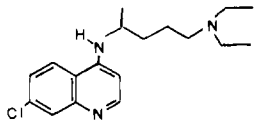
Synthetic Models Related to DNA Intercalating Molecules: Comparison between Quinacrine and Chloroquine in Their Ring-Ring Interaction with Adenine and Thymine

Jean Bolte,[1a] Colette Demuynck,[1a] M. France Lhomme,[1b] Jean Lhomme,*[1b] Jacques Barbet,[1c] and Bernard P. Roques[1c]

Contribution from the Laboratory de Chimie Organique Biologique, Université de Lille I, ERA CNRS 827, Associé à l'Enscl, 59655 Villeneuve D'ascq Cedex, and Departement de Chimie, Université R. Descartes, ERA CNRS 613 and SCN 21 Inserm, 75270 Paris Cedex 06, France. *Received November 19, 1979*

Abstract: Stacking interactions between the acridine ring of the antimalarial drug quinacrine and the nucleotide bases, adenine and thymine, have been studied by preparing and examining models in which the acridine is linked to the base by a trimethylene bridge. The degree of intramolecular ring-ring stacking interaction in water has been determined by hypochromism measurement in the UV and by 270-MHz ^1H NMR spectroscopy. We show that the acridine ring exhibits very high affinity for the two bases and in particular for the adenine ring, the corresponding model being essentially stacked in the whole temperature range studied. These results are compared to previous data obtained for the quinoline ring present in the antimalarial drug chloroquine. They indicate the stronger nature of the interaction in the case of the acridine ring, a conclusion which is in agreement with the well-known greater ability of acridines to interact with DNA.

Chloroquine (**1**) and quinacrine (**2**) are structurally related



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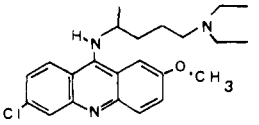

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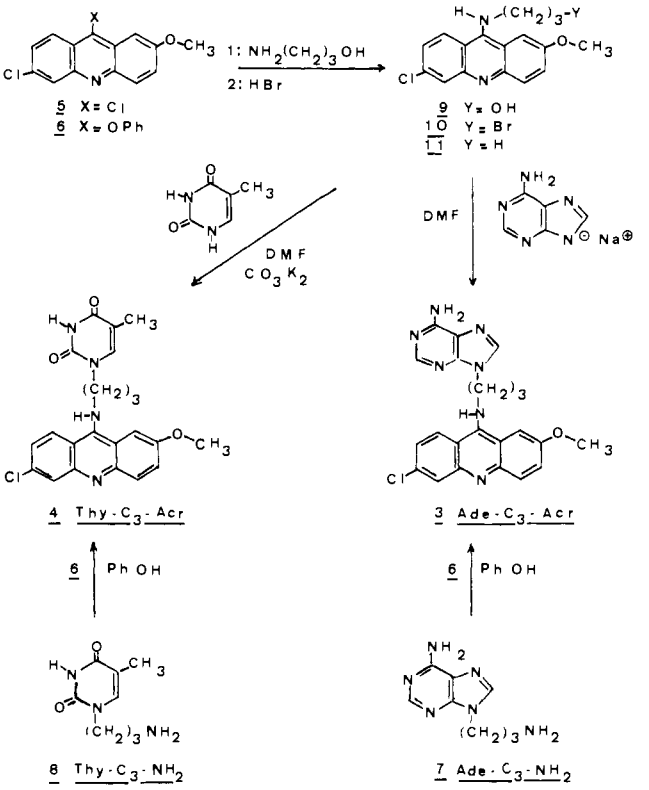
antimalarial drugs which inhibit the DNA replication and RNA transcription in susceptible cells.[2,3] In vitro they form reversible complexes with polynucleotides in which the identical protonated amino aliphatic side chains interact ionically with the phosphate groups of the polymer while the aromatic rings are involved in stacking interactions with the nucleotide bases. A pure intercalation scheme is widely accepted for the acridine drug quinacrine which exhibits high affinity for polynucleotides.[4] However for chloroquine, which only possesses two of the three aromatic rings present in the latter, weaker drug-DNA interactions are observed and a binding scheme including both internal and external modes has been proposed.[3,4] These differences between chloroquine and quinacrine may be due to variations in the strength of the ring-ring stacking interactions between the drug and the bases.

In preceding work we studied such interactions between quinoline and the nucleotide bases by preparing models in which the two interacting aromatic moieties are linked by a flexible trimethylene bridge.[6] The position of the equilibrium between the folded and unfolded forms in aqueous solution, established by UV and NMR experiments, was used as a measure of the interaction. We describe here results obtained for the corresponding acridine-base systems, **3** and **4**, in which the acridine ring is linked respectively to adenine and thymine. The comparison between the quinoline and the acridine models should furnish quantitative information about the role of the additional ring present in the acridine nucleus.

Scheme I



Synthesis. Model compounds Ade-C_3-Acr (**3**) and Thy-C_3-Acr (**4**) were obtained by two different routes. 6-Chloro-2-methoxy-9-phenoxyacridine (**6**), prepared from the corresponding dichloride **5**, was caused to react in phenol at 120 °C with equimolar 9-(3-aminopropyl)adenine (**7**)[8] to give Ade-C_3-Acr with

(1) (a) Department de Chimie, Université de Clermont 63170 Aubière, France. (b) Université de Lille I. (c) Université R. Descartes.

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(7) Abbreviations used: Ade for aden-9-yl, Thy for thym-1-yl, Acr for 6-chloro-2-methoxy-9-(-yl)aminoacridine, Q for 7-chloro-4-(-yl)aminoquinoline, and C_3 for *n*-propyl, according to the IUPAC-IUB symbols (*Biochemistry* **1970**, *9*, 4022) and to the symbols proposed by: Cohn, W. E.; Leonard, N. J.; Wang, S. Y. *Photochem. Photobiol.* **1974**, *19*, 89.

