residue was taken up with water and extracted with ethyl acetate. The organic layer was washed with water, dried, and evaporated. The crude oil was chromatographed on silica gel, eluting with chloroform–methanol (97:3). Pure **77** was obtained and recrystallized from ethyl ether (6.6 g, 94%, mp 115–116 °C): NMR ($CDCl_3$) δ 1.13 (d, 6 H, J_{CH-CH_3} = 6.5, $(CH_3)_2CH$), 2.60 (tt, 2 H, $J_{CH_2-CH_2}$ = 5.5–0.5, CH_2-7), 2.77 (t, 2 H, CH_2-6), 3.00 (sept, 1 H, $CH(CH_3)_2$), 3.73 (t, 2 H, CH_2-4), 5.27 (s, 2 H, CH_2Ph), 7.0–7.7 (m, 9 H, arom). Compounds **76–79** were obtained similarly.

Registry No. 1, 87628-26-0; **2**, 87628-31-7; **3**, 87628-33-9; **4**, 87628-25-9; **5**, 87628-32-8; **6**, 87628-27-1; **7**, 87628-30-6; **8**, 87628-29-3; **9**, 87628-28-2; **10**, 87628-34-0; 11, 87628-38-4; **12**, 87628-74-8; **13**, 87628-87-3; **14**, 87628-77-1; **15**, 87628-73-7; **16**, 87628-76-0; **17**, 87628-82-8; **18**, 87628-90-8; **19**, 87628-91-9; **20**, 87628-92-0; **21**, 87628-93-1; **22**, 87628-94-2; **23**, 87629-00-3; **24**, 87629-01-4; **25**, 95936-04-2; **26**, 87629-10-5; **27**, 87628-78-2; **28**, 87628-75-9; **29**, 87628-50-0; **29**·2HCl, 87642-29-3; **30**, 87628-43-1; **31**, 87628-45-3; **32**, 87642-31-7; **33**, 87628-44-2; **34**, 87628-52-2; **34**·HCl, 95936-12-2; **35**, 87642-33-9; **35**·2HCl, 87642-32-8; **36**, 87628-42-0; **37**, 87628-51-1; **37**·HCl, 95936-13-3; **38**, 87628-46-4; **39**, 87628-48-6; **40**, 87642-37-3; **40**·CHO_3S, 87642-36-2; **41**, 87629-19-4; **42**, 95936-05-3; **42**·$C_7H_8O_3S$, 87629-13-8; **43**, 87629-16-1; **44**, 87629-12-7; **45**, 87629-18-3; **45**·CH_4O_3S, 87629-17-2; **46**, 87642-56-6; **46**·CH_4O_3S, 87642-55-5; **47**, 87642-58-8; **47**·CH_4O_3S, 87642-57-7; **48**, 87642-70-4; **48**·CH_4O_3S, 87642-69-1; **49**, 87642-72-6; **49**·CH_4O_3S, 87642-71-5; **50**, 87642-39-5; **50**·HCl, 87642-38-4; **51**, 87642-52-2; **51**·CH_4O_3S, 87642-51-1; **52**, 87642-54-4; **52**·CH_4O_3S, 87642-53-3; **53**, 87642-62-4; **53**·CH_4O_3S, 87642-61-3; **54**, 87629-31-0; **54**·HCl, 95936-14-4; **55**, 87629-15-0; **56**, 87629-26-3; **56**·HCl, 95936-15-5; **57**, 95936-06-4; **57**·$C_6H_8O_7$, 87628-59-9; **58**, 87628-63-5; **59**, 95936-07-5; **59**·$C_6H_8O_7$, 87628-61-3; **60**, 95936-08-6; **60**·$C_7H_8O_3S$, 87628-65-7; **61**, 87629-37-6; **61**·CH_4O_3S, 87629-36-5; **62**, 87629-39-8; **62**·CH_4O_3S, 87629-38-7; **63**, 87628-06-6; **63**·CH_4O_3S, 87628-05-5; **64**, 87628-08-8; **64**·CH_4O_3S, 87628-07-7; **65**, 87629-84-3; **65**·CH_4O_3S, 87628-18-0; **66**, 87628-21-5; **66**·CH_4O_3S, 87628-20-4; **67**, 87642-74-8; **67**·CH_4O_3S, 87642-73-7; **68**, 87628-02-2; **69**, 87628-04-4; **69**·HCl, 87628-03-3; **70**, 87628-12-4; **70**·HCl, 87628-11-3; 71, 87629-48-9; 71·CH_4O_3S, 87629-47-8; **72**, 87628-69-1; **72**·HCl, 95936-16-6; **73**, 95936-09-7; **73**·$C_3H_4O_4$, 87628-67-9; **74**, 95936-10-0; **74**·$C_3H_4O_4$, 87628-70-4; **75**, 95936-11-1; **76**, 87629-61-6; **76**·CH_4O_3S, 87629-60-5; **77**, 87628-22-6; **78**, 87629-66-1; **78**·HCl, 95936-17-7; **79**, 87628-24-8; **79**·HCl, 87628-23-7; *p*-FC_6H_4COCl, 403-43-0; N_2H_4, 302-01-2; $PhCH_2Br$, 100-39-0; *p*-BrC_6H_4F, 460-00-4; $(CH_3)_2CHBr$, 75-26-3; morpholine, 110-91-8; 1-acetyl-3-(4-fluorobenzoyl)-4-piperidinone, 87642-26-0; *N*-acetyl-4-piperidinone, 32161-06-1.

