Topography of Nucleic Acid Helices in Solutions. A Nuclear Magnetic Resonance Study of the Interactions of Reporter Molecules with Deoxyribonucleic Acid. Evidence for Hydrogen-bonding Interactions[1]

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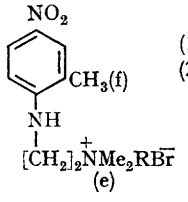
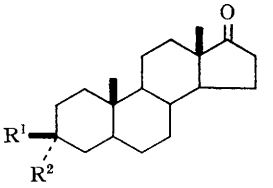
Summary The ^{1}H n.m.r. signals of the methyl protons of tertiary ammonium salts' complexes with DNA are considerably more broadened than those of the corresponding quaternary ammonium salts, and strongly suggestive of H-bonding interactions of DNA with the tertiary ammonium salts (**1**), (**3**), and (**5**).

IF the rate of molecular tumbling of molecules in solutions is lower than the typical Larmor frequencies w_0 (of the order of 10^8—10^9 rad sec^{-1} for protons in the conventional magnetic field) then T_2, the transverse relaxation time, is considerably diminished, leading to substantial line broadening of the proton signal.[2] This situation is obtained if the proton is contained in a rigid macromolecule, *e.g.* DNA,[3] or if the proton is contained in a slowly tumbling small molecule bound to a macromolecule.[4,5]

We report the temperature-dependent ^{1}H n.m.r. spectra of the free and DNA-bound reporter molecules, (**1**)—(**6**). The results in terms of chemical shifts from sodium 2,2-dimethyl-2-silapentanesulphonate (DSS) and line width in Hz at half height, $\Delta\nu_{\frac{1}{2}}$, are summarized in the Table. Several interesting points may be made. (i) The ^{1}H n.m.r. signal of the N·CH_3 protons (e-CH_3) of the tertiary ammonium salts, reporters (**1**), (**3**), and (**5**)/complexes of DNA is considerably broadened and is indistinguishable from baseline noise (SUBLN) at 25°, 35°, and 35°, respectively. These results may be explained in terms of restricted rotations of the $Me_2\overset{+}{N}H$ as compared with the quaternary



(**1**) R=H
(**2**) R=Me



(**3**) R^1=$^+$NHMe$_2$ (e), R^2=H
(**4**) R^1=$^+$NMe$_3$ (e) R^2=H
(**5**) R^1=H, R^2=$^+$NHMe$_2$ (e)
(**6**) R^1=H, R^2=$^+$NMe$_3$ (e)

Chemical shifts (p.p.m.) from DSS (±0·01) and line width at half-height in Hz ($\Delta\nu_{\frac{1}{2}}$) of free and DNA-bound reporter molecules (**1**)—(**6**) *at various temperatures.*[a,b]