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a 60% yield. Reaction of 1-(3-aminopropyl)thymine hydrochloride (Thy-C_3-NH_3^+,Cl^-, **8**)[9] with equimolar phenoxyacridine (**6**) in phenol at 120 °C afforded, after several recrystallizations, the monohydrated model compound **4** (Thy-C_3-Acr). A shorter path to **3** and **4** was devised starting from a common ((hydroxypropyl)amino)acridine **9** (Acr-C_3-OH) which was obtained by treatment of **6** with an excess of aminopropanol in Me_2SO. Treatment of **9** with $HBr-H_2SO_4$ afforded the labile bromo derivative **10**. Displacement of bromine in **10** by sodium adenylate was accomplished at 25 °C in DMF to give model **3**. Model **4** was obtained in a comparable substitution by thymine in Me_2SO in the presence of K_2CO_3. This second pathway proved to be more rapid, but great difficulties were encountered in the final purification of **3** and **4**, probably due to the lability of the bromo precursor **10**.

The reference compounds 9-propyladenine (Ade-C_3) and 1-propylthymine (Thy-C_3) had been prepared before.[9] The propylacridine (Acr-C_3) **11** was obtained by refluxing the 9-phenoxyacridine **6** in propylamine.

In all cases the final products were scrupulously purified by recrystallization and chromatography, and their purity was checked by microanalysis and by thin-layer chromatography using several solvent systems. The structures of the products were confirmed by their UV and NMR spectra.

Experimental Section

All melting points are uncorrected. Infrared spectra were determined on a Perkin-Elmer Model 377 spectrometer. Electronic absorption spectra were obtained by using a Cary 15 spectrometer. Routine NMR spectra were recorded on a JEOL C 60 H spectrometer using tetramethylsilane as internal standard. Analyses were performed by the "Service Central de Microanalyse du Centre National de la Recherche Scientifique". Liquid chromatography was performed on silica "MERCK Kieselgel 60" or neutral alumina MERCK 90 (activity II-III). Homogeneity of the compounds was established by TLC on silica gel with fluorescence indicator (Kieselgel PF 254).

UV Spectroscopy. The quantitative ultraviolet spectrometric measurements were made as described previously.[6] Spectral grade ethanol and deionized water, distilled under nitrogen, were used. Samples of each compound (about 50 ± 1- or 5 ± 0.01-mg) were dissolved in absolute ethanol and then diluted with water to give (1–6) × 10^{-5} M solutions having a maximum optical density of 0.8 and containing either 1% or 5% ethanol. The pH was adjusted by using phosphate buffer. Spectra were determined in 10-mm cells, except for the check experiments (study of the absorption as a function of concentration) where 50-mm cells were used. Each spectrum was run at least three times and on more than one sample.

The percent of hypochromism (% *H*) was calculated according to the general equation % $H = \{1 - f(\text{B-C}_3\text{-Acr})/[f(\text{B-C}_3) + f(\text{Q-C}_3)]\}100$, where f is the oscillator strength of the transition, $f = (4.32 \times 10^{-9}) \int \epsilon(\lambda)/\lambda^2 \, d\lambda$.

The f values were obtained from optical densities measured every 2.5 nm by application of the Simpson's rule. We calculated % *H* in the 350–500-nm region of the spectrum, where the bases do not absorb; i.e., the term $f(\text{B-C}_3)$ is suppressed in the calculation of % *H*.

^{1}H NMR Study. Proton magnetic resonance spectra were recorded at 270 MHz with a Bruker WH 270 spectrometer operating in the Fourier transform mode and locked to the deuterium resonance of the solvent D_2O or Me_2SO-d_6. The residual water resonance was reduced by standard homonuclear gated decoupling. Probe temperature was regulated to ±1 °C by a Bruker B ST 100/700 controller. Stock solutions of the studied compounds were prepared in sodium deuteroacetate buffer (0.04 M, pD 5.5) containing disodium ethylenediaminetetracetate (10^{-4} M). Chemical shifts were measured with respect to an internal reference, HMDS, at saturation in D_2O, and are reliable to ±0.01 ppm. The geometry of the folded form of Ade-C_3-Acr was determined from isoshielding curves.[10] The acridine geometry was taken from crystal data.[11] Theoretical proton shifts for both the acridine and the adenine were estimated for a fixed orientation of the acridine relative to the adenine ring, as already described.[12] The self-association constants (K) were calculated according to Dimicoli and Helene,[13] with the assumption that the compounds form vertically stacked dimers or n-mers without cooperativity. B_0 being the total concentration of the compound studied, a plot of $(\Delta\delta/B_0)^{1/2}$ vs. $\Delta\delta$, for each proton, gives a straight line with slope $s = (K/2\Delta\delta B_2)^{1/2}$. Each line intercepts the x axis at $x_0 = 2\Delta\delta B_2$, allowing the determination of the chemical shift difference between the free molecule and the stacked dimer ($\Delta\delta B_2$), for each proton. The association constant ($K = x_0 s^2$) was computed from the different protons, and the given values correspond to the average. All these calculations were performed on a Hewlett-Packard HP-85 desk calculator, using a nonlinear optimization program based on Newton method, allowing direct plotting of the theoretical curves.[14]

The assignment of the signals corresponding to the acridine protons was made by double-irradiation experiments as already described,[15] and the H-2 and H-8 protons of adenine were attributed from exchange experiments with D_2O at pD ~11, conditions under which H-8 is slowly exchanged with deuterium.

