Synthetic Models of Deoxyribonucleic Acid Complexes with Antimalarial Compounds. Comparative Ultraviolet and Proton Magnetic Resonance Study of Quinoline–Base, Quinoline–Quinoline, and Base–Base Stacking Interactions†

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ABSTRACT: Stacking schemes have been proposed for the mode of binding of the antimalarial drug chloroquine with nucleic acids. In relation to this problem, we have devised synthetic models to evaluate, in the absence of the complicating factors present in the DNA–chloroquine complex, the relative stacking tendency of various aromatic rings involved in the binding process, i.e., the purine nucleotide bases, adenine and guanine, and the aromatic part of chloroquine and 7-substituted analogues. In the model compounds, the rings under study are linked by a flexible trimethylene chain. These systems exhibit in solution a folded ⇌ unfolded equilibrium, which reflects the strength of the interaction. This equilibrium was studied by hypochromism measurement in the UV and by Fourier transform magnetic resonance spectroscopy. We thus show that in our models the ring–ring stacking interaction between the aromatic ring of chloroquine (4-amino-7-chloroquinoline) and the monomeric nucleotide bases, adenine and guanine, is of the same order of magnitude as that observed between two identical aminoquinoline rings and that between two adenine molecules. In the analogous series of 7-substituted 4-aminoquinolines, our results point to a higher tendency for the 7-Cl compound than for the 7-Br and 7-H analogues to stack with purines. These results are discussed in view of the different binding schemes proposed for the chloroquine–DNA complex.

A number of studies have shown that many planar, positively charged heteroaromatic molecules reversibly bind with nucleic acids, thereby influencing their physicochemical and biological properties (Hahn, 1971; Le Pecq & Paoletti, 1967; Le Pecq et al., 1975; Müller & Crothers, 1975; Waring, 1970, 1975). The binding has been frequently interpreted in terms of the "intercalation model" first proposed by Lerman (1961), in which the interacting molecule is intercalated between two adjacent base pairs (Patel, 1974; Tsai et al., 1975; Krugh & Reinhardt, 1975). This model has been proposed for the antimalarial drug chloroquine (**1**) (Figure 1) (O'Brien & Hahn, 1965) which has been shown, by a variety of biophysical methods (Parker & Irvin, 1952; Kurmick & Radcliffe, 1962; Stollar & Levine, 1963; Cohen & Yielding, 1965; O'Brien et al., 1966; Blodgett & Yielding, 1968; Morris et al., 1970), to bind with nucleic acids and polynucleotides. Yielding et al. (1971) proposed a possible alternate (or additional) model in which chloroquine stacks into the major groove of DNA by electrostatic interaction between the side-chain amino and backbone phosphate groups and by ring stacking both between adjacent chloroquine molecules and between chloroquine and the bases as they protrude into the major groove. These two model hypotheses were tested by Waring (1970, 1975) using drug-induced local unwinding of closed circular DNA. This author concluded that the behavior of chloroquine is qualitatively consistent with intercalation, but the apparent unwinding angle measured led the author to propose that a certain proportion of bound drug molecules were in an externally bound, nonintercalated state.

Whatever the model proposed, the binding implies a situation in which the aromatic part of the drug and the nucleotide bases must compete to participate in ring stacking: the drug may either self-associate and/or associate with the bases and intercalate (partially or totally). The comparative strengths of these ring–ring interactions (drug–drug, base–base, and drug–base), independently of the ionic phosphate nitrogen contributions, appear to be difficult to attain by the usual intermolecular approach (effect of added chloroquine to DNA, polynucleotides, mononucleotides, or monomeric base residues).

In order to investigate this specific problem of ring–ring stacking interaction, we have prepared compounds in which two interacting rings are linked by a flexible hydrocarbon chain. In preceding papers (Bolte et al., 1976, 1977a,b), we have thus described the stacking between the chlorinated ring of chloroquine and the nucleotide bases, taken successively, by preparing and examining models of type **2a**, **3a**, and **6a**, in which the quinoline and the base are bridged by a trimethylene chain which allows, but does not impose, the intramolecular ring–ring stacking. The degree of folding of such molecules, is an indication of the relative stacking tendency of the rings, at least in the geometrical arrangement allowed by the bridge. It appeared that quinoline exhibits in water greater attractive interactions for purines than for pyrimidines, as reflected by the degree of folding of the corresponding models Ade-C_3-Q(Cl)[1] (**2a**) and Gua-C_3-Q(Cl) (**3a**) folded to ca. 100% in water, at 25 °C, as compared to ca. 40% for Thy-C_3-Q(Cl).

We report here the study of compounds **2–8** prepared with the aim of evaluating the relative stacking interactions between

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[1] Abbreviations used: Ade, aden-9-yl; Gua, guan-9-yl; Q(X) for *N*-4-(aden-9- or guan-9-yl)amino-7-X-quinoline, X being Cl, Br, or H and C_3 representing *n*-propyl, according to the IUPAC–IUB Commission (1970) symbols and to the symbols proposed by Cohn et al. (1974).

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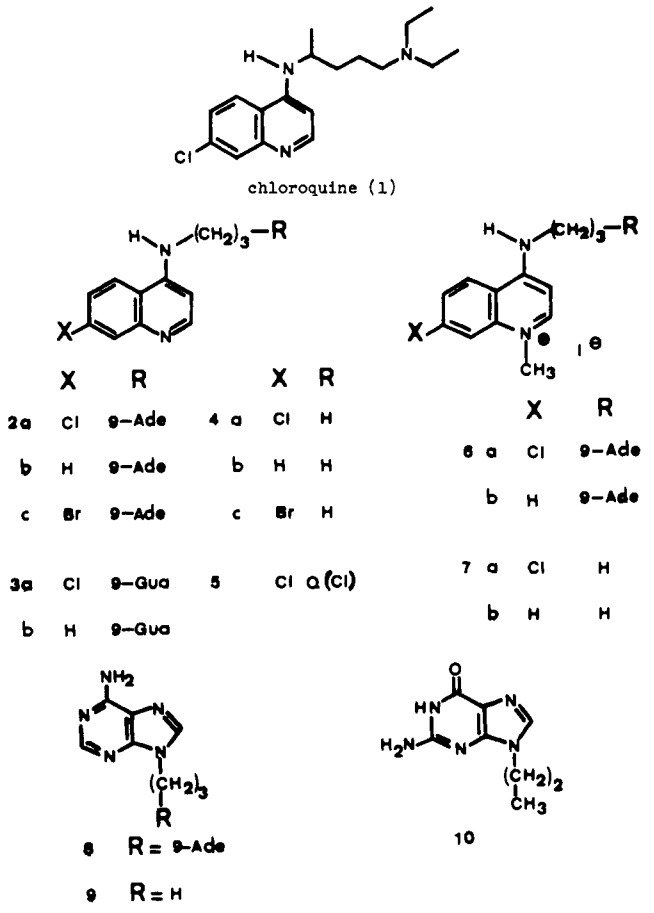

