formations observed for the propionic acid group in the above crystal structures and taking into account the modeling of the cyclooxygenase/2-arylpropionic acids interaction proposed by Sankawa et al.,[22] who concluded that the carboxylic acid group must interact with the C(15) oxygenation site on the cyclooxygenase, our results suggest that in a restricted hydrophobic pocket, such as the enzyme active site, steric considerations orient the carboxylic acid groups of the *R* and *S* isomers in different directions, so that only the *S* can interact with the C(15) oxygenation site on the enzyme.

Conclusions

(*R*)-(−)- and (*S*)-(+)-fenoprofens adopt different packing arrangements inside the β-CD dimer, the *R* being head-to-head and the *S* head-to-tail. The differences are due to the effects of formation of the complex dimer and crystal-packing effects such as the means of satisfying the hydration requirements of the carboxylic acid group. Steric restrictions on the meta-substituted propionic acid group, such as the inability of the methyl group to approach the plane of ring 1 and the restricted dihedral angles of the planes of ring 1 and ring 2, result [in the case of the (*S*)-FP] in hydrogen bonds from a guest molecule to secondary hydroxyl groups on the β-CD, which have not been previously observed in crystal structures of β-CD dimer complexes. In the case of (*R*)-FP, the less favorable hydrogen bonding of the carboxylic acid groups to water as compared with CD primary or secondary groups for the (*S*)-FP is to some extent compensated for by more favorable parallel, phenyl/phenyl packing of rings 2 in the hydrophobic CD dimer interface versus methyl/phenyl packing for the (*S*)-FP.

The difference in binding of the *R* and *S* isomers to the β-CD makes it possible that β-CD in the crystal form is stereoselective for one or the other isomer of fenoprofen but provides no evidence that the stereoselectivity also exists in solution.

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Registry No. (*R*)-(−)-FP-CD complex, 114185-91-0; (*S*)-(+)-FP-CD complex, 114185-92-1.

Supplementary Material Available: Tables of anisotropic thermal parameters, hydrogen positional parameters, and individual bond lengths and angles for CD molecules (22 pages); tables of observed and calculated structure factors (87 pages). Ordering information is given on any current masthead page.

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UV-Visible and Carbon NMR Studies of Chloroquine Binding to Urohemin I Chloride and Uroporphyrin I in Aqueous Solutions

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Abstract: Interactions of the antimalaria drug chloroquine with urohemin I and uroporphyrin I have been studied in aqueous solutions at pH 6.0 and 22 ± 1 °C with UV-visible and natural abundance carbon NMR spectroscopies. Both tetrapyrroles are water soluble and were chosen because their aggregation properties are understood and can be regulated by concentration and ionic strength. Chloroquine binding to urohemin I monomer has a stoichiometry of two urohemin molecules to one chloroquine molecule with an apparent association equilibrium constant of $(7.8 \pm 0.4) \times 10^8$ M^{-2} at pH 6.0 and a urohemin concentration of 10^{-6} M. This stoichiometry is identical with that recently reported for complexes of urohemin I with another antimalarial, quinine. In that case, the binding was found to be cooperative, whereas in this case the drug binding is noncooperative. Uroporphyrin I binds to chloroquine with 1:1 stoichiometry and an apparent equilibrium constant of $(2.8 \pm 0.2) \times 10^6$ M^{-1} at a uroporphyrin concentration 10^{-6} M. Carbon NMR spectroscopy and optical methods best describe these complexes as cofacial $\pi-\pi$ dimers with structures different from the quinine complexes of the same tetrapyrroles.

Chloroquine and quinine (Figure 1) are perhaps the most common clinical antimalarials in use today. Their demonstrated association with the malaria pigment found in parasitized erythrocytes, which is composed of heme that is presumably released from protease degraded hemoglobin, lead to the idea that the drug's physiological efficacy involves a ferriprotoporphyrin IX-chloroquine complex.[1-5] Several studies of the ferriprotoporphyrin IX-chloroquine interaction resulted from such observations, and the dimensions of the chloroquine membrane receptor were shown to be similar to those of the heme moiety.[2,6-12]

The apparent relevance of heme-malaria drug interactions recently lead us to a study of quinine interacting with two species

