NMR Titration Experiments. Most amines used in these studies were commercially available (Aldrich) and were used as received. The amino acid ester **3b** was prepared by standard methods[11] and liberated from HCl just prior to use. Typically, 500 μL of a 5.0×10^{-3} M $CDCl_3$ solution of **1** prepared as described[3,12] was treated with small aliquots (1–5 μL) of a 0.5 M $CDCl_3$ solution of amine, and the spectrum was recorded after each addition. Chemical shift values for **1** and various amines at selected stoichiometries are reported in Table I. Experiments with the β-arylethylamines in $CD_3OD/CDCl_3$ (1:1, v/v) also showed the upfield shifts characteristic of stacking interactions. Spectra at low temperatures were complex but generally indicated that two different acidine subunits are present in the complexes.

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UV–Visible and Carbon NMR Studies of Quinine Binding to Urohemin I Chloride and Uroporphyrin I in Aqueous Solution

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Abstract: Quinine binding by urohemin I and uroporphyrin I in aqueous solution at pH 6.0–6.4 has been studied by UV-visible and natural-abundance carbon NMR spectroscopies. Our results show that the soluble uroporphyrin–quinine complex has 1:1 stoichiometry characterized by an apparent overall association equilibrium constant $K_A = (4.2 \pm 0.2) \times 10^4$ M^{-1} at a uroporphyrin concentration of 10^{-6} M (22 ± 1 °C). From a combination of optical methods (Job, Scatchard, and Hill formulations) and carbon NMR spectroscopy, the complex is best formulated as a cofacial π–π dimer. In contrast, quinine interacts cooperatively with urohemin, yielding a Hill parameter of 2. Also, different from the uroporphyrin complex, the urohemin complex has a stoichiometry shown by Job's method to be 2:1 (urohemin to quinine). It is characterized by an apparent overall $K_A = (3.8 \pm 0.4) \times 10^8$ M^{-2} at 10^{-6} M urohemin and 22 ± 1 °C. NMR spectra of the quinine–urohemin I complex are consistent with iron ion coordination by the quinine 9-position hydroxyl group accompanying π–π type bonding between the heme ring and quinoline ring. For the uroporphyrin, where metal coordination is precluded, a different geometry involving primarily a π–π complex formation is indicated.

The interaction of certain malaria drugs with hemes has been studied in both aqueous and nonaqueous media[1–6] as a consequence of suggestions that protoheme IX may function as the receptor for antimalarials in *Plasmodium*-infected erythrocytes.[3,7] In this view, protoheme IX, originating from protease-degraded hemoglobin in the parasitized cell, may become available for complex formation with malaria drugs, resulting in the formation of malaria pigment clumps.[7,8] Indeed, it has been postulated that protoheme IX liberated from hemoglobin, in association with the erythrocyte membrane, may even act as the specific malaria drug receptor.[3,9]

In view of the seeming relevance of heme–malaria drug interactions, we have undertaken a study of quinine interaction with urohemin I [(uroporphyrin I)iron(III) chloride] and free base uroporphyrin I in aqueous solution. These structures are shown in Figure 1. Our motivation for this study is twofold. First, previous studies of this type in aqueous solution have utilized protohemin IX, a heme known to aggregate to varied extents in aqueous media depending upon pH and concentration,[11,12] so that with one exception[3] little quantitative data have been derived from such studies. Second, although an aqueous environment is, perhaps, more relevant to the physiological chemistry of malaria drug chemotherapy, an extremely interesting recent study was performed in nonaqueous solution.[5] The results of that work suggested that quinine is capable of axially coordinating the dimethyl ester of protohemin IX [chloroiron(III)protoporphyrin IX dimethyl ester] and (tetraphenylporphinato)iron(III) cations containing different counterions. Those results were extremely significant and stand in contrast to conclusions drawn by others who performed similar experiments with protohemin IX and uroporphyrin I in aqueous solution.[4] These latter workers concluded that axial coordination by quinine in aqueous solution was not evident.

In an attempt to further clarify the aqueous chemistry of quinine in the presence of hemes we have chosen to study this drug's interaction individually with urohemin I and uroporphyrin I near neutral pH. Previous work has characterized the aggregation dynamics of both of these hemes,[12–20] making it possible to derive equilibrium constants, stoichiometries, and Hill parameters by UV-visible methods. The choice of pH 6.0 as an appropriate value

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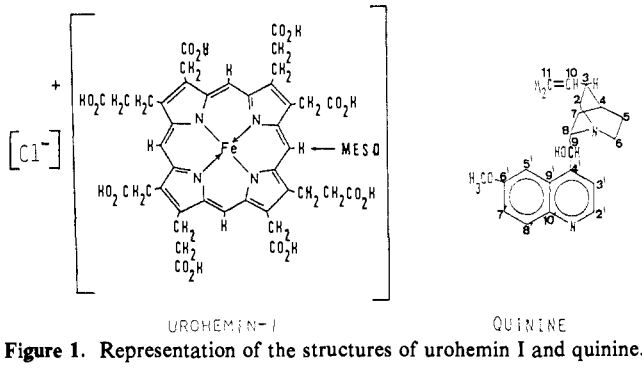

Figure 1. Representation of the structures of urohemin I and quinine.

was made not only for its physiological relevance but because previous studies have shown that the degree of urohemin I self-aggregation increases as the pH increases.[17,18] The region between pH 5.5 and 6.5 is a narrow range wherein urohemin I and uroporphyrin I remain soluble but where heme self-aggregation is minimized. Such a restriction precludes pH-dependence studies of drug binding.