System	Temp	δ p.p.m. from DSS and line width ($\Delta\nu_{\frac{1}{2}}$) e-CH_3[c]	f-CH_3	C-18 and C-19 CH_3	S.P.[d]
(**1**)	25	2·95 (2·0)	2·17 (2·2)	—	—
(**1**)	90	2·97 (1·5)	2·22 (2·0)	—	—
(**1**) DNA	25	SUBLN[e]	SUBLN	—	—
(**1**) DNA	33	2·92 (12)	1·40 (>20)	—	—
(**1**) DNA	51	2·96 (7)	1·50 (>20)	—	—
(**1**) DNA	70	2·95 (4)	1·64 (12)	—	—
(**1**) DNA	88	2·98 (2)	2·10 (3)	—	—
(**2**)	25	3·23 (2)	2·16 (2·5	—	—
(**2**)	90	3·24 (2)	2·21 (2·0)	—	—
(**2**) DNA	25	3·22 (18)	SUBLN	—	—
(**2**) DNA	51	3·22 (5)	1·60 (>20)	—	—
(**2**) DNA	88	3·26 (2)	2·18 (2)	—	—
(**3**)	35	2·84 (1·8)	—	0·86 (2·3); 0·90 (2·0)	1·00—2·20
(**3**)	90	2·84 (1·5)	—	0·83 (1·5); 0·87 (1·5)	1·00—2·20
(**3**) DNA	35	SUBLN	—	SUBLN	SUBLN
(**3**) DNA	50	2·77 (7·0)	—	SNCBLN[f]	SNCBLN[f]
(**3**) DNA	88	2·81 (2·2)	—	0·63 (3·0); 0·73 (3·0)	0·80—2·00
(**4**)	35	3·03 (2·0)	—	0·85 (2·0); 0·88 (2·0)	1·00—2·20
(**4**)	90	3·05 (1·5)	—	0·84; 0·87	1·00—2·20
(**4**) DNA	35	3·00 (15)	—	SUBLN	SUBLN
(**4**) DNA	50	3·00 (6·0)	—	SNCBLN	SNCBLN
(**4**) DNA	88	3·03 (2·4)	—	0·68 (3); 0·76 (3)	0·80—2·00
(**5**)	35	2·87 (1·7)	—	0·88 (4)	1·00—2·20
(**5**)	90	2·90 (1·5)	—	0·87 (1·8)	1·00—2·20
(**5**) DNA	35	SUBLN	—	SUBLN	SUBLN
(**5**) DNA	50	2·85 (14)	—	SNCBLN	SNCBLN
(**5**) DNA	88	2·89 (2·0)	—	0·73 (3); 0·77 (3)	0·80—2·00
(**6**)	35	3·03 (2·0)	—	0·85 (2); 0·88 (2)	1·00—2·20
(**6**)	90	3·05 (2·0)	—	0·85; 0·87	1·00—2·20
(**6**) DNA	35	3·00 (12)	—	SUBLN	SUBLN
(**6**) DNA	50	3·01 (7)	—	SNCBLN	SNCBLN
(**6**) DNA	88	3·02 (3·2)	—	0·71 (2); 0·77 (3)	0·80—2·00

[a] Sonicated low-molecular-weight salmon-sperm DNA (M <500,000) used for the ^{1}H n.m.r. experiments was prepared according to the procedure of Strauss, *et al.*[6] from native SS DNA (Worthington Co.). Nucleic acid solutions were prepared in D_2O in 10^{-4}M-sodium phosphate buffer, pD 7·00 ± 0·2 at 75 mg./ml. and diluted 4 : 5 with D_2O or 0·1M-reporter molecule in D_2O. Final concentrations of (**1**)—(**6**) and nucleic acid phosphate are 0·02M and 0·16M, respectively. Under these conditions the reporter molecules are fully bound.[7] [b] All spectra were determined on a Varian HA-100. [c] *N*-Methyl protons (e-CH_3). [d] Steroidal protons in A, B, C, and D rings. [e] Proton signal is indistinguishable from baseline noise (SUBLN). [f] Proton signal is not clearly distinguishable from baseline noise (SNCBLN).

ammonium group, Me_3N^+, of reporters (**2**), (**4**), and (**6**) since the resonance signals of the latter are clearly distinguishable from the baseline noise. These results are strongly suggestive of the hydrogen-bonding interactions between the $^+$N–H and H-bond acceptor in DNA. (ii) The ring methyl signal (f-CH_3) of the DNA-reporters (**1**) and (**2**) complexes is indistinguishable from the baseline noise at 25° Similarly, the proton signals of the steroidal nucleus including the C-18 and C-19 methyl groups are broadened at 35°. These results suggest that the rate of tumbling about the plane of the 2-methyl-4-nitroaniline ring and the steroidal nucleus is considerably slowed in the DNA complex presumably due to steric hindrance. (iii) Increasing temperatures cause a deshielding and sharpening of the resonance lines for the f-CH_3 of reporters (**1**) and (**2**), and the protons of the steroidal nucleus. For example, at 88° where partial melting of the DNA helix has occurred, sharp resonance lines characteristic of the free reporter molecule are observed. More rapid tumbling and efficient averaging of chemical shift environment is indicated. At this temperature melting of the DNA helix also occurs as evidenced by the appearance of the thymine-methyl peaks at 1·88 and 1·76 p.p.m.[5] It is also clear that denatured DNA at 88° still binds the reporter molecules as indicated by the different chemicals shifts of the f-CH_3 and steroidal protons including C-18 and C-19 methyls as compared with the free molecules.

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[1] For previous paper in this series, see E. J. Gabbay, B. L. Gaffney, and L. A. Wilson, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 854.
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