The Intercalation of 6-Chloro-substituted-9-[[3-(dimethylamino)propyl]amino]acridines with DNA

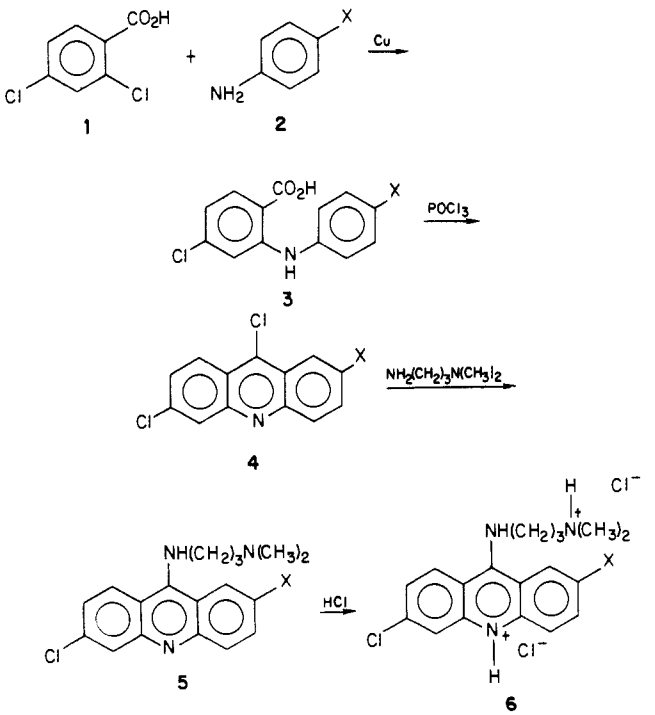
S. E. Kitchen, Yueh-Hwa Wang, A. L. Baumstark, W. D. Wilson,* and D. W. Boykin*

Department of Chemistry and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303–3083. *Received October 15, 1984*

A series of 6-chloro-2-substituted-9-[[3-(dimethylamino)propyl]amino]acridines has been prepared. The binding affinities and the unwinding angles for the acridine derivatives, relative to ethidium, were determined from viscometric titrations with ccs-DNA. The binding affinities were the same, within experimental error, ca. 2.0×10^{-5}. Similarly, with the exception of 11, the unwinding angles were close to 17°. For 11 the unwinding angle (12°) was smaller than the other derivatives. The general insensitivity of the apparent binding constants to substituent effects is attributable to a masking effect of the formal charge on the ring. The smaller unwinding angle for 11 is believed to arise from its relative dissymmetry, resulting in a "wedge" effect upon intercalation.

The intercalation of planar aromatic molecules with the DNA double helix[1,2] is considered to be important in mutagenesis, carcinogensis,[3] and the medicinal action of antibacterial,[4] antiparasitic,[5] and antineoplastic drugs.[1-3] The interaction of acridines with DNA is important for medicinal,[1-5] cytogenetic,[6] and intercalation modeling[7] studies. Quinacrine and closely related analogues are important in treating malaria[5] and in chromosome-banding studies in cytogenetics.[6] A series of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide analogues has exhibited excellent anticancer activity and DNA has been identified as a probable primary bioreceptor in their antineoplastic action.[8]

Scheme I

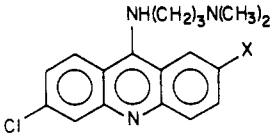


Although acridines such as proflavin and quinacrine were among the first compounds used by Lerman in de-

