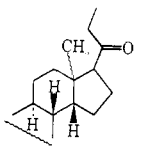
Rev., **74**, 219 (1974)). Several small vpc peaks (*ca.* 2-4% of the total) were trapped and examined; since **28** was not present, it was assumed that they were diastereomers of **23** and **24** in which the cyclizing side chain had epimerized, *e.g.*



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Effect of Aromatic Cations on the Tertiary Structure of Deoxyribonucleic Acid[1]

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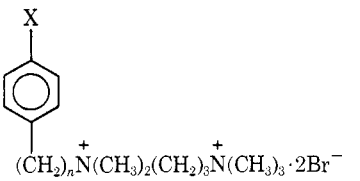
Abstract: The synthesis of several aromatic substituted diammonium cations and their interaction specificity with DNA have been examined. The results of the temperature-dependent proton magnetic resonance (pmr), viscometric, and melting temperature studies are presented. It is found that significantly different effects on the tertiary structure of DNA may be caused by slight modifications in the aromatic substituted diammonium cations. A "wedge" model is proposed whereby the aromatic ring of the latter is either "partially" or "fully" inserted between base pairs thus leading to either a decrease or increase, respectively, in the effective length of the DNA helix.

The mechanism(s) by which histone and nonhistone proteins influence(s) the tertiary structure of DNA in condensed chromatin has been the subject of considerable interest in many laboratories.[3-10] Hanlon and coworkers[6] have suggested that supercoiling of DNA may occur in nucleohistone *via* alternating B and C conformations of the DNA duplex whereby the latter conformation is induced *via* histone binding. Recently, Bartley and Chalkley[3] have proposed that the histone proteins may act as a spring, *i.e.*, an α-helical segment of the polypeptide chain may be involved in connecting the protein to two or more binding sites along the DNA helix, thus causing the latter to bend and assume a supercoil condensed form. Recent work from this laboratory[11,12] on the interactions of oligopeptides with DNA has shown that the peptides which contain aromatic amino acids at the C terminus cause a dramatic decrease in the specific viscosity, η_{sp}, of the DNA solution. The above data together with the proton magnetic resonance studies of oligopeptides-DNA complexes led Gabbay, *et al.*,[11,12] to propose a nonclassical model of intercalation whereby the aromatic residue of the oligopeptides is partially inserted between base pairs of DNA thus leading to a bend of the helix at the point of intercalation.

In order to investigate the effect of partial and/or total insertion of an aromatic residue between base pairs on the tertiary structure of DNA, the following compounds were synthesized. It is reasoned that at low values of *n*, total insertion of the aromatic ring of I may not occur. On the



I

X = H; **1**($n = 1$); **2**($n = 2$); **3**($n = 3$); **4**($n = 4$)
X = NO_2; **5**($n = 1$); **6**($n = 2$); **7**($n = 3$); **8**($n = 4$)
X = CH_3; **9**($n = 2$)

other hand, at higher values of *n* and in the presence of para substituents, *i.e.*, NO_2 and/or CH_3 groups, the aromatic ring may fully insert itself between base pairs of DNA to cause a net increase in the helix length (Figure 3). The results of our studies are consistent with the above interpretation.

