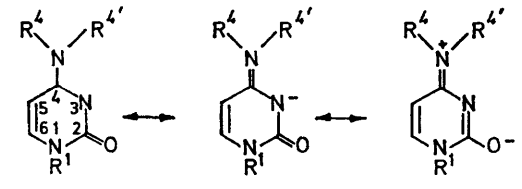
# Restricted Rotation about the Exocyclic C–N Bond in Nucleic Acid Bases

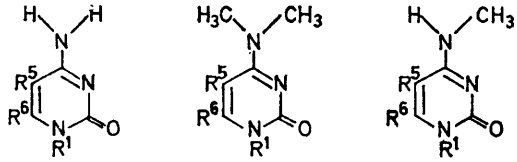
By K. Gurudath Rao and C. N. Ramachandra Rao,* Department of Chemistry, Indian Institute of Technology, Kanpur-16, India

Barrier heights to rotation of amino- and methylamino-groups in several cytosine and guanine derivatives have been estimated by CNDO/2 calculations. The results are in accord with the n.m.r. studies recently reported on three cytosine derivatives. The present studies are of relevance in understanding the stabilities and interactions of methylated nucleic acids. Hindered rotation of the amino-group is also found in adenine.

THE amino-group in purine and pyrimidine bases has generally been presumed to undergo relatively free rotation about the exocyclic C–N bond. The amino-group in cytosine derivatives is, however, likely to have significant amide character due to contributions from structures (Ia—c). The barrier to rotation of the



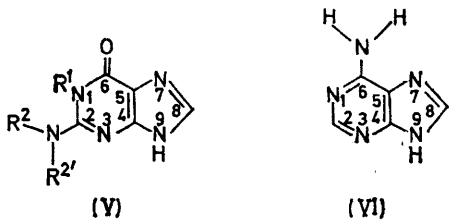
(Ia) (Ib) (Ic)



(II) (III) (IV)

amino-group of nucleotide bases is of significance in understanding the configuration and interaction of polynucleotides. While either coplanar conformation of the unsubstituted amino-group in (II) can participate in base pairing, neither conformation is capable of pairing in the dimethylamino-derivative (III). In the monomethyl derivative (IV) only one rotomer can form base pairs. Restriction to rotation in the individual bases will also be present in the polymers although the equilibrium would be affected by helix formation. Recently, n.m.r. spectral evidence for the restricted rotation of the amino group in cytosine has been provided by Shoup *et al.*[1] These workers have also determined the rotation barriers of the dimethylamino-group in cytosine derivatives[2] and have noted that there may be restricted rotation in the monomethylamino-derivative as well. It is relevant to note here that the importance of methylated bases in determining the structure and biological functions of nucleic acids has been emphasized in the literature.[3,4] Many of the methylated nucleic acids essentially retain the stability and order of the parent acids[3-5] and this is undoubtedly dependent on the magnitude of the barrier height to rotation about the exocyclic C–N bond.

We have carried out MO calculations employing the CNDO/2 method[6] on several amino-, methylamino-, and dimethylamino-derivatives of cytosine with the primary purpose of evaluating barrier heights to rotation about the exocyclic C–N bond, since this method has been found to be quite successful in estimating barrier heights to rotation about C–N bonds in simple primary and secondary amides.[7-9] We have extended these calculations to three guanine derivatives (V) and also to adenine (VI). For purpose of comparison, we have carried out extended Hückel calculations[10] on a few of the derivatives since the EHT method has been



(V) (VI)

fruitfully employed for estimating barrier heights in amides.[8,9]

## METHOD

The details of the CNDO/2 and EHT methods employed in our calculations are given in earlier papers.[7,8,11,12] The structural parameters of cytosine and guanine were

