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THE EFFECT OF INTERCALATOR STRUCTURE ON BINDING STRENGTH AND BASE-PAIR SPECIFICITY IN DNA INTERACTIONS

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The interaction of naphthothiophene, phenanthrene and anthracene ring systems, which have amide and ester side chains with cationic groups (synthesized from the aromatic acid chlorides and appropriate amines and alcohols), with calf thymus DNA has been investigated by using viscometric titrations, spectrophotometric binding experiments and ^{1}H-, ^{31}P- and ^{17}O-NMR methods. The viscosity and NMR experiments suggest that all of these compounds bind to DNA by intercalation. These experiments and spectrophotometric binding studies, however, indicate that there is considerable variation in the interaction of these compounds with DNA. These variations can all be explained by the geometry of the ring systems, the position of protons adjacent to the side chains, and the relative sizes of the amide and ester side chains. With the naphthothiophene ester and amide, for example, the planar amide cannot rotate into the plane of the naphthothiophene ring whereas the smaller planar ester can. With this ring system the ester has a significantly higher binding constant than the amide derivative. Additional binding studies with poly[d(A-T)$_2$] and poly[d(G-C)$_2$] have shown that all of these compounds bind more strongly to the A-T- than the G-C-containing polymer. Since the ester compounds do not have hydrogen bond donating groups proximate to the aromatic ring, these results suggest a model for the A-T specificity of these compounds that involves a solvent-mediated hydrogen bond between the C-2 carbonyl of thymine and the carbonyl group of the intercalators.

1. Introduction

Recognition of specific DNA sequences is responsible for selective expression of chromosomal genes due to environmental changes [1]. It is essential to understand this recognition process at the molecular level, and this understanding should also lead to the ability to design rationally drugs which selectively bind to specific DNA sequences. One approach to this problem involves detailed investigations of the interaction of specific model compounds with DNA. As part of our efforts in this area, we have recently shown that it is possible to design intercalators with quite high A-T base-pair binding preferences [2]. Intercalators **1–3** (fig. 1) are all A-T specific and **2** has a K_{AT}/K_{GC} ratio (ratio of the binding constant of **2** to poly[d(A-T)] · poly[d(A-T)]/the binding constant to poly[d(G-C)] · poly[d(G-C)]) of almost 50 [2]. Since essentially all intercalators investigated to this time have had from little to high G-C pair specificity, this finding of A-T specificity can lead to a new understanding of the origin of base-pair specific interactions.

Postulated mechanisms for intercalator G-C binding preferences have ranged from direct hydrogen bonding with G-C base pairs in specific cases such as actinomycin [3] to a general dependence on polarizability of the intercalators [4]. For the large A-T specificity observed for **1–3**, we

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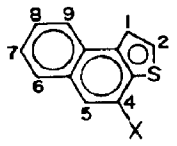

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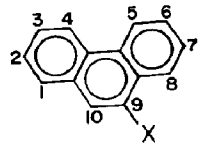
4 X = R_2
7 X = R_3



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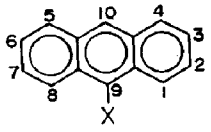

3 X = R_1
6 X = R_2
9 X = R_3

$R_1 = -CHCH_2N(CH_2CH_2OH)_2$ (OH)

$R_2 = -CNH(CH_2)_3N(CH_3)_2$ (O)

$R_3 = -CO(CH_2)_3N(CH_3)_2$ (O)

Fig. 1. Structures of the naphthothiophene, phenanthrene, and anthracene compounds with alcohol (R_1), amide (R_2) and ester (R_3) side chains. HCl salts of the compounds were used in all experiments.

have proposed two possible limiting mechanisms: (i) a direct hydrogen bond between the α-hydroxy group on the side chain of **1–3** and the C-2 oxygen of thymine or (ii) a solvent-mediated hydrogen bond between the C-2 oxygen of T and the side chain of **1–3** [2]. The latter model could also explain the A-T specificity of tilorone [5], which does not have α-hydroxy substituents, and fits well with the idea of Dickerson and co-workers [6] that A-T regions in DNA have a hydrogen-bonded water lattice in the minor groove. The water lattice could be disrupted by G-C specific intercalators, as it is by G-C base-pairs, leading to less favorable interactions at A-T base-pairs.