Material Synthesis. 9-Propyladenine and 1-propylthymine had been prepared before according to N. J. Leonard et al.[9]

6-Chloro-2-methoxy-9-[(3-(aden-9-yl)propyl)amino]acridine (3). To a solution of 1.2 g (6.2 mmol) of 9-(3-aminopropyl)adenine (**7**)[8] in phenol (50 mL) was added 2.0 g (6.2 mmol) of 6-chloro-2-methoxy-9-phenoxyacridine (**6**). The resulting solution was heated at 120 °C for 2 h. The mixture was then poured with stirring into 500 mL of a 2% sodium hydroxide solution, and the precipitate was collected by filtration and washed with water. The solid obtained (2.4 g, 89%) was crystallized successively from ethanol and methanol: mp 228–229 °C; NMR (Me_2SO-d_6) δ 8.6–7.5 (m, 8 H, aromatic H of Acr and Ade), 4.6 (m, 2 H, Ade-CH_2), 4.2. (s, 3 H, OCH_3), 4.0 (m, 2 H, Acr-CH_2), 2.6 (m, 2 H, Acr-CH_2-CH_2); IR (KBr) 3300, 3250, 3150, 2920, 1640, 1625, 1590, 1575, 1560, 1470, 1460, 1415, 1335, 1310, 1265, 1250, 1215, 1200, 1165, 1100, 1070, 1025, 920, 885, 850, 830, 850, 775 cm^{-1}; UV (ethanol) λ_{max} 450 (ε 8800), 426 (9100), 343 (4400), 327 (3300), 275 (48 300), 263 (48 300) nm. Anal. Calcd for $C_{22}H_{20}N_7OCl$: C, 60.89; H, 4.65; N, 22.59. Found: C, 61.12; H, 4.68; N, 22.58.

6-Chloro-2-methoxy-9-[(3-(thym-1-yl)propyl)amino]acridine (4). A solution of 6-chloro-2-methoxy-9-phenoxyacridine (1.0 g, 3.1 mmol) and 1-(3-aminopropyl)thymine hydrochloride (**8**) (0.70 g, 3.2 mmol) in phenol (30 g) was heated at 120 °C for 2 h. After being cooled, the mixture was poured into 200 mL of 2% aqueous sodium hydroxide. The precipitate was collected by filtration and washed with 2% aqueous sodium hydroxide. The residue was suspended in 100 mL of chloroform, and the mixture vigourously stirred. This operation was repeated twice. The chloroform extract contained a complex mixture while the solid residue was almost pure **4** (0.60 g, 46%) which was recrystallized successively in methanol and acetone to give the analytically pure compound. **4** crystallized with one molecule of water: mp 221–222 °C; NMR (Me_2SO-d_6) δ 11.4 (brs 1 H, Acr-NH), 8.3 (d, 1 H, J = 9 Hz, Acr-H-8), 8–7.1 (m, 7 H, Acr-H-1, -3, -4, -5, -7, Thy-H_6, and Thy-NH), 3.95 (s, 3 H, OCH_3), 3.8 (br, 4 H, Acr-CH_2-CH_2-CH_2-Thy), 2.1 (br, 2 H, Acr-CH_2-CH_2), 1.7 (s, 3 H, Thy-CH_3); IR (KBr) 3650, 3350, 3150, 3050, 2840, 1695, 1675, 1635, 1560, 1520, 1480, 1440, 1390, 1370, 1350, 1340, 1270, 1250, 1225, 1205, 1150, 1120, 1080, 1030, 990, 935, 880, 850, 840 cm^{-1}; UV (ethanol) λ_{max} 448 (ε 9800), 424 (10 200), 342 (4100), 327 (2100), 280 (54 200), 270 (sh) (47 300) nm. Anal. Calcd for $C_{21}H_{21}N_4ClO_3 \cdot H_2O$: C, 59.66; H, 5.20; N, 12.65. Found: C, 59.68; H, 5.20; N, 12.53.

6-Chloro-2-methoxy-9-[(3-hydroxypropyl)amino]acridine (9). A 1.0-g (4-mmol) portion of 6-chloro-2-methoxy-9-phenoxyacridine (**6**) was added to 0.59 g (8 mmol) of 3-amino-1-hydroxypropane in Me_2SO (25 mL). The mixture was heated for 12 h at 110 °C. After being cooled, the solution was poured into 100 mL of ice–water and left overnight at 0 °C. The solid was filtered, washed with water, and crystallized from chloroform (1.02 g, 90%). **9** crystallized with one molecule of water: mp 184–185 °C; NMR (Me_2SO-d_6) δ 8.5–7 (m, 6 H, aromatic H of Acr), 6.8 (brs, 1 H, Acr-NH), 4.6 (brs, 1 H, Acr-$(CH_2)_3$-OH), 3.9 (s, 3 H, OCH_3), 3.8–3.3 (m, 4 H, Acr-N-CH_2(CH_2)-CH_2OH), 1.88 (m, 2 H, Acr-N-CH_2-CH_2-CH_2-OH); IR (Nujol) 3500, 3000, 1630, 1600 cm^{-1}; mass spectrum, m/e (relative intensity) 335 ($M^+ + H_2O$, 25.7), 317 (M^+, 100), 301 (6.35), 285 (7.95), 271 (31.8), 255 (15.9), 243 (19.1), 240

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(23.8), 228 (19.1), 200 (39.8), 159 (27.6).

6-Chloro-2-methoxy-9-[(3-bromopropyl)amino]acridine (10). To a stirred solution of 47% hydrobromic acid (50 mL) and sulfuric acid (15 mL) was added 0.93 g (3 mmol) of 6-chloro-2-methoxy-9-[(3-hydroxypropyl)amino]acridine (**9**). The mixture was heated under nitrogen at 70 °C during 4 h. After the mixture was cooled, an ice-cold solution of potassium hydroxide was added under vigourous stirring to neutrality. The solution was extracted with dichloromethane. The extracts were washed with water and dried with sodium sulfate. Removal of the solvent in vacuo left the solid desired compound (0.37 g, 30%), mp 181–182 °C (with decomposition), which could not be purified further. The product rapidly decomposed as revealed by TLC analysis and was used immediately after obtention.

6-Chloro-2-methoxy-9-[(3-(aden-9-yl)propyl)amino]acridine (3). Alkylation of Adenine. A 0.22-g (9-mmol) portion of sodium hydride was suspended in 40 mL of dry dimethylformamide, and 1.22 g (9 mmol) of adenine was added. After 3 h of stirring under nitrogen was added a solution of crude 6-chloro-2-methoxy-9-[(3-bromopropyl)amino]acridine (**10**) (1.8 g, 6 mmol) in dimethylformamide (20 mL). The resulting mixture was stirred for 48 h at room temperature under nitrogen. Solvent was removed in vacuo. The residue (2.1 g) was chromatographed on neutral alumina using a 1:1 mixture of chloroform–acetone plus approximately 0.5% of NH_4OH as eluent. The product obtained was crystallized several times from methanol–water to give a product, mp 228–229 °C, identical with that already described.

