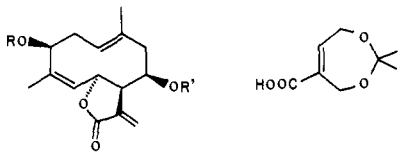

16 17

($C_5H_5NH^+OTs^-$, MeOH, $HOCH_2CH_2OH$, 54%) to yield **1**. Synthetic eucannabinolide thus prepared was found by IR, ^1H NMR (270 MHz), TLC, and CD to be identical with a sample of authentic **1** kindly supplied by Professor Koji Nakanishi here at Columbia.[14]

Registry No. 1, 38458-58-1; **2**, 84066-29-5; **3**, 84066-30-8; **4**, 38425-58-0; **5**, 84066-31-9; **6**, 84066-32-0; **7**, 84066-33-1; **8a**, 84066-34-2; **8b**, 84107-75-5; **9**, 84066-35-3; **10**, 84066-36-4; **11**, 84142-53-0; **12**, 84066-37-5; **13**, 84066-38-6; **14**, 84066-39-7; **15**, 84066-40-0; **16** (R, R' = TMS), 84066-41-1; **16** (R = Ac; R' = H), 84066-42-2; **17**, 84066-43-3; (+)-carvone, 2244-16-8.

Supplementary Material Available: Infrared and 250- or 270-MHz proton NMR spectra for all numbered compounds, X-ray structure of **15** shown as an ORTEP stereopair, and molecular mechanics structures and energies for compounds **8-13** are included (22 pages). Ordering information is given on any current masthead page.

(13) This compound was prepared from methyl tiglate as follows: (1) NBS, CCl_4, cat. $(PhCOO)_2$ (48%); (2) NaOAc, Ac_2O (72%); (3) LiOMe, MeOH (56%); (4) $CH_3C(OCH_3)=CH_2$, cat. $C_5H_5NH^+OTs^-$, CH_2Cl_2 (88%); (5) (a) NaOH, H_2O, (b) pH 2.5 (76%).

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Effect of Intercalating Drugs and Temperature on the Association of Sodium Ions with DNA: Sodium-23 NMR Studies

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The conformations and physical properties of many biological polymers in aqueous solutions are strongly influenced by small counterions.[1-5] Record and co-workers have shown that cationic peptides and proteins that bind strongly at specific sites on DNA release an equivalent amount of associated counterion.[1,3,6] We have demonstrated similar effects with the intercalators quinacrine[7,8] and ethidium.[8,9] Sodium-23 NMR spectroscopy has been useful in studies of synthetic and natural polymers.[10] Record and co-workers have examined association of sodium and other simple counterions with DNA by using ^{23}Na line widths.[11,12] In the work reported here we have, for the first time, monitored sodium ion release from DNA as a result of binding of an intercalating drug, ethidium bromide, to DNA, observed sodium ion release from native DNA on denaturation, and by varying the charge density on DNA, obtained information on the relaxation of $^{23}Na^+$ associated with DNA. The ability to vary the charge density on DNA in a quantitative manner is especially significant because it allows direct comparison of ^{23}Na ion relaxation when associated with DNA to predictions from polyelectrolyte theory. With synthetic polyanions it has been shown both theoretically and experimentally that the $^{23}Na^+$ relaxation rate has a quadratic dependence on the degree of neutralization (α) above α values of approximately 0.3, which is the range of ion condensation.[10,12-14] Since the charge density of native DNA cannot be significantly varied in a pH titration, we show in this work that titration of DNA with cationic intercalators can be particularly useful in evaluation of relaxation mechanisms.

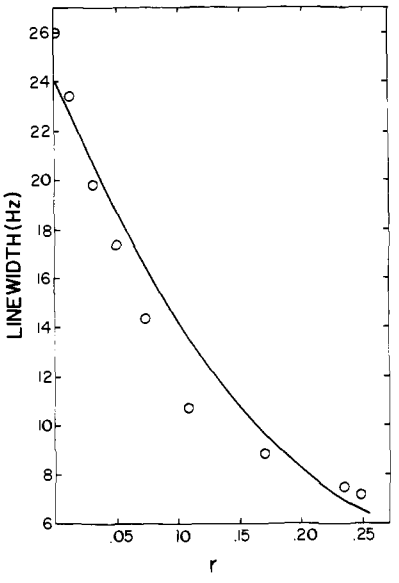

Figure 1. ^{23}Na NMR titration of DNA with ethidium bromide at 25 °C. The ^{23}Na ion line width is plotted vs. r, the ratio of moles of ethidium bound per mole of DNA phosphate. The open circles are experimental points, and the solid line was calculated as indicated in the text.