To test these two limiting proposals for the A-T specificity of **1–3** and to evaluate changes in molecular geometry on binding strength, we have synthesized **4–9** (fig. 1) which have the same three-ring systems but do not have the hydroxy groups on the side chain. The interaction of these compounds with natural DNA and DNA polymers has been investigated by using viscometric titrations, visible spectral shifts, spectrophotometric binding experiments, ^{1}H-, ^{31}P- and ^{17}O-NMR methods.

2. Experimental

2.1. Materials

Compounds **1–9** were synthesized as previously described [7–9]. For ^{17}O-NMR studies, **5** was also prepared as follows with an ^{17}O-labeled carbonyl group. A solution of phenanthrene-9-carboxylic acid (1.11 g, 5 mM) and thionyl chloride (29 ml, 0.4 M) was refluxed for 3 h. The excess thionyl chloride was distilled and the last traces of it were removed by co-distillation with toluene. The resulting acid chloride was dissolved in dry acetonitrile (25 ml), water (^{17}O, 45%, 114 μl) was added, and the reaction mixture was refluxed for 24 h under a nitrogen atmosphere. The acetonitrile and excess water were distilled and finally co-distilled with toluene. The crude, enriched phenanthrene-9-carboxylic acid was then converted to its acid chloride as described above. The resulting enriched acid chloride was mixed with 3-dimethylaminopropylamine (10 ml) and refluxed for 2 h. The reaction mixture was diluted with ice/water and extracted with ether (2 × 100 ml). The combined ether extracts were washed with water (2 × 100 ml) and with brine solution (1 × 50 ml). The ether solution was dried over anhydrous magnesium sulfate and the ether was evaporated under reduced pressure. Finally, the residue was dried in vacuo at room temperature. The product was crystallized from ethyl acetate/hexanes and pale-yellow crystals (0.71 g, 46%) were obtained, m.p. 97–99°C; ^{17}O-NMR (acetonitrile), 339.7 ppm. Analysis: Calcd. for $C_{20}H_{22}ON_2$: C, 78.39; H, 7.23; N, 9.14. Found: C, 78.26; H, 7.28; N, 9.13. Mass spectral results indicated that the compound is 17% enriched.

DNA samples were sonicated, filtered, phenol and ether extracted, ethanol precipitated, dialyzed

into Pipes buffer (0.01 M Pipes, 10^{-3} M EDTA, adjusted to pH 7 with NaOH, $[Na^+] = 0.007$ M), and characterized as previously described [10]. Samples for binding and viscosity studies were sonicated for shorter periods and had an average length of 500–600 base-pairs. Samples for NMR experiments were sonicated for longer times and had an average length of 150–200 base-pairs. All sonications were done with application of pulse power at near 0°C in Pipes buffer with 0.5 M NaCl added.

2.2. Spectrophotometric methods

Spectra in the absence and presence of DNA, free and bound extinction coefficients, were determined as previously described with a Cary 219 spectrophotometer interfaced to an Apple IIe microcomputer [2]. Absorbance measurements were used to determine equilibrium constants as described [2].

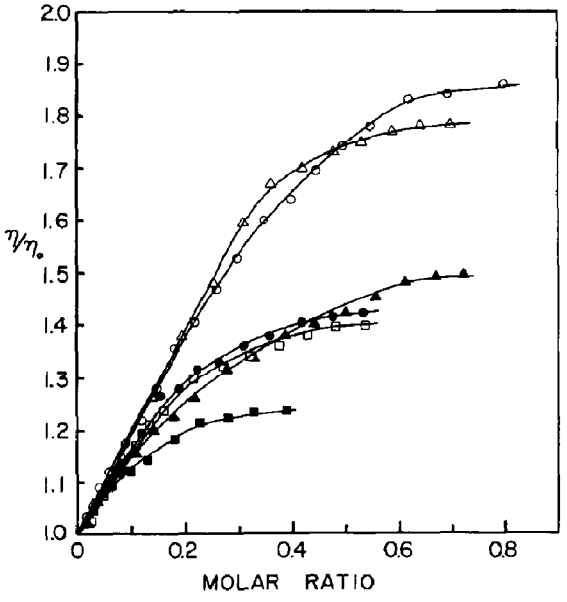

Fig. 2. Viscometric titrations of sonicated calf thymus DNA with **4–9**. Esters are denoted by open symbols: (△) **7**, (○) **8**, (□) **9**. Amides are represented by closed symbols: (▲) **4**, (●) **5**, (■) **6**. Naphthothiophenes (**4**, **7**) are indicated by triangles, phenanthrenes (**5**, **8**) by circles and anthracenes (**6**, **9**) by squares. The reduced specific viscosity ratio, η/η_0, is plotted as a function of the molar ratio of intercalator to DNA base-pairs. The titrations were at 28°C in Pipes buffer.