[1] R. R. Shoup, E. D. Becker, and H. T. Miles, *Biochem. Biophys. Res. Comm.*, 1971, **43**, 1350.
[2] R. R. Shoup, E. D. Becker, and H. T. Miles, *J. Phys. Chem.*, 1972, **76**, 64.
[3] P. R. Srinivasan and E. Borek, *Science*, 1964, **145**, 548.
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[5] R. L. C. Brimacombe and C. B. Reese, *J. Mol. Biol.*, 1966, **18**, 529.
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[7] C. N. R. Rao, K. G. Rao, A. Goel, and D. Balasubramanian, *J. Chem. Soc.* (*A*), 1971, 3077.
[8] A. S. N. Murthy, K. G. Rao, and C. N. R. Rao, *J. Amer. Chem. Soc.*, 1970, **92**, 3544.
[9] J. F. Yan, F. A. Momany, R. Hoffmann, and H. A. Scheraga, *J. Phys. Chem.*, 1970, **74**, 420.
[10] R. Hoffmann, *J. Chem. Phys.*, 1963, **39**, 1397.
[11] C. N. R. Rao, A. Goel, K. G. Rao, and A. S. N. Murthy, *J. Phys. Chem.*, 1971, **75**, 1744.
[12] A. Goel, A. S. N. Murthy, and C. N. R. Rao, *J. Chem. Soc.* (*A*), 1971, 190.

taken from the literature.[13,14] The exocyclic C–C and methylamino C–N distances were taken to be 1·50 and 1·47 Å respectively. The energy of the molecule concerned was calculated as a function of the angle of rotation ($\theta$) of the energy difference between that of the planar conformation ($\theta = 0$ or 180°) and the transition state ($\theta = 90°$) was taken as the barrier height $E_a$. In the case of the methylamino-derivatives of cytosine (IV) and guanine (V; $R^{2} = H$, $R^{2'} = CH_3$), we have listed the barrier heights with respect to the stable *syn*-conformer.

All calculations were carried out with an IBM 7044/1401 computer.

## RESULTS AND DISCUSSION

The results of the MO calculations on cytosine derivatives are summarised in Tables 1 and 2. We see that there is restricted rotation of the amino-group in cytosine as suggested by Shoup *et al.*[1] based on high resolution n.m.r. studies.* The calculated barrier height to rotation is quite large (105 kJ mol$^{-1}$). The barrier height is sensitive to methyl substitution in the 1-, 5-, and 6-positions, being smallest when the 5-position is substituted (Table 1). In nucleosides where the sugar residue is present, the barrier height may therefore be expected to be slightly different from the value in the corresponding methylated derivative.

TABLE 1

CNDO/2 Estimates of barrier heights to rotation in cytosine derivatives (I)

| $R^4$ | $R^{4'}$ | $R^1$ | $R^5$ | $R^6$ | $E_a$/kJ mol$^{-1}$ |
|---|---|---|---|---|---|
| H | H | H | H | H [a] | 105 |
| H | H | Me | H | H | 84 |
| H | H | H | H | Me | 84 |
| H | H | H | Me | H | 67 |
| H | Me | H | H | H [b] | 75 |
| H | Me | Me | H | H | 75 |
| H | Me | H | H | Me | 75 |
| Me | Me | H | H | H [c] | 71 |
| Me | Me | Me | H | H | 71 |

[a] EHT calculations give a value of 63 kJ mol$^{-1}$ for $E_a$ for this derivative. [b] $E_a$ from EHT is 71 kJ mol$^{-1}$, but the energy difference between the *syn*- and *anti*-conformer is large (*ca.* 9 kJ mol$^{-1}$). [c] $E_a$ from EHT is 67 kJ mol$^{-1}$.

The barrier height to rotation of the methylamino-group in (IV) is lower compared to that of the unsubstituted amino-group and the barrier height is not sensitive to methyl substitution in 1- and 6-positions. In the case of the 5-methyl derivative, the conformation where the 4-NMe group is *syn* to N-3 [as in structure (IV)] is very much more stable than the *anti*-conformation (by *ca.* 56 kJ mol$^{-1}$) in accordance with the n.m.r. results;[2] the energy of the non-planar conformation ($\theta = 90°$) lies between the energies of these two conformers. The *syn*-conformation is more stable than the *anti*-conformation in the other 4-methylamino-derivatives as well but the energy difference is very small (0·4—0·8 kJ mol$^{-1}$). The abundance of the *syn*-conformer in these derivatives is consistent with the n.m.r. results[2] as well as with the known information on the *cis–trans* isomerism in simple secondary amides.