FIGURE 1: The structure of the chloroquine molecule and of the synthetic model compounds.

rings involved in the complexation process: quinoline–base, quinoline–quinoline, and base–base. In the quinoline–base systems **2**, **3**, and **6**, the 7-Cl substituent of quinoline (which is determinant for antimalarial activity) (O'Brien & Hahn, 1965) is successively replaced by bromine and hydrogen. In the present systems the differently substituted quinolines are linked to adenine and guanine molecules, as these purines are the preferred binding sites in nucleic acids (Cohen & Yielding, 1965) and exhibit, as already mentioned, the highest binding affinity for 7-chloroquinoline in the models. The bisquinoline compound **5** was prepared to examine the self-stacking properties of the drug and the bisadenine model **8** [already reported by Leonard & Ito (1973)] was taken as an example of base–base interactions.

Materials and Methods

Materials. The following compounds had been prepared and described before: Ade-C_3-Ade (**8**) and Gua-C_3 (**10**) (Brown et al., 1968), Ade-C_3-Q(Cl) (**2a**), Gua-C_3-Q(Cl) (**3a**), and Ade-C_3-Q(Cl)CH_3^+ (**6a**) (Bolte et al., 1977a,b). We report separately the synthesis of the other new compounds. Ade-C_3-Q(H) (**2b**): UV (H_2O, pH 6.9) λ_{max} 233 nm (ϵ 17800), 331 (12600), 343 (12700). Ade-C_3-Q(Br) (**2c**): λ_{max} 238 (ϵ 22400), 259 (23100), 334 (12700), 346 (13300). C_3-Q(H) (**4b**): λ_{max} 233 (ϵ 17500), 328 (17500), 342 (18000). C_3-Q(Br) (**4c**): λ_{max} 237 (ϵ 19500), 258 (19000), 331 (18000), 344 (19500). Gua-C_3-Q(H) (**3b**): λ_{max} 245 (ϵ 20900), 330 (12500), 343 (12700). Ade-C_3-Q(H)$^+CH_3$ (**6b**): λ_{max} 252 (ϵ 20300), 337 (15100), 350 (15600). C_3-Q(H)-CH_3^+ (**7b**): λ_{max} 228 (ϵ 28200), 335 (18900), 348 (19600).

All compounds were tested for purity by a variety of methods including microanalyses, ^{1}H NMR spectroscopy, and thin-layer chromatography in different solvent systems.

UV Spectroscopy. The quantitative ultraviolet spectrometric measurements were made with a Cary Model 15 spectrophotometer as described previously (Bolte et al., 1976, 1977a,b), using dilute aqueous solutions (approximately 5×10^{-5} M). Spectral-grade ethanol (Prolabo) and deionized water distilled under nitrogen were used. The pH was adjusted with hydrochloric acid, sodium hydroxide, potassium dihydrogen phosphate, or disodium hydrogen phosphate solutions. The spectroscopic properties were not affected by the nature of the buffer used.

The hypochromic effect was calculated according to the equation

$$H = 1 - \frac{f_{B\text{-}C_3\text{-}Q}}{f_{B\text{-}C_3} + f_{Q\text{-}C_3}}$$

where f is the oscillator strength of the transition, i.e., a measure of the intensity of the absorption: $f = (4.32 \times 10^{-9})\epsilon(\lambda)/\lambda^2 d\lambda$, where ϵ is the molecular extinction coefficient. The f values were obtained from optical densities measured every 2.5 nm by application of the Simpson's rule, as described in precedent publications (Bolte et al., 1976, 1977a,b).

The UV spectra were recorded at various temperatures by using thermostated cells, the temperature values being known to ±0.5 °C.

^{1}H NMR Studies. ^{1}H NMR spectra were recorded at 90 MHz (Bruker WH 90) or 270 MHz (Bruker WH 270) operating in the Fourier transform mode and locked on the deuterium of the solvent (D_2O). Probe temperature was regulated to ±0.1 °C and monitored by using ethylene glycol. Solutions were made in a deuterioacetate buffer (pD ~5). The apparent pD values of the solutions were determined with a Tacussel TS60/N pH meter according to Glasoe & Long (1960).

The self-association constant K was determined by using the equation (eq 1) of Dimicoli & Helene (1973) according

$$(\Delta\delta/B_0)^{1/2} = (K/2\Delta\delta_{B_2})^{1/2}(2\Delta\delta_{B_2} - \Delta\delta) \qquad (1)$$

to Barbet et al. (1976). $\Delta\delta$ = difference between an extrapolated chemical shift at infinite dilution and the actual chemical shift at a given concentration; B_0 is the total concentration of the compound. A plot of $(\Delta\delta/B_0)^{1/2}$ vs. $\Delta\delta$ gives a straight line of which slope (S) and x-axis intercept (x_0) are respectively $(K/2\Delta\delta_{B_2})^{1/2}$ and $2\Delta\delta_{B_2}$. $\Delta\delta_{B_2}$ is the induced shielding in the dimer. Therefore, $\Delta\delta_{B_2}$ for each proton is directly obtained from these plots, and the association constant K is calculated ($K = x_0^2$).

