side chain in the monocation (protonated at N-3) folds back to form an intramolecular hydrogen bond between N-3 and N-2.

(31) (a) Y. Iitaka and Y. Huse, *Acta Crystallogr.*, **18**, 110 (1965); (b) Y. Huse and Y. Iitaka, *ibid.*, **25**, 498 (1969).

(32) A. A. Bothner-By, *Advan. Magn. Resonance*, **1**, 195 (1965).

(33) N. S. Angerman, S. S. Danyluk, and T. A. Victor, manuscript in preparation.

A much more detailed picture of the CQ conformation emerges from the pseudocontact-induced shift data. In this approach the induced shift changes can be directly related to proton positions provided any contribution from the contact term is negligible, as has been shown for the present system. Combining all of the data it is possible to construct a complete model showing the time-averaged conformation of CQ of the CQ–Pr(tmh)₃ complex in solution, Figures 10 and 11. Although the conformations deduced in Figures 10 and 11 are not strictly those for the free CQ molecule, it is unlikely that the latter will be appreciably different in view of the weak nature of the interaction between CQ and Pr(tmh)₃. This view is substantiated by the lack of any coupling constant changes between free CQ and CQ in the presence of [Pr(tmh)₃]_T = 0.20 M.

The solution conformation reveals several interesting features, some of which differ from the crystal conformation. An out-of-plane (with respect to the quinoline ring) location is observed for both H-2–N and C-11 atoms. In consequence the side-chain orientation with respect to the quinoline ring differs markedly from that in the crystal. An even more striking difference between the crystal and solution structures is observed for the side chain itself. Whereas the bonds along the backbone (taken as C-12 to N-3) are in an all *trans* form in the crystal this is clearly not the case in solution. Here the time-averaged conformation is one in which backbone bonds adopt a staggered form. The net overall effect amounts to a curling of the side chain into a more compact form in which the N-3 ethyl groups lie more directly over the center of the quinoline ring. An idea of the compactness of the solution structure is given by the N-1–N-3 separation of 7.39 Å at 20°, a distance almost 7.0 Å less than in the crystalline state. The distance increases to 9.33 Å at 48°, indicating a significant opening up of the structure with increasing thermal motion; however, the separation at this temperature still suggests a residual buckling of the side chain.

The conformational differences between the crystal and solution structures are not surprising since the

former was determined for the diprotonated form of CQ (N-1 and N-3 protonated) while the latter relates to the free base. Electrostatic repulsion between the positive charges would tend to force the CQ molecule into a more open structure with an extended side chain. Interaction with phosphate groups further acts to stabilize the extended form in the cation. On the other hand the unusual buckling of the side chain in solution may arise in part from an intramolecular dipole-induced dipole type of interaction between the N-3 ethyl groups and the π -electron system of the quinoline ring. Such an interaction has its counterpart in the well-known intermolecular dipole-induced dipole interactions between polar aliphatic amides, amines, etc., and various aromatic ring systems. The key factors favoring this type of interaction, namely a polar acceptor and a well-defined π -electron donor ring, are both present in CQ.

3. Chloroquine Interaction with DNA. Several attempts have been made to relate the conformational features of CQ to its binding interaction with double-stranded DNA. Hahn and O'Brien³⁴ have recently made the interesting suggestion, based on studies of the effect of CQ on the thermal stability of DNA, that the

(34) R. L. O'Brien and F. E. Hahn, *Antimicrob. Ag. Chemother.*, 315 (1966).

CQ dication stabilizes the DNA structure by electrostatic interaction between the positive charges on CQ and the phosphate groups on DNA. In this complex the side chain of CQ is visualized as forming a bridge across the minor groove of the double helix. Since the distance across the minor groove, $\sim 10.5 \text{ \AA}$, is larger than the maximum possible extended distance between N-2 and N-3, 7.5 \AA , and much larger than the distance observed in the crystal, 5.5 \AA , a N-2,N-3 complex across the minor groove would appear to be a quite unfavorable possibility. Moreover, the pmr protonation data³³ point to a dication in which positive charges are located at N-1 and N-3 rather than N-2 and N-3. The N-1-N-3 distance in this case is appreciably larger in the crystal, 14.1 \AA , and in solution, $6.7 (20^\circ)$ and $8.1 \text{ \AA} (48^\circ)$, than N-2-N-3, and a bridging complex would appear to be more favorable. However, the present solution data, though determined for the free base form of CQ, do suggest that the side chain has considerable conformational flexibility and is influenced by a variety of factors including solvent medium, temperature, and pH.

