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THE CRYSTAL AND MOLECULAR STRUCTURE OF 3-METHYLGUANINE, A POTENTIALLY MISCODING BASE

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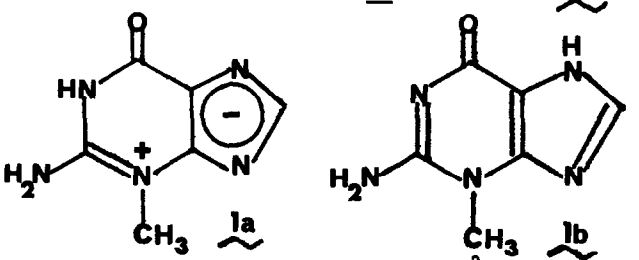
The structure of 3-Methylguanine (3 MeG) has been held in question with differences of opinions reported by three groups: Pullman[1], Townsend and Robins[2], and Coburn and Carapellotti[3]. Two structural forms have been proposed for 3 MeG: one is the mesoionic, 1H tautomer, (1a)[2] and the other is the covalent 7H tautomer[1] (1b).



In their initial communication, Townsend and Robins[2] concluded that 3 MeG must exist to some degree in the mesoinic form due to its observed chemical properties. These workers discovered 3 MeG to be resistant to attack by nitrous acid and that it hyrolyzed readily in aqueos base to form 3-methylxanthine (both reactivities are unlike those observed in guanine). Therefore, they concluded that a positive charge must reside in the pyrimidine ring with a negative charge on the imidazole. This can be achieved only if the proton on the N(1) atom of guanine were to remain in this position after methylation at the three position.

Pullman[1] on the other hand concluded via molecular orbital calculations that 3 MeG probably exists as the N(7)-H tautomer. His calculations predicted the mesoionic tautomer proposed for this compound to be about 50 kcal/mole less stable than any of the usual tautomers of guainine, including the N(7)-H tautomer for 3 MeG. Besides, these calcualtions also predicted a very high dipole moment for the N(1)-H tautomer. While no information is available on its dipole moment, Pullman points out that the absorption spectrum for 3 MeG (λ max = 264 nm) does not exhibit an expected bathochromic shift.

Coburn and Carapellotti[3] however concluded that based on their studies on mesoionic compounds, one cannot exclude the existence of the mesoionic tautomer solely on the basis of ultraviolet spectroscopic data.

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†Anyone wishing computer drawn depictions of the molecule and its interactions with itself and H_2O, as well as coordinates etc., should write the primary authors for photo copies.

We undertook the determination of the crystal structure of 3 MeG in order to establish its naturally existing tautomer in the solid state. We were also interested in comparing the intermolecular interactions of the alkylated base with those of guanine from which one might gain information on possible miscoding mechanisms by such residues on normal Watson-Crick base pairing in DNA.

EXPERIMENTAL SECTION

Long, prismatic needle-shaped crystals of 3 MeG were grown from an aqueous solution. Preliminary oscillation and Weissenberg photographs indicated that the crystals were monoclinic mounted about the unique axis. The space group was determined from diffractometer measurements to be the centrosymmetric $P2_1/c$. The cell parameters are $\underline{a}$ = 10.204 (2), $\underline{b}$ = 10.555 (2), $\underline{c}$ = 16.222 Å (4), and β = 92.7° (3). Since only one suitable crystal was available, no density measurments were made. However, assuming a reasonable density of 1.4, at least two molecules per asymmetric unit were indicated. Final solution of crystal structure showed two molecules of 3 MeG and three water molecules per asymmetric unit. Three dimensional Cukα graphite-monochromated X-ray data were collected using a θ 2 θ scan on the Nonius CAD-4 diffractometer. Reflections with indices hkl and $\bar{h}$kl were collected. Of 3537 independent X-ray intensities measured, 2919 were found to be three standard deviations above background and were thus considered significant.

The crystal structure was determined by a combination of direct methods, using the X-ray 70 link, Phase [4], and Fourier methods. The positions of the hydrogen atoms were determined from difference Fourier maps obtained after several cycles of least squares refinement of the heavy atom parameters. Full matrix least squares techniques were used throughout the refinement of the structure and the final cycle included a Cruickshank-type weighting scheme giving a final R value for all reflections of 0.056.

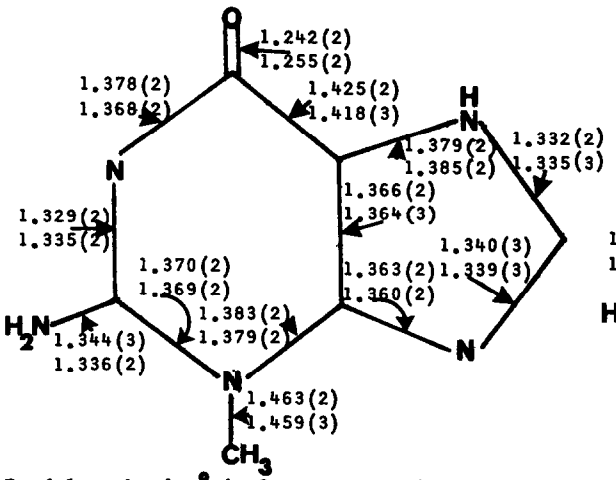
RESULTS AND DISCUSSION

The results of the crystal structure analysis clearly show that 3 MeG in this crystal state exists as the N(7) tautomer proposed by Pullman [1]. The evidence for this conclusion comes from the direct determination of the position of the proton on N(7) by difference Fourier synthesis and from the implications which one can draw from the observed bond lengths and angles.