A clear advantage in the use of urohemin I is its characterization as a monomer under conditions employed in this work. In addition to optical work, solution NMR studies yield insights to the mode of quinine binding to both hemes. These results support the conclusion of Behere and Goff[5] that quinine can coordinate to heme iron through the 9-hydroxyl group. Our results are consistent with this interpretation for quinine interacting with urohemin I in aqueous solution. Furthermore, a reanalysis of the results of Moreau et al.[4] indicates that their data are, in fact, consistent with 9-hydroxyl coordination to protohemin IX in aqueous solution.

Experimental Section

Urohemin I [chloroiron(III) uroporphyrin; Figure 1] and uroporphyrin I were purchased from Porphyrin Products (Logan, UT) and were purified by chromatography on Sephadex G-25 (Sigma) at pH 14 (0.1 M NaOH, Fisher). Most samples were then precipitated from aqueous solution near their isoelectric points (pH 4–4.5) by acidification (HCl, Fisher). Solutions of both were adjusted to the correct pH for the studies described herein with minimal amounts of HCl or NaOH. Solutions were made up in distilled water, which was further deionized and cleaned by a two-stage filtering system (Barnstead PCS). Care was taken throughout to work in the lowest ion concentrations possible in view of the proclivity of urohemin and uroporphyrin to aggregate at high ion concentrations.[12-20] An accurate estimate of the ionic strength of these solutions depends upon such unknown parameters as the ionization state of the heme acid substituents; thus, we hesitate to make what would necessarily be a rather poor estimate of ionic strength. However, unbuffered solutions were used throughout in order to assure that heme monomer species would predominate in solution. The confirmation that no substantial higher order aggregates were present was by UV-visible and NMR spectroscopies, which have been shown to be sensitive to heme aggregation.[12-20] This also necessitated careful, repeated monitoring of the pH throughout all procedures, which was accomplished with a Beckman pH Φ60 meter and a combination electrode (Fisher). In general, once the solution pH's stabilized following appropriate adjustment, they remained stable throughout the immediately following data collection operation. Fresh solutions were used for all such operations. Quinine hydrochloride was purchased from Sigma and used as obtained. Solutions of quinine hydrochloride gave pH values of 6.0 (±0.1), which is the pH range in which we worked.

UV-visible spectroscopy was carried out on a Perkin-Elmer 559A dual-path spectrophotometer using variable-temperature cell holders thermostated to 22 °C. Quartz cuvettes (Fisher) of various sizes, ranging between 1-mm and 10-cm path lengths, were used, depending upon solution concentration. Equilibrium binding studies were carried out according to the Hill formalism.[21,22] Scatchard plots[23] were constructed as a means of determining cooperativity, and stoichiometry was defined with Job's method.[24-26] Care was taken to satisfy the conditions necessary for valid use of Job's method (method of continuous variations);[26] namely all solutions were (1) dilute and apparently obeyed the Debye-Hückel limiting law in that linear Beer's law behavior was observed[26] and (2) in the proposed equilibria the free heme (porphyrin) and complexed species are of the same charge type. Overall apparent equilibrium constants were obtained initially from the graphical data. It should be emphasized that the constants reported in Table I are "apparent association equilibrium constants", which are strictly valid only at the reported pH. Studies at various pH's were precluded by urohemin and uroporphyrin aggregation at higher pH values and precipitation at lower pH values. Once the actual stoichiometry was defined, a nonlinear least-squares calculation from the equation that defines the equilibrium process for the 1:1 complex involving uroporphyrin was employed as the most accurate determination of K_A(app). The tabulated values presented here and those calculated graphically were in excellent agreement. For the 2:1 complex involving urohemin, satisfactory fits to the binding data could not be achieved by using nonlinear fitting based on sample binding models. However, graphical treatment of the data, independent of a precise model, gave quite satisfactory results (Figure 3) for the pH at which the data were obtained.

