

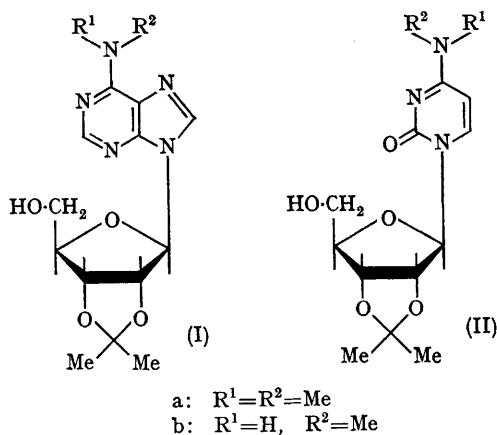
## Restricted Rotation in Methylated Derivatives of Adenosine and Cytidine

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WE recently reported that poly-N(6)-methyladenylic and polyuridylic acids form a 1:1 complex,<sup>1</sup> and that certain co-polymers of cytidylic and N(4)-methylcytidylic acids form 1:1 complexes with polyinosinic acid.<sup>2</sup> In both cases, the N-H protons of the methylamino-groups must essentially all be orientated in the same direction so that hydrogen-bonding with the uracil† or hypoxanthine residues may occur. As these complexes<sup>1,2</sup> form readily at *ca.* 5°, it was assumed that rotation of the appropriate 4- or 6-methylamino-groups could occur freely at this temperature. To determine whether or not this assumption is justified,‡ we have investigated the temperature dependence of the n.m.r. spectra of some suitably methylated derivatives of adenosine and cytidine.

N-methyl protons of 2',3'-O-isopropylidene-N(6),-N(6)-dimethyladenosine (Ia) at -60°, 0°, and 40°, are shown in Figure 1. It can be seen that the two sharp signals at -60° (325, 372 c./sec.) coalesce at *ca.* 0°, and that a single sharp signal with an approximately average chemical shift (346 c./sec.) is observed at 40°. This is a clear example of hindered internal rotation,<sup>3</sup> with slow exchange occurring below, and fast exchange occurring above, 0°. By plotting the appropriate



(i) *Adenosine derivatives.* The effect of temperature change on the n.m.r. spectra of deuteriochloroform solutions of the 2',3'-O-isopropylidene derivatives of N(6),N(6)-dimethyladenosine (Ia) and N(6)-methyladenosine (Ib) has been studied.§ The absorption regions (300–400 c./sec.) of the

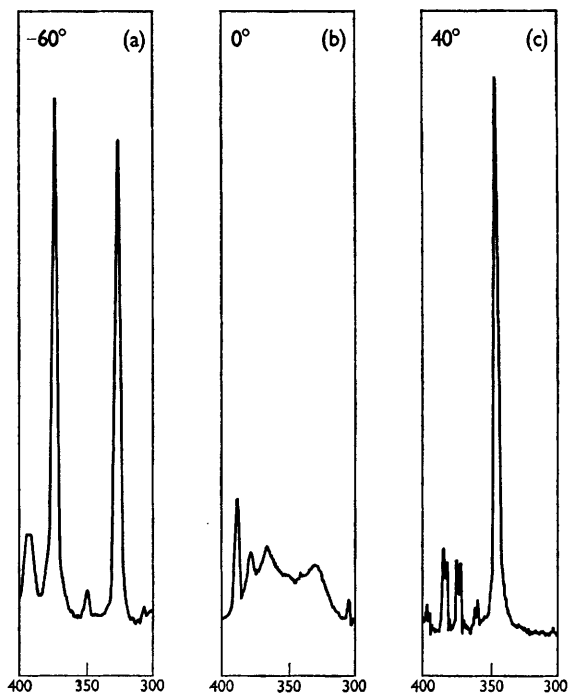


FIGURE 1. Absorption region of N-methyl protons of 2',3'-O-isopropylidene-N<sup>6</sup>,N<sup>6</sup>-dimethyladenosine (Ia) at (a) -60°, (b) 0°, and (c) 40°. Abscissa values are in c./sec. downfield from Me<sub>4</sub>Si.

† The hydrogen-bonding in the poly-N(6)-methyladenylic:polyuridylic acid complex need not be of the Watson-Crick type; it could involve N(7) [instead of N(1)] of the 6-methylaminopurine residues (G. Felsenfeld, D. R. Davies, and A. Rich, *J. Amer. Chem. Soc.*, 1957, **79**, 2023).

‡ We previously found (ref. 1) that the n.m.r. spectrum of N(6)-methyladenosine, in deuterium oxide solution at 35°, displayed only one signal attributable to the resonance of the N-methyl protons.