Results and Discussion

UV Study. The binding of chloroquine with DNA and synthetic polynucleotides has been most thoroughly studied by examining the UV spectrum of the drug molecule when present in solutions containing the relevant polynucleotide. The spectrum is characterized by a strong decrease in intensity (hypochromic effect), a slight shift to the blue in the region of the absorption maximum, and a small increase in absorption at longer wavelength [see, for example, Cohen & Yielding (1965)]. This technique was therefore chosen to study the ring–ring interaction in our models, allowing comparisons to be made with the results obtained for the biological system. The electronic absorption spectra of the models B-C_3-Q were compared quantitatively with the summation of the spectra of the constituent aromatic units (B-C_3-Q vs. B-C_3 + Q-C_3). The spectra were recorded in dilute aqueous solution (5×10^{-5} M), at concentrations low enough to avoid any intermolecular

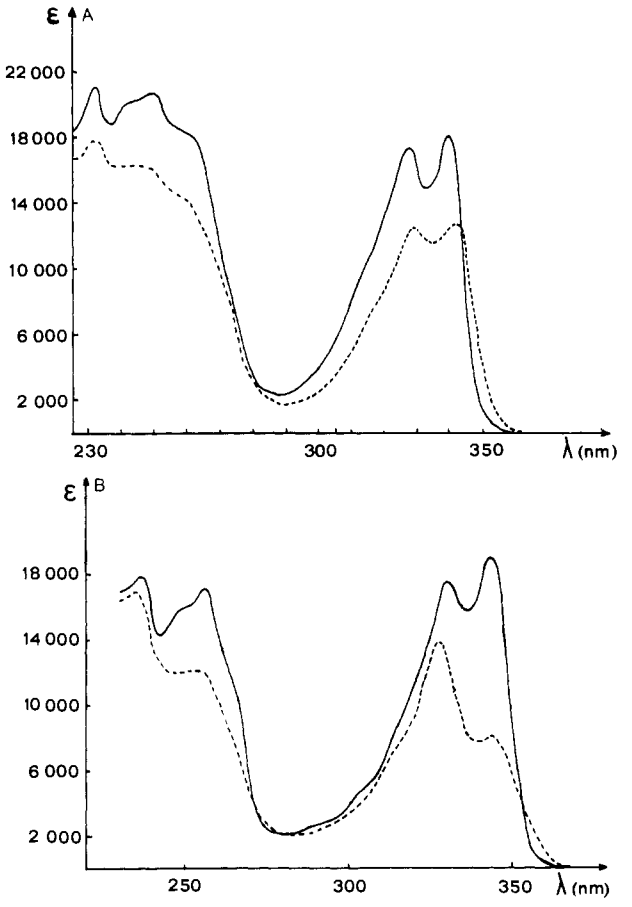

FIGURE 2: (A) Comparative electronic absorption spectra of Ade-C_3-Q(H) (**2b**) (---) and Ade-C_3 + C_3-Q(H) (**9** + **4b**) (—) in water, pH 6.9 (phosphate buffer), 25 °C, 5 × 10^{-5} M in each chromophore. (B) Spectra of C_3-Q(Cl) (**4a**) (—) and Q(Cl)-C_3-Q(Cl) (**5**) (---) in water, pH 6.9, 25 °C, 5 × 10^{-5} M for **4a** and 2.5 × 10^{-5} M for **5**.

interaction (Beer's law is followed in this concentration range), and in different conditions of pH (pH 1, 6.9, and 13).

It appears that the spectra of the quinoline models **2**, **3**, and **6** exhibit perturbations which are quite analogous to those reported for chloroquine in its interaction with nucleic acids, being characterized by a strong decrease in absorbance (for example, see Figure 2A). This "hypochromic effect" reflects the stacking of the two chromophores in the molecules. It can be quantitatively expressed by the percent hypochromism, % *H*, which is generally considered as a measure of the interaction.

The applicability of this criterion, % *H*, to assess intramolecular stacking has been amply demonstrated in the past (Mutai et al., 1975). The percent hypochromism, % *H*, was calculated for the 230–300-nm range where both quinoline and base show an absorption and for the 300–380-nm absorption band where quinoline is the only absorbing chromophore (Table I).

Table I: Computed Percent Hypochromism Values (% *H*) for the 230–300-nm and 300–380-nm Absorption Bands of Model Compounds in Water, 25 °C, at Different pH Values

model compd	no.		pH 1[c]	pH 6.9[c]	pH 13[c]
Ade-C_3-Q(Cl)	2a	*a*	16	20	21
		b	19.5	25.5	23
Ade-C_3-Q(H)	2b	*a*	13	16	17
		b	19	22.5	19
Ade-C_3-Q(Br)	2c	*a*	11	16	19
		b	18.3	22.7	22.7
Gua-C_3-Q(Cl)	3a	*a*	13	16	11
		b	22.4	25.9	13
Gua-C_3-Q(H)	3b	*a*	9	10	7
		b	18	20	6
Q(Cl)-C_3-Q(Cl)	5	*a*	21	21	
		b	28	28	
Ade-C_3-Q(Cl)CH_3^+	6a	*a*	12	18	15
		b	17	23	18
Ade-C_3-Q(H)CH_3^+	6b	*a*	10	13	
		b	12	14	
Ade-C_3-Ade	8	*a*	2.9	14.8[d] 14.5[e]	15.7

[a] 230–300-nm range (quinoline and base absorption); precision, ±2. [b] 300–380-nm range (quinoline is the only absorbing chromophore); precision, ±1. [c] pH 1, 0.1 N HCl; pH 6.9, phosphate buffer; pH 13, 0.1 N NaOH. [d] Leonard & Ito (1973). [e] This work.

Adenine Models. Ade-C_3-Q(X) (**2**) *and* (**6**). In the adenine series Ade-C_3-Q(X), where X = Cl, Br, or H, the three models exhibit a strong hypochromic effect (Table I). At pH 6.9, the percent hypochromism, % *H*, decreases when the 7-Cl substituent of quinoline is replaced by a bromine or an hydrogen atom. % *H* diminishes from 25.5 for chlorine to 22.7 for bromine and to 22.3 for hydrogen in the 300–380-nm band (the relative values of % *H* are quite comparable in the 230–300-nm region, being respectively 20, 16, and 16). However, the differences observed between the extreme values are small, and it is necessary to question their significance in terms of the relative degree of stacking, as the percent hypochromism is known not to be a precise measure of the interaction, being dependent both upon the thermodynamic strength of the interaction (Poland et al., 1966) and upon the relative orientation of the transition moments (Tinoco, 1960, 1961). Following the methodology previously described for **2a** (Bolte et al., 1976), the variation of % *H* as a function of temperature was studied. Figure 3 shows the difference of behavior of the three models: % *H*, which is proportional to the degree of stacking, increases when the temperature is lowered. For Ade-C_3-Q(Br) (**2c**) and Ade-C_3-Q(H) (**2b**), the increase is observed along the whole of the temperature range