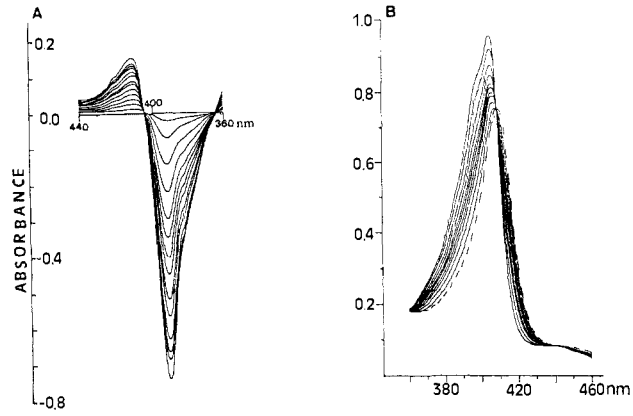

Figure 2. Optical titrations: A, urohemin–quinine recorded in the difference mode, 3 mL of [urohemin] = 5×10^{-6} M and 5–130-μL additions of 3.23×10^{-3} M quinine; B, uroporphyrin–quinine recorded in the absolute mode, 3 mL of [uroporphyrin] = 5×10^{-6} M followed by 1–50-μL additions of 1.5×10^{-2} M quinine. The pH was monitored at 6.0 throughout both experiments, and the temperature remained constant at 22 °C.

Natural-abundance carbon NMR spectra were acquired at 8.5 T (90 MHz) with 12-mm sample tubes. Broad-band, noise-modulated proton decoupling was employed, and all data (700–24000 transients) were acquired at 21 ± 1 °C. Quinine possesses several nonprotonated carbons (4′, 6′, 9′, 10′) whose spin-lattice relaxation times are quite long (4–5 s, data not shown); however, we chose to accept partial saturation of these resonances by employing a 45° observation pulse and a total recycle time of 2.2 s. At the maximum concentrations employed for these NMR studies (2×10^{-3} M), uroporphyrin shows slight evidence of dimerization whereas urohemin does not.[12-20] Carbon spectra were referenced against external $CHCl_3$ (77.0 ppm), which was contained in a small concentric tube.

Results and Discussion

Unlike the interaction of quinine with hemes in nonaqueous media,[5] the interactions of quinine with both urohemin I and uroporphyrin I are characterized by an isosbestic point in the Soret region (near 400 nm) of the spectrum (Figure 2). The UV-visible spectral characteristics of the individual heme solutions indicate that principally monomer units are present at pH 6.0. Spectroscopic criteria for uroporphyrin and urohemin dimerization and aggregation have previously been described,[12,15-18] and we point out here that the position of the Soret absorption maximum indicates the monomer form. The monomer forms are expected at the concentrations used to study equilibrium binding (10^{-8}–10^{-5} M), and addition of salt to solutions of both urohemin I and uroporphyrin I at pH 6 results in spectral changes characteristic[12,15] of aggregation (data not shown). Furthermore, these solutions

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Table I. Apparent Association Equilibrium Constants K_A(app) (22 °C) at pH 6.0 and Hill Parameters for Quinine Binding to Urohemin I and Uroporphyrin I

Urohemin I–Quinine			
hemin concn, M	$10^{-8}K_A$(app), M^{-2}	Hill param	repetitions
5×10^{-7}	(2.8 ± 0.2)	1.97 ± 0.03	2
5×10^{-6}	(3.8 ± 0.4)	2.00 ± 0.05	3
5×10^{-5}	(1.0 ± 0.3)	2.03 ± 0.05	2
Uroporphyrin I–Quinine			
porphyrin concn, M	$10^{-4}K_A$(app), M^{-1}	Hill param	repetitions
5×10^{-7}	(6.6 ± 0.5)	1.05 ± 0.02	2
5×10^{-6}	(4.2 ± 0.2)	0.95 ± 0.05	3
5×10^{-5}	(3.4 ± 0.2)	1.10 ± 0.05	2

Table II. Peak Heights[a] for Individual Carbon Resonances of Quinine in the Absence (0%) and Presence (1%) of Urohemin I, Measured as a Percent of the Height of the External Chloroform Resonance

	urohemin I			urohemin I	
C posn	0%	1%	C posn	0%	1%
6′	27.75	13.43	5′	60.92	17.69
2′	48.44	15.80	9	82.14	18.67
4′	30.98	11.25	8	77.81	22.07
10′	26.17	12.84	M	72.47	24.22
10	64.16	34.56	2	68.42	35.30
8′	63.65	22.47	6	73.18	28.16
9′	21.00	14.69	3	71.64	36.03
3′	83.22	23.85	4	62.66	25.63
7′	70.06	21.65	5	46.09	30.23
11	73.76	45.09	7	41.83	17.06

[a] All heights obtained with broad-band proton decoupling.

at pH 6 conform to Beer's law over the 3 orders of magnitude concentration changes (from 10^{-8} to 10^{-5} M) that were employed in the UV–visible work.

Quinine clearly perturbs the spectrum of both urohemin I and uroporphyrin I (Figure 2), indicating that an interaction takes place between the drug and each heme type. Titrating increasing amounts of quinine into solutions of either heme achieves conversion of the spectrum of each to a form with lower Soret molar absorptivity and a Soret band maximum shifted to longer wavelength (Figure 2). Both absolute (2B) and difference (2A) methods were employed in this study. These spectral changes are similar to those observed for molecular complex formation between several metallouroporphyrins and aromatic molecules.[13,20] The conclusion is that similar complexes are formed here, for both quinine and urohemin I and quinine with uroporphyrin I, in aqueous, unbuffered, low-salt solutions at pH 6.