§ N.m.r. spectra were measured at 100 Mc./sec. with a Varian H100 spectrometer. Chemical shifts are given in c./sec. downfield from tetramethylsilane. Isopropylidene derivatives of nucleosides were used to promote solubility in a low-freezing solvent.

function<sup>¶</sup> of the line width against the absolute temperature, an Arrhenius plot was obtained with both slow- and fast-exchange points lying on the same straight line. The height of the energy barrier to rotation ( $E_a$ , see Table) was found to be 11.8 kcal./mole.

TABLE

Data relating to restricted rotation in NN-dimethyladenosine and -cytidine

Compound	$\Delta\nu^a$ (c./sec.)	$T_c^b$ (° c)	$E_a$ (kcal./mole)	$\log_{10} A^c$
(Ia) ..	47	0	11.8	11.5
(IIa) ..	12	30	8.6	7.8

<sup>a</sup> Separation of N-Me proton resonances under conditions of slow exchange; <sup>b</sup> Coalescence temperature; <sup>c</sup> Frequency factor.

It was not possible to apply the same treatment to 2',3'-O-isopropylidene-N(6)-methyladenosine (Ib), as fast exchange occurred at the lowest temperature examined ( $-60^\circ$ ). However, it may be concluded that the height of the energy barrier to rotation in (Ib) is not greater than 11.8 kcal./mole in deuteriochloroform solution. Therefore, unless the change of solvent has an unforeseen effect, it would seem reasonable to assume that the methylamino-group can rotate freely under the conditions required<sup>1</sup> for complex formation between poly-N(6)-methyladenylic and polyuridylic acids.

(ii) *Cytidine derivatives.* The temperature dependence of the n.m.r. spectra of 2',3'-O-isopropylidene derivatives of N(4),N(4)-dimethylcytidine (IIa) and N(4)-methylcytidine (IIb), in deuteriochloroform solution, was studied in the same way. The absorption regions (280–340 c./sec.) of the N-methyl protons of 2',3'-O-isopropylidene-N(4),N(4)-dimethylcytidine (IIa), at 5°, 30°, and 50°, are shown in Figure 2. As before [with (Ia)] an Arrhenius plot was obtained, with both slow- and fast-exchange points lying on the same straight line. The height of the energy barrier to rotation (see Table) was found to be 8.6 kcal./mole. A quantitative treatment was

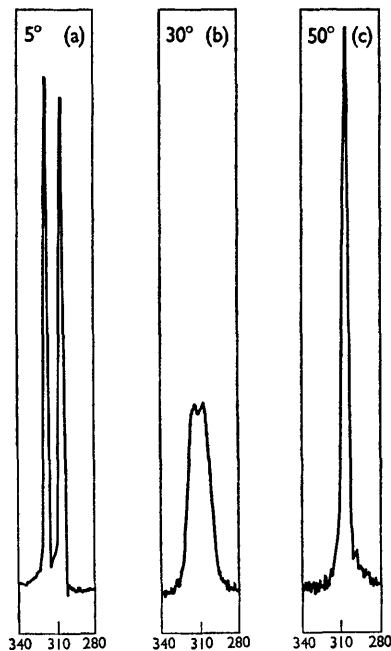


FIGURE 2. Absorption region of N-methyl protons of 2',3'-O-isopropylidene-N<sup>4</sup>,N<sup>4</sup>-dimethylcytidine (IIa) at (a) 5°, (b) 30°, and (c) 50°. Abscissa values are in c./sec. downfield from Me<sub>4</sub>Si.

again excluded for the methylamino-compound (IIb) as fast exchange occurred at the lowest temperature examined ( $-60^\circ$ ).<sup>\*\*</sup> It is thus concluded that the height of the energy barrier to rotation in (IIb) is not greater than 8.6 kcal./mole and that, subject to the above reservation, the methylamino-group can rotate freely enough at 5° to account for the observed stoichiometry<sup>2</sup> of the complex formation between polyinosinic acid and co-polymers of cytidylic and N(4)-methylcytidylic acids.

We thank Dr. Andrew McLachlan for very helpful discussions. One of us (D.M.G.M.) thanks the S.R.C. for the award of a research studentship.

(Received, October 16th, 1967; Com. 1110.)

<sup>¶</sup> Depending on whether slow or fast exchange is occurring (ref. 3).

<sup>\*\*</sup> E. D. Becker, H. T. Miles, and R. B. Bradley (*J. Amer. Chem. Soc.*, 1965, **87**, 5575) have examined the temperature dependence of the n.m.r. spectrum of the conjugate acid of the corresponding derivative of 1-methylcytosine, and have observed slow exchange below 100°.

<sup>1</sup> B. E. Griffin, W. J. Haslam, and C. B. Reese, *J. Mol. Biol.*, 1964, **10**, 353.

<sup>2</sup> R. L. C. Brimacombe and C. B. Reese, *J. Mol. Biol.*, 1966, **18**, 529.

<sup>3</sup> A. Carrington and A. D. McLachlan, "Introduction to Magnetic Resonance," Harper and Row, New York, 1967, p. 204 *et seq.*