Acknowledgments. The authors wish to acknowledge Dr. Fred E. Hahn for helpful discussions on the properties of antimalarial drugs. We also wish to thank Dr. J. M. Stewart for kindly providing the crystal coordinate data.

Communications to the Editor

Boat and Chair Transition States of 1,5-Hexadiene¹

Sir:

The stereospecific Cope rearrangement of acyclic 1,5-dienes continues to attract theoretical interest²⁻⁵ of increasingly more quantitative ambitions.^{4,5} Supplementing the previous stereochemical² and kinetic⁶ investigation, we here report discovery of a second degenerate rearrangement of 1,5-hexadiene—one that requires an additional 5.8 kcal/mol in ΔG^\ddagger (at 250°) but 11.2 kcal/mol more in ΔH^\ddagger .^{2,6}

To simplify subsequent comparison with theory,⁴ we tentatively assign the "boat" stereochemistry to this new, higher energy transition state and accept the "chair" assignment for the lower energy one.^{2,6,7} The

(1) Taken in part from the Ph.D. Thesis of M. S. Benzon, Cornell University, Dec 1971.

(2) W. von E. Doering and W. R. Roth, *Tetrahedron*, 18, 67 (1962).

(3) (a) R. Hoffmann and R. B. Woodward, *J. Amer. Chem. Soc.*, 87, 4389 (1965); (b) R. B. Woodward and R. Hoffmann, *Angew. Chem.*, 81, 797 (1969); *Angew. Chem., Int. Ed. Engl.*, 8, 781 (1969).

(4) (a) K. Fukui and H. Fujimoto, *Tetrahedron Lett.*, 251 (1966); (b) M. Simonetta, G. Favini, C. Mariani, and P. Gramaccioni, *J. Amer. Chem. Soc.*, 90, 1280 (1968); (c) A. Brown, M. J. S. Dewar, and W. Schoeller, *ibid.*, 92, 5517 (1970).

(5) (a) M. J. S. Dewar and W. W. Schoeller, *ibid.*, 93, 1481 (1971); (b) M. J. S. Dewar, *Fortschr. Chem. Forsch.*, 23, 1 (1971); (c) M. J. S. Dewar and D. H. Lo, *J. Amer. Chem. Soc.*, 93, 7201 (1971).

(6) W. von E. Doering, V. G. Toscano, and G. H. Beasley, *Tetrahedron*, 27, 5299 (1971).

(7) Both assumptions are more critically examined in the succeeding communication.⁵

(8) M. J. Goldstein and M. S. Benzon, *J. Amer. Chem. Soc.*, 94, 7149 (1972).

experimental approach then also becomes more apparent. This begins with the racemic mixture of *ERSZ*⁹ and *ESRZ* enantiomers of 1,5-hexadiene-*d*₄, efficiently obtained by stereospecific cleavage of bicyclo-[2.2.0]hexane-*exo-d*₄ at $135\text{--}180^\circ$ (Figure 1).¹⁰ Diene stereointegrity (measured in part by oxidation to the exclusively *meso*-dideuteriosuccinic acid) is retained throughout 22 half-lives⁶ of Cope rearrangement at a somewhat higher temperature (233°).

The graphical analysis of Figure 2 then precludes any other structural assignment. It also suggests that leakage into the *ESSE-ERRE-ZSSZ-ZRRZ* quartet might yet be achieved through a "boat" transition state. In practice, identical oxidation of samples recovered at four, still higher temperatures ($259\text{--}295^\circ$) also provided *meso*-dideuteriosuccinic acid but now increasingly contaminated with its racemic diastereomer. The three characteristic pmr areas of the diene remained equal. Fifteen such samples were each oxidized; the derived succinic acid was recrystallized from acetonitrile, accurately weighed ($w \approx 600 \pm 5 \mu\text{g}$), dispersed in KBr, and the racemic content assayed by absorbance (*A*) at 1215 cm^{-1} .^{10,11} An appropriate nonlinear least-squares analysis then provided the best values of either (a) $[(A/w)_\infty - (A/w)_0]$ and λ (eq 1) or (b) $[(A/w)_\infty -$

(9) E. L. Eliel, *J. Chem. Educ.*, 48, 163 (1971).

(10) M. J. Goldstein and M. S. Benzon, *J. Amer. Chem. Soc.*, 94, 5119 (1972).

(11) C. R. Childs and K. Bloch, *J. Org. Chem.*, 26, 1630 (1961).