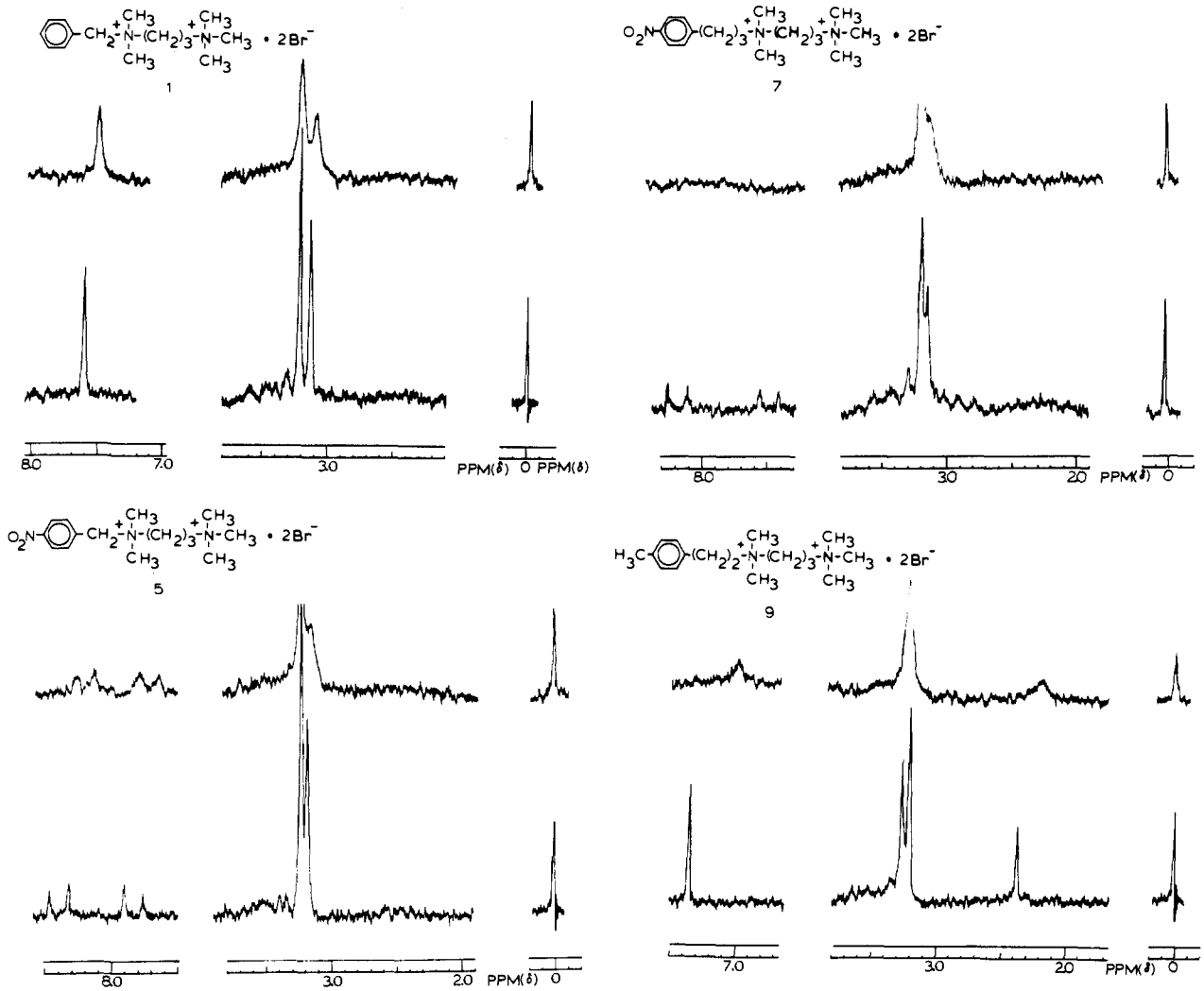
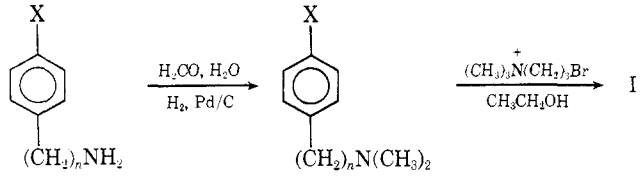


Figure 1. The partial proton magnetic resonance spectra of several cations I in the absence (lower spectrum) and presence (upper spectrum) of sonicated salmon sperm DNA at 55°.

Material and Methods

Analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. Infrared spectra were recorded on either a Perkin-Elmer Model 337 or a Perkin-Elmer IR-10. Pmr spectra were recorded on Varian A-60 spectrometer equipped with a variable-temperature probe. Viscosity studies were performed with a low-shear Zimm viscometer from Beckmann Instrument Co. Ultraviolet and visible absorption measurements were recorded on either a Cary 15 or a Gilford 240 spectrophotometer. DNA melting studies were carried out as previously described.[11] Compounds **1–9** were synthesized from appropriate starting materials (Aldrich Chemicals) according to the following scheme. All final products were chromatographed on silica gel and recrystallized. The elemental analyses and proton magnetic resonance spectra are found to be consistent with the assigned structures.



Salmon sperm DNA was obtained from Worthington Biochemicals (8BA, ϵp^{260} 6500) and is found to contain less than 0.2% protein as estimated by fluorescamine reaction.[12,13]

Results

Pmr Studies. Temperature-dependent pmr studies of the free and DNA-bound reporter molecules in D_2O were conducted according to previously published procedure[11] using 20 and 80 m*M* of reporter and DNA-phosphate per liter, respectively, at pD of 7.0 ± 0.1.