(1) Wilson, W. D.; Jones, R. L. In "Intercalation Chemistry"; Whittingham, M. S., Jacobson, A. J., Eds.; Academic Press: New York, 1981; Chapter 14.
(2) Wilson, W. D.; Jones, R. L. In "Advances in Pharmacology and Chemotherapy"; Hawking, F., Ed.; Academic Press: New York, 1981; pp 117–222.
(3) Waring, M. J. *Annu. Rev. Biochem.*, **1981**, *50*, 159.
(4) Waring, M. J. In "The Molecular Basis of Antibiotic Action", 2nd Ed.; Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. J., Eds.; Wiley: London, 1981.
(5) Steck, E. A. In "The Chemotherapy of Protozoan Diseases"; U.S. Government Printing Office: Washington, DC, 1973; pp 23, 77–123, 177.
(6) Comings, D. E. *Annu. Rev. Genet.* **1978**, *12*, 25.
(7) Lopp, I. G.; Wilson, W. D. *Biopolymers* **1979**, *18*, 3025.
(8) Bagulay, C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170, 520.

0022-2623/85/1828-0940$01.50/0

Table I. 2-Substituted 6-Chloro-9-(diamino-substituted)acridines



no.	X	mp, °C	% yield	formula[c]	λ_{max}, nm	(ϵ, M^{-1} cm^{-1})[d]
7	CH_3	120–121	37	$C_{19}H_{22}ClN_3$	413	(9735)
8	Cl	122–123	23	$C_{18}H_{19}Cl_2N_3$	415	(11520)
9	OCH_3	256–258[a]	66	$C_{19}H_{22}ClN_3O\cdot 2HCl\cdot 2H_2O$	421	(8950)
10	F	236–237	43	$C_{18}H_{19}ClFN_3\cdot HCl\cdot H_2O$	414	(8300)
11	H	228–230[b]	53	$C_{18}H_{20}ClN_3\cdot 2HCl$	409	(9115)
12	SCH_3	250–252	12	$C_{19}H_{22}ClN_3S\cdot 2HCl\cdot H_2O$	436	(7200)

[a] Lit.[29] mp 225 °C. [b] Lit.[30] mp 232–233 °C. [c] Anal. C, H, N. [d] Value calculated from a linear least-squares program using data obtained from a Beer's law determination.

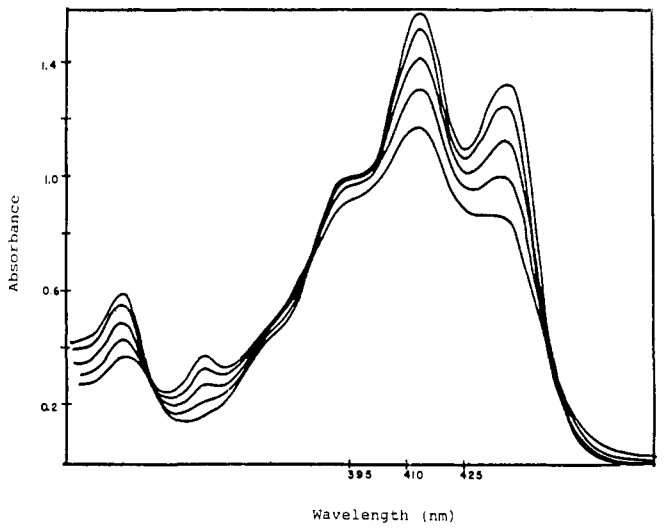


Figure 1. Spectrophotometric titration of compound 10 with NaOH. The absorption spectrum of the acridine at pH 4.0 is that having the greatest absorbance at 414 nm.

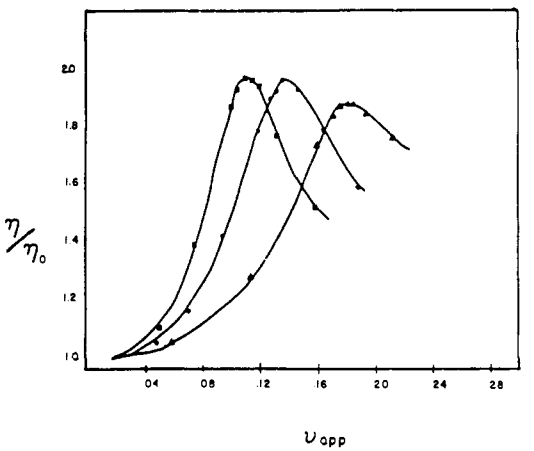


Figure 2. Viscometric titration of closed circular superhelical DNA with compounds 11 (▲), 8 (■), and 7 (●). The reduced specific viscosity ratio (η/η_0 where $\eta = \eta_{sp}/c$) is plotted as a function of moles of acridine added per moles of DNA base pairs (ν_{app}).

fining the intercalation binding mode with DNA,[9] much remains to be learned about their specific interactions with DNA and how these interactions are perturbed by substituent changes on the acridine nucleus. Studies to define these factors should be of significant benefit in designing medically useful acridines. For this purpose we have synthesized quinacrine analogues, 6-chloro-2-substituted-9-[[3-(dimethylamino)propyl]amino]acridines (Table I), which vary in substituent size and electronic character. Using viscometric titrations with closed circular superhelical DNA, we have determined the effects of these substituent changes both on the complex structure using the sensitive method of unwinding angle determinations and on the intercalation binding constant.