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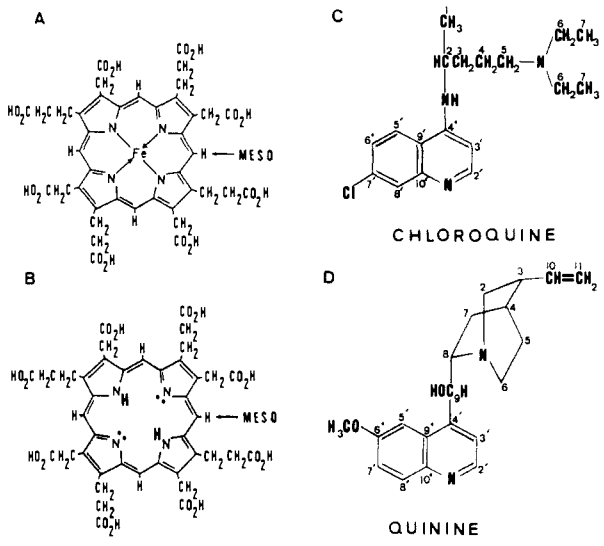

Figure 1. Chemical structure of A, urohemin I; B, uroporphyrin I; C, chloroquine, D, quinine.

related to protoheme IX.[13] These were urohemin I (uroporphyrin(I)iron(III) chloride; Figure 1) and uroporphyrin I, the corresponding free base macrocycle. These hemes were chosen because they exhibit a variable and controllable degree of self-aggregation,[13-20] which protoheme does not.[21-24] Since their solution chemistry is well defined, equilibrium binding studies could be successfully carried out.[13]

For these reasons, we have chosen to use these same tetrapyrroles in a comparison study with chloroquine, a drug whose structure is similar to quinine in that both contain the quinoline ring (Figure 1). The work presented here was completed under conditions identical with the quinine study.[13] It identifies two interesting differences in the association of chloroquine with urohemin I compared to the quinine results. First, the binding is not cooperative as it was for quinine, even though the stoichiometry of the resulting complex is identical. Second, the apparent solution structure of the resulting complexes is different for each drug.

For the same reasons that we previously enumerated,[13] we have chosen to study this equilibrium at pH 6.0. This choice was made for its physiological relevance and because previous studies have shown that urohemin I and uroporphyrin I both precipitate as the pH is lowered.[17,19,20] Furthermore, in the case of urohemin I, self-aggregation becomes increasingly severe as the pH is raised above 6.5.[19,20] This precludes variable-pH studies of binding for these hemes and restricts us to a narrow region of pH at 6.0 in order for our hemes to be soluble monomers.

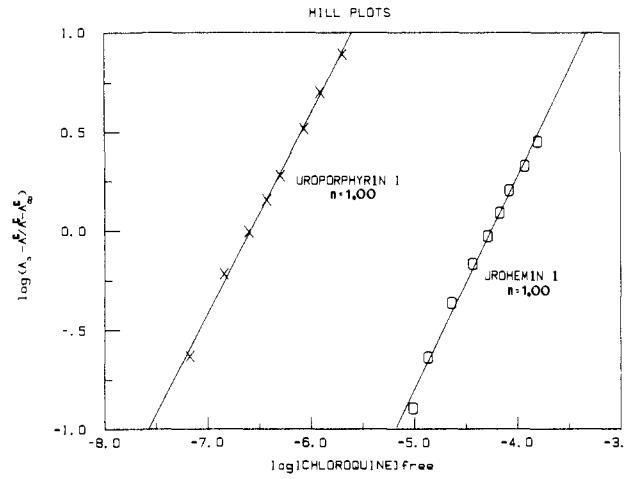

Figure 2. Hill plots of the uroporphyrin I–chloroquine binding (X) at 5×10^{-7} M uroporphyrin concentration and urohemin–chloroquine binding (O) at 5×10^{-6} M urohemin concentration. The pH was monitored at 6.0 for all experiments, and the temperature was constant at 22 °C; n corresponds to the individual slope or Hill parameter.