It should be emphasized that although the binding affinities, K_a, of the dications **1–9** to DNA have not been measured, it is safe to assume that it is ≥1000 since the binding affinity of the unsubstituted dication, 1,3-propanediamine dihydrochloride, is approximately 3.4×10^3.[14] Under the experimental conditions of the pmr experiment, *i.e.*, 20 and 80 mmol of I and DNA-P/l., respectively, it can be shown *via* simple calculation that the dications, **1–9,** are bound to the extent of >98% if $K_a \geq 1000$.

Figure 1 shows the typical spectra obtained at 55° with compounds **1, 5, 7,** and **9** in the presence and absence of sonicated (mol wt < 500,000) salmon sperm DNA. The results of the pmr studies for compounds **1–9** are summarized in Table I. It should be noted that considerable broadening and upfield chemical shift, Δδ Hz, of the pmr signals of the aromatic protons of I is observed in the presence of DNA. In addition, the extent of upfield chemical shift and signal broadening increases as follows. For compounds **1–4,** where X = H, the upfield chemical shift, Δδ Hz, increases as *n* (the number of methylene carbons between the aromatic ring and the adjacent quaternary ammonium group) increases from 1 to 4 in the presence of DNA at 37 and 55° (Table I). Similar results are also obtained with heat-denatured DNA.

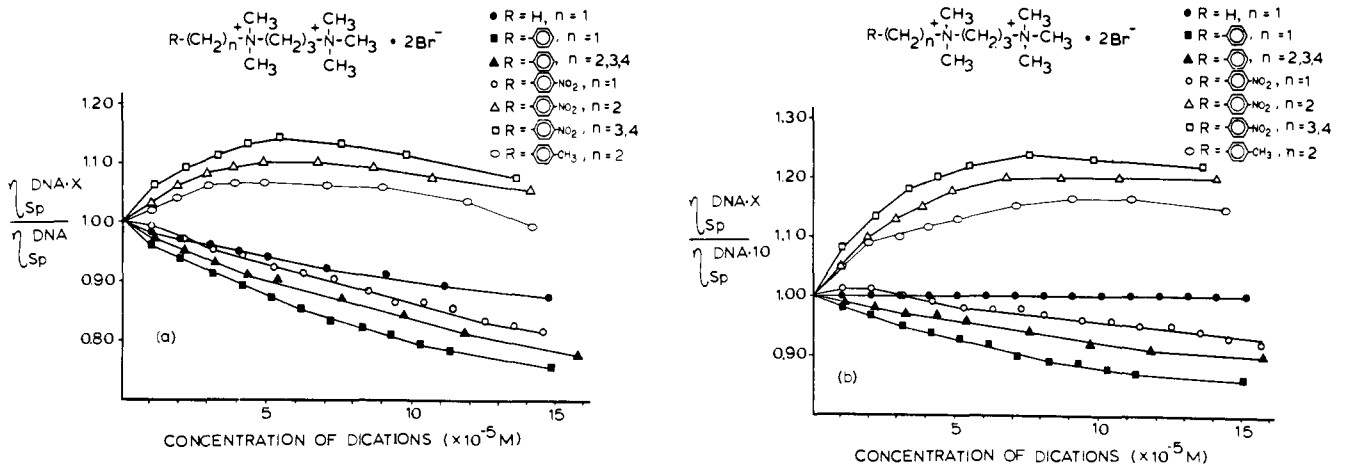


Figure 2. (a) The effect of increasing concentrations of cations I on the relative specific viscosity of near infinitely dilute solution of salmon sperm DNA. (b) Normalization of the data presented in (a) with respect to the relative specific viscosity of DNA–**10** complex, the ionic standard.

For the *p*-nitrophenyl-substituted cations **5–8,** the pmr signals of aromatic protons of the ortho and meta protons (which appear as an AA′BB′ pattern for the free compounds) are observed to be extensively broadened in the presence of DNA. More specifically, the AA′BB′ pattern for the aromatic protons cannot be observed for n = 2, 3, and 4 (Figure 1, Table I). However, for n = 1, *i.e.,* compound **5,** broadened and chemically upfield shifted pmr signals are observed in the presence of DNA. It should be noted that the pmr signals of the protons adjacent to the NO_2 group of **5,** *i.e.,* the ortho protons, are observed to undergo a higher upfield chemical shift ($\Delta\delta$ = 10 Hz at 37°) than the corresponding meta protons ($\Delta\delta$ = 6 Hz) in the presence of DNA. Similar results are also observed at higher temperature, *i.e.,* 55° with native DNA and at 37° with denatured DNA (Table I). It should be noted that the total line broadened pmr signals which are observed at 37° for the aromatic protons of **6–9** in the presence of denatured DNA are not surprising since at this temperature the latter is known to have considerable secondary structure, *i.e.,* stacked single-stranded helix. Thus, insertion of the aromatic rings of I between bases of denatured DNA would not only lead to totally broadened pmr signals (because of slow exchange and/or slow tumbling rates) but could also result in upfield chemical shifts due to aromatic ring currents of the neighboring bases.