Results

Chemistry. The 6-chloro-2-substituted-9-[[3-(dimethylamino)propyl]amino]acridines were synthesized in a four-step process as outlined in Scheme I. Condensation of para-substituted anilines with 2,4-dichlorobenzoic acid in the presence of copper bronze gave the corresponding substituted diphenylamine carboxylic acids.[10,11] The diphenylaminecarboxylic acids on treatment with phosphorus oxychloride were converted into substituted 9-chloroacridines in good yields.[11] The desired 9-[[3-(dimethylamino)propyl]amino]acridines were obtained by the reaction of the 9-chloroacridines with 3-(dimethylamino)propylamine in phenol; usually the final compounds were isolated as hydrochloride salts.

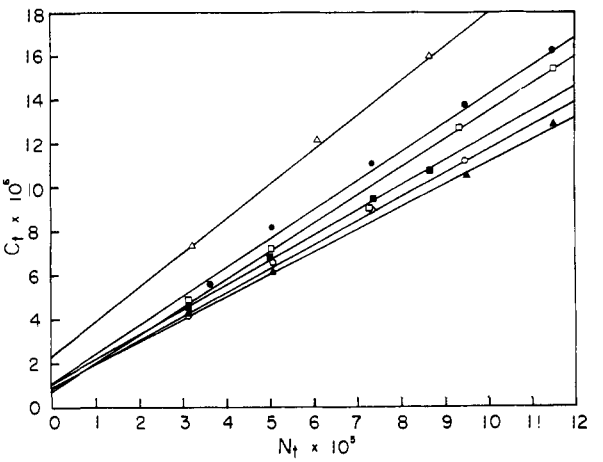


Figure 3. A plot of C_t vs. N_t according to eq 1 for compounds 11 (△), 12 (●), 7 (□), 10 (■), 9 (○), and 8 (▲). The slopes and intercepts were calculated using a linear regression computer program. C_t is the total acridine concentration and N_t is the DNA concentration in nucleotides.

Closed Circular DNA. The viscometric titration profiles for compounds 7, 8, and 11, as examples, with ccs-DNA are illustrated in Figure 2. All three compounds

(9) Lerman, L. S. *J. Mol. Bio.* **1961**, *3*, 18.
(10) Varma, R. S.; Whisenant, L. K.; Boykin, D. W. *J. Med. Chem.* **1969**, *12*, 913.
(11) Albert, A., "The Acridines"; 2nd Ed.; St. Martin's Press: New York, 1966; p 50.

Table II. Unwinding Angles and Calculated Apparent Equilibrium Binding Constants for Substituted Acridines

compd	ν^a	ϕ^b	$10^{-5}K_{app}{}^c$ (ω = 1.00)
7	0.128	15.7	2.9
8	0.102	19.6	1.6
9	0.109	18.3	2.1
10	0.114	17.6	1.6
11	0.160	12.5	1.3
12	0.130	15.4	2.0
quinacrine[16]	0.126	15.9	
ethidium	0.077	26	

[a] The error is approximately ±0.01 and represents the 95% confidence limits calculated from a linear least-squares computer program and is used to calculate an error of approximately ±1° in the unwinding angle, ϕ. Correlation coefficient >0.997 in all cases. [b] Determined according to eq 2, assuming ϕ = 26° for ethidium bromide. [c] Apparent equilibrium binding constants were calculated using the ν and C_f values obtained from Figure 3 and eq 1.

give the typical maxima seen for intercalating ligands.[1-4,12] From the figure it can be seen that the maxima occur at different [drug]/[DNA] ratios. The DNA and drug concentrations at the peak for each curve define the values for N_t and C_t, respectively, in eq 1 (Experimental Section). The N_t and C_t values from Figure 2 are plotted in Figure 3, from which ν and C_f are obtained by eq 1[12] (Experimental Section). The ν values determined from Figure 3 allow calculation of unwinding angles for acridine derivatives relative to ethidium according to eq 2 (Experimental Section). The results from Figure 3 are collected in Table II.