6-Chloro-2-methoxy-9-[(3-(thym-1-yl)propyl)amino]acridine (4). Alkylation of Thymine. A mixture of 0.37 g (1 mmol) of 6-chloro-2-methoxy-9-[3-(bromopropyl)amino]acridine (**10**), 0.50 g (4 mmol) of anhydrous potassium carbonate and 0.38 g (3 mmol) of thymine in 25 mL of dimethyl sulfoxide was stirred at room temperature under nitrogen for 48 h. After filtration, the mixture was poured with stirring into water and extracted with dichloromethane. The extracts were washed with water and dried over sodium sulfate. After removal of the solvent in vacuo, the residue was chromatographed over alumina. The 1:1 mixture of methanol–acetone eluates 0.45 g (75%) of a solid which was recrystallized from methanol and shown to be identical with authentic **4**.

6-Chloro-2-methoxy-9-(propylamino)acridine (11). A solution of 6-chloro-2-methoxy-9-phenoxyacridine (2.0 g, 6 mmol) and propylamine (0.73 g, 12 mmol) in phenol was warmed at 120 °C for 2 h. After being cooled, the mixture was poured into a 2% sodium hydroxide solution (500 mL) and extracted with dichloromethane. The organic phase was washed with aqueous sodium hydroxide and with water and dried over sodium sulfate, and the solvent was removed in vacuo. The residue was treated with the following mixture ethanol (100 mL), water (100 mL), 12 N hydrochloric acid (2 mL), leaving 1.8 g (45%) of solid (**11**) which was recrystallized twice from acidic methanol (0.1 N): mp 248 °C (dec); NMR (Me_2SO-d_6) > 10.2 (br s, 1 H, NH-10), 8.6 (d, 1 H, J = 9.5 Hz, *H*-8), 8.2-7.7 (m, 5 H, H-1, -3, -4, -5 and Acr-N*H*), 7.55 (dd, 1 H, J = 9.5 and 1.5 Hz, H-7), 4.2 (m, 2 H, Acr-CH_2), 4.05 (s, 3 H, OCH_3), 2.1 (m, 2 H, Acr-CH_2-CH_2), 1.05 (t, 3 H, J = 9 Hz), CH_2-CH_3); IR (KBr) 3300, 3230, 3030, 2960, 1630, 1595, 1560, 1500, 1475, 1330, 1275, 1255, 1240, 1180, 1100, 1040, 940, 840, 775 cm^{-1}; UV (ethanol) λ_{max} 448 (ϵ 10090), 423 (10500), 342 (4260), 326 (3400), 282 (55700) nm. Anal. Calcd for $C_{16}H_{18}N_2Cl_2O$: C, 59.09; H, 5.58; N, 8.61. Found: C, 59.06; H, 5.62; N, 8.67.

Results and Discussion

The ring–ring intramolecular stacking properties of models **3** and **4** were investigated by hypochromism measurement in the UV and by 270-MHz ^1H NMR spectroscopy.

Hypochromism. The binding of quinacrine to DNA and synthetic polynucleotides has been thoroughly studied by examining the UV spectrum of the drug molecule in the presence of the relevant polynucleotide.[16,17] Intercalation of the acridine moiety of the drug between the nucleotide bases results in a strong decrease of the absorption intensity of the quinacrine chromophore ("hypochromic effect") accompanied by a slight shift to the blue in the region of the absorption maximum (see, for example A. Krey and F. Hahn in ref 16a). We therefore selected this method to detect the ring–ring interaction in our models and to compare the results with those reported for the drug–DNA interaction. In addition, the value (and the limits) of this "hypochromism" technique to assess stacking interactions has been amply demonstrated, notably by N. J. Leonard,[18] in a series of nucleic acid bases and coenzymes. We have also used it to evaluate the interaction between aminoquinolines and nucleotide bases.[6] Consequently we compared, quantitatively, the electronic absorption spectra of the models with the summation of the spectra of the constituent aromatic units, i.e., Ade-C_3-Acr vs. Ade-C_3 + Acr-C_3 and Thy-C_3-Acr vs. Thy-C_3 + Acr-C_3. Illustratively, Figure 1 represents the absorption spectrum of Ade-C_3-Acr compared with that of Acr-C_3 in the 350–500-nm region (where acridine is the only absorbing chromophore) using equimolar concentrations of each compound. The spectra were determined at concentrations low enough (usually 5×10^{-5} M)[19] to avoid intermolecular association (Beer's law was obeyed in this range). Due to the poor solubility of the compounds in water, solutions in 5% ethanol–95% water were employed after we had ascertained that the added ethanol did not alter significantly the extinction coefficients of the compounds (no significant differences were observed for Ade-C_3-Acr examined in both 5:95 and 1:99 ethanol–water solutions). In all cases a phosphate buffer (pH 6.9) was used to ensure total protonation of the aminoacridine ring, as is the case for quinacrine at physiological pH. As illustated in Figure 1, the spectra of the models Ade-C_3-Acr and Thy-C_3-Acr exhibit a strong decrease in the absorption and a shift to long wavelength. These changes are quite comparable to those observed for quinacrine intercalated in DNA.[16] This "hypochromic effect" is direct evidence of intramolecular ring–ring stacking. Hypochromism is observed when two chromophores are stacked one on top of other.[20] Quantitatively expressed by the percent of hypochromism (% *H*), it can be used as a measure of the interaction. The % *H* values for Ade-C_3-Acr and Thy-C_3-Acr, at 25 °C, were calculated for the long wavelength band of the acridine chromophore, as the absence of absorption of the nucleic acid bases in this region renders the experiments and calculations more precise. The values are given in Table I. A first observation is that both models exhibit an important hypochromic effect, indicative of intramolecular interaction between acridine and the base and corresponding to folded conformations. Comparison of the two systems