The interaction represented by the titration behavior in Figure 2 can be analyzed by the Hill method[21,22] in order to determine the Hill parameter, which is an indication of cooperativity. The so-called Hill plots[21,22] of our binding data are shown in Figure 3 for uroporphyrin I–quinine and for urohemin I–quinine at the 5×10^{-6} M urohemin I and uroporphyrin I concentration. Similar results (presented in Table I) were obtained for both urohemin I and uroporphyrin I at the two other concentrations used in this work. The slopes of these linear graphs are (within experimental error) 1.0 and 2.0, respectively. The slope is a measure of the Hill parameter, which describes the extent of cooperativity for ligand binding processes, or the extent to which multiple ligand binding sites interact.[21,22] In the cases under consideration here, the overall equilibrium interaction between drug (Q) and macrocycle (M) monomer is studied by observing changes in the characteristic heme Soret band near 400 nm. Anticipating the results, the simplest overall equilibrium process of quinine binding to a heme, which may involve more than one heme (M) is described in general by eq 1. Therefore, the interpretation of $n =$

$$Q + mM \rightleftharpoons QM_m \qquad m = 1, 2, \ldots \qquad (1)$$

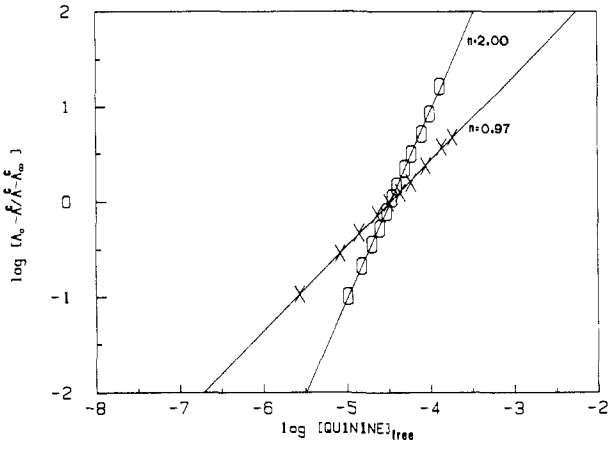

Figure 3. Hill plots of the (×) uroporphyrin–quinine association at 5×10^{-6} M uroporphyrin concentration and of the (□) urohemin–quinine association at 5×10^{-6} M urohemin concentration. The pH was monitored at 6.0 for all experiments, and the temperature was constant at 22 °C; n corresponds to the individual slope. Graphs similar to these were obtained for the other urohemin and uroporphyrin concentrations.

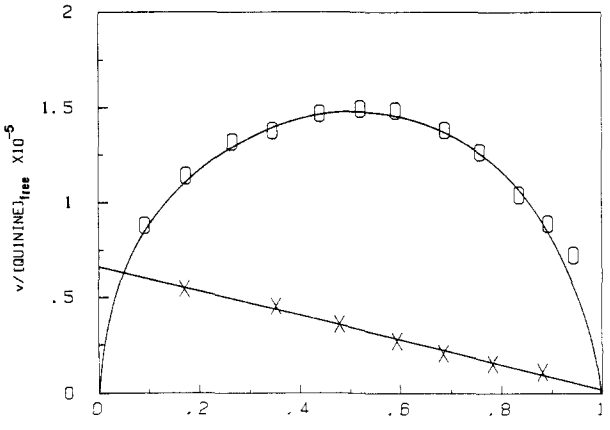

Figure 4. Scatchard plots of the (×) uroporphyrin–quinine association at 5×10^{-7} M uroporphyrin concentration and of the (□) urohemin–quinine associations at 5×10^{-6} M urohemin concentration. The same data used to construct Figure 3 were used for these plots. Graphs similar to these were obtained for the other urohemin and uroporphyrin concentrations.

1 for the uroporphyrin–quinine binding is that (a) m, in eq 1, may be 1 (an apparent stoichiometry of 1:1) or (b) m may be larger (i.e., more than one porphyrin binds to a quinine), but all quinine binding sites on the porphyrins are identical and independent of each other (multiple sites, no cooperativity). It should be noted at this point that structural comparisons of porphyrin macrocycles and quinine suggest only 2:1 or lower (1:1, 1:2) ratios for quinine to macrocycle stoichiometry are probable[1,4,20] as the basic unit.

In the case of urohemin I–quinine complex formation, observation of $n = 2$ (Figure 3) indicates that two interacting sites are available on a particular urohemin I for quinine binding. In other words, quinine binding to urohemin I is a cooperative process. The minimum stoichiometry for such a complex is 1:1, in which case both the quinine and urohemin I would possess two inequivalent interacting sites.

A further test for cooperativity in molecular systems such as these is the Scatchard plot[23] and the results for uroporphyrin I–quinine and urohemin I–quinine at the 5×10^{-6} M macrocycle concentration are shown in Figure 4. Similar plots were obtained (not shown) for the other macrocycle concentrations (5×10^{-7} and 5×10^{-5} M). The straight line indicates the absence of cooperativity for the uroporphyrin I–quinine complex while, for urohemin I–quinine, a curved graph is observed, indicating that this interaction is confirmed as cooperative and involves nonidentical interacting binding sites.