2.3. NMR methods

Proton (270 MHz) and phosphorus (109 MHz) NMR spectra were obtained on a Jeol GX270 spectrometer as previously described [2]. ^{17}O spectra were recorded on a Jeol GX-270 spectrometer equipped with a 10 mm broad-band probe operated at 36.5 MHz in the proton non-decoupled mode. The instrument settings were: 30 kHz spectral width, 2 K data points zero-filled to 8 K, 90° pulse angle (28 μs pulse width), 5–200 μs acquisition delay, 34.5 ms acquisition time. The signal-to-noise ratio was improved by applying a 100 Hz exponential broadening factor to the free induction decay prior to Fourier transformation. Chemical shifts are reported relative to the solvent water signal.

2.4. Viscometric methods

Viscometric titrations of sonicated DNA by **1–9** were conducted at 30°C in Pipes buffer as previously described [2].

3. Results

3.1. Viscometric titrations

The effect of addition of **4–9** on the viscosity of sonicated calf thymus DNA is shown in fig. 2. All compounds cause the pronounced increases in DNA viscosity expected of intercalators [11] but there are significant ring- and side chain-dependent variations. For the three-ring systems, the phenanthrenes and naphthothiophenes give similar titration results. The anthracene derivatives cause significantly smaller increases in DNA viscosity when compared to the phenanthrenes and naphthothiophenes with the same side chains. A similar ordering of viscosity results was seen in titration of calf thymus DNA with **1–3** [2]. In comparing the side chains, the ester substituent on each of the three-ring systems causes larger viscosity increases than the amide side chain on the

same ring systems. Compounds **1–3** with α-hydroxy side chains caused viscosity increases between those of the ester and amide side chain compounds.

3.2. Visible spectral titrations

Titrations of **4–9** with calf thymus DNA were monitored in the region above 300 nm where DNA absorption does not significantly interfere. In all cases addition of DNA causes a shift of the spectra to longer wavelengths and a general reduction in extinction coefficient. As examples, titrations of the naphthothiophene, phenanthrene and anthracene esters **7–9** are shown in fig. 3. As can be seen in fig. 3, isosbestic points were obtained in all titrations: at 334, 363, and 350 nm with **7**, at 317 and 355 nm with **8**, and at 314 and 393 nm with **9**. Similar titrations were obtained with **1–3** [2] and **4–6** (results not shown).

3.3. Spectrophotometric binding

Using extinction coefficients of the intercalator free and bound to DNA, as well as apparent extinction coefficients of the compound in the presence of DNA at various concentrations, a binding isotherm can be constructed [2]. A fairly complete isotherm can be generated by titrating

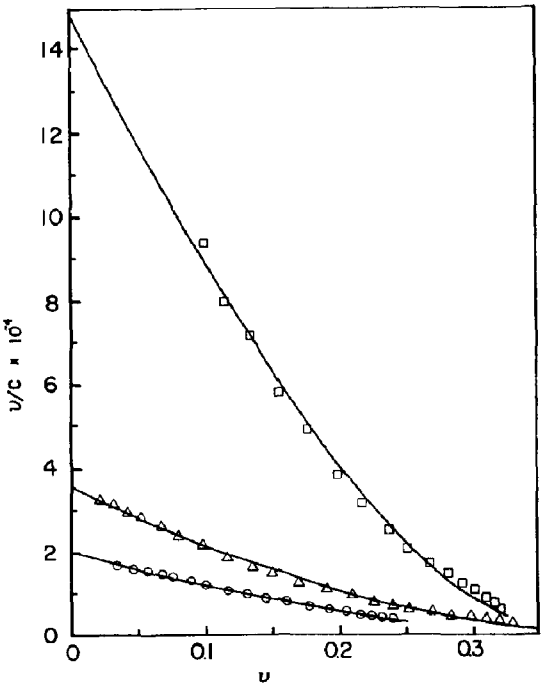

Fig. 4. Scatchard plots for the binding of **4** to different DNA samples in Pipes buffer at 25°C: (□) poly[d(A-T)]·poly[d(A-T)], (△) calf thymus DNA, (○) poly[d(G-C)]·poly[d(G-C)]. The points in the figure are the nonlinear least-squares best-fit values to the points using eq. 1. Several titrations were conducted for each DNA sample and the average values are collected in table 1.