As can be seen from Figure 3 and Table II, ν values for this series of acridine compounds fall in the range 0.115 ± 0.015, with the exception of the larger ν (and therefore smaller unwinding angle) for the acridine 11 unsubstituted in the 2-position. The free drug concentration (C_f) in equilibrium with the complex is obtained from the intercept on the C_t axis in Figure 3. The fact that straight lines are obtained in Figure 3 suggests that eq 1 is valid for these compounds over the range of these experiments.

Linear DNA. Because of the significant differences in unwinding angles found between 8 and 11, viscometric titrations were carried out with sonicated calf thymus DNA. The results of this study are shown in Figure 4. The 2,6-dichloro compound 8 is seen as causing a greater change in the η/η_0 value as a function of ν compared to the 2-H compound 11; however, the difference is not as pronounced as the difference in unwinding angles seen with superhelical DNA. At ν values above 0.6, the viscosity enhancement, as reflected in the η/η_0 values, is seen to plateau due to saturation of binding sites.

pK_a Determinations. The pK_a determinations were made spectrophotometrically by following absorbance as a function of pH as previously described.[13] Absorption spectra on all compounds studied were obtained in the visible range. The λ_{max} of the compounds are listed in Table I. Spectra shifts of the substituted acridine dihydrochlorides obtained upon titration with NaOH are illustrated in Figure 1. Periodic scans of the absorbance spectrum were performed to ensure isosbestic behavior. Loss in isosbestic behavior that was commonly encountered when a pH above 9.5 was attained may be attributed to aggregation of the uncharged acridine rings as well as approaching the pK_a of the tertiary amine present in the aliphatic side chain. Data outside of the isosbestic region were not used in calculating pK_a values. The pK_a determinations were performed on the compounds that would provide the widest range in electronic substituents at the 2-position of the acridine ring. As evidenced from the results for compounds 8–10, pK_a's 7.8, 8.1, and 7.7, respectively, substituents at the 2-position of the acridine ring have a small effect on the basicity of the ring nitrogen within the range of substituents that were studied.

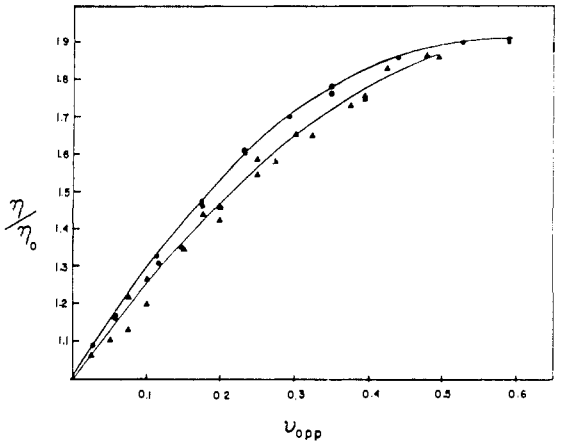


Figure 4. Viscometric titrations of sonicated calf thymus DNA with compounds 8 (▲) and 11 (●). The reduced specific viscosity ratio (η/η_0 where $\eta = \eta_{sp}/c$) is plotted as a function of moles of acridine per mole of DNA base pairs (ν_{app}).

Binding Constants. For all compounds studied, approximate binding affinities were calculated by using eq 3 (Experimental Section) from the ν and C_f values determined from Figure 3. The results are presented in Table II. All binding affinities are near 2×10^5 M^{-1}, and there is no correlation with σ.

Discussion

The method developed by Vinograd and co-workers[14] for evaluating data obtained from viscometric experiments performed with ccs-DNA allows the determination of ν and C_f according to eq 1 (Experimental Section). With values for ν and C_f, apparent binding constants and unwinding angles can be calculated by using eq 3 and 2, respectively. Examination of Figure 3, a typical Vinograd plot, reveals that there is little difference in the intercepts of these compounds, which indicates that the amount of free drug in solution (C_f) was roughly the same for each. Also, the slopes (ν values) of the compounds were similar, indicating that the amount of drug bound at any given DNA concentration varied very little for this series. Thus, as Table II shows, the binding constants for these drugs did not vary significantly from one another. This finding indicates that the electronic nature of the substituents in the 2-position have, within experimental error, no influence on the binding affinity of these acridines for DNA. It can be concluded that substituent effects are being masked, perhaps by the formal charge present on the ring nitrogen.