Elucidation of the true stoichiometry of these complexes was carried out by Job's method.[24-27] The results are shown in Figure

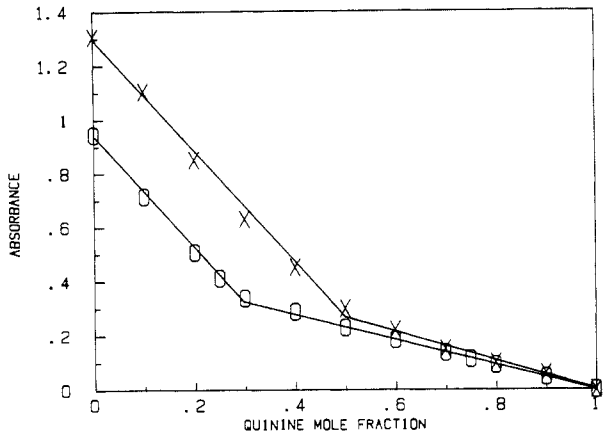

Figure 5. Job plots of (×) uroporphyrin–quinine at 1×10^{-5} M uroporphyrin, monitoring the absorbance at 405 nm, and of (□) urohemin–quinine at 8×10^{-6} M urohemin, in which absorbance was monitored at 393 nm. Both experiments were at 22 °C, and the pH was 6.0.

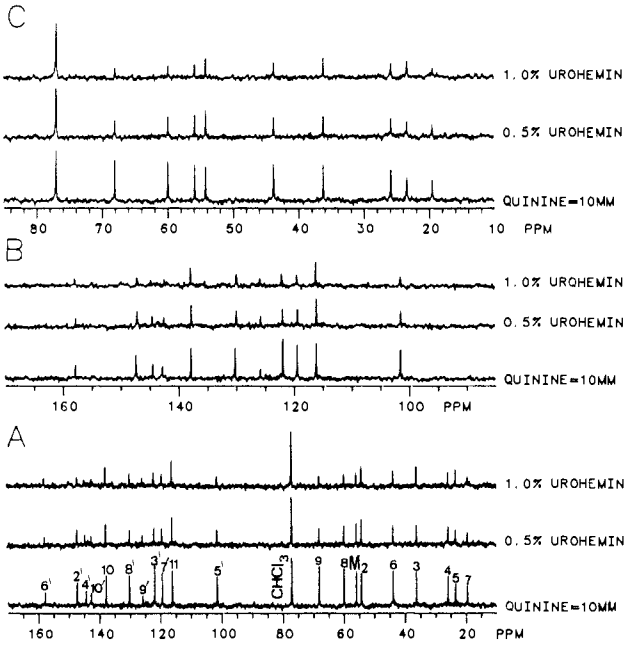

Figure 6. ^{13}C NMR of quinine as a function of urohemin addition in mole percent at pH 6.0, 22 °C, 90 MHz, and 20% D_2O used for lock. All peaks were referenced to external $CHCl_3$ (77.0 ppm). A shows the entire spectrum, while B shows only the aromatic region and panel C only the aliphatic region. All spectra were broad-band proton decoupled.

5. By comparison with the predicted forms of such graphs for various stoichiometries,[24-27] the uroporphyrin I–quinine complex exhibits 1:1 stoichiometry and the urohemin I–quinine complex exhibits 2:1 (urohemin to quinine) stoichiometry.

Apparent equilibrium association constants K_A(app) that define the drug binding process were calculated as described in the Experimental Section. In the method that we employed, experimental slopes of 0.95–1.10 were interpreted in conjunction with Job's method results as indicating $n = 1$, since fractional binding sites are unrealistic for this system. Similarly, experimentally determined slopes of 1.97–2.03 were interpreted as indicating $n = 2$. These idealized values of n were then employed to calculate K_A(app) from the binding data. The values of K_A(app) are reported in Table I. It is interesting to note the similarity between the value of a K_A determined by equilibrium dialysis for a related antimalarial, chloroquine [$K_A = (K_D)^{-1} = 2.9 \times 10^8$ M^{-2}], interacting with aqueous protohemin IX[3] with the values determined here (Table I). Furthermore, we note that the reported protohemin IX–chloroquine stoichiometry (2:1)[3] is identical with our results for the urohemin I–quinine complex.