For the *p*-tolyl-substituted dication **9,** the pmr signals of the aromatic and *p*-CH_3 protons are not observed in the presence of native DNA at 37°. However, at the higher temperature, 55°, the pmr signals of the aromatic and CH_3-group protons appear broadened and upfield shifted by 21 and 5 Hz, respectively (Figure 1). By comparison, the pmr signal of the aromatic protons of unsubstituted analog of **9,** *i.e.,* dication **3,** can still be observed at 37° and is upfield shifted by 10 Hz at 55°.

Viscometric Studies. The effect of increasing concentrations of compounds **1–9** and the parent unsubstituted dication, $(CH_3)_3N^+(CH_2)_3N^+(CH_3)_3$ (**10**), on the relative specific viscosity of the DNA solution, $\eta_{sp}^{complex}/\eta_{sp}^{DNA}$, is shown in Figure 2a. It is noted that increasing concentrations of the parent dication **10** cause a decrease in the η_{sp} of DNA solution. A more pronounced decrease is observed in the presence of the unsubstituted phenyl dications **1–4** (where n = 1–4, X = H) and the *p*-nitrophenyl dication **5** (where n = 1). On the other hand, the *p*-nitrophenyl dications **6–8** and the *p*-CH_3-phenyl dication **9** cause an increase in the relative specific viscosity, $\eta_{sp}^{DNA\text{-}X}/\eta_{sp}^{DNA}$. Figure 2b shows the viscometric data normalized with respect to the parent dication, **10,** *i.e.,* a plot of $\eta_{sp}^{DNA\text{-}I}/\eta_{sp}^{DNA\text{-}10}$ *vs.* the concentration of I.

Table I. Chemical Shifts (Hz) from DSS at 60 MHz of the Aromatic Protons of Free and DNA-Bound Dication Systems of Variable Temperature[a]

Dication	T, °C	δ_1[b]	$\Delta\delta_1$[c]		
1	37	455			
1 + DNA	37	451	4		
1 + DNA	55	450	5		
1 + d-DNA	37	446	9		
3	37	442			
3 + DNA	37	434	8		
3 + DNA	55	432	10		
3 + d-DNA	37	428	14		
4	37	440			
4 + DNA	37	431	9		
4 + DNA	55	428	12		
4 + d-DNA	37	424	16		
Dication	T, °C	δ_1[d]	δ_2[d]	$\Delta\delta_1$	$\Delta\delta_2$
5	37	503	469		
5 + DNA	37	493	463	10	6
5 + DNA	55	491	461	12	8
5 + d-DNA	37	485	458	18	11
6, 7, 8	37	(493–491)	(450–448)		
6, 7, 8 + DNA	37	[e]	[e]		
6, 7, 8 + DNA	55	[e]	[e]		
6, 7, 8 + d-DNA	37	[e]	[e]		
Dication	T, °C	δ_1	δ_3[f]	$\Delta\delta_1$	$\Delta\delta_3$
9	37	441	142		
9 + DNA	37	[e]	[e]		
9 + DNA	55	420	137	21	5

[a] 2,2-Dimethyl-2-silapentanesulfonic acid. [b] Chemical shift of phenyl aromatic protons. [c] Difference in chemical shifts of the phenyl aromatic protons. [d] δ_1 and δ_2 represent the lower and higher portions of the AA′BB′ signal for the aromatic protons of the *p*-nitro-substituted aromatic rings. [e] The proton signal is not distinguishable from base-line noise. [f] δ_3 is the chemical shift of the aromatic methyl protons. All spectra were taken on a Varian A60A spectrometer equipped with a variable-temperature probe. Sonicated salmon sperm DNA was used at 0.16 *M* of DNA-P/l. in D_2O at pD of 7.0 ± 0.1. Denatured DNA (d-DNA) was obtained by heating native DNA at 100° for 10 min followed by quenching in ice–water at 0°.