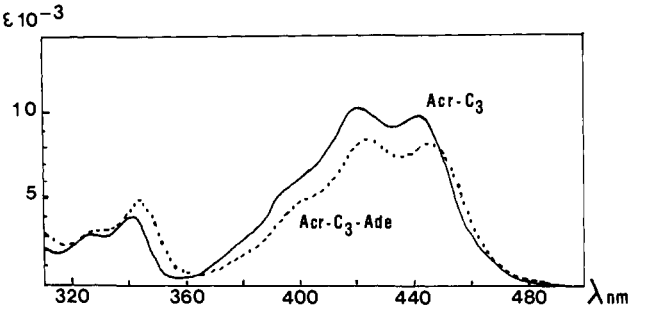

Figure 1. Comparative ultra-violet spectra of Ade-C_3-Acr (---) and Acr-C_3 (—), in H_2O–EtOH (99:1), at the same concentration of the acridine chromophore (0.5×10^{-5} M, pH 6.9, 25 °C).

Table I. Computed Percent Hypochromism (% *H*) for the 365–500-nm Absorption Band of Model Systems **3** and **4** at 25 °C

compd	solvent	% *H*
	water[a]	20
Ade-C_3-Acr (**3**)	ethanol	9.8
	water[a]	14
Thy-C_3-Acr (**4**)	ethanol	−2.0

[a] 95% water–5% ethanol, phosphate buffer (pH 6.9). The % *H* values are reproducible within ±0.5; they should be regarded with a tolerance of ±1%.

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(19) The experiments were run both at 5×10^{-5} and 0.5×10^{-5} M. No change could be detected. This absence of intermolecular contribution is further confirmed by the NMR experiments.

(20) For review, see: Bush, C. A. In "Basic Principles in Nucleic Acid Chemistry"; Ts'o, P. O., Ed.; Academic Press: New York, 1974; p 102.

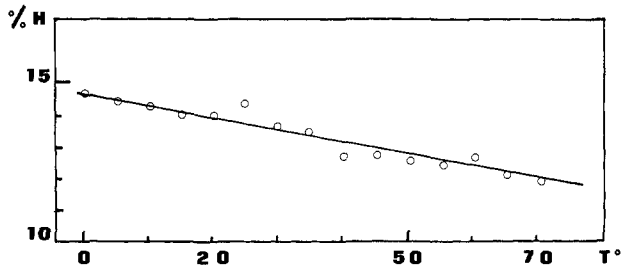

Figure 2. Variation of the percent hypochromism (% *H*) with temperature for Thy-C_3-Acr in H_2O–EtOH (95:5), phosphate buffer (5×10^{-5} M, pH 5.5) (% *H* obtained in the same conditions for Ade-C_3-Acr shows a quasi constant value of 20 in this temperature range). % *H* is computed for the 365–500-nm absorption band.

indicate that % *H* is higher for Ade-C_3-Acr than for Thy-C_3-Acr, which suggests that the adenine model is stacked (folded) to higher extent than the latter. This, indeed, corresponds to the well-known ability of purines to stack to a higher degree than pyrimidines.[20] However, this direct comparison cannot be quantitatively evaluated in terms of folding of the corresponding models since the magnitude of % *H* is not only dependent on the degree of stacking but also on the orientation of the transition moments.[21] However an important additional result is given by the investigation of the influence of temperature on the magnitude of % *H*. This technique has proved to be quite useful in the past,[6a] showing that % *H* usually decreases when the temperature is increased, which corresponds to the unfolding (or opening) of a stacked model with temperature. With Ade-C_3-Acr we came to the first example for which % *H* remains almost constant over the temperature range examined, from 0 to 80 °C. This, as confirmed by NMR experiments below, can be considered as convincing evidence that *the system remains essentially folded (or stacked) in water solution, even at 80 °c.*

The behavior of the pyrimidine model Thy-C_3-Acr is slightly different. It also exhibits appreciable, although smaller hypochromism at 20 °C, and the effect seems to decrease with increasing temperature. This may correspond to an unfolding of the system, and it is an indication of the weaker character of the acridine–thymine interactions as compared to the above adenine case. However, one must take note of the very weak influence of temperature on the magnitude of % *H* which diminishes only 2 units between 20 and 80 °C (% *H* = 14 and 12, respectively, at those temperatures). A reasonable interpretation could be that the system approaches 100% folding in the region examined. This would be in accordance with the NMR results, in which no significant variations can be observed with temperature.

^{1}H NMR Study. Investigation into the Self-Association of Ade-C_3, Thy-C_3, Acr-C_3, Ade-C_3-Acr, and Thy-C_3-Acr in Aqueous Solution. Mutual interactions between the two aromatic moieties in compounds of the type Ade-C_3-Acr and Thy-C_3-Acr should be readily observable by using ^{1}H NMR spectroscopy since both nucleotide bases, particularly purines,[10a] and acridines[10b] exhibit strong aromatic shielding effects as witnessed by their isoshielding curves. Indeed folding of Ade-C_3-Acr or Thy-C_3-Acr will give rise to upfield shifts of the proton signals with respect to the corresponding chemical shifts in the bases and the acridine residue alone. The experimental shielding effects would reflect the proportion of folded molecules. However, such direct measurements require an accurate determination of the proton chemical shifts of the free bases and the acridine system and, consequently, independent investigations into the self-association phenomena manifested by each of the two moieties involved in the folding $\rightleftharpoons$ unfolding process.