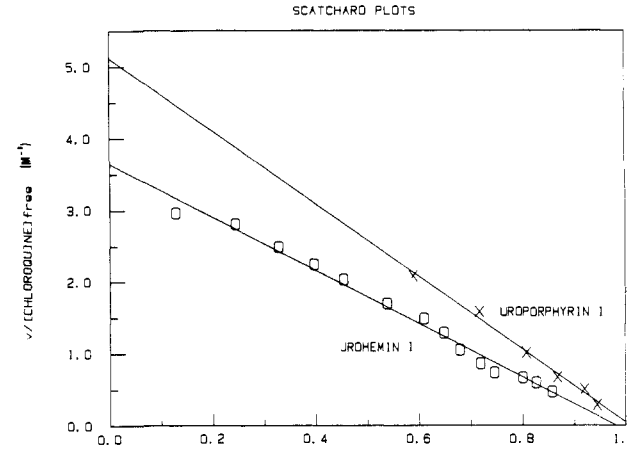

Figure 3. Scatchard plots of the uroporphyrin I–chloroquine (X) and urohemin I–chloroquine (O) binding at 5×10^{-6} M tetrapyrrole concentration. For the uroporphyrin I–chloroquine plot, the y axis should be multiplied by 10^{-6}; for the urohemin I–chloroquine plot, it should be multiplied by 10^{-4}.

Experimental Section

The details of the experiments designed to elucidate apparent equilibrium constants and the stoichiometry of chloroquine binding to urohemin I and uroporphyrin I are precisely those that have been previously described,[13] using published methods.[25-29] Urohemin I and uroporphyrin I were purchased from Porphyrin Products (Logan, UT) and further purified as before.[13] Chloroquine diphosphate (Sigma) was used as obtained. Freshly prepared solutions were used in all experiments. Previously the necessity of working at minimal ion concentrations was emphasized due to the ability of salts to induce heme aggregation. All methods previously described in that respect and those necessary for valid use of Job's method[27-29] were strictly followed in this study.[13]

Natural abundance carbon NMR spectra were acquired at 8.5 T with 12-mm sample tubes. Broad-band, noise-modulated proton decoupling was employed, and all spectra were acquired at 22 ± 1 °C. Chloroquine possesses several nonprotonated carbons (4', 7', 9', 10') whose spin–lattice relaxation times are quite long (4–5 s, data not shown); however, we

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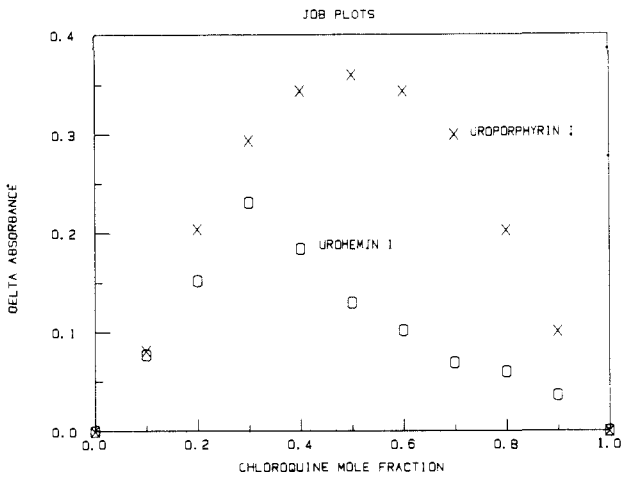

Figure 4. Job plots of (X) uroporphyrin I–chloroquine and (O) urohemin I–chloroquine at 5×10^{-7} M macrocycle concentrations. For the uroporphyrin experiment, the absorbance was monitored at 405 nm, while for the urohemin experiment it was monitored at 393 nm. Both experiments were performed at pH 6.0 and 22 °C. Identical plots were obtained for the other two tetrapyrrole concentrations.

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 (5×10^{-4} M), uroporphyrin showed slight evidence of dimerization, whereas urohemin I did not.

Results and Discussion

(A) Optical Experiments. In aqueous solution, the interaction of chloroquine with urohemin I and uroporphyrin I perturbs the UV-visible spectra of the porphyrins in the same way quinine does.[13] Titrations of urohemin I and uroporphyrin I with chloroquine produce sequential spectra with similarly well defined isosbestic points in the Soret region (400–415 nm, data not shown). The spectroscopic changes are a red shift and a decrease in intensity of the heme or porphyrin Soret band. The position of the Soret band maximum is indicative of heme–heme aggregation,[14-20] and our heme and porphyrin solutions gave spectra indicating the presence only of monomer units. The red shift of the Soret, which is induced by chloroquine binding, is characteristic of molecular complex formation between aromatic molecules and various metallouroporphyrins.[14,16,17] The conclusion indicated by these results is that molecular complexes of chloroquine and each tetrapyrrole are being formed in these solutions.