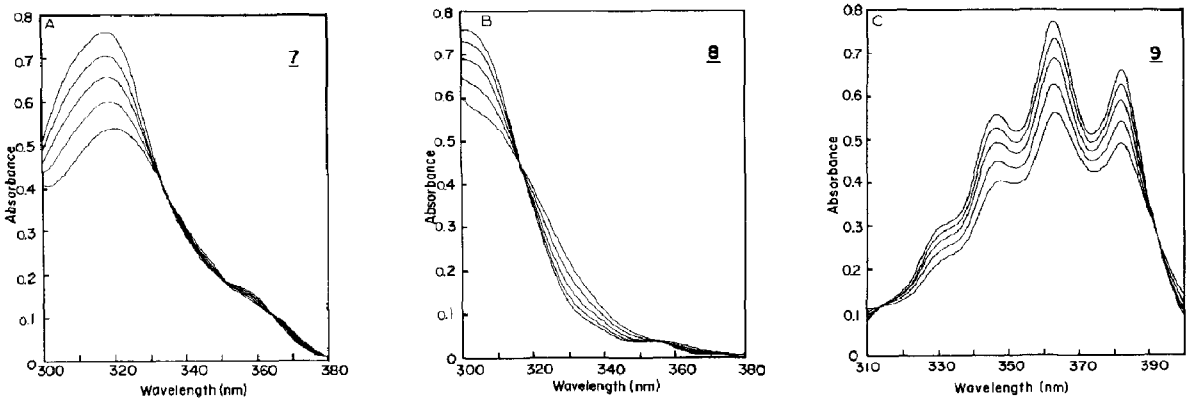

Fig. 3. Spectrometric shifts of the esters, **7–9**, on addition of DNA. The intercalator concentration in each case was approx. 1×10^{-5} M. In each set of spectra the DNA concentration is zero in the top spectrum and increases in the order of descending spectra. Scans were taken in a 10 cm cell on a Cary 219 spectrophotometer at 25°C.

Table 1

Spectrophotometric binding results [a]

Compound	Poly[d(A-T)]·poly[d(A-T)]		Calf thymus DNA		Poly[d(G-C)]·poly[d(G-C)]		K_{AT}/K_{GC} [c]
	K ($\times 10^{-3}$)	n	K ($\times 10^{-3}$)	n	K ($\times 10^{-3}$)	n	
1 [b]	34.7	2.6	21.4	2.9	8.2	2.8	4.2
2 [b]	1050	2.5	129	2.6	23.0	2.3	46
3 [b]	216	2.7	57.2	2.8	34.6	2.6	6.4
4	148	2.6	34.6	2.5	19.9	2.6	7.0
5	525	2.4	40.6	2.7	10.9	2.7	48
6	54.3	2.7	12.7	2.7	6.7	2.5	8.2
7	1290	2.4	222	2.7	155	2.6	8.0
8	648	2.4	112	2.8	51.0	2.7	12
9	150	3.0	32.9	3.5	9.1	3.7	16

[a] Nonlinear least-squares best-fit K and n values using eq. 1.
[b] Values from ref. 2.
[c] The ratio of binding constants for the A-T and G-C polymers.

DNA in a cuvette with the ligand of interest and repeating the titration at several DNA concentrations. These titrations can be conducted with DNA samples differing in base-pair composition to determine base-pair binding specificity [2]. Isotherms for the binding of naphthothiophene amide, **4**, to calf thymus DNA and the DNA polymers poly[d(A-T)$_2$] and poly[d(G-C)$_2$] are shown, as examples, in fig. 4. The points in the figure are experimental and the solid lines are the nonlinear least-squares best-fit values using the excluded-site model of McGhee and Von Hippel [12]:

$$\nu/c = K[1 - n\nu][(1 - n\nu)/(1 - (n-1)\nu)]^{n-1} \quad (1)$$

where ν is the number of moles of compound bound per DNA base-pair, c the free compound concentration, K the binding equilibrium constant, and n the number of base-pairs per binding site.