Melting Temperature Studies. The effect of the dications **1–10** on the T_m of the helix-coil transition is shown in Table II. It is noted that in all cases a net stabilization of the helix occurs. In addition, it is found that dications **6–8** (*p*-nitrophenyl, n = 2–4) cause a greater stabilization of the

Table II. Effect of Various Concentrations of the Dications **1–10** on the T_m of the Helix–Coil Transition of Salmon Sperm DNA[a]

System	ΔT_m, °C	
	20 μM	40 μM
1	4.3	6.5
2	3.1	5.3
3	3.1	5.0
4	3.3	5.5
5	5.9	7.5
6	7.4	10.2
7	7.3	10.4
8	7.8	11.4
9	4.0	5.1
10	3.0	4.6

[a] $\Delta T_m = T_m - T_{m_0}$, where T_m and T_{m_0} are the melting temperatures in the presence and absence of **1–10**. T_m studies were carried out in 10 mM 2-(*N*-morpholino)ethanesulfonate (Mes) buffer (pH 6.2) and 5 mM Na^+ using 60 μmol of DNA-P/l. and 20 and 40 μmol of **1–10**. T_{m_0} is found to be 61.5 ± 0.3°.

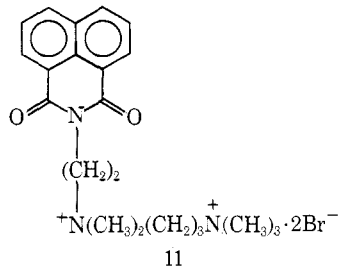
helix than unsubstituted phenyl dications **1–4** (where n = 1–4) and the *p*-nitrophenyl cation **5** (where n = 1). The T_m studies were carried out using 60 μM salmon sperm DNA-P/l. and 20–40 μM of the dications **1–10.**

Discussion

Proton Magnetic Resonance Studies. The line widths and chemical shifts of the pmr signals of a small molecule bound to a macromolecule often reveal considerable information concerning the nature of the binding process. For example, three types of binding may be distinguished. Type I binding, which is characteristic of a rigid macromolecule–small molecule complex, leads to a total line broadening of the pmr signals. This effect has been observed for molecules which intercalate between base pairs of DNA.[15,16] In such a case, the small molecule experiences strongly restricted tumbling in the DNA complex, leading to an unaveraged chemical shift of the individual protons and total line broadening.[17] The *p*-nitrophenyl dications **6–8** (n = 2–4) exhibit this type of behavior in the presence of native DNA at 37 and 55°. The *p*-tolyl dication **9** also shows totally broadened pmr signals of the aromatic and *p*-CH_3 protons at 37° (Table I).

Type II binding, which is characteristic of molecules exhibiting a line broadened pmr signal as well as an upfield chemical shift, is noted with the unsubstituted phenyl dications **1–4** and the *p*-nitrophenyl dication **5** (n = 1). Such effects have also been observed for peptides and peptide amides which contain an aromatic amino acid near the C terminus of the peptide, *e.g.*, L-Lys-L-PheNH_2, L-Lys-L-TyrNH_2, and L-Lys-L-TrpNH_2,[11,12] upon binding to DNA. It should be noted that this effect may arise by two distinctly different mechanisms, *i.e.*, (1) weak restriction of molecular tumbling of the aromatic ring in the DNA complex and/or (2) slow rate of exchange between the various DNA binding sites and the unbound state.[17] The latter mechanism is highly unlikely since it has been shown that the kinetics of the on-rate[18] and off-rate[19] of a DNA intercalating molecule (which is structurally related to the dications **1–10**), *i.e.*, the naphthylimide dication **11,** is substantially faster than the pmr time scale. For example, the half-life for the formation and dissociation of the DNA–**11** complex is found to be ≤0.1 msec.

Type III binding to DNA is noted for molecules which have a high affinity to nucleic acids but exhibit no line broadening and upfield chemical shift of the pmr signals. Among these are the polyammonium salts including the dication **10,** *i.e.*, $R_3N^+(CH_2)_nN^+R_3 \cdot 2Br^-$, where R = H, CH_3, and n = 2–6,[20] and the oligopeptides which contain the simple aliphatic and polar amino acids, *e.g.*, glycine, lysine, histidine, serine, etc.[11]