Measurements of the concentration dependence of the aromatic proton chemical shifts were performed under identical conditions for each of the compounds investigated. The positions of the proton signals of Ade-C_3 and Thy-C_3 were not significantly altered

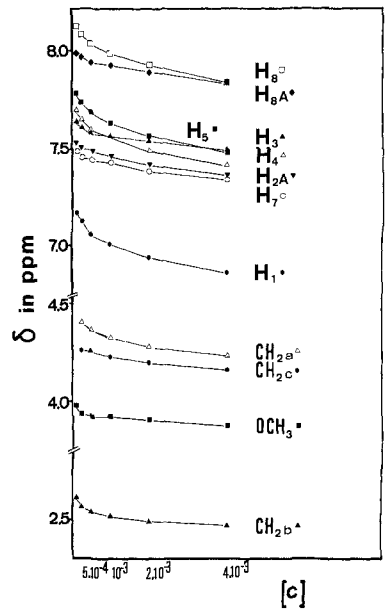

Figure 3. Concentration dependency for the proton chemical shifts of Ade-C_3-Acr in D_2O, 0.04 M sodium deuteroacetate buffer (pD 5.6, *T* = 21 °C).

on dilution of 10^{-2} M solutions to 10^{-4} M. These findings indicate the absence of self-association phenomena under these conditions. The very limited solubility of Acr-C_3 does not allow us to obtain an accurate determination of the self-association constant (K_{assoc}) of this compound. Nevertheless, the large shielding of the chemical shift in a narrow concentration range (2×10^{-4} to 5×10^{-5} M) permits us to estimate a value on the order of 400 L·M^{-1} for K_{assoc}.

The relatively good solubility of Ade-C_3-Acr and Thy-C_3-Acr facilitated the observation of the chemical shift variations of all protons, including the bridging methylene protons, over a large concentration range. As shown in Figure 3, all signals from Ade-C_3-Acr are deshielded with increasing dilution. These results demonstrate the presence of intermolecular stacking interactions involving both the acridine and the adenine rings. Since Ade-C_3 does not self-associate under similar conditions it is concluded that the chemical shift variations of adenine H-2 and H-8 are due to the stacking of an acridine residue of one Ade-C_3-Acr molecule onto the adenine moiety of another. If such is the case, various types of intermolecular associations would occur with the consequence that the Dimicoli and Helene relationship could not be used to calculate the self-association constants.[13]

The occurrence of several types of complexes in Ade-C_3-Acr is confirmed by the results obtained for Thy-C_3-Acr (Figure 4). For this compound only the acridine protons are deshielded at low concentrations. Consequently, only dimeric associations involving the stacking between two independent acridine rings seem to occur. Such a dimerization process gives rise to an association constant, K_{assoc} of 60 ± 10 L·M^{-1}. The values of the chemical shift differences ($\Delta\delta B_2$) for such a process can also be deduced from the corresponding $2\Delta\delta B_2$ data (Figure 4b). It is very interesting to note the similarities between these $\Delta\delta B_2$ values and those observed for stacked dimers of 7-chloro-2-methoxy-9-((dimethylamino)propyl)acridine.[15] All these features are in accordance with a dimerization process involving folded molecules and demonstrate the inferior stacking ability of pyrimidines compared to that of acridine and purine molecules.

Table II lists the ^{1}H chemical shift values extrapolated to infinite dilution for Ade-C_3, Thy-C_3, Acr-C_3, Ade-C_3-Acr, and Thy-C_3-Acr.

Investigation into the Intramolecular Stacking of Ade-C_3-Acr and Thy-C_3-Acr. The principal purpose of this study was to compare the strength of the stacking interactions between acridine and quinoline with pyrimidine and purine bases through a comparison of the folding tendencies of the different model compounds. Three types of experiments can afford evidence of such behavior.

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Table II. ^{1}H Chemical Shifts δ_0 (Ppm) Extrapolated to Zero Concentration for Ade-C_3, Thy-C_3, Acr-C_3, Ade-C_3-Acr, and Thy-C_3-Acr (D_2O, Sodium Deuterioacetate Buffer (0.04 M, pD 5.6, 21 °C)) and Differences between the δ_0's in the Corresponding Residues for Ade-C_3-Acr ($\Delta\sigma A$) and Thy-C_3-Acr ($\Delta\sigma B$)

	H-1	H-3	H-4	H-5	H-7	H-8	OCH_3	H-2A	H-8A	H_T	CH_3T
Ade-C_3								8.30	8.22		
Thy-C_3										7.53	1.90
Acr-C_3	7.69	7.67	7.78	7.82	7.51	8.39	4.04				
Ade-C_3-Acr	7.21	7.66	7.14	7.84	7.52	8.17	3.99	7.55	8.01		
Thy-C_3-Acr	7.62	7.67	7.79	7.87	7.53	8.32	4.02			7.13	1.46
$\Delta\sigma A$	0.48	0.01	0.04	−0.02	−0.01	0.22	0.05	0.75	0.21		
$\Delta\sigma B$	0.07	0.00	−0.01	−0.05	−0.02	0.07	0.02			0.40	0.44

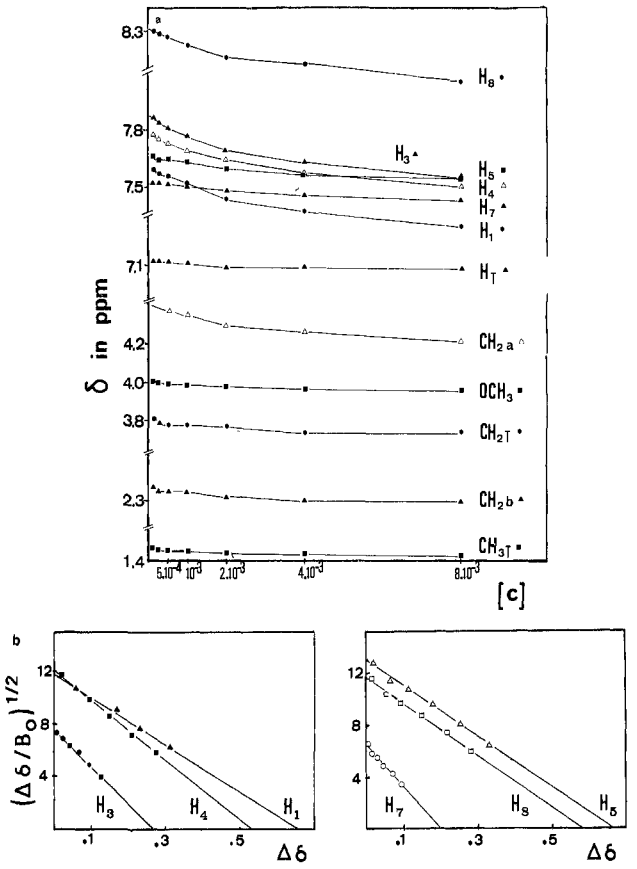

Figure 4. (a) Concentration dependency for the proton chemical shifts of Thy-C_3-Acr in D_2O, 0.04 M sodium deuteroacetate buffer (pD 5.6, T = 21 °C). (b) Self-association parameters of Thy-C_3-Acr obtained from curves of B_0 is the total concentration of Thy-C_3-Acr and $\Delta\delta$ is the difference between the extrapolated chemical shifts at zero concentration and the actual shifts at a given concentration. The x axis intercept gives $2\Delta\delta B_2$. $\Delta\delta B_2$ is the shielding of the acridine protons in the stacked dimer of Thy-C_3-Acr. The slope $(K/2\Delta\delta B_2)^{1/2}$ gives the association constant K.