Values for binding results of **4–9** with calf thymus DNA, poly[d(A-T)$_2$] and poly[d(G-C)$_2$] are collected in table 1. As with **1–3** (included in table 1 for comparison), all of the amides and esters show significantly higher binding to poly[d(A-T)$_2$] than to poly[d(G-C)$_2$]. Values of K_{AT}/K_{GC} are also shown in table 1. The magnitude of the specificity varies with both the ring system and the side chain. The phenanthrenes as a class show the greatest specificity with both the alcohol and the amide having K_{AT}/K_{GC} between 45 and 50. The naphthothiophenes have the least specificity with $K_{AT}/K_{GC} < 10$ for all three side chains. There is no obvious correlation of side chain with specificity. The phenanthrene alcohol and amide have similar specificity as do the naphthothiophene amide and ester. The anthracene ester has the highest specificity for that ring system.

The binding constants cover a large range, from approx. 10^4 to 10^6 on a molar scale. For the compounds **4–9** the ester derivative with each ring system binds better than the amide derivative. This fact is particularly apparent with the naphthothiophene ring where the amide, **4**, has a medium binding constant with all three DNA samples, but the ester is the strongest binding compound in the entire group with all of the DNAs.

3.4. NMR experiments

The ^{31}P-NMR signal of DNA was monitored on titration with **4–9**. All compounds cause similar downfield shifts and the shifts are similar to those observed for the DNA ^{31}P signal on titration with **1–3** [2]. All compounds cause some increase in linewidth of the DNA resonance but only one peak was observed under all conditions with all compounds. An example titration for the phenanthrene amide, **5**, is shown in fig. 5 (all NMR

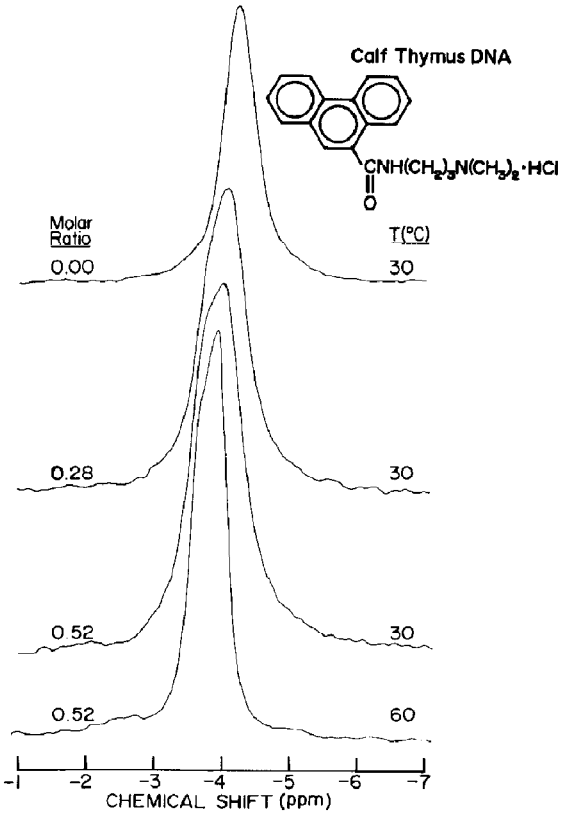

Fig. 5. ^{31}P-NMR (24.15 MHz) spectra of intercalation complexes of **5** with calf thymus DNA as a function of ratio (**5** to base-pairs) and temperature. The samples (1.5 ml in volume) in 10-mm tubes were 20 mM in DNA phosphate molarity in $^{2}H_2O$-Pipes buffer and contained trimethyl phosphate as internal reference. The number of scans was 6700 (2 h of total accumulation time) for all the spectra.