These results may be relevant in understanding the nature of interaction found with 4-*N*-methylpolycytidylic acid (4*N*-MeC). It is known the copolymer (3 : 2) of polycytidylic acid (C) and 4*N*-MeC interacts with polyinosinic acid.[5] The stoicheiometry indicates that there is paring to 4*N*-MeC as well as C residues. Apparently, once the helix formation is initiated by the unsubstituted C residues, the energetic advantage may be sufficient to overcome the steric interference between the *N*-methyl group and the 5-proton. While methylation may affect physical properties like phase transition temperatures, it appears that it does not destroy the ordered structure of nucleic acids.[3,4]

The barrier height to rotation in the dimethylamino-derivative (III) (71 kJ mol$^{-1}$) is in accord with the experimental value.[2] Methyl substitution in the 1-position of (III) does not affect the barrier height, consistent with n.m.r. observations.[2] It is noteworthy that the barrier heights to rotation about the exocyclic C–N bond in cytosine derivatives varies in the order 4-$NH_2$ > 4-MeNH > 4-$NMe_2$. This trend is significant

TABLE 2

CNDO Net charges in cytosine derivatives

| Cytosine | | C-2 | C-4 | C-5 | C-6 | N-1 | N-3 | 4-N | 2-O | $\mu$/D [a] |
|---|---|---|---|---|---|---|---|---|---|---|
| (II; $R^1 = R^5 = R^6 = H$) | σ | 0·219 | 0·105 | 0·018 | 0·050 | −0·515 | 0·018 | −0·420 | 0·087 | 7·7 |
| | π | 0·206 | 0·220 | −0·204 | 0·143 | 0·331 | −0·363 | 0·175 | −0·507 | |
| (II; $R^1$ = Me; $R^5 = R^6 = H$) | σ | 0·206 | 0·109 | 0·032 | 0·043 | −0·521 | 0·022 | −0·421 | 0·099 | 6·2 |
| | π | 0·190 | 0·213 | −0·202 | 0·129 | 0·381 | −0·352 | 0·180 | −0·478 | |
| (II; $R^6$ = Me; $R^1 = R^5 = H$) | σ | 0·218 | 0·110 | 0·017 | 0·118 | −0·524 | 0·020 | −0·421 | 0·094 | 7·3 |
| | π | 0·200 | 0·218 | −0·212 | 0·088 | 0·321 | −0·356 | 0·180 | −0·489 | |
| (II; $R^5$ = Me; $R^1 = R^6 = H$) | σ | 0·219 | 0·099 | 0·077 | 0·042 | −0·519 | 0·017 | −0·421 | 0·087 | 8·7 |
| | π | 0·204 | 0·212 | −0·179 | 0·124 | 0·335 | −0·357 | 0·176 | −0·506 | |
| (IV; $R^1 = R^5 = R^6 = H$) | σ | 0·218 | 0·101 | 0·016 | 0·051 | −0·515 | −0·015 | −0·391 | 0·082 | 7·9 |
| | π | 0·026 | 0·213 | −0·202 | 0·141 | 0·330 | −0·362 | 0·211 | −0·508 | |
| (III; $R^1 = R^5 = R^6 = H$) | σ | 0·217 | 0·100 | 0·017 | 0·051 | −0·515 | 0·013 | −0·367 | 0·087 | 7·6 |
| | π | 0·205 | 0·207 | −0·200 | 0·139 | 0·329 | −0·362 | 0·248 | −0·509 | |

[a] Dipole moment.

* Spin-lattice relaxation measurements on crystalline cytosine (Adams, Jones, and Thomas, *Trans. Faraday Soc.*, 1970, **66**, 1854) also seem to indicate the presence of hindered rotation of the amino-group.

[13] D. Voet and A. Rich, *Progr. Nucleic Acid Res.*, 1970, **10**. 183.
[14] E. J. O'Brien, *Acta Cryst.*, 1967, **23**, 92.

in explaining the stabilities and interactions of methylated nucleic acids since a lower barrier height to rotation in methylated bases would facilitate proper orientation of the base sites.

TABLE 3

CNDO/2 Estimates of barrier heights to rotation in guanine derivatives (V) [a]

| $R^2$ | $R^{2'}$ | $R'$ | $E_a$/kJ mol$^{-1}$ |
|---|---|---|---|
| H | H | H [b] | 67 |
| H | H | Me | 84 |
| H | Me | H [c] | 63 |

[a] $R^8 = R^9 = H$ in all cases. [b] EHT calculations give a value of 55 kJ mol$^{-1}$ for $E_a$. [c] The energy difference between the conformers is 0·4 kJ mol$^{-1}$.