(a) Comparative Study of ^{1}H Chemical Shifts in Ade-C_3, Thy-C_3, Acr-C_3, Ade-C_3-Acr, and Thy-C_3-Acr. The folding of the acridine ring onto the adenine or thymine moieties gives rise to large shielding of the base protons as compared to their respective positions in Ade-C_3 and Thy-C_3. Similarly, the aromatic purine ring induces strong shielding effects on the H-1 and H-8 acridine protons in Ade-C_3-Acr (Figure 5). These effects may be evaluated more quantitatively by $\Delta\sigma$, the difference in the chemical shifts of the corresponding protons extrapolated to infinite dilution (Table II). Under these conditions, the effects of self-aggregation of the various compounds are eliminated. As expected, the stronger induced diamagnetic field exhibited by purines with respect to pyrimidines results in a larger shielding of the acridine protons, H-1 and H-8, in Ade-C_3-Acr than in Thy-C_3-Acr.

Furthermore, in the spectrum of Ade-C_3-Acr the adenine H-1 and H-8 signals are significantly broadened at 21 °C (Figure 5), a phenomenon not encountered in either Ade-C_3-Q[6e,7] or Thy-C_3-Acr. This very interesting occurrence is not related to the

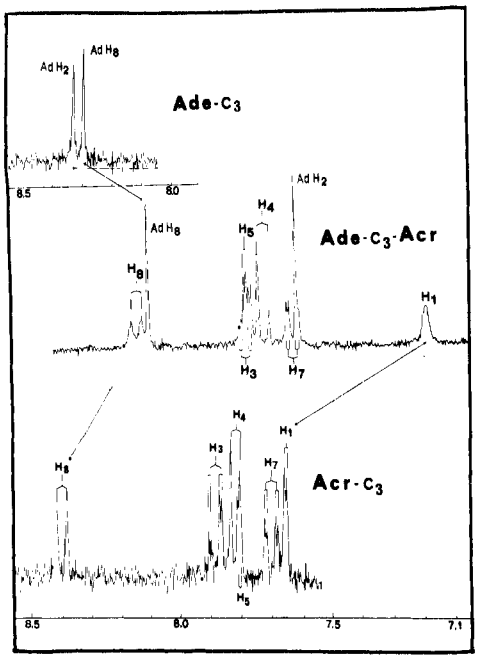

Figure 5. 270-MHz ^{1}H NMR spectra of Ade-C_3 (top), Ade-C_3-Acr (middle), and Acr-C_3 (bottom). All spectra were recorded at 5×10^{-4} M concentration in deuterioacetate buffer (pD 5.6, 21 °C).

presence of paramagnetic impurities since it remains unchanged after the addition of large amounts of EDTA. Moreover, the line widths of H-1 and H-8 are both dependent on the temperature and sensitive to the addition of organic solvents to the aqueous solution. Such dependence of the above phenomena on the conditions is highly indicative of intramolecular stacking processes.

Preliminary T_1 and NOE measurements indicate that the above selective signal broadening could be associated with a lowering of the motion of the methylene groups of the bridging chain in the folded conformation.

(b) Temperature Dependency of the Chemical Shifts in Ade-C_3-Acr and Thy-C_3-Acr. The existence of an equilibrium between the folded and unfolded forms can be corroborated by an investigation into the temperature dependency of the chemical shifts of the shielded protons. For both Ade-C_3-Acr and Thy-C_3-Acr the population of the folded forms appears to be quasi-independent of temperature since the differences in the chemical shifts between the corresponding protons in acridine, bases, and linked species remain almost constant in the temperature range 15–92 °C.

(c) Solvent Effect on the Folding Equilibrium. It is now well-established that organic solvents such as methanol, ethanol, and especially dimethyl sulfoxide are able to disrupt inter- and/or intramolecular complexes.[22] This phenomenon is probably due to a decrease of the van der Waals interactions induced by the aqueous medium.

Whereas the addition of small amounts of Me_2SO induces considerable unfolding of Ade-C_3-Q,[6e,7] the opening of Ade-C_3-Acr requires large quantities of this solvent as illustated by the variation

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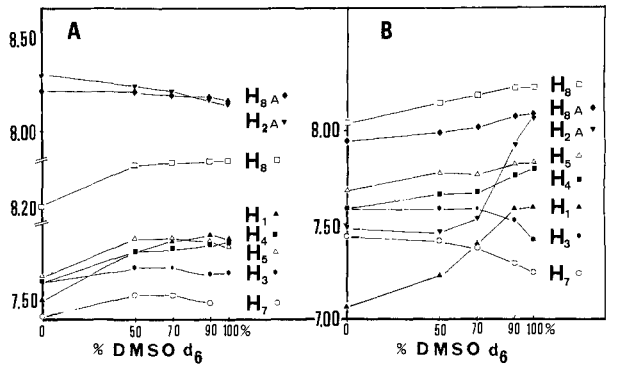

Figure 6. Variations of the proton chemical shifts of Ade-C_3, Acr-C_3 (A) and Ade-C_3-Acr (B) in mixed D_2O–Me_2SO-d_6 solvent. The spectra were recorded at 21 °C and 5×10^{-4} M.