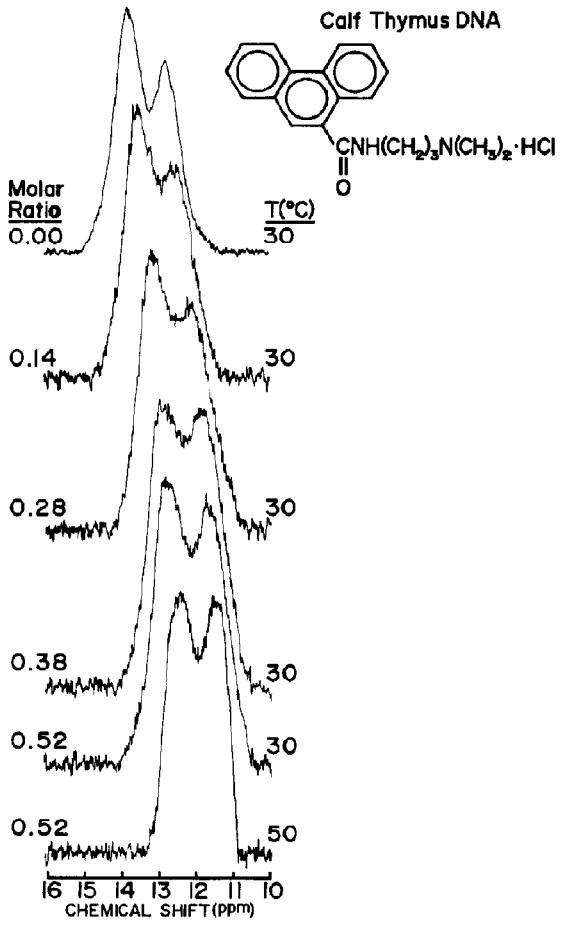

Fig. 6. Imino ^{1}H-NMR (270 MHz) spectra of intercalation complexes of **5** with calf thymus DNA as a function of ratio (**5** to base-pairs) and temperature. The samples (0.825 ml in volume) in 5-mm NMR tubes were 20 mM in DNA phosphate molarity in 9% $^{2}H_2O$) in H_2O-Pipes buffer and contained TSP as reference. The number of scans was typically 15000 (2 h of total accumulation time) for all the spectra.

experiments will be illustrated with **5**).

An NMR titration experiment was also conducted by monitoring the DNA imino proton peaks in the 12–14 ppm chemical shift range. The hydrogen-bonded imino proton is at 13.7 ppm for A-T and 12.7 ppm for G-C base-pairs in DNA at 30°C. A titration of calf thymus DNA with **5** is illustrated in fig. 6. Both of the imino proton resonances experience large upfield shifts on addition of **5** and reach limiting shifts of 12.6 and 11.6 ppm for the A-T and the G-C resonance, respectively, in the complex. Compounds **4** and **6–9** show similar large upfield shifts as do **1–3** [2].

In a titration in the reverse direction, the ^{17}O-NMR signal of the ^{17}O-labeled phenanthrene amide, **5**, was monitored during a titration by addition of DNA. Spectra are shown with increasing amounts of DNA in fig. 7. The original signal of the free compound is at 302 ppm in water. On titration with DNA no significant change in the chemical shift occurs but there is a slight increase in linewidth which could be due to viscosity increases as DNA is added. The most striking

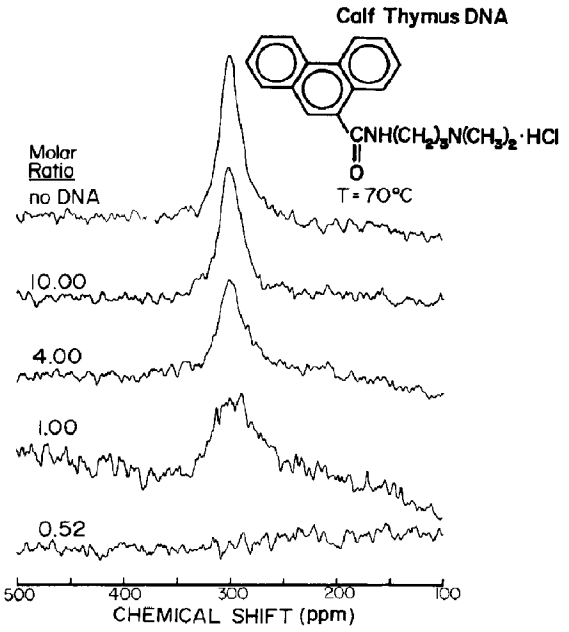

Fig. 7. ^{17}O-NMR (36.5 MHz) spectra of **5** as a function of added calf thymus DNA (ratio = **5**/base-pairs) at 70°C. The samples (1.5 ml in volume) in 10-mm tubes were 10 mM in **5** in ^{17}O-depleted water-phosphate buffer containing 15 mM NaH_2PO_4, 0.1 mM EDTA, 0.1 M NaCl. The number of scans was typically 120000 (2 h of total accumulation time) for all the spectra. Chemical shifts are reported relative to the residual $H_2^{17}O$ in the solvent.