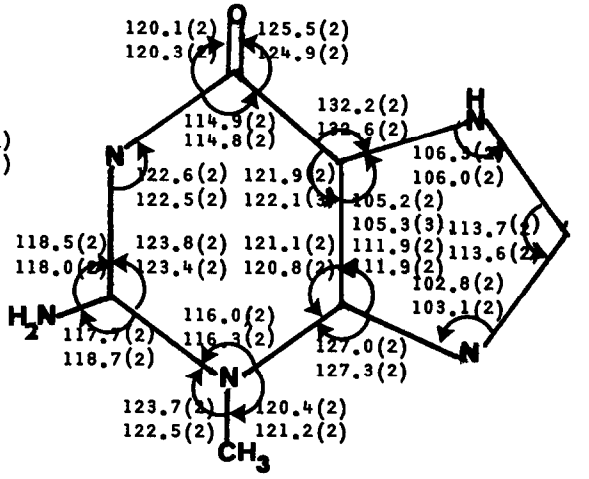
The latter arguments based on observed bond lengths and angles are summarized below. The C(2)-N(1) bond in the crystal is observed to be much shorter than the C(2)-N(3) bond which is consistant with structure 1b. Next the bond angles around atoms N(1), N(7), and N(9) are also consistant with the N(7)-H tautomer proposed by Pullman. For example it has been observed that the heterocyclic nitrogen valency angles decrease or increase depending on the relative partial charge on the nitrogen atom. In the adenine example protonation at the N(1) position causes the C(6)-N(1)-C(2) angle to open. In our present work we observe that compared to guanine the C(6)-N(1)-C(2) angle in 3 MeG is significantly smaller. This agrees with having no proton on N(1) for 3MeG. Also the shift of the larger angle from around N(9) in guanine to N(7) in 3 MeG implies that the proton normally found attached to N(9) in guanine is now attached at the N(7) position in 3 MeG.

Although both molecules (A and B) in the asymmetric unit exhibit the same N(7)-H tautomer,

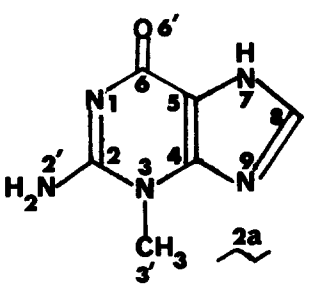
some minor but significant differences are observed in some of the interatomic distances and angles in the pyrimidine ring. The C-O bond is observed to be longer in molecule B, the N(1)-C(6) bond is longer in molecule A, the N(1)-C(2) bond is longer in molecule B, and the C(2)-N(2′) bond is longer in A . These significant differences vary from twice the standard deviation to five times the standard deviation in the observed bond lengths. Together with other observations, which will be discussed below, these variation in the two structures qualitatively suggest a relative difference in the predominant resonance forms in the two molecules. Molecule A appears to have more influence from the resonance form, 2a, whereas molecule B appears to have greater influence from resonance from 2b. The observed differences in the bond lengths in the pyrimidine ring of molecules A and B apparently may be rationalized by these differences in resonance contributions.



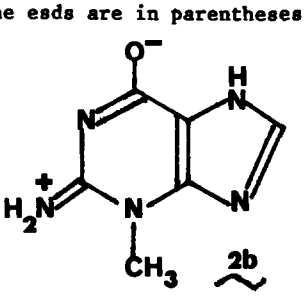
Bond lengths in Å in 3 MeG. The first numbers refer to molecule A, the second to B. The esds are in parentheses.



Bond angles in (°) for 3 MeG. The first numbers refer to molecule A, the second to B. The esds are in parentheses.



2a



2b

Two other observations may bolster the assumption regarding the differences in the predominant resonance forms between the two independant molecules. First is the fact that the atom O(6′) of molecule B is involved in two hydrogen bonds, the geometry of which is approximately tetrahedral. This is consistent with resonance form 2b, which predicts a relatively more significant amount of sp^3 hybridization around O(6′). Second, it is observed that the hydrogen atoms of the N(2′) amino group of molecule A are more deviated from the pyrimidine plane as compared to that of molecule B. This observation is consistent with resonance form 2a, where a more significant amount of sp^3 hybridization is predicted around the exocyclic N(2′) atom.

The hydrogen bonding between molecules A and B is very similar to that observed in guanine[6], 8-azaguanine [7], and almost identical to guanosine[8], except in 3 MeG a proton resides on N(7) and thus N(1) serves as the hydrogen bond acceptor. Also methylation at N(3) disrupts the hydrogen bonding observed between N(9) and N(3) in guanine and 8-azaguanine. Both N(9) atoms in 3 MeG accept hydrogen bonds from the water molecules.

BIOLOGICAL IMPLICATIONS

It has been shown [9] that relatively small amounts of alkylation at the N(3) atom of guanine can occur in nucleic acids and poly (G) if these are treated with agents of both the S_N2 and S_N1 type at neutral pH. Other sites viz., at N(7) and O(6), are apparently restricted to alkylation by S_N1 agents. This paper deals with the N(3) alkylation product, (3 MeG) which can be considered as a potentially miscoding base. Its formation in DNA may provide an explanation for the relatively weak mutagenic activity of the S_N2 alkylating agents.[9] The N(3) methylated base may also serve as a purine antagonist at other levels of guanine activity.[2] The hydrogen bonding as observed in the crystal structure could be of possible biological interest. Compared to guanine, 3 MeG lacks the proton at the N(1) position which is involved in the Watson-Crick type of base pairing and N(1) is now a potential hydrogen bond acceptor. In the Watson-Crick scheme of base pairing, guanine donates its N(1) hydrogen to cytosine. Removal of this proton (as a result of N(3) methylation) would naturally alter this interaction giving rise to possible miscoding or deletions in the original code.

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