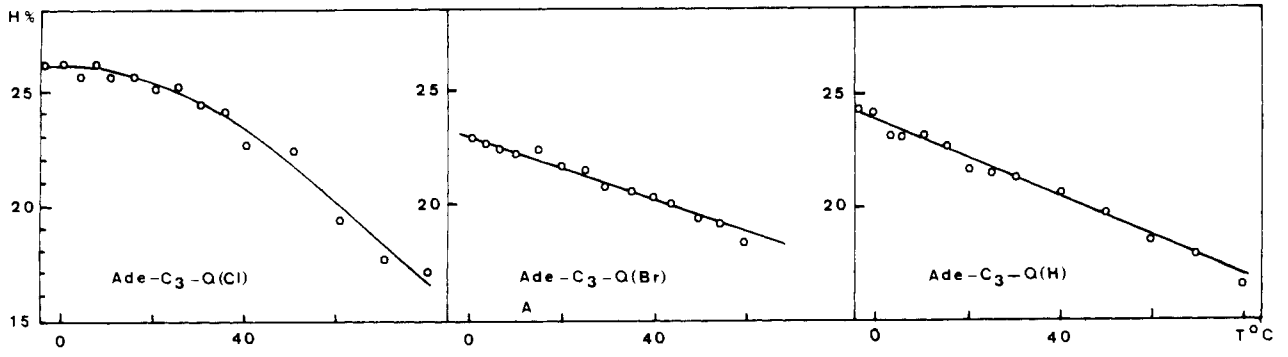

FIGURE 3: Variation of the percent hypochromism (% *H*) as a function of temperature, measured in water, pH 5.5 (phosphate buffer), 5 × 10^{-5} M, for the adenine models **2**.

studied, while % *H* reaches a maximum asymptotic value near 20 °C for Ade-C_3-Q(Cl) (**2a**). Interpreted in terms of stacking, this means that the degree in intramolecular stacking increases at low temperatures and reaches 100% for the 7-Cl model near 20 °C, while the 7-Br and 7-H compounds seem not to be completely stacked even at lower temperatures. These results parallel the previous conclusion obtained from the direct comparison of the % *H* values at 25 °C that the 7-chloroquinoline exhibits a higher affinity for adenine in the models than the 7-Br and 7-H analogues.

This is equally confirmed by the comparison of models Ade-C_3-Q(Cl)CH_3^+ (**6a**) and Ade-C_3-Q(H)CH_3^+ (**6b**) where the proton present on the ring nitrogen of quinoline (at pH 6.9) is formally replaced by a methyl group, leaving a comparable electronic distribution in the molecule. The value of % *H* decreases from 23 to 14 and 18 to 13, according to the absorption band considered, when chlorine is substituted by hydrogen.

It is also of interest to compare the stacking of the models in different conditions of pH. At pH 1, when both quinoline and adenine are protonated, the results are not very conclusive. % *H* is clearly higher for the 7-Cl derivative in the 230–300 absorption band (16 to be compared with 11 and 13 for the 7-Br and 7-H analogues, respectively) while the value is roughly the same in the 300–380-nm range. In the *N*-methyl series, % *H* is higher in the two bands for the 7-chloro compounds. In basic medium, when the two half-units of the models are neutral, no important variation is observed, but once again the 7-Cl derivative gives the highest % H value.

It seems, therefore, that, in all conditions studied, the 7-chloroquinoline exhibits a ring–ring affinity for adenine in the models which is higher than that for the other analogues.

Guanine Models. Gua-C_3-Q(X) (**3**). The 7-H quinoline model **3b** was prepared and compared to the previously reported 7-Cl model **3a**. Under neutral conditions, where quinoline is protonated and guanine is neutral, the highest % *H* was found in the two absorption bands for the 7-chloro model. This is equally the case at pH 1 (quinoline and guanine protonated) and at pH 13 (quinoline neutral, guanine negatively charged).

Bisquinoline Model Q(Cl)-C_3-Q(Cl) (**5**). The spectrum of this dimeric compound shows very large perturbations as compared to that of the monomer (Figure 2B). Besides a strong hypochromic effect, one observes a change in the relative intensity of the maxima in the 300–380 region. Accordingly, the difference spectrum cannot be rigorously interpreted in terms of hypochromic effect. However, if one estimates the perturbation, i.e., the decrease of the absorption intensity as was previously carried out for the percent hypochromism, one finds a value of 26% for the 300–380-nm region. The study of the variation of this "percent hypochromism" as a function of temperature gives the curve indicated in Figure 4, which is quite comparable to that which we have previously observed for the models in which 100% intramolecular stacking was reached near 20 °C. It seems, therefore, that the self-stacking of quinoline in the model is close to 100% in water at 20 °C.[2]

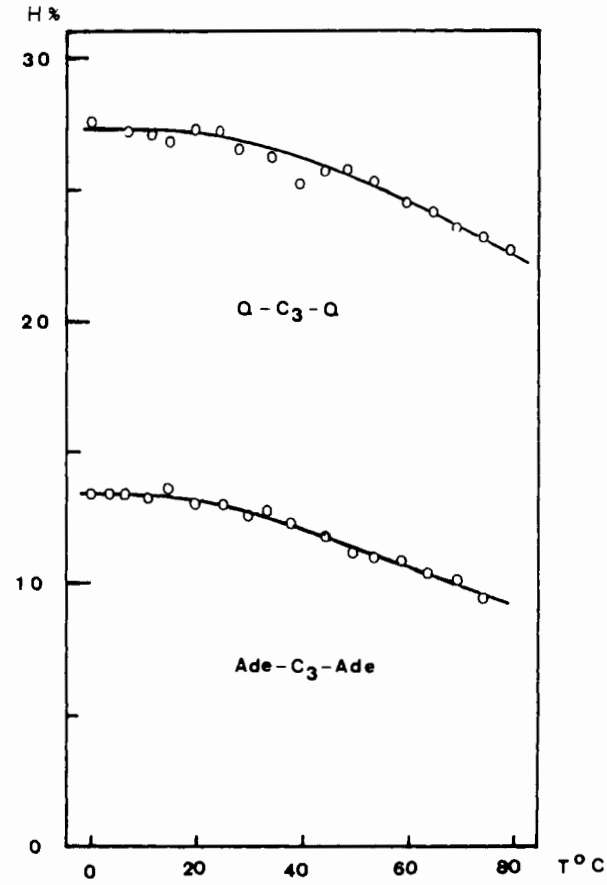

FIGURE 4: Variation of the percent hypochromism (% *H*) as a function of temperature for Q(Cl)-C_3-Q(Cl) (**5**) in water, 2.5×10^{-5} M, pH 5.5 (phosphate buffer), and for Ade-C_3-Ade (**8**) in water, 2.5×10^{-5} M, pH 6.9 (phosphate buffer).