The raw binding data were analyzed by the Hill,[25,26] Scatchard,[26] and Job[27-29] graphical methods in order to determine the degree of cooperativity for binding, the apparent equilibrium association constants, and the true stoichiometry of the resulting soluble complexes. We have chosen to present representative graphical results since differences with the quinine data[13] are more easily compared.

The degree of cooperativity was determined by the Hill and Scatchard plots. Representative data are shown in Figures 2 (Hill plot) and 3 (Scatchard plot). The Hill parameter (n, Figure 2) of 1.0 and the straight lines in the Scatchard plot indicate that chloroquine binding to both urohemin I and uroporphyrin I is noncooperative. Previously, quinine binding to urohemin I was found to be cooperative.[13]

Elucidation of the true stoichiometry of these complexes was performed by using Job's method.[27] The results for one tetrapyrrole concentration are presented in Figure 4. Similar plots were found for the remaining concentrations (data not shown). Comparing the form of these graphs with those predicted for various stoichiometries[27] leads to the conclusion that uroporphyrin I–chloroquine exhibits 1:1 stoichiometry, whereas urohemin I–chloroquine exhibits 2:1 (urohemin–chloroquine) stoichiometry. These stoichiometries are exactly those found for quinine binding to the respective tetrapyrroles.[13]

Apparent equilibrium association constants K_A(app) that define the drug binding process at pH 6.0 were calculated as described

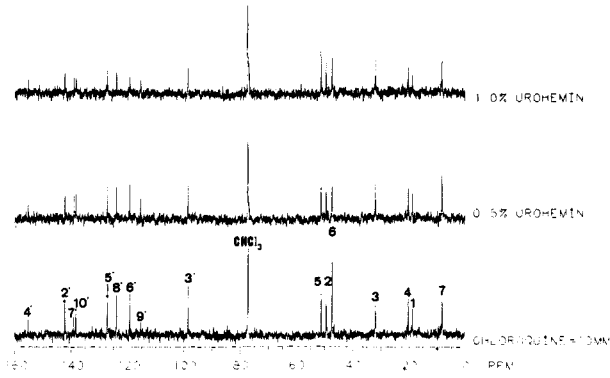

Figure 5. ^{13}C NMR of broad-band proton decoupled chloroquine as a function of urohemin additions (mole percent) at pH 6.0 and 22 °C, taken at 90 MHz, 20% D_2O was used for lock, and all peaks are referenced to external $CHCl_3$ (77.0 ppm).

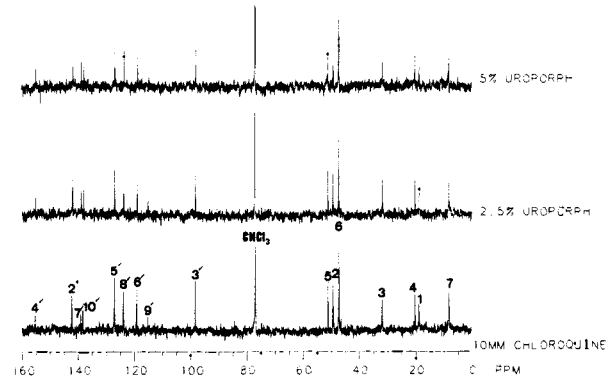

Figure 6. ^{13}C NMR of broad-band proton decoupled chloroquine as a function of uroporphyrin additions (mole percent) at pH 6.0 and 22 °C, taken at 90 MHz, with 20% D_2O for lock purposes. All resonances are referenced to external $CHCl_3$ (77.0 ppm).