Others have reported useful insights into the solution geometry of quinine–heme complexes by utilizing NMR spectroscopy,[4,5] and it was of interest to compare results in aqueous solution with the previous reports. Consequently, titrations of urohemin I (Figure 6) and uroporphyrin I (Figure 7) into aqueous solutions of quinine were observed by ^{13}C NMR. The resonance assignments for quinine have been previously reported, although they are reiterated in each figure. There are slight shift differences for the carbon spectrum of quinine in water, compared with chloroform solutions; however, resonance assignments shown in this figure were confirmed by comparison of coupled and decoupled spectra, by concentration behavior, and by comparison with the previous assignments.[5,31-33]

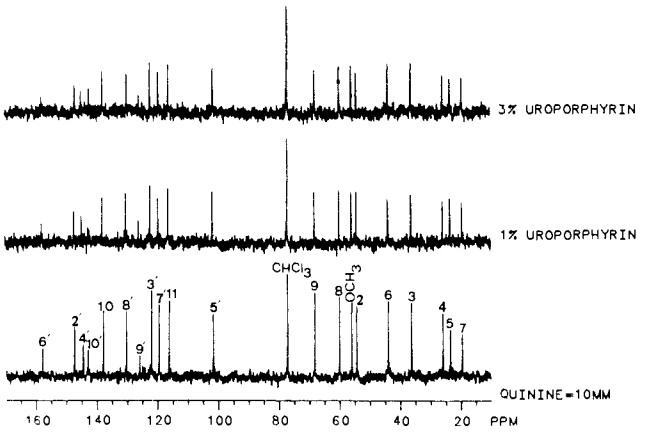

Figure 7. ^{13}C (broad-band, proton-decoupled) NMR of quinine as a function of uroporphyrin addition in mole percent at pH 6.0, and 22 °C, 90 MHz, and 20% D_2O used for lock. All peaks are referenced to external $CHCl_3$ (77.0 ppm).

Table III. ^{13}C Induced Chemical Shifts[a] (ppm) of Quinine upon Complexation with Urohemin and Uroporphyrin

	quinine–uroporphyrin[b]		quinine–urohemin[b]	
peaks	1%	3%	0.5%	1%
6′	0.068	0.053	−0.007	−0.007
2′	−0.178	−0.428	−0.159	−0.262
4′	0.258	0.444	0.142	0.241
10′	−0.254	−0.574	−0.233	−0.412
10	−0.003	−0.016	−0.039	−0.037
8′	−0.190	−0.454	−0.187	−0.337
9′	0.042	−0.012	−0.027	−0.050
3′	0.141	0.195	0.066	0.113
7′	0.007	−0.064	−0.029	−0.037
11	0.019	−0.002	−0.008	−0.009
5′	0.030	−0.034	−0.051	−0.086
9	0.018	−0.038	−0.004	−0.020
8	−0.003	−0.056	−0.022	−0.034
OCH_3	0.005	−0.006	−0.023	−0.007
2	0.002	−0.023	−0.027	−0.025
6	0.003	−0.016	−0.027	−0.012
3	0.015	0.005	−0.001	−0.002
4	−0.008	−0.026	−0.022	−0.028
5	0.002	−0.020	−0.016	−0.019
7	0.018	−0.035	−0.008	−0.027

[a] A 5-Hz line broadening was used so that changes in the induced shifts by ±0.030 ppm are within the experimental error. The negative signs indicate that the induced shifts are upfield. [b] Mole percent urohemin I or uroporphyrin I.

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Figure 6 shows that, in the presence of urohemin I, virtually all of the quinine aromatic carbon resonances that appear downfield from 100 ppm (Figure 6A,B) broaden and lose intensity relative to most of the aliphatic carbon resonances (Figure 6A,C). Slight upfield shifts of less than 0.1 ppm are experienced by most quinine resonances in the presence of 1 mol % urohemin I (Figure 6A; Table III). This mole fraction is the highest ratio practically achievable without causing extensive precipitation in the solution at the concentrations required for NMR studies. The largest induced shift (Table III) is observed at the 2′-, 8′-, 10′-position carbons, implying that they occupy a position at the heme periphery where induced shifts as a result of combined effects of ring current and iron paramagnetism are larger. Notable also is the larger relative intensity loss of the aliphatic 8- and 9-carbon resonances (60–70 ppm, Figure 6A,C), in comparison with other aliphatic carbon resonances (for example carbons 3, 5, 10, 11; see also Table II). This parallels the behavior of the aromatic carbon resonances where the intensity loss is more dramatic. The intensity loss indicates resonance broadening, and the extent of this is shown in Table II. This effect is similar to that observed previously in nonaqueous solution by Behere and Goff[5] and has been interpreted by them as indicating that the hydroxyl-bearing 9-carbon strongly interacts with the paramagnetic heme core. It is a result that is certainly consistent with the 9-hydroxyl acting as an axially coordinating ligand to the urohemin I iron ion. Despite serious efforts, we have not been successful in defining directly whether the 9-hydroxyl group is protonated or deprotonated in this complex.