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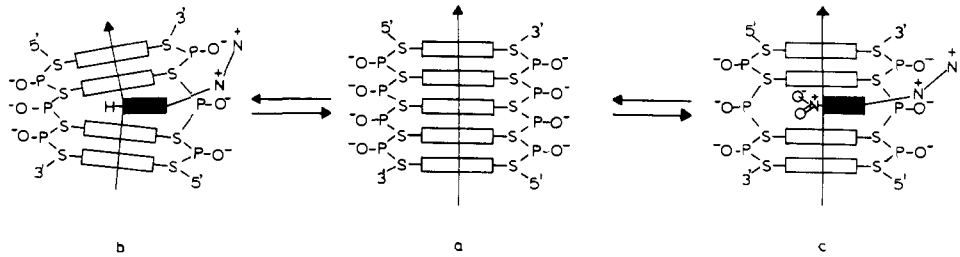
In summary, the pmr data indicate that the *p*-nitrophenyl dications **6–8** and the *p*-tolyl dication **9** exhibit type I binding to DNA which is characteristic of restricted molecular tumbling and is consistent with an intercalation mode of binding. The viscometric titration studies (discussed below) are consistent with the above interpretation since it is found that the same dications which exhibit *type I binding, i.e.,* **6–9,** also cause an increase in the specific viscosity, η_{sp}, of the DNA solution which is indicative of a net increase in the helix length.

The pmr results obtained with DNA complexes of dications **1–5,** which show a broadened and chemically upfield shifted pmr signals of the aromatic ring protons, *i.e.*, type II binding, are very revealing. For example, as the number of methylene carbons, n, increases from 1 to 4 (*i.e.*, compounds **1–4**), the upfield shift, $\Delta\delta$, increases from 5 to 12 Hz in the DNA complex at 55°. Similar results are also obtained with native DNA and denatured DNA at 37°. The results are consistent with the interpretation that as n increases, the geometrical flexibility of the phenyl ring of the dications **1–4** is enhanced to allow the latter to stack with DNA bases and experience a ring current anistropy effect.[17] However, the data indicate that merely increasing n is not sufficient to obtain *total insertion* between base pairs of DNA, *e.g.*, the pmr results obtained with n = 3 and 4 are nearly identical. In other words, *total insertion* which is being defined as characteristic of an intercalation mode of binding would lead to restriction of molecular tumbling and enhanced viscosity of the DNA solution. Neither effects are observed with the unsubstituted dications **1–4** and the *p*-nitrophenyl dication **5** (where n = 1). On the other hand, the presence of a para substituent on the aromatic ring and where n > 1, *i.e.*, compounds **6–8** (n = 2, 3, 4 and *p*-NO_2 substituent) as well as the dication **9** (n = 2 and *p*-CH_3 substituent), appear to be necessary conditions for total insertion. Figures 3 and 4 schematically illustrate the two types of postulated binding: (1) type I (full insertion exhibited by molecules which cause a net increase in the helix length, *i.e.*, "classical" intercalation mode of binding) and (2) type II (partial insertion exhibited by molecules which cause a net decrease in the helix length, *i.e.*, "nonclassical" intercalation mode of binding). The observations that peptides which contain aromatic amino acids, *e.g.*, Phe, Tyr, and Trp, exhibit only type II binding to DNA suggest that insufficient flexibility is available to the aromatic ring to undergo total insertion between base pairs.[11,12]

Viscometric Studies. In order to compare the effect of the dications **1–10** on the hydrodynamic property of DNA, attempts were made to determine the intrinsic viscosity, $[\eta]$, of the complexes. However, the results are found to be uninformative since the value of the intrinsic viscosity at infinite dilution in the presence of other molecules will and does approach the value of the intrinsic viscosity of free DNA at infinite dilution; *i.e.*, since the binding constant of the small molecule to DNA is finite, the complex will be dissociated



b a c

Figure 3. Schematic illustration of a segment of DNA duplex (a) which can either partially intercalate a molecule to give structure b or fully intercalate a molecule to give structure c. These two processes will either decrease or increase, respectively, the effective length of the DNA duplex.