Adenine–Adenine Model. Ade-C_3-Ade (**8**). This compound belongs to the series of "spectroscopic models" developed by N. J. Leonard [see, for example, Leonard & Ito (1973)], who prepared a number of dinucleotide analogues in which the different bases are linked by a trimethylene chain. These authors examined the intramolecular ring–ring interaction notably by determining the percent hypochromism. They found that Ade-C_3-Ade is one of the models which exhibits the highest % *H* (% *H* = 14.5; H_2O, 25 °C, pH 6.9) in the series. Consequently, we chose this compound as a reference to compare the quinoline–base interaction to the base–base interaction.

The variation of the percent hypochromism as a function of temperature shows (Figure 4) that % *H* reaches a maximum value near 20 °C. This is interpreted as an indication that this compound exists under an almost completely stacked conformation at this temperature. One should note the resemblance of the diagram with the curves observed for Ade-C_3-Q(Cl) (**2a**) and Q(Cl)-C_3-Q(Cl) (**5**). Using a two-state model (which constitutes a crude approximation) to analyze the folding–unfolding process, as was done in previous work (Bolte et al., 1977a,b), we found the thermodynamic parameters to be $\Delta H° = -10$ kcal/mol, $\Delta S° = -24$ eu.

^1H NMR Studies. By virtue of the extreme sensitivity of the method to detect structural and conformational changes within molecules, ^1H NMR spectroscopy has been successfully applied to the study of the folded ⇌ unfolded equilibrium in dinucleotides (Chan & Nelson, 1969), NAD (Mac Donald et al., 1972), or intercalating drugs (Barbet et al., 1976). Moreover, the differences in the chemical shifts of the protons between the completely folded and the unfolded forms give

[2] In this case the thermodynamic parameters cannot be extracted safely, as the spectrum is too complex and as a hyperchromic contribution in the nonstacked conformation cannot be excluded. We noted, for example (and contrarily to what was observed for quinoline–purine models (Bolte et al., 1977a,b), that the spectrum of Q(Cl)-C_3-Q(Cl) in EtOH, pH 1, is superimposable to that of C_3-Q(Cl) except for an increase in the absorbance at the 345-nm maximum. This can be compared to the observations made by Craig et al. (1972) for comparable two-charged systems existing in a nonstacked conformation.

information about the geometry for the stacked dimer. The investigation of the variation of this equilibrium with temperature leads to an evaluation of the thermodynamic parameters.

An examination into such phenomena necessitates a preliminary investigation into the self-association properties of the monomeric units.

Self-Association of C_3-Q(Cl) and Ade-C_3. The concentration dependence of the proton chemical shift values of C_3-Q(Cl) at 27 °C between 5×10^{-4} and 2×10^{-2} M is in accordance with the formation of vertically stacked *n*-mers. The self-association constant K was found to be ~ 80 M^{-1} (mean value from the ring protons). This value is in the same range as those for acridine (Barbet et al., 1976) or ethidium bromide (Roques et al., 1976) but clearly smaller than for ellipticine (Delbarre et al., 1976).

In the case of Ade-C_3, the attribution of the two aromatic signals at 7.95 and 7.87 ppm was facilitated by the exchange property of the proton located on the imidazole ring. Thus, the signal at 7.87 ppm had disappeared after 2 h at 80 °C in a deuterioacetate buffer, and it can therefore be assigned to H_8.

Due to the low solubility of Ade-C_3 in D_2O the self-association constant cannot be determined with accuracy. However, the concentration dependence of the chemical shift values between 2×10^{-3} and 2×10^{-4} M is very small (H_2 appears respectively at 7.93 and 7.95 ppm at those two concentrations and H_8 appears at 7.86 and 7.87 ppm).

Similarly, compared to the chemical shifts of the protons in pure Ade-C_3 or C_3-Q(Cl), no significant change occurs in a mixture of these compounds at the same concentration (2×10^{-3} M; 27 °C; pD $\simeq$5.5). This result provides evidence that in these conditions no interaction occurs between the adenine and quinoline rings.

Investigation into the Conformational Behavior of the Symmetric Dimers Q(Cl)-C_3-Q(Cl) and Ade-C_3-Ade. (*1*) *Q(Cl)-C_3-Q(Cl).* The conformational behavior of Q(Cl)-C_3-Q(Cl) has been studied in water at low concentrations (2×10^{-3} M) in order to minimize the intermolecular associations. As compared to the reference compound C_3-Q(Cl), large upfield shifts are observed for the ring protons H_5, H_6, and H_8 (H_5, 0.61; H_6, 0.36; H_8, 0.31 ppm) while protons H_2 and H_3 are much less affected (H_2, 0.14; H_3, 0.01 ppm)(Figure 5). In addition, the aliphatic methylene protons of the linking chain now appear as broad signals. These spectacular upfield shifts of the aromatic protons are an evidence of the intramolecular stacking of the rings, confirmed by the broadening of the methylene signals, which reflects restrictions in the rotational motion around C–C bonds in the "immobilized" bridging chain.

The degree of folding of the dimer was evaluated by investigating the variation of the quinoline proton chemical shifts as a function of temperature. Figure 6 shows the temperature dependence of the chemical shift differences in the monomer and in the dimer for the H_2, H_5, H_6, and H_8 protons. As expected, for the existence of a folded $\rightleftharpoons$ unfolded equilibrium, the chemical shift differences decrease as the temperature is increased, in the range 30–85 °C, while no significant changes are observed below ca. 30 °C. This can be interpreted as an indication of the presence of a completely folded conformation below 30 °C. It is interesting to note that, even at 87 °C, all protons still resonate at higher field than the corresponding protons in C_3-Q(Cl). This result indicates a very large residual ring–ring interaction at this temperature which is in accordance with the UV experiments. In the case of a two-state model