Table I. Apparent Association Equilibrium Constants Obtained from Optical Titrations of Chloroquine with Urohemin I and Uroporphyrin I at pH 6.0

hemin concn, M	K_A, M^{-2} (app)	Hill parameter	repetition
	Urohemin I–Chloroquine		
5×10^{-5}	$(2.9 \pm 0.2) \times 10^8$	1.09 ± 0.02	4
5×10^{-6}	$(7.8 \pm 0.4) \times 10^8$	1.00 ± 0.01	10
porphyrin concn, M	**K_A, M^{-1} (app)**	**Hill parameter**	**repetition**
	Uroporphyrin I–Chloroquine		
5×10^{-5}	$(6.5 \pm 0.7) \times 10^6$	1.10 ± 0.05	3
5×10^{-6}	$(2.8 \pm 0.2) \times 10^6$	1.10 ± 0.02	3
5×10^{-7}	$(3.8 \pm 0.3) \times 10^6$	1.00 ± 0.01	3

previously.[13] The values of K_A(app) reported in Table I are strictly valid only under our conditions at pH 6.0. We call attention to the similarity of the equilibrium constants (Table I) and stoichiometry for urohemin I–chloroquine with that determined for the binding of chloroquine to ferriprotoporphyrin IX.[2]

(B) NMR Experiments. Carbon-13 NMR has proven useful in previous studies of malaria drug–heme interactions,[13,30] and it was of interest to compare results of chloroquine binding to urohemin I and uroporphyrin I in aqueous solution to the results obtained for quinine in both aqueous[13] and nonaqueous[30] media. The resonance assignments for chloroquine have been published but are reiterated on the spectra in Figures 5 and 6 (also refer to Figure 1).[31,32]

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Table II. Peak Heights[a] for Individual Carbon Resonances of Chloroquine as a Function of Urohemin Concentration, Measured as a Percent of the External Chloroform Resonance

carbon position	no urohemin	0.5% urohemin	1.0% urohemin
4′	18	12	13
2′	44	20	16
7′	20	18	13
10′	26	20	13
5′	59	29	23
8′	46	26	23
6′	46	31	16
9′	13	13	13
3′	56	26	27
5	56	43	44
2	54	33	35
6	110	82	77
3	36	33	35
4	41	29	27
1	31	23	16
7	41	35	35

[a] Relative intensities measured at 22 °C; percentages indicate mole percent; all heights obtained with broad-band proton decoupling.

Figures 5 and 6 reveal the results of titrating urohemin I (Figure 5) and uroporphyrin I (Figure 6) into aqueous solutions of chloroquine (pH 6.0). Figure 5 reveals that, in the presence of urohemin I, the aromatic region (100–160 ppm) of the chloroquine ^{13}C spectrum displays significant loss of relative intensity compared to the aliphatic region (0–60 ppm) and the external $CHCl_3$ reference. As previously demonstrated for experiments with quinine,[13,30] this loss of intensity is consistent with line broadening induced by dynamic association of the drug with urohemin I. The quantitative intensity reduction for each carbon position on the drug structure is shown in Table II. The aromatic resonances also exhibit slight upfield shifts (less than 0.1 ppm), whereas the aliphatic carbon resonances show no detectable shift in the presence of 1.0 mol % urohemin I.

We observe significant intensity reductions only for protonated carbons (Table II) for the reason that our pulse repetition rate was fast enough to saturate the nonprotonated carbon resonances (see the Experimental Section). The percent intensity reductions observed here (0–40%) are similar to those found in the quinine study,[13] with two notable exceptions. In the quinine case, the 8- and 9-position carbons (Figure 1) exhibited unusually large relative intensity decreases (56% and 64%, respectively), which were notable in their magnitude and structural localization (Figure 1). This information supported the concept advanced by Behere and Goff[30] that the 9-OH group (see quinine, Figure 1) could act as an iron ion axial ligand.[13] In the case of chloroquine, no such uniquely large intensity decreases occur. This correlates with the structurally based incapacity of chloroquine for iron coordination in a manner similar to that proposed for quinine. Figure 1 shows that no group corresponding to the quinine 9-OH exists in chloroquine. Model building studies indicate that neither nitrogen in chloroquine (Figure 1) can be suitably positioned for heme iron ion coordination.