at the lower concentrations. Instead, the effect of increasing concentrations of the dications I on the η_{sp} of DNA solution at *near infinite dilution* of the latter, *i.e.*, at 47.5 μM DNA-P/l., was studied (Figure 2). In order to interpret these data, it is important to note that the η_{sp} of a DNA solution is inversely dependent on ionic strength.[21] Presumably, the effect is due to shielding of neighboring negatively charged phosphate groups by the positively charged counterions which would lead to electrostatic constriction of the DNA polymer. Such an effect is observed for the parent dication **10** as well as the other dications **1–9** (Figure 2a). In order to correct for the electrostatic constriction due to ionic strength and to compare the effect of increasing concentration of the dications **1–9** on the η_{sp} of DNA, the viscometric titration data were normalized with respect to the parent compound **10.** The results shown in Figure 2b indicate that the dications I may be divided in two groups. Group A dications show an increase in the η_{sp} of DNA solution, *e.g.*, compounds **6–9.** Group B dications (**1–5**) show a decrease in the η_{sp} of DNA which cannot be explained on the basis of an ionic effect alone. It should be noted that group A dications not only show an increase in the η_{sp} of DNA but also exhibit total line broadening of the pmr signals of the aromatic protons in the native DNA complex (Table I). On the other hand, group B dications, which cause a greater decrease in the η_{sp} of DNA than the parent compound **10,** exhibit line broadening and upfield shift of the pmr signals of the aromatic protons in the DNA complex. Since the viscometric study was carried out at near infinite dilution of the DNA, the relative values of the ratio of η_{sp} of the DNA–I complex to the η_{sp} of the DNA–**10** complex ($\eta_{sp}^{\text{DNA-I}}/\eta_{sp}^{\text{DNA-10}}$) are close approximations of the relative values of the intrinsic viscosity of DNA–I to DNA–**10** complexes ($[\eta]^{\text{DNA-I}}/[\eta]^{\text{DNA-10}}$).[22,23] In addition, it is well known that the intrinsic viscosity of a rod-like polymer, such as DNA, increases with increasing length.[23] It is therefore reasonable to conclude that group A dications increase the effective length, and group B dications decrease the effective length of the DNA helix. Figures 3 and 4 illustrate these effects, *i.e.*, lengthening of the helix as a result of intercalation of group A dications and shortening of the effective length of the helix as a result of *partial* intercalation of group B dications. The latter effect is presumably due to *bending* of the helix as a result of partial insertion of the aromatic ring of group B dications between DNA base pairs. The above hypothesis, *i.e.*, bending of the helix at the intercalation sites, has also been invoked to account for the interaction of peptides containing aromatic amino acids[11,12] as well as other aromatic cations.[16]

Melting Temperature Studies. The effect of the dications **1–10** on the melting temperature, T_m, of the helix–coil transition of DNA was studied, and the results are summarized in Table II. Two points may be made from these data. (i) The dications are found to stabilize the DNA helix toward heat denaturation. (ii) Group A dications **6–8** (with the exception of the dication **9**) are found to increase the

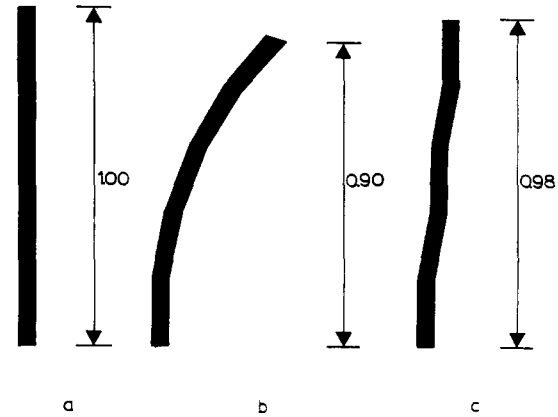


Figure 4. Two-dimensional schematic representations illustrating the decrease in helical length due to partial intercalation of an aromatic molecule. Structure a represents a unit length of helix. Structure b represents that same unit length of helix with consecutive 10° helical bends in the same direction. Structure c represents that unit length of helix with consecutive 10° helical bends of opposite direction. Hence b and c represent maximum and minimum effects, respectively.

T_m of the DNA to a greater extent than the corresponding group B dications **1–5.** Unfortunately, it is not clear how to interpret the T_m data since they are complicated by the fact that they involve relative interaction of the dications I with the helix and the random coil. However, it may be significant to note that the T_m data are consistent with pmr and viscometric studies in at least one respect, *i.e.*, it also differentiates between the effects of group A and B dications on DNA.

In summary, the synthesis and interaction specificity of aromatic substituted dications with DNA have been examined. It is shown *via* the use of pmr spectroscopy and viscometric titration studies that significantly different effects on the tertiary structure of DNA may be caused by slight modifications of small molecules. The results can be satisfactorily explained in terms of a reasonable model.