The binding of intercalators to DNA, unlike simple counterions, affects the charge density of the double helix in two potential ways: (i) the charge on the intercalator neutralizes some of the anionic charge of DNA, and (ii) insertion of the aromatic ring of the intercalator between base pairs of DNA increases both the local and the average phosphate to phosphate distances and, therefore, also decreases the DNA charge density.[7] In agreement with this prediction, the intercalation binding constant has been shown to depend on the counterion concentration,[7-9] indicating that, in the thermodynamic measurements, the total associated counterion is approximately linearly dependent on the amount of intercalator bound to DNA. It should be emphasized that the total amount of sodium ion associated with the double helix in the thermodynamic sense may be different from that monitored by the ^{23}Na NMR method. In Figure 1 results of ^{23}Na line width measurements in the presence of DNA at varying ratios of ethidium bromide to DNA phosphate are shown.[15] As a first approach

(1) (a) Record, M. T., Jr.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103. (b) Manning G. S. *Ibid.* **1978**, *11*, 179.
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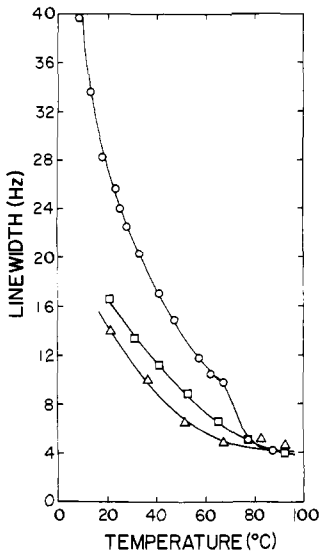

Figure 2. ^{23}Na ion line width plotted as a function of temperature for native DNA (○), denatured DNA (□), and an ethidium–DNA complex at an r value of 0.072 (△).

to treating these results, the solid line in Figure 1 has been plotted by assuming that there is a quadratic dependence of the bound-sodium relaxation rate on the nucleic acid charge density and that the $^{23}Na^+$ relaxation rate can be separated into a term due to the free solution relaxation of $^{23}Na^+$ and another term due to relaxation of sodium ions associated with the DNA double helix.[13,14,17] Similar effects have been seen in this α range in pH titrations of anionic synthetic polymers.[13,14,18] Although the theoretical line in Figure 1 is a reasonable fit to the points, the actual line width decrease is obviously steeper than predicted. This agrees with the finding that larger amounts of Na^+ are released by intercalators binding to DNA than would be expected from their charges.[7-9] Errors in the theory such as the assumed constancy of the Na^+–DNA average separation distance, constancy of the quadrupole coupling constant, and the cylindrical smeared charge model for point charges in the DNA–ethidium complex could also contribute to the systematic difference between the experimental results and the theoretical prediction in Figure 1.

In Figure 2 $^{23}Na^+$ line widths in native, denatured, and ethidium–DNA solutions are shown as a function of temperature. For native DNA there is a continuous decrease in line width up to the denaturation region of the double helix (the T_m is approximately 63 °C under these conditions) at which point a break in the curve occurs with a more pronounced decrease in the line width. This is a direct illustration of the cooperative conformational change, the release of Na^+ ions, and mobility changes that occur on DNA denaturation. When the heated sample was quickly cooled to yield denatured DNA at low temperature and then reheated, a smooth change in relaxation rate was observed with no breaks in the curve (Figure 2). The decrease in line width as temperature is increased also is important in illustrating that Na^+ is in the fast-exchange region under these conditions[2] and agrees with other results obtained on heating native DNA.[19]

These experiments using intercalators and heating illustrate the utility of ^{23}Na NMR in monitoring DNA conformational changes and binding interactions.

Acknowledgment. This work was supported by NIH Grant GM 30267 (W.D.W.) and a seed research grant from Atlanta University (Y.H.M.). Financial support for the Bruker WM-250 spectrometer from NIH (RR-8006) and the NSF Resource Center for Science and Engineering at Atlanta University (78-11955) is acknowledged.