Figure 6 shows the ^{13}C NMR results of similar experiments in which chloroquine is titrated with uroporphyrin I. In this case, solutions containing up to 5 mol % uroporphyrin I were achievable. Although line broadening indicated by intensity decreases are observed for all resonances, it is the shifts in resonance positions (Table III) that are most informative. The data presented in Table III reveal that significant shifts are induced by uroporphyrin I only in the case of carbons belonging to the quinoline aromatic rings of chloroquine (Figure 1). Virtually identical results were obtained for quinine and the present interpretation of this effect is precisely the same.[13] Table III reveals that the largest induced shifts occur for the chloroquine aromatic (quinoline) carbons, whereas small and even negligible induced shifts occur for carbons of the aliphatic chain.

This information provides clues to the geometry of the uroporphyrin I–chloroquine complex. Calculations of heme ring current shifts[33] were cited in our analysis of the quinine–uroporphyrin I complex.[13] Similar arguments may be applied here, and the most important feature is that upfield shifts are indicative of atoms in molecules located over the center of the porphyrin. The shift pattern reported in Table III supports a model where the chloroquine quinoline ring is stacked directly over the porphyrin in a manner generally indicated in Figure 7B.

Table III. Induced ^{13}C Chemical Shifts (in ppm) of Chloroquine upon Complexation with Uroporphyrin I[a]

	uroporphyrin content[b]	
chloroquine carbons	2.5%	5%
4′	−0.279	−0.550
2′	−0.433	−0.654
7′	−0.220	−0.410
10′	−0.433	−0.699
5′	−0.213	−0.385
8′	−0.272	−0.481
6′	−0.374	−0.586
9′	−0.342	−0.571
3′	−0.228	−0.389
5	0.016	0.028
2	−0.074	−0.154
6	−0.020	−0.031
3	−0.110	−0.169
4	−0.020	−0.011
1	−0.116	−0.159
7	−0.001	−0.006

[a] The experiment was performed at 22 °C, pH 6.0, and a chloroquine concentration of 10 mM in 20% ^2H_2O. A 5-Hz line broadening was used so that changes by ±0.030 ppm are within experimental error. Negative signs indicate upfield shifts. [b] Mole percent.

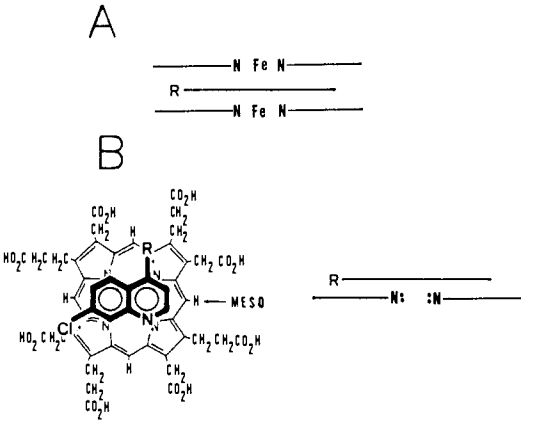

Figure 7. Proposed models derived from our data. (A) model for the urohemin I–chloroquine complex and (B) top and side view of the uroporphyrin I–chloroquine model.

The quinine–uroporphyrin I complex and the chloroquine complex share the common feature of being π–π type complexes. However, the induced-shift pattern of the quinoline carbons is different for each drug, leading to different complex structures. Quinine interacts at the porphyrin periphery[13] because its quinoline carbons exhibit both upfield and downfield induced shifts. Chloroquine lies at the porphyrin center (Figure 7) because all of its quinoline carbons exhibit upfield induced shifts. The most likely reason for this difference is steric hindrance. In quinine (Figure 1), the quinuclidine part of the molecule is much more rigid than the aliphatic part (carbons 1–7) of chloroquine. Model building shows that the aliphatic chain is flexible and may adopt orientations that do not sterically interfere with chloroquine's positioning over the heme. In contrast, for quinine, peripheral positioning seems to minimize steric interferences from the quinuclidine moiety.