Results with a limited series of naphthothiophene-ethanolamine derivatives suggested that the binding constant of these intercalators significantly increases as the electron-withdrawing character of ring substituents increases.[15,16] Le Pecq and co-workers[17] have analyzed the

(12) Jones, R. L. "The effect of Ligand Structure and Ionic Strength Upon DNA–Ligand Unwinding Angles"; Masters Thesis, Georgia State University, Atlanta, GA, 1979.
(13) Jones, R. L.; Wilson, W. D. *Biopolymers* **1981**, *20*, 141.
(14) Revet, B. M.; Schmir, M.; Vinograd, J. *Nature (London), New Biol.* **1971**, *229*, 10.
(15) Panter, J. W.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem.* **1973**, *16*, 1366.
(16) Olmstead, E. J.; Panter, J. W.; Boykin, D. W.; Wilson, W. D. *Biochemistry* **1975**, *14*, 521.

binding of several 9-substituted ellipticine derivatives with DNA; no correlation between their DNA binding results and the electronic character of the ellipticine substituents is apparent. Cain and co-workers[8] have synthesized a series of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide analogues with varying electronic substituents. They qualitatively measured affinity by determining the amount of compound needed to decrease the fluorescence of ethidium bound to poly[d(A·T)] by 50%. There seems to be no significant correlation between their relative binding results and the electronic character of the substituents on the acridine ring (other parameters were checked by Cain and co-workers[8] and no good correlations between any parameter or parameter set and the relative binding results could be found). No analysis of intercalation or whether all of these molecules do bind to DNA by intercalation was made. Müller and Crothers[18] in their study of G·C specificity measured the relative binding affinity for four series of aromatic heterocycles containing oxygen, nitrogen, and sulfur in the same location within the heteroaromatic ring; a correlation between polarizability and the G·C binding specificity of these heterocycles was noted.

The effect that the various substituents had on the unwinding angles are shown in Figure 3 and tabulated in Table II. As can be seen, with the exception of 11, the unwinding angles for this series of acridines are 17 ± 2°. This finding agrees with earlier investigations conducted in our laboratories which suggested that most acridines unwound ccs-DNA by an amount that was rather constant, usually by approximately 17°.[12,19] These studies were conducted on acridine rings that were similarly substituted, with substituents positioned in a manner that imparted some degree of symmetry to the molecule. It has been noted previously that a dissymmetrically substituted acridine exhibits an unwinding angle significantly lower than that of its symmetrical counterpart.[12] Proflavin was found to have an unwinding angle of 20.4 (under identical ionic strength conditions as the experiments reported herein), while *N*,*N*-dimethylproflavin had an unwinding angle of 14.5.[12] Thus, the steric environment that is present when intercalation occurs appears to have a measurable influence on the unwinding angle, and this may also account for the lower value for 11. Experimental findings similar to these have been observed previously;[20-22] Gabbay and co-workers have proposed that dissymmetrically substituted molecules may bend the double helix upon intercalation and result in an unwinding angle that is aberrant from that usually observed.[12] The more highly substituted systems, particularly ones with the substituents symmetrically positioned, were observed to effect the largest enhancement in viscosity upon binding to DNA. Considering the "thickness"[22] of the aromatic ring to be dependent on the position and the size of the substituents, Gabbay proposed that nonuniform thickness of the aromatic ring results in a "wedge" effect upon intercalation, which causes a bending in the DNA and a relative decrease in the degree of viscosity enhancement.

Comparison of the unwinding angles for the various compounds in this acridine series reveals that the compound that most closely approximates being "symmetrical", the 2,6-dichloro compound 8 was found to have the largest unwinding angle. Conversely, the compound with the most nonuniform ring thickness, the 6-chloro 2-H compound 11, has the smallest unwinding angle. The remaining compounds have unwinding angles that closely approximate that of quinacrine. These results appear to be consistent with the proposal by Gabbay and also with the unwinding behavior demonstrated by symmetrically and asymmetrically substituted proflavin compounds.[19]