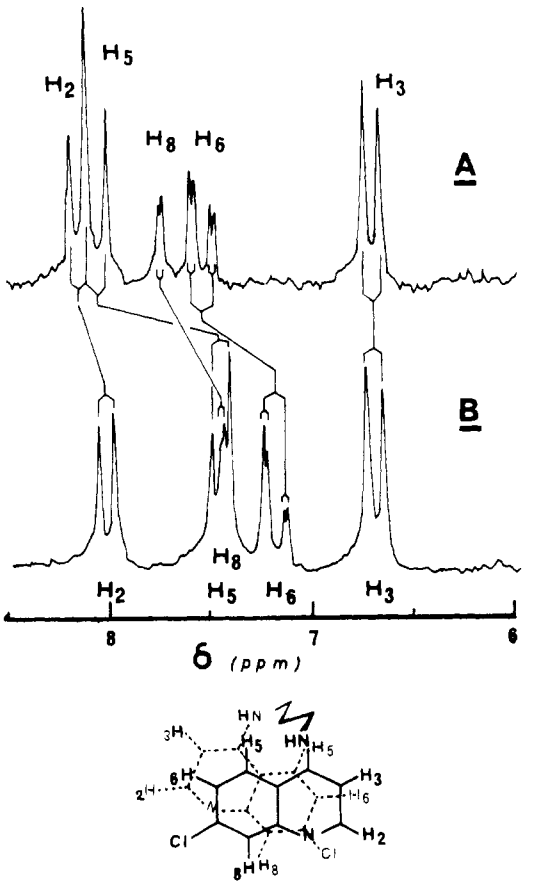

FIGURE 5: Spectra at 27 °C of 2×10^{-3} M solutions (pD $\sim$5.5) of C_3-Q(Cl) (A) and Q(Cl)-C_3-Q(Cl) (B). Possible stacked conformation of Q(Cl)-C_3-Q(Cl) using the difference ($\Delta\delta_{m-d}$) in the chemical shifts (in ppm) of the corresponding protons between the completely folded dimer, d, Q(Cl)-C_3-Q(Cl) at 20 °C (2×10^{-3} M; pD $\sim$5.5) and the monomer, m, C_3-Q(Cl) in the same conditions. $\Delta\delta_{m-d}$ values: H_2 = 0.14; H_3 = 0.01; H_5 = 0.61; H_6 = 0.36; H_8 = 0.31.

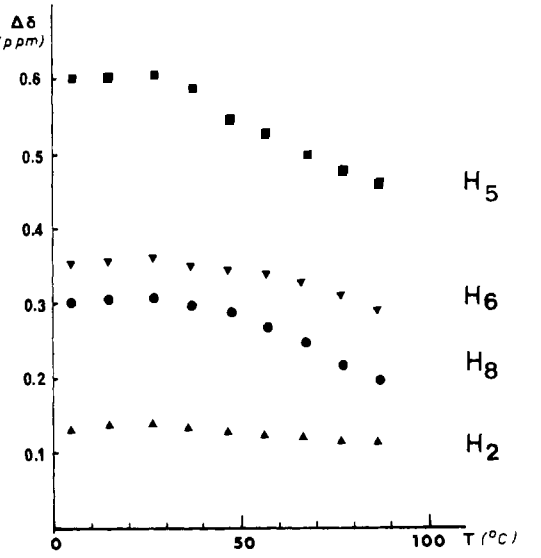

FIGURE 6: Q(Cl)-C_3-Q(Cl) and C_3-Q(Cl). Variation of the chemical shift difference $\Delta\delta_{m-d}$ for the H_2, H_5, H_6, and H_8 protons with temperature in degrees Celsius. The spectra of the monomer, m, C_3-Q(Cl) and the dimer, d, Q(Cl)-C_3-Q(Cl) were performed in the same conditions (2×10^{-3} M; pD $\sim$5.5).

of folded $\rightleftharpoons$ unfolded equilibrium, it is customary to extract thermodynamic parameters of the process from a van't Hoff plot. The equilibrium constant K can be calculated at each temperature from the relationship $K_i = \delta_i/\Delta_i$ where δ_i is the

chemical shift difference for a proton i between monomer and dimer at each temperature and Δ_i is the difference between the monomer and the dimer at 5 °C. At this temperature, the dimer is assumed to be completely folded. The values of $\Delta H_0 = -9.5$ kcal/mol and $\Delta S_0 = -25$ eu from H_5 and $\Delta H_0 = -10.8$ kcal/mol and $\Delta S_0 = -29$ eu from H_8 can thus be obtained.

All these results are in good agreement with the UV data. In addition, a geometry for the average preferred stacked conformation can be proposed from the differences in the shielding of the aromatic protons of Q(Cl)-C_3-Q(Cl). In order to explain the large upfield shift of H_5, H_6, and H_8 protons compared to H_2 and H_3, it is necessary to superimpose the phenyl rings of the quinoline moieties on the corresponding pyridine rings of the opposing quinoline molecule (Figure 5). Such a disposition, in which the H_2 and H_3 protons are not greatly affected, requires an inverted disposition of the quinoline moieties.

(*2*) *Ade-C_3-Ade*. As was carried out for Ade-C_3, the assignments of H_2 and H_8 in Ade-C_3-Ade were achieved by heating a deuterioacetate buffer solution (2.5×10^{-4} M; pD 5) of this dimer at 80 °C during 4 h. From the two signals at 7.63 and 7.56 ppm, only the latter disappeared; it was consequently assigned to the H_8 proton (Schweizer et al., 1964).

The very low water solubility of Ade-C_3-Ade does not permit a definitive investigation of its intermolecular association. However, no changes in the position of H_2 and H_8 occur between 5×10^{-4} and 10^{-4} M. This indicates that no aggregation occurs in this concentration range. Consequently, the difference in the chemical shifts of H_2 ($\Delta\delta = 0.32$) and H_8 ($\Delta\delta = 0.31$) between Ade-C_3 and Ade-C_3-Ade at 2.5×10^{-4} M (pD ~5.22) can be due solely to the occurrence of a folding in the dimer. The variation of the equilibrium between the folded and the unfolded forms as a function of temperature, which was monitored by following the changes in $\Delta\delta$ H_2 and $\Delta\delta$ H_8, indicates the high stability of the intramolecular stacked form since no changes in $\Delta\delta$ occur between 5 and 25 °C whereas $\Delta\delta$ H_2 and $\Delta\delta$ H_8 decrease respectively from 0.32 and 0.31 ppm (100% folded form) at 22 °C to 0.27 ppm (~85% folded forms) at 82 °C. From these values, the following thermodynamical parameters can be derived: $\Delta G° = -2.4 \pm 0.2$ kcal/mol at 295 K; $\Delta H° = -8.7 \pm 0.2$ kcal/mol; $\Delta S° = -21 \pm 2$ eu.