change in the spectra is the loss of area as the ratio of compound to DNA decreases. At high ratios in fig. 7, there is little change in the spectra. Saturation with this compound should occur at ratios of compound to DNA base-pairs of approx. 0.4 (table 1). There is significant signal left in the spectra at all ratios above the saturation point (10–1.0 in fig. 7) but at a ratio of 0.5, which is essentially at the saturation point, the signal has completely disappeared. This is characteristic behavior for slow exchange of ligand between bound and free states with essentially total broadening of the ^{17}O signal of the bound ligand due to a very short T_2 value [13,14]. We have collected spectra at very short delay times to ensure that we are not simply losing signal due to relaxation in the delay between the irradiation pulse and acquisition. At the intermediate ratio values (10–1.0) this causes a slight increase in signal intensity as expected (accompanied by baseline roll) but at the 0.5 ratio, no signal could be detected even at the shortest delay time.

4. Discussion

Although some compounds which do not intercalate can cause small increases in linear DNA viscosity, the generally large viscosity increases seen in fig. 2 are characteristic of intercalation [11]. NMR results are particularly useful for distinguishing intercalation versus outside binding modes for molecules which interact with the double helix. Outside or groove binding compounds, for example, have been shown to cause upfield shifts in ^{31}P-NMR experiments while intercalators give downfield shifts [15–18]. DNA imino protons experience large upfield shifts when intercalators bind to the double helix [16,17,19] while groove binding compounds cause downfield shifts for these protons [15,20]. The sonicated DNA viscosity increases, downfield ^{31}P-NMR, and large upfield ^{1}H imino proton chemical shifts on titration with **4–9** indicate that these compounds, as well as **1–3** [2], bind to DNA by intercalation. The isosbestic points obtained in the spectral titrations (fig. 3) suggest that, under these conditions, the intercalation complex is the only one present in significant amounts for these compounds.

Analysis of the base-pair binding specificity of **4–9** compared to **1–3** (table 1) illustrates that an α-hydroxy group, as present in **1–3**, is not necessary for a pronounced preference for binding to A-T base-pairs. In the phenanthrene series, for example, the K_{AT}/K_{GC} ratio is greater than 40 for both the α-hydroxy and amide compounds, **2** and **5**, respectively, and is 12 for the ester derivative, **8**. Both the amide and ester derivatives have greater A-T binding preference than the α-hydroxy compounds in the naphthothiophene and anthracene series. In **4–6** the α-hydroxy substituent of **1–3** is replaced by an amide group adjacent to the aromatic ring. The amide has an N-H group which, as with the α-OH group of **1–3**, is potentially capable of forming a hydrogen bond with the thymine C-2 carbonyl oxygen in the minor groove and, thus, directly conferring A-T binding specificity to these compounds. With the ester

derivatives, **7–9**, this potential hydrogen bond donor is not present and no direct hydrogen bond to the thymine carbonyl is possible. The ester derivatives, however, maintain pronounced A-T binding specificity and, in fact, in the naphthothiophene and anthracene series, the esters have the highest K_{AT}/K_{GC} ratios (table 1). The model requiring a direct hydrogen bond for A-T specificity [2] in this series is clearly not required. The model postulating a solvent-mediated hydrogen bond between the thymine C-2 carbonyl and an acceptor group on an intercalator bond adjacent to the thymine containing base-pair must be considered more likely for the origin of A-T specificity of **1–9**. The majority of intercalators may simply displace water from the minor groove at A-T base-pairs, giving less favorable energetics for interaction at A-T relative to G-C base-pairs. With **1–9** and similar intercalators such as tilorone, the water lattice would be modified to include the intercalator but favorable interactions would be maintained and A-T specificity generated. At A-T and G-C base-pairs there are gradations of specificity and other factors such as electrostatic interactions, polarizability, dipole interactions, etc., must be included in the final analysis of the K_{AT}/K_{GC} ratio. We are currently attempting to synthesize derivatives of **1–9** in which the hydrogen bond acceptors are absent from the side chain position adjacent to the intercalating ring system to test the above ideas further. It should also be noted that hydrogen bond donors or acceptors on the side chain farther from the intercalating ring system are unlikely to generate A-T binding specificity since many intercalators have these groups and show no or G-C base-pair binding specificity [2,4].