Registry No. Sodium, 7440-23-5; ethidium bromide, 1239-45-8.

(15) ^{23}Na NMR measurements were performed on a Bruker WM-250 spectrometer equipped with a broad-band (10 mm) tunable probe at 66.13 MHz. Typically, a sweep width of 5000 Hz, 8K data points, a 90° pulse and 400–600 transients were used. All measurements were made under nondecoupling conditions. Line width values are bandwidths at half-peak intensity determined digitally. Temperature control and measurements were made by utilizing a Bruker VT1000 unit and were maintained to within ±0.5 °C. DNA sample preparation was as specified previously.[16] For titration experiments, microliter aliquots of a known concentration (determined by using ϵ_{480} 5750 M^{-1} cm^{-1})[9] of a stock solution of ethidium bromide were added to a DNA sample in an NMR tube directly and measurements taken. DNA samples were prepared at 0.01 M DNA phosphate in a PIPES buffer adjusted to pH 7 with NaOH and containing 0.0125 M NaCl, 10^{-3} M PIPES, 10^{-4} M EDTA, and 20% D_2O.

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(17) For the analysis of the results of Figure 1, we have used the theory developed by Leyte and co-workers for $^{23}Na^+$ relaxation in the presence of a polyanion and simple salt.[13,14] The fractions of bound- and free-sodium ions at varying ethidium concentrations were calculated by using the following results for ethidium–DNA interactions:[9] neighbor exclusion binding with an equilibrium constant of 10^6 under these conditions and 0.8 Na^+ ion released per bound ethidium. The average charge density of an ethidium binding site on the DNA double helix was calculated by assuming four changes per two base pairs (6.8 Å) or 1.7 Å between charges. This gives the literature value of 4.2 for the charge density parameter (ξ) for DNA.[1] On addition of ethidium to these two base pairs, the distance is increased to 10.2 Å and the charge is reduced by one unit so that the average spacing becomes 3.4 Å between charges and ξ is reduced to 2.1. Other average charge densities and ξ values were calculated by assuming a linear change between these two values as the fractional saturation of ethidium sites is increased from 0% to 100%. For comparisons with pH titrations this corresponds to a change in α from 1.0 (free DNA) to 0.5 for DNA saturated with ethidium. A linear correspondence between α and fractional saturation with ethidium is assumed. Constants in the equation for relaxation rate[13,14] were grouped together, and this single constant was used as a variable parameter to obtain the best-fit line to the data points in Figure 1. A free solution line width of 7 Hz for $^{23}Na^+$ was determined experimentally. The r values in the figure were calculated for each fractional saturation value for ethidium. Several experiments have indicated that with synthetic polymers the ^{23}Na relaxation behavior becomes more complex below α values of approximately 0.3,[13,14,18] perhaps due to conformational changes in the highly neutralized polymers. We have not been able to go below an α of 0.5 with ethidium, but the fact that the line width at this low α value approaches the value for free sodium ions suggests that such complicating effects may not be as significant with conformationally more rigid polymers like DNA.

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Electron Spin Resonance Spectroscopy of the Triplet State of *m*-Xylylene

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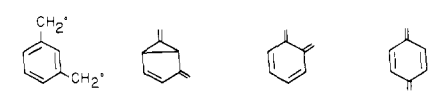
m-Xylylene (1,3-benzoquinodimethane, **1**) has no Kekulé



1 **2** **3** **4**

structure that fully satisfies the valency of every carbon without recourse to the formation of a highly strained compound such as **2**.[1] It is fundamentally different in electronic structure than the ortho and para isomers **3** and **4**.[2]

(1) Studies of *m*-xylylene as a reactive intermediate in solution are quite limited. The first cyclophane synthesis was accomplished by treatment of dibromide **9** with sodium, through the possible intervention of **1**: Pellegrin, M. *Recl. Trav. Chim Pays-Bas* **1899**, *18*, 458. The most convincing modern study of a simple analogue of **1** concerns the tetramethyl derivative: Gajewski, J. J.; Chang, M.-J.; Stang, P.-J.; Fisk, T. E. *J. Am. Chem. Soc.* **1980**, *102*, 2096.

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