For the urohemin I–chloroquine complex, the description of a general solution structure relies on the resonance intensity diminutions reported in Table II. Clearly, the most sizable intensity decreases are for the carbons of the quinoline ring. This would

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be a consequence of these carbons being positioned near to the paramagnetic heme iron ion, as previously discussed.[13] It implies, again, that the primary interaction between chloroquine and urohemin I is also a $\pi-\pi$ type complex between aromatic moieties. Furthermore, considering the 2:1 stoichiometry (heme-drug) revealed by Job's method, a sandwich-type complex (Figure 7A) is consistent with all of our data. This structure resembles the structure for the quinine-urohemin I complex, which has identical stoichiometry.[13]

Urohemin I association with quinine and chloroquine results in solution complexes of identical stoichiometry. The quinine association is cooperative (Hill parameter = 2),[13] while the chloroquine association is noncooperative (Hill parameter = 1). For quinine, the source of cooperativity, consistent with the other spectroscopic data, was postulated to be the 9-OH group coordination to the heme iron, in addition to the $\pi-\pi$ association. This accounted for two inequivalent binding sites on urohemin I. The data presented here add further support to this view, which was first identified by Behere and Goff,[30] by revealing that chloroquine, a structurally related drug that has no donor group capable of coordinating to heme iron simultaneous with $\pi-\pi$ association, does not exhibit cooperativity.

Finally, a comparison of the overall apparent equilibrium constants determined under identical conditions for chloroquine (Table I) and quinine[13] is warranted. If hemes are truly the drug receptors, the data of Table I suggest that differences in the efficacy of the two drugs is not attributable to different binding constants. For a common heme (urohemin I), K_A(app) has the same order of magnitude for both drugs. Such is not the case for binding to uroporphyrin I where chloroquine exhibits an affinity approximately 2 orders of magnitude higher than quinine does. We attribute this to the more complete $\pi-\pi$ type interaction that is possible for chloroquine in our model of the complex (Figure 7B) rather than the peripheral complex model, with less $\pi-\pi$ overlap, found for quinine.

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Registry No. Chloroquine, 54-05-7; urohemin I chloride, 92284-96-3; uroporphyrin I, 607-14-7; urohemin I-chloroquine complex, 114422-76-3; uroporphyrin I-chloroquine complex, 114422-77-4.

Monolayer Properties of Eight Diastereomeric Two-Chain Surfactants at the Air/Water Interface: A Resultant of Intramolecular and Intermolecular Forces

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Abstract: Force-area isotherms, equilibrium spreading pressures, free energies of activation to viscous flow, and surface viscosities are reported for four pairs of diastereomeric surfactants made by joining two pentadecanoic acid units by a carbonyl group at the 3,3′-, 6,6′-, 9,9′-, and 12,12′-positions. The eight compounds are four pairs of diastereomers (meso, *dl*) that differ only by the position of the carbonyl cross-link. Accordingly, any differences in their surface properties must have a stereochemical origin. In all cases the meso monolayers are compressed more easily than their *dl* diastereomers. This is attributed to the more facile aggregation (easier packing) of the former as a result of their lowest energy conformations, which give good lateral alignment of the hydrocarbon chains about the meso plane of symmetry when both carboxylate groups are in the water surface. In contrast, the *dl* compounds are forced into a higher energy conformation if the chains are brought into alignment while the carboxylate groups are in the surface, since the lowest energy *dl* conformation is one in which the head groups are separated by the largest possible longitudinal distance in the surface of the water. This results in resistance of the *dl* films to compression and reduces the attraction between their chains as compared to meso films. This is to our knowledge the first systematic study of the monolayer properties of a diastereomeric series.

A decade ago we initiated an investigation of the effects of introducing chiral centers into surfactants on their intermolecular forces as reflected by their properties as monolayers at the air/water interface. There were virtually no published accounts on the topic available at that time. All of the work we have done in the interim shows clearly that, with the notable exception of the phosphatidylcholines,[1] essentially all of the surface properties of all of the surfactants we have studied to date are strongly stereoselective in that they show chiral discrimination between the properties of pure enantiomers and their racemic mixtures. In the course of another project, reported elsewhere,[2] four pairs of diastereomeric diacids consisting of two *n*-pentadecanoic acids bonded together at the 3-, 6-, 9-, and 12-carbons by a carbonyl group were synthesized. When they were epimerized in aqueous base at 60 °C, *at concentrations above the critical micelle concentration (CMC)*, all of them showed a preference for the meso form in accordance with predictions from molecular mechanics. However, epimerization in homogeneous media (e.g., benzene solution, TsOH catalyst) or in water ($^-$OH catalyst) below the CMC gave 50:50 mixtures of meso and *dl* diastereomers. It was thus concluded that hydrophobic forces perturb the equilibrium

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