The results from this study offer several particularly significant findings. First, from the small differences seen in the DNA binding constants for this series of acridines, it is concluded that, under our experimental conditions, the intercalating binding interaction was relatively insensitive to the influence of different electronic substituents in the 2-position of the acridine nucleus (maximum free energy difference of less than 0.5 kcal). The minor variation between the side chain present on the compounds in this study and that on quinacrine appeared to have little effect on the apparent binding constants or the unwinding angle, based on comparison of the behavior of quinacrine and its 2-methoxy 6-chloro analogue.[9] Another finding was the effect of substitution on the unwinding angles for these compounds. The rather large variation in unwinding angle for the 2-H compound 11 was unexpected on the basis of our previous studies with acridines. The results of this study suggest that acridine rings bearing substituents with significantly different steric requirements exhibit different unwinding angles from those systems with substituents of similar (more symmetrical) steric character. Finally, Cain and co-workers have shown that a large series of antitumor acridines act at the DNA level in vivo.[8] In agreement with the results described here, they have shown that electronic substituent effects in the acridine ring are not well correlated with DNA interactions or biological effects.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra on all new compounds were recorded with a Perkin-Elmer 710B spectrometer. ^1H and ^{13}C spectra were recorded on a Varian 360L or a JEOL-FX60Q instrument. All spectra were in accord with the structures assigned. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA, and were within 0.4% of theoretical values. Listed below are typical synthetic procedures used to prepare the acridines required for study.

Typical Procedure for the Preparation of 5-Chloro-4′-substituted-diphenylamine-2-carboxylic Acids 3. Dry K_2CO_3 (45 g) was slowly added to a solution of 2,4-dichlorobenzoic acid (28.7 g, 0.15 mol), *p*-fluoroaniline (20.8 g, .19 mol), and isoamyl alcohol (125 mL); copper bronze (1.5 g, washed with ether and stored under hexane) was added, and the contents were allowed to reflux for 3 h. The isoamyl alcohol was removed by steam distillation and the mixture was poured into 1 L of hot water. The mixture was acidified with concentrated HCl. The greenish-brown precipitate which formed was filtered, washed with hot water, and collected. The crude acid was dissolved in aqueous NaOH solution, treated with animal charcoal (1 h), and acidified with concentrated HCl. The greenish-yellow acid was then filtered, washed with hot water, and dried (31.5 g, 79% yield). Recrystallization from a 10% aqueous methanol solution yielded yellow needles (21 g, 56%), mp 215–216 °C (lit.[23] mp 212–213 °C).

(17) Le Pecq, J. B.; Le Bret, M.; Gosse, C.; Paoletti, C.; Chalvet, O.; Dat-Xuong, N. In "Molecular and Quantum Pharmacology"; Bergmann, E., Pullman, B., Eds.; D. Reidel Publishing Co.: Dordrecht, Netherlands, 1974; pp 515–535.
(18) Müller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267.
(19) Wilson, W. D.; Jones, R. L.; Lanier, A. C.; Keel, R. A. *Nucleic Acids Res.* **1980**, *8*, 1613.
(20) Gabbay, E. J.; Adawadkar, P. D.; Kapicak, L.; Pearce, S.; Wilson, W. D. *Biochemistry* **1976**, *15*, 146.
(21) Gabbay, E. J.; Adawadkar, P. D.; Wilson, W. D. *Biochemistry* **1976**, *15*, 152.
(22) Gabbay, E. J.; Scofield, R. E.; Baxter, C. S. *J. Am. Chem. Soc.* **1973**, *95*, 7850.
(23) Bradlow, H. L.; Vandewerf, C. H. *J. Am. Chem. Soc.* **1948**, *70*, 654.

The physical constants for the diphenylamine-2-carboxylic acids were in accord with literature values: 4'-Cl,[24] 4'-F,[23] 4'-CH_3,[25] 4'-H,[25] and 4'-SCH_3.[26]

Typical Preparation of 6,9-Dichloro-2-substituted-acridines (4). A mixture of 5-chloro-4'-fluorodiphenylamine-2-carboxylic acid (6 g, 0.023 mol) and 30 mL of $POCl_3$ was heated under reflux for ca. 2 h, and then the contents were slowly added to a large excess of ice-NH_4OH. Care was taken to maintain alkalinity until all the $POCl_3$ had undergone hydrolysis. The product was filtered, washed with dilute NH_4OH, promptly dried in a desiccator over KOH, and recrystallized from benzene (5.3 g, 87%), mp 168–169 °C. Anal. ($C_{13}H_6Cl_2FN$) C, H.

The physical constants for the 6,9-dichloro-2-substituted-acridines were in accord with literature values: 2-Cl,[27] 2-CH_3,[28] 2-H,[25] 2-SCH_3.[26] The 2-OCH_3 compound was commercially available (Aldrich).