From the adenine isoshielding curves of Giessner-Prettre & Pullman (1976) and the somewhat similar upfield shifts on H_2 and H_8 occurring in Ade-C_3-Ade, a maximum overlap of the two adenine rings on each other can be proposed. Such preferential geometry could explain the strong stabilization of the folded form.

Study of the Adenine–Quinoline Models. Ade-C_3-Q(Cl) and Ade-C_3-Q(Br). In Ade-C_3-Q(Cl) and Ade-C_3-Q(Br) the adenine protons were assigned as before, by exchange of the H_8 protons with D_2O at 80 °C.

In contrast to what was observed for Ade-C_3 and Ade-C_3-Ade, the H_8 proton is more deshielded than H_2 in each compound (as already found for 5′-AMP). The concentration dependency of the chemical shifts has been studied at 270 MHz between 2×10^{-3} and 10^{-4} M, but no significant variation could be observed. This excludes the possibility of intermolecular aggregation in this concentration range.

Comparison of the chemical shift values of the protons present in the dimers Ade-C_3-Q(Cl) and Ade-C_3-Q(Br) with those of the corresponding monomers at the same concentration (5×10^{-4} M; pD ~5) indicates an upfield shift for all

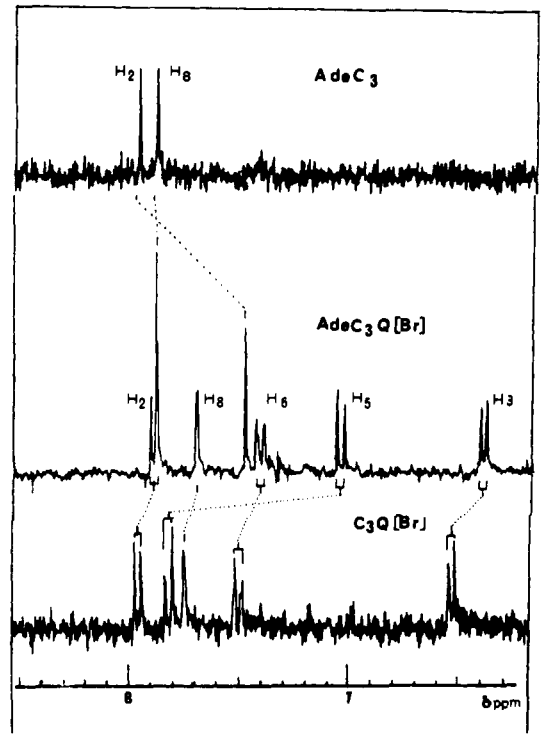

FIGURE 7: NMR spectra (recorded at 270 MHz) of Ade-C_3, Ade-C_3-Q(Br), and C_3-Q(Br) in D_2O, pD 5.5, 5×10^{-4} M.

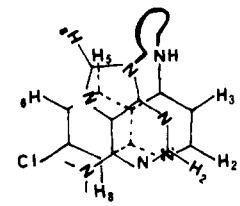

FIGURE 8: Stacked conformation of Ade-C_3-Q(Cl) using the adenine isoshielding curves of Giessner-Prettre & Pullman (1976). Differences ($\Delta\delta_{m-d}$) in the chemical shifts of the corresponding protons between the dimer Ade-C_3-Q(Cl) and the monomers Ade-C_3 and C_3-Q(Cl) (270 MHz; 5×10^{-4} M; pD 5.5; 20 °C): H_2Ade = 0.51; H_8Ade = 0.13; H_2Qui = 0.11; H_3Qui = 0.19; H_5Qui = 0.81; H_6Qui = 0.11; H_8Qui = 0.10 ppm.

protons and particularly for H_5 (0.81 and 0.76 ppm, respectively) in the quinoline part and for H_2 in the adenine moiety (0.55 and 0.54 ppm, respectively) (Figure 7).

These upfield shifts are due to the respective ring-current effects of the two stacked moieties. Moreover, as for Q(Cl)-C_3-Q(Cl), a large broadening of the signals of the aliphatic bridge protons was observed. Due to the lack of precise isoshielding curves for the quinoline moiety, it is difficult to propose a precise geometry for the folded form of Ade-C_3-Q(Cl) or its bromo analogue. However, taking account of both the geometric constraints brought about by the linking chain and of the large upfield shift of H_5 due to the adenine ring current, a model with a parallel orientation of the quinoline C_7–Cl and adenine C_4–NH_2 bonds can be proposed (Figure 8).

A quantitative investigation of the equilibrium between folded and unfolded forms can be obtained from the temperature dependencies of the proton chemical shifts in Ade-C_3-Q(Cl) and Ade-C_3-Q(Br) relative to the corresponding ones in Ade-C_3, C_3-Q(Cl), and C_3-Q(Br) under the same experimental conditions (270 MHz; 5×10^{-4} M; pD ~5). However, such a quantitative study requires the knowledge of the proton chemical shifts of the dimers both in the pure

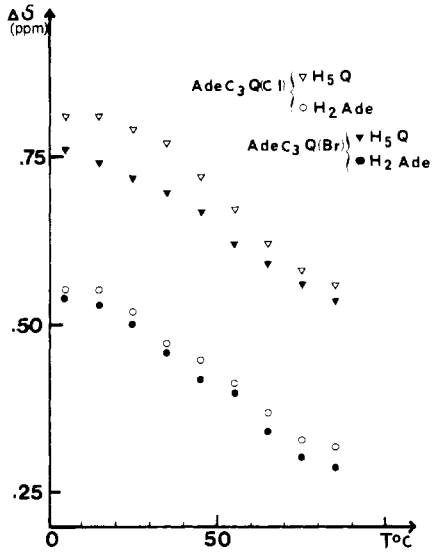

FIGURE 9: Variation of the chemical shift difference $\Delta\delta_{m-d}$ for the H_2 adenine and H_5 quinoline protons with temperature in Ade-C_3-Q(Cl) and Ade-C_3-Q(Br). The spectra of the monomers Ade-C_3, C_3-Q(Cl), and C_3-Q(Br) and the dimers are monitored in the same conditions (270 MHz; 5×10^{-4} M; pD 5.5).

folded and in the unfolded forms. It is generally assumed that the chemical shifts in the unfolded dimers are the same as in the corresponding monomers.