This view is strengthened by considering the effect upon the quinine spectrum caused by the presence of uroporphyrin I. In this case, the absence of a porphyrin-coordinated (paramagnetic) ferric ion means large line broadening should not be observed, and the possibility for 9-hydroxyl ligation is removed as well. One expects in this case that the 9-carbon resonance should undergo perturbation comparable with the other aliphatic resonances. Titrations of quinine solutions with uroporphyrin I (up to 3 mol % porphyrin) show that the carbon spectrum is perturbed (Figure 7) in the following manner. Large shifts induced by porphyrin ring currents as a result of association are demonstrated by most of the quinine aromatic carbon resonances (Table III). The aromatic carbon resonances of the 5′-, 6′-, 7′-, and 9′-positions exhibit small shifts in comparison with the large shifts of the 4′ (+0.44 ppm), 8′ (−0.45 ppm), 2′ (−0.43 ppm), 10′ (−0.57 ppm) and 3′ (+0.20 ppm) carbon resonances. This pattern of induced shifts is interpretable in terms of a predictable solution geometry for the complex as discussed later. In comparison with the large shifts experienced by these central carbons of the aromatic rings, the aliphatic carbon resonances display smaller shifts, ranging between 0 and −0.05 ppm. In this case the 9-carbon resonances behaves like its neighbor aliphatic carbons (8, 6), exhibiting a shift of only −0.04 ppm. The smooth variation of the carbon shifts indicates that this system is in dynamic, fast chemical exchange between complexed and free entities on the NMR time scale.

The NMR results may be logically interpreted by turning attention first to the ring current induced shifts experienced by quinine upon titrating it with increasing amounts of uroporphyrin I, up to a 3% uroporphyrin solution. Attempts at achieving higher mole percentages of uroporphyrin I resulted in extensive precipitation of the solution so that 3% was the practical limit at the concentrations required for carbon NMR spectroscopy. Previous calculations of heme ring current induced shifts have centered on proton resonances,[4,34] but we may use those results as qualitative predictors for carbon behavior. Those studies indicate that resonances of atoms in molecules that are located over the central part of the heme experience upfield shifts that are expected to vary with precise relative location (cylindrical coordinates). Resonances of atoms in molecules associated with a porphyrin, which lie at the porphyrin periphery, may exhibit little induced

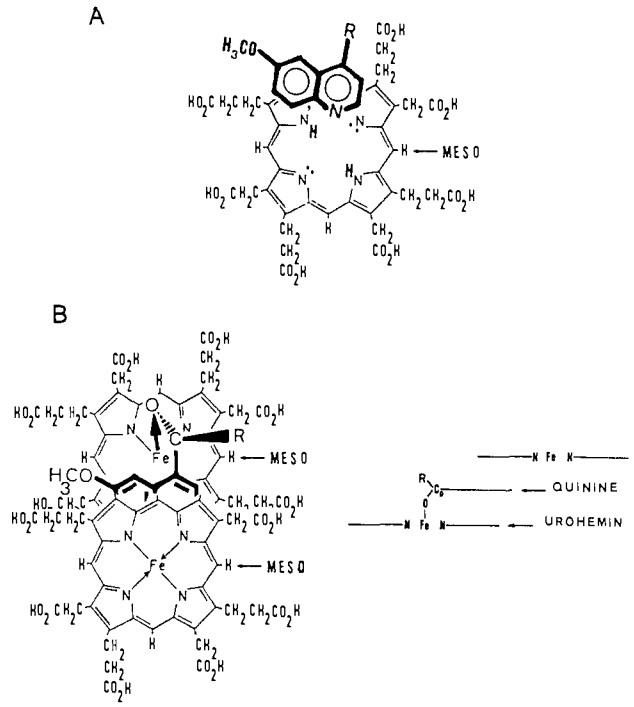

Figure 8. Proposed models derived from our data: A, model for the uroporphyrin–quinine complex; B, views of the model for the urohemin–quinine complex, perpendicular to the heme and parallel to the heme (edge on). Note that one or more propionic and acetic acid groups have been deleted from the models for illustration purposes.

shift or even downfield shifts (positive in our nomenclature). Ring current induced shifts for protons may be sizable, for example, ranging between approximately +0.1 and −8 ppm for groups in well-characterized proximity to the heme in diamagnetic heme proteins.[34] Parallel shifts are expected in the carbon spectrum. The large upfield shifts observed for the quinine aromatic 2′-, 8′-, and 10′-carbons, the sizable downfield shifts for the aromatic 3′- and 4′-carbons, and the small shifts for the aromatic 5′–7′- and 9′-carbons suggest that quinine adopts a specific geometry when it is complexed to uroporphyrin I. This observation supports a model where the quinoline ring is stacked above the periphery of the porphyrin, in a $\pi-\pi$ type interaction with the 2′, N, 10′, 8′ edge facing the center of the porphyrin. With this geometry, upfield shifts of these carbons are expected. Furthermore, the downfield shifts observed for the peripheral quinine 3′- and 4′-carbon resonances indicate that these atoms lie close to the porphyrin periphery. The aliphatic side chain hangs away from the ring current effects of the porphyrin and experiences little induced shifts. The gross features of a model of this complex constructed from our carbon data are presented in Figure 8A. It bears little similarity to that previously proposed on the basis of proton NMR.[4] This observation is important, since it emphasizes the dissimilarity in structure of the urohemin and uroporphyrin complexes (vide infra, Figure 8B) and demonstrates that the 9-OH group is appropriately positioned for coordination only when iron(III) ion is present in the porphyrin core.