References and Notes

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Crystal and Molecular Structure of 2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane, $C_{10}H_{11}Cl_7$, a Toxic Component of Toxaphene Insecticide

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Abstract: Toxaphene insecticide contains over 175 distinct C_{10}-chloro compounds. Two of the components, one a $C_{10}H_{10}Cl_8$ compound and the other a $C_{10}H_{11}Cl_7$ compound, appear to contribute more than any others to the acute toxicity of toxaphene to mice treated intraperitoneally. The structure of the $C_{10}H_{11}Cl_7$ component has been determined by X-ray methods. The crystals are orthorhombic, space group $P2_12_12_1$, $a = 8.603$ Å, $b = 21.384$ Å, $c = 7.608$ Å, and $Z = 4$. Data were collected with an automatic diffractometer, over one-quarter of the reflection sphere with Ni-filtered, Cu Kα radiation. The molecule is 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane; the bond angles and distances agree with published values for similar compounds. The 100-MHz proton magnetic resonance spectrum of this compound has been analyzed and is shown to be completely consistent with the X-ray deduced structure. Several spin–spin couplings are discussed in relation to this structure. No isomeric impurities were detected.

Although toxaphene has been in commercial use for about 25 years and approximately one billion pounds have been applied to crops and livestock for pest insect control, very little is known about the composition of this material. Toxaphene is produced by chlorination of camphene to *ca.* 67–69% chlorine by weight, yielding a reproducible but very complex mixture of compounds with an overall average elemental composition of $C_{10}H_{10}Cl_8$.[2] No individual component, toxic or otherwise, has been previously isolated in pure form.

Preliminary examination of fractions from silica gel column chromatography by combination gc–mass spectroscopy reveals that toxaphene is a complex mixture of more than 175 compounds, mostly $C_{10}H_{18-n}Cl_n$ derivatives where the chlorine number (n) is 6, 7, 8, or 9.[3a] By use of mouse intraperitoneal acute toxicity as the monitoring criterion, two components have been isolated in crystalline form with empirical formulas $C_{10}H_{11}Cl_7$ and $C_{10}H_{10}C1_8$. These two components are 6- and 14-fold, respectively, more toxic to mice than technical toxaphene. Their toxicity to houseflies treated topically is also greater by twofold for the $C_{10}H_{11}Cl_7$ component and fourfold for the $C_{10}H_{10}Cl_8$ component. The $C_{10}H_{11}Cl_7$ component is pure based on a variety of spectral and chromatographic criteria.[3b]

The determination of the crystal and molecular structure of the $C_{10}H_{11}Cl_7$ component is described in this paper. This component is a heptachlorobornane and it is likely that the majority of the other $C_{10}H_{18-n}Cl_n$ compounds in technical toxaphene are also polychlorobornanes.[3a]

Experimental Section

The heptachlorobornane was isolated by use of a partition column with β-methoxypropionitrile and heptane, followed by a silica gel–hexane absorption column. A repetition of these two steps in sequence was followed by preparative gc; the component was further purified by crystallization.[3b]

A Varian HR-100 equipped with an internal field-frequency lock provided 100-MHz proton magnetic resonance (pmr) spectra.[4] To clearly resolve the weaker peaks of complex multiplets, it was necessary to average as many as 200 scans using a 1000-channel time-averaging computer. Overlapping peaks constituted a major problem which was minimized through the use of carbon tetrachloride as the solvent. However, the doublet-split triplet resonance of H(4) is almost completely obscured in this solvent while it is resolved clearly in acetone. No unassigned impurity resonances could be detected in the spectral range of 2.3–4.8 ppm. It is, therefore, estimated that the isomeric purity of this compound exceeded 98%. High field resonances arising from the gc column coating or the crystallization solvents appeared to varying extents in different preparations.

Crystals suitable for X-ray analysis were grown from hexane–ether in the ratio of 5:1. The crystals were colorless prisms elongated along c. Weissenberg and precession photographs were used to determine the unit cell dimensions and space group. The unit cell is orthorhombic; the only systematic absences were $(h\,0\,0)$, $(0k0)$, and $(00l)$ when h, k, and l were odd. The crystallographic data are summarized as follows: $C_{10}H_{11}Cl_7$, formula wt = 379.37, orthorhombic, space group $P2_12_12_1$, $a = 8.603 \pm 0.005$ Å, $b = 21.384 \pm 0.009$ Å, $c = 7.608 \pm 0.005$ Å, $Z = 4$, $F(000) = 760$, $\rho_c = 1.80$ g cm^{-3}, λ(Cu Kα) = 1.5418 Å, $\mu = 127$ cm^{-1} (for Cu Kα).

The density of the crystal could not be measured accurately be-