This assumption was confirmed in the case of Ade-C_3-Q-(Br). In the mixed solvent Me_2SO–D_2O, 75:25, where the compound is present solely in the unfolded form (Bolte et al., 1977a,b), all proton chemical shifts of the dimer are identical with those found for the monomer units, Ade-C_3 and C_3-Q(Br), in the same solvent. The proton chemical shifts in the totally folded forms can be obtained only in the case of Ade-C_3-Q(Cl) where the chemical shifts are unchanged from 5 to 15 °C, whereas such a plateau is not observed in Ade-C_3-Q(Br) (Figure 9). Therefore, in accordance with UV measurements, we can assume that Ade-C_3-Q(Cl) is 100% folded below 15 °C while Ade-C_3-Q(Br) is not completely stacked even at 5 °C.

For Ade-C_3-Q(Cl), as in the cases of Q(Cl)-C_3-Q(Cl) and Ade-C_3-Ade, the quantitative treatment of the chemical shift differences ($\Delta\delta$) is easily carried out on the quinoline H_5 and the adenine H_2 protons which are the most affected by folding of the molecule. A plot of log K against $1/T$ furnishes the thermodynamic parameters of the equilibrium: $\Delta G° = -1.75 \pm 0.25$ kcal/mol at 298 K; $\Delta H° = -9.9 \pm 1.1$ kcal/mol; $\Delta S° = -27 \pm 3$ eu.

Conclusion

We have examined models to evaluate the relative ring–ring stacking interactions between the monomeric aromatic molecules involved in the binding of chloroquine with nucleic acids. The intramolecular interaction in the model compounds was determined by UV and ^{1}H NMR measurements. The use of these two techniques proved to be quite fruitful in their complementarity to abstract detailed information on such an intramolecular phenomenon.

One of the principal conclusions is that important attractive interactions do exist in water for all couples of rings examined, as reflected by very high degrees of folding of the corresponding models.

For the model compounds in which differently substituted aminoquinolines are linked to purine residues, the percent hypochromism, % H, measured at 25 °C is higher for the 7-Cl than for the 7-H derivatives. This is observed in the adenine model and also for that in the guanine series. The study was extended in the adenine series to the 7-Br derivatives and to N-methylated analogues with the 7-Cl and 7-H quinolines. In all cases the % H value is the highest for the 7-Cl compounds. The temperature dependence of % H confirms these observations as the 7-Cl model Ade-C_3-Q(Cl) is the only one which exhibits 100% folding near 25 °C. The ^{1}H NMR study of the variation of the chemical shifts of the quinoline and adenine protons with temperature in Ade-C_3-Q(Br) and Ade-C_3-Q(Cl) confirms these observations. As a consequence, although the difference of behavior between the different quinoline analogues is small, all our results point to a higher tendency for the 7-chloroquinoline ring to stack with purines in the models.

More important, however, is the comparison of the three models Ade-C_3-Ade, Q(Cl)-C_3-Q(Cl), and Ade-C_3-Q(Cl). The UV and ^{1}H NMR data (and notably the variations observed as a function of temperature) indicate clearly a very similar behavior. The degree of folding approaches 100% in the 15–30 °C temperature range for the three compounds. This means that the stacking binding energies between two quinolines, between two purine bases, and between one quinoline and one purine base are all of the same order of magnitude. The comparison of the thermodynamic parameters is particularly instructive: $\Delta H_0 = -9.5$, -8.7, and -9.9 kcal/mol and $\Delta S_0 = -25$, -21, and -27 eu respectively for Q(Cl)-C_3-Q(Cl), Ade-C_3-Ade, and Ade-C_3-Q(Cl).

Although rigorously this is valid only for the systems described, i.e., when the rings are covalently linked by a trimethylene chain which imposes limitations to the geometry of the complex, it is of interest to consider these results in view of the problem of the binding of chloroquine to nucleic acids. In such a situation the quinoline ring of the drug, which is ionically bound to DNA by the diethylamino chain, may either intercalate between the nucleotide bases or/and self-associate. Considering the problem of the possible stacking interactions which may take place, purely from the thermodynamic point of view, our results clearly indicate that both the intercalation and the self-stacking of the drug are phenomena which are equally favored.

The binding of chloroquine with nucleic acids has been frequently studied by examination of the UV spectrum of the drug in the presence of polynucleotides. One could speculate as to the nature of the spectral changes which should result from a binding process involving both intercalation and self-stacking of the drug. From our models one can note that the stacking of quinoline with purines is characterized by a strong hypochromic effect (Figure 2A), while the self-stacking of quinoline induces both hypochromism and large changes in the shape of the absorption curve, notably a strong decrease of the absorption maximum at 345 nm (inversion of the relative intensities of the two maxima at 335 and 345 nm; Figure 2B). Figure 10 presents a spectrum calculated for a solution containing a 75:25 mixture of Ade-C_3-Q(Cl) and Q(Cl)-C_3-Q(Cl), i.e., a spectrum which indicates changes in the absorption originating from both base–quinoline (base-stacking) and quinoline–quinoline (self-stacking) systems. As expected, the relative intensities of the maxima are inverted relative to the reference C_3-Q(Cl). This can be compared to the absorption curve of chloroquine in its interaction with DNA, as reported inter alia by Cohen & Yielding (1965) where a comparable inversion of the maxima is observed when the drug becomes totally bound, i.e., when the spectrum is characteristic of the bound species without the spectral contribution of free chloroquine.

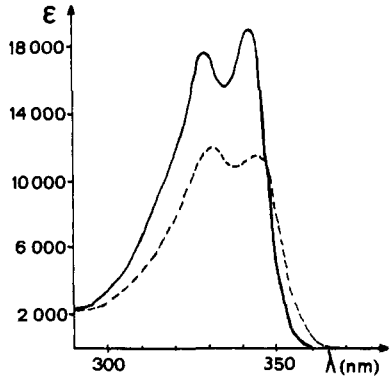

FIGURE 10: Calculated ultraviolet addition spectrum of a mixture of purine–quinoline model Ade-C_3-Q(Cl), 75%, and quinoline–quinoline model Q(Cl)-C_3-Q(Cl), 25% (---), compared to the absorption spectrum of C_3-Q(Cl) (—). Identical concentration of the quinoline chromophore for the two curves (5×10^{-5} M).

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