The ^{17}O-NMR results of fig. 7 allow us to evaluate potential hydrogen bonding interactions at the α-carbonyl group of **5**. Titration of the compound with DNA does not result in significant chemical shift changes or line broadening of the original signal until the peak area is lost at the point where the compound is essentially completely bound to DNA. This suggests that there are two species present in slow exchange on the ^{17}O-NMR time scale: the unbound species present with the original shift and linewidth (modified only by the slightly increased viscosity in the presence of sonicated DNA) and an unobserved bound species with very large linewidth (essentially so broad that it is not separable from the baseline noise). These results are consistent with the carbonyl group being involved in a hydrogen bond [21] when bound to DNA.

In addition to binding specificity, it is also interesting to compare variations in overall binding strength for the closely related derivatives **4–9** (see table 1, for example). For the three-ring systems, the anthracene derivatives have the lowest binding constants. Comparing the amide and ester side chains, the esters always have stronger binding to DNA. From fig. 2 we also note that the anthracene derivatives cause a smaller increase in DNA viscosity than the naphthothiophene and phenanthrene derivatives, and in each ring system, the ester derivative produces a larger viscosity increase than the corresponding amide. In comparing the ratio of ester to amide binding constants, we find the ratio is less than 3 for anthracenes binding to poly[d(A-T)$_2$], calf thymus DNA and poly[d(G-C)$_2$]; less than 5 for the phenanthrenes binding to the same three DNA samples; and greater than 5 for the naphthothiophenes with the three DNAs. The ratio, for example, is 9 for binding of napthothiophenes to poly[d(A-T)$_2$] and 8 for the binding ratio when using poly[d(G-C)$_2$]. The conclusions from these results are that the anthracenes have smaller viscosity increases, weaker binding and less difference between the amide and ester substituent than the phenanthrene and naphthothiophene compounds. The naphthothiophene derivatives have the largest binding difference between the amide and ester side chains.

Analysis of CPK molecular models for the amides and esters, **4–9**, reveals several significant differences. With the anthracene derivatives, **6** and **9**, the protons at positions 1 and 8 of the anthracene ring sterically prevent the planar ester and amide groups from rotating into the plane of the anthracene ring. This creates a partial steric block to intercalation at one side of the anthracene aromatic ring system and may cause a slight bending of the DNA on intercalation as has previously been seen with asymmetrically substituted

phenanthrolines and quinolines [22,23]. This would also decrease the binding of the anthracenes relative to similar intercalating systems without the same steric constraints. The ester substituent is smaller than the amide but this does not cause a large improvement in binding in the anthracene system. With the phenanthrene ring system, the amide and ester substituents are less sterically hindered and have more rotational flexibility, but the protons at positions 8 and 10 of the phenanthrene ring prevent these groups from assuming a planar conformation with the phenanthrene ring system. The smaller ester side chain improves the binding more with this ring system than with the similarly substituted anthracenes.

The naphthothiophene derivatives, **4** and **7**, present the most interesting results. The amide side chain is sterically prevented from assuming a planar conformation with the aromatic ring system. With the carbonyl group of the ester oriented in the direction of the thiophene ring, however, this side chain can rotate into the same plane as the naphthothiophene ring system. A larger increase in binding is, therefore, predicted in going from the amide to the ester side chain in the naphthothiophene system than with either the phenanthrene or anthracene derivatives and that is the result obtained experimentally (table 1). All of the derivatives **4–9** are sterically restricted in varying degrees in their ability to slide into a binding site and optimize the energetics of interactions with base-pairs on both sides of the intercalation site. The ester derivatives in all ring systems are the least restricted and the naphthothiophene ester, **7**, is significantly less restricted than the esters **8** and **9** and has the highest binding constant of any compound in this group (table 1). These conclusions are reinforced by NMR work on the shifts in the aromatic protons of **4–9** on addition of DNA [24]. The protons on **7** are all shifted approximately the same amount on addition of DNA and are shifted more than the same protons on **4** in the presence of DNA.

These compounds dramatically illustrate the methods that can be used both to generate base-pair specificity in intercalation binding and the importance of stacking interactions and steric effects, in general, in DNA-intercalation binding interactions and conformational changes.

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