Typical Preparation of 6-Chloro-2-substituted-9-[[3-(dimethylamino)propyl]amino]acridines (5) and Their Hydrochlorides (6). A mixture of 2-fluoro-6,9-dichloroacridine (3.5 g, 0.013 mol), 3-(dimethylamino)propylamine (1.46 g, 0.014 mol), and 33 mL of dry phenol ws heated at ca. 95–100 °C for 1.5 h. The reaction mixture was poured into a beaker containing ca. 400 mL of 1 M NaOH. The product appeared as a reddish-brown, gummy precipitate, which was subsequently extracted into methylene chloride. The methylene chloride phase was recovered and subsequently subjected to a series of extractions consisting of CH_2Cl_2/aqueous NaOH, CH_2Cl_2/H_2O, and CH_2Cl_2/saturated aqueous NaCl. The methylene chloride phase displayed marked fluorescence. Frequently, the 9-aminoacridines displayed limited solubility in methylene chloride and required the addition of a small amount of chloroform or methanol to clear the solution, which appeared to be colloidal. HCl gas was bubbled into the methylene chloride and a yellow crystalline precipitate formed. Methanol was added to dissolve the salt. A small amount of hexane was then added, and yellow crystals slowly appeared, which were filtered and collected (2.3 g, 43%), mp 236–237 °C. Data for the 6-chloro-2-substituted-9-[[3-(dimethylamino)propyl]-amino]acridines are listed in Table I.

Viscometric and Binding Measurements. Linear and ccs-DNA were prepared and viscometric titrations with these DNA's were conducted at 25 °C in Pipes buffer (0.01 M Pipes, 10^{-3} M EDTA, 0.1 M NaCl, pH 7.0) in Cannon-Ubbelohde semimicro capillary viscometers with electronic timing as previously described.[19] The viscometric titrations with DNA were conducted as a function of DNA concentration and the DNA (N_t) and drug (C_t) concentrations at the maxima in the titration (e.g., Figure 2) were plotted (Figure 3) as suggested by eq 1. The slope of

$$C_t = \nu N_t + C_F \quad (1)$$

this plot gives ν the moles of drug bound per DNA base pair at the point at which all superhelical turns are removed from the DNA and the intercept give the free drug concentration, C_f, in equilibrium with the complex. The unwinding angle for an unknown drug, ϕ_u, can be determined if ν_u is measured along with ν_s for a standard of known unwinding angle, ϕ_s:

$$\nu_s\phi_s = \nu_u\phi_u \quad (2)$$

The binding isotherm of intercalators and ccs-DNA is described by

$$\nu/C_f = K\frac{(1 - 2\nu)^2}{(1 - \nu)} \exp[-a(\nu - \nu') - b(\nu - \nu')^2] \quad (3)$$

where a and b are constants, ν' in this equation represents the moles of drug bound per DNA base pairs at the maximum in a viscometric titration, and K is the observed binding constant. At the maximum in a viscometric titration $\nu = \nu'$ and the exponential term becomes equal to 1. Since ν and C_f values can be determined at the maximum in a titration from Vinograd plots, as illustrated in Figure 3, a value for K can be determined. Since equilibrium constants determined in this way are not from complete binding isotherms and are not, for example, corrected for cooperativity in binding, they should be viewed essentially as relative binding affinities for a series of compounds under identical experimental conditions.

Acknowledgment. This work was supported by NSF Grant PCM83-09575 and the Georgia State University Research Fund. W.D.W. is the recipient of an American Cancer Society Faculty Research Award. A.L.B. is a fellow of the Camille and Henry Dreyfus Foundation, 1981–1986.

Registry No. 1, 50-84-0; 2, 371-40-4; 3 (X = F), 2367-04-6; 4 (X = F), 394-88-7; 7, 96394-30-8; 8, 96394-31-9; 9, 59962-52-6; 10, 96394-32-0; 11, 35604-84-3; 12, 96394-33-1; $NH_2(CH_2)_3NMe_2$, 109-55-7.

(24) Mietzsch, F.; Mauss, H. *Fortschr. Teerfarbenfabr.* **1930**, *19*, 1167.
(25) Albert, A.; Dyer, F. J.; Linnell, J. *Q. J. Pharm. Pharmacol.* **1937**, *10*, 649.
(26) Magidson, O. Y.; Travin, A. I. *Ber.* **1936**, *69*, 537.
(27) Magidson, O. Y.; Grigorovski, A. M.; Galperin, E. P. *J. Gen. Chem. USSR (Engl. Transl.)* **1938**, *8*, 339; *Chem. Abst.* **1938**, *32*, 5405.
(28) Magidson, O. Y.; Travin, A. I. *Ber.* **1936**, *69*, 396.
(29) Mauss, H.; Mietzsch, F. German Patent 553 072, 1930; *Chem. Abstr.* **1932**, *26*, 4683.
(30) Horowsko, B.; Ledochowski, A. *Rocz. Chem.* **1974**, *45*, 1447.