The CNDO/2 charges on the various atoms in the cytosine (Table 2) are similar to those found by Pullman and his co-workers.[15] Among the amino-, 4-methylamino-, and 4-dimethylamino-derivatives, the main change is found at the C-4 and 4-H positions; the variations of the net $\sigma$ and $\pi$ charges on the C-4 and 4-N atoms appear to be consistent with the decreased barrier height in the methylamino-derivatives. The variation in the charges on methyl substitution in the 1-, 5-, or 6-positions are in the expected direction. In Table 2 we have listed the charges in all the methyl derivatives of (II) since they may be of value in understanding the role of these bases in biology.

The barrier heights from MO calculations on guanine derivatives (V) are shown in Table 3. The barrier height to rotation of the amino-group about the exocyclic C–N bond of guanine is comparable to that in some of the cytosine derivatives. The barrier height is slightly lower in the 2-methylamino-derivative, but is appreciably enhanced when there is methyl substitution in 1-position. The CNDO/2 charges on the various atoms of guanine derivatives are given in Table 4; the charges in the parent base are in agreement with those found by Pullman and his co-workers.[15]

The barrier to the rotation of the amino-group of adenine (VI) about the exocyclic C–N bond is estimated to be 67 kJ mol$^{-1}$ by the CNDO/2 method and 50 kJ mol$^{-1}$ by the EHT method. The barrier is appreciable compared to simple aromatic amines. The charges on the various atoms of adenine are shown in Table 4; these are in agreement with those found by Pullman and his co-workers.[15]

We see that both EHT and CNDO/2 methods predict the presence of restricted rotation about the exocyclic C–N bond in cytosine and guanine derivatives.

TABLE 4

CNDO Charges in guanine derivatives (V) and adenine (VI)

| Compound | | C-2 | C-4 | C-5 | C-6 | C-8 | N-1 | N-2 | N-3 | N-7 | N-9 | 6-O | $\mu$/D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (V; $R^2 = R^{2'} = H$; | $\sigma$ | 0·189 | 0·130 | −0·129 | 0·164 | 0·184 | −0·504 | −0·413 | 0·062 | −0·019 | −0·494 | 0·079 | 7·2 |
| $R^1 = H$) | $\pi$ | 0·200 | 0·080 | −0·232 | 0·191 | −0·047 | 0·282 | 0·164 | −0·393 | −0·130 | 0·348 | −0·464° | |
| (V; $R^2 = R^{2'} = H$; | $\sigma$ | 0·182 | 0·134 | 0·126 | 0·165 | 0·186 | −0·501 | −0·380 | 0·057 | −0·031 | −0·484 | 0·080 | 7·6 |
| $R^1 = Me$) | $\pi$ | 0·189 | 0·085 | −0·237 | 0·192 | −0·041 | 0·284 | 0·210 | −0·384 | −0·140 | 0·354 | −0·468 | |
| (V; $R^2 = R^1 = H$; | $\sigma$ | 0·184 | 0·130 | 0·129 | 0·164 | 0·184 | −0·506 | −0·392 | 0·056 | −0·019 | −0·494 | 0·083 | 7·4 |
| $R^{2'} = Me$) | $\pi$ | 0·194 | 0·081 | −0·232 | 0·191 | −0·047 | 0·284 | 0·196 | −0·389 | −0·130 | 0·349 | −0·465 | |
| (VI) | $\sigma$ | 0·097 | 0·154 | 0·099 | 0·112 | 0·159 | −0·031 | | −0·021 | −0·033 | −0·479 | −0·386 [a] | 2·5 |
| | $\pi$ | 0·111 | 0·058 | −0·154 | 0·155 | 0·001 | −0·254 | | −0·223 | −0·181 | 0·339 | 0·148 [a] | |

[a] These charges are for 6-N of adenine.

The barrier heights from the two methods vary slightly with EHT values generally being lower, but the results provide a bracketing range to be expected from more rigorous calculations.[9] Amongst three parent bodies, both the methods predict the highest barrier to rotation of the unsubstituted amino-group in cytosine; the EHT method shows the trend in barrier height to be cytosine > guanine > adenine, a trend which is understandable in terms of the double bond character of the C–N bonds.

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[2/1950 *Received*, 15*th August*, 1972]

[15] B. Pullman and A. Pullmann, *Progr. Nucleic Acid Res.*, 1969, **9**, 328.