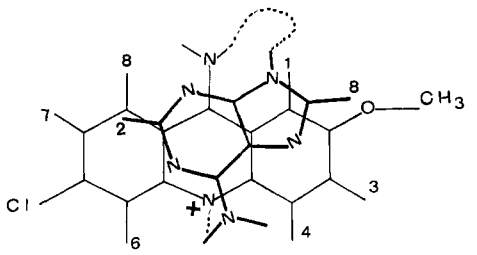

Figure 7. Proposed geometry for the stacked form of Ade-C_3-Acr, deduced from the comparison between the upfield shifts computed from isoshielding curves, $\Delta\delta$(comp), and the experimental shifts, $\Delta\delta$(exptl), extrapolated to zero concentration. The best fit (see materials and methods) gives the following $\Delta\delta$(comp) and $\Delta\delta$(exptl) values (ppm) for each proton, respectively: H-1, 0.55, 0.48, H-3, 0.09, 0.01, H-4, 0.11, 0.04, H-5, 0.07, 0.01, H-7, 0.02, 0.01, H-8, 0.26, 0.22, for acridine; H-2, 0.81, 0.75, H-8, 0.32, 0.21 for adenine.

in the chemical shifts of Ade-C_3-Acr as a function of the Me_2SO/D_2O ratio (Figure 6). Although the same solvent change study was carried out for Ade-C_3 and Acr-C_3, the curves reported in Figure 6 cannot be analyzed quantitatively, since several phenomena occur simultaneously (destacking of intermolecular aggregates, differences in solvation between Ade-C_3-Acr and its free moieties, etc.). However, it must be emphasized that only the adenine H-2 and H-8 and the acridine H-4 and H-5 protons in Ade-C_3-Acr exhibit the same chemical shifts in the corresponding free species. The persistence of shielding effects (~0.25 ppm) on the acridine H-1, -3, -7, and -8 protons in Ade-C_3-Acr in pure Me_2SO-d_6 might be due to residual ring–ring interactions involving only the acridine moieties.

Geometry of the Stacked Complexes. In the case of Thy-C_3-Acr the presence of the folded species is confirmed by the large shifts induced in the thymine ring and methyl protons by the large ring current of the acridine. The ring current of the thymine is very small, and the acridine protons are virtually unshifted. Given this paucity of data the shape of the folded form could not be established precisely. However in the case of Ade-C_3-Acr, comparison between the proton chemical shifts in Ade-C_3-Acr, Ade-C_3, and Acr-C_3 extrapolated to infinite dilution with the values calculated from the isoshielding curves of adenine[10a] and acridine[10b] established the geometry of the stacked form which is shown in Figure 7.

Taking into account the restriction of motion of the two rings, only one stacked geometry gives a good fit between the experimental and computed values. This folded form is characterized (1) by a superposition of the NH_2 group of adenine onto the positively charged nitrogen of acridine and (2) by a greater overlap of the ring bearing the methoxy group than the opposite one bearing the chlorine substituent.

We considered the usefulness of nuclear Overhauser effects in confirming the proposed geometry. With the exception of Ade H-2 and Acr H-6, all protons of the complex are relaxed by proximate H or CH_2 groups in the same plane. Moreover the distance between these protons, one in the adenine and one in the acridine, in the folded form is expected to be at least 3.4 Å, which is about the limit for detecting an NOE in dilute solution in a medium containing residual HOD protons.[23,24] In the event, irradiation of Acr H-8 led to a 6% increase in the integral of Ade H-2 which is in accord with the proposed model.

It is interesting to notice that this kind of geometry is also found in the crystal of minihelical complexes between proflavine and CpG.[25] It was also recently evidenced by ^1H NMR spectroscopy in D_2O in the intercalated complexes of 6-chloro-2-methoxy-9-aminoacridine derivatives with the autocomplementary ribonucleoside monophosphate CpG.[12b] Thus it seems that in Ade-C_3-Acr the stacking between the intercalating compound and the base already mimics the situation found at the DNA minihelix level.

Conclusion

We have observed acridine–nucleic acid bases stacking interactions in water by means of hypochromism in the UV and by ^1H NMR spectroscopy. We show that these two techniques are quite complementary to obtain detailed information about the complex.

In the past we ran a comparable study in the chloroquine series by preparing models of the type base-$(CH_2)_3$-quinoline.[6] Comparison of the properties of those very simplified models, in the acridine and in the quinoline families, leads to very clear results which are quite in accordance with the known intercalation properties of the corresponding molecules. The acridine–purine interactions are surprisingly strong in water, as indicated by the total folding of Ade-C_3-Acr, even at 80 °C. Moreover, the tendency of the rings to associate is also indicated by the persistance of intramolecular stacking in the presence of organic solvents such as ethanol or Me_2SO.

By comparison, in the corresponding quinoline model Ade-C_3-Q, we observed a stacking of 100% in water at 20 °C, which decreased to about 70% at 60 °C and disappeared in Me_2SO.[6c] Furthermore weaker interactions were also observed for quinoline linked to the thymine ring.

The acridine ring is known to participate quite effectively in ring–ring stacking interactions[26] and a number of derivatives, including proflavine, acridine orange, ..., are typical intercalators in polynucleotides.[4,27] Our results demonstrate the much stronger nature of the forces involved in the interaction of the larger quinacrine ring with the nucleotide bases, as compared to those exhibited by the quinoline ring in chloroquine. As a consequence, purely from the thermodynamic point of view of the strength of the drug–nucleotide base interactions, all our observations agree that acridines are more prone to intercalate in nucleic acids than quinolines.

Registry No. 1, 54-05-7; **2**, 69-05-6; **3**, 79953-24-5; **4**, 79953-25-6; **6**, 7478-26-4; **7**, 21708-31-6; **8** HCl, 54517-89-4; **9**, 79953-26-7; **10**, 79953-27-8; **11**, 79953-28-9; adenine, 73-24-5; thymine, 65-71-4; 3-amino-1-hydroxypropane, 156-87-6; propylamine, 107-10-8; Ade-C_3, 707-98-2; Thy-C_3, 22919-49-9.

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