The dramatic line broadening indicated by intensity loss (Table II) accompanying the titration with urohemin I is a consequence of the ferric ion's paramagnetism.[35] Since the urohemin I chloride monomer in aqueous solution (pH 6.0, 1×10^{-3} M) has been identified as a high-spin ferric species, a highly symmetric ^6A orbital ground state is anticipated.[17,18] The significant line broadening exhibited by quinine aromatic carbons is understandable on this basis, although further detailed consideration of these effects is not warranted until clearer comparisons with chloroquine, a related drug incapable of axial coordination, are completed.[36]

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Summary

The experimental results presented here may be recapitulated as follows. For uroporphyrin I interacting with quinine, one to one stoichiometry and noncooperative binding is found. The ring current shifts indicate a $\pi-\pi$ type dimer is formed in solution with a structure like that shown in Figure 8A.

For urohemin I interacting with quinine, the minimum stoichiometry is two urohemins bound to one quinine. Consistent with the general carbon NMR shift pattern of the uroporphyrin–quinine complex and the NMR line broadening, a π type sandwich structure is postulated. However, this system exhibits cooperativity when quinine binds to urohemin I, indicating two nonequivalent interacting sites for quinine binding on a given urohemin molecule. One of these sites must be characterized by the aromatic ring–ring interaction that contributes to the sandwich structure postulated here and to the protohemin IX–quinine complex previously identified.[4] The other site is most likely axial iron ion coordination by the quinine via its 9-hydroxyl group to one of the urohemins, as has been shown to exist in other systems.[5] Our data give no evidence for deciding whether the coordination is carried out by an alkoxide ($-O^-$) or a hydroxyl (–OH) moiety. Note that cooperativity is absent in quinine binding to uroporphyrin I where axial coordination is an impossibility. Again, the line broadening of the 9- and 8-position quinine carbons is consistent with this postulate, and our results indicate no other simple possibilities that can account for all of the data presented here. The minimal unit structure for this complex that fits our data is shown in Figure 8B.

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Additional support for this concept comes from completed work[36] with the urohemin I–chloroquine complex. Chloroquine has a structure similar to quinine in that both possess the quinoline type aromatic rings. However, the aliphatic substituents at position 4′ are different, and in the case of chloroquine, the 9-position carbon (C–OH in quinine) is replaced by a nitrogen, rendering axial ligation impossible. Our results show that chloroquine binding to urohemin is noncooperative.[36]

In view of these results the proton NMR characterization of quinine interacting with aqueous protohemin IX and the conclusion reached that iron ion coordination was not indicated require additional scrutiny.[4] It seems obvious to us that the reported strong perturbation of the quinine 9-, 2-, 6-, and 8-position proton resonances upon addition of increasing amounts of protohemin IX[4] can easily be interpreted as indicating 9-OH coordination to heme iron. It is clear from model building studies (and noted by Behere and Goff[5]) that conformations of quinine exist in which the quinuclidine 2-, 6-, and 8-position proton resonances would be perturbed owing to 9-OH coordination to the heme iron ion.

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Registry No. Quinine, 130-95-0; urohemin I chloride, 92284-96-3; uroporphyrin I, 607-14-7.

Interactions between DNA and Mono-, Bis-, Tris-, Tetrakis-, and Hexakis(aminoacridines). A Linear and Circular Dichroism, Electric Orientation Relaxation, Viscometry, and Equilibrium Study

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Abstract: The interaction between DNA and a series of mono-, bis-, tris-, tetrakis-, and hexakis-intercalating 9-aminoacridines has been studied with flow linear dichroism (LD), circular dichroism (CD), electric orientation relaxation (EOR) techniques, and with viscometry and equilibrium analyses. The orientation of the 9-aminoacridine ligand relative to the average orientation of the DNA bases, measured by LD, shows that with both 9-aminoacridine and the bis(acridines) the in-plane short axes of the acridine ligands are oriented perfectly parallel to the planes of the DNA bases, as expected for classical intercalation, whereas the long axes are found to be significantly tilted. This is supported by the DNA lengthening measured by EOR, which for 9-aminoacridine is 1.5 base-pair units, compared with 1.0 for ethidium bromide. Also in case of the tris(acridines) LD, CD, viscometry, and equilibrium data indicate that all acridine ligands are intercalated. The binding analysis shows an increasing degree of cooperativity in the sequence 9-aminoacridine < bis(acridines) < tris(acridines), and the corresponding binding densities, 4, 8, and 11–14, respectively, are in good agreement with those expected from the nearest-neighbor exclusion principle. The LD and CD measurements show that the tetrakis- and hexakis(acridines), despite long and flexible links, bind to DNA with only three of the acridine ligands intercalated.

The DNA intercalation of polycyclic aromatic compounds has been the subject of much interest due to their potential as cytostatic agents and as probes in molecular biology.[1-5]

Binding to DNA by intercalation was originally explained by Lerman[6] as a process whereby the intercalator was sandwiched

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