BASE STACKING IN NUCLEIC ACID COMPONENTS: THE CRYSTAL STRUCTURES OF GUANINE, GUANOSINE AND INOSINE*

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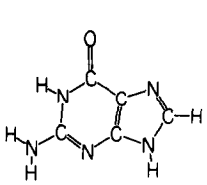
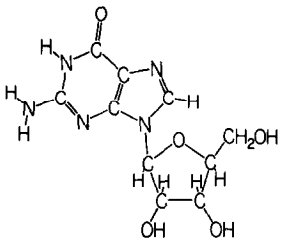
Interactions among the constituents of the nucleic acids are believed to be of major importance in genetic replication and protein synthesis. These interactions appear to be primarily of two types: hydrogen bonding and base stacking. Since the role of hydrogen bonding in nucleic acids was first suggested by Watson and Crick (Watson and Crick, 1953), much evidence supporting its importance has been accumulated. Recently, however, a number of investigations have suggested that the dominating force of interactions between nucleic acid constituents in aqueous solution involves parallel stacking of the purine and pyrimidine bases (Chan Et.Al., 1964; Chan Et.Al., 1966; Solie and Schellman, 1968; Ts'o Et.Al., 1963; Ts'o Et.Al., 1964). We report here the crystal structures of guanine (I), guanosine (II) and inosine (III), which suggest that parallel stacking of purine bases is an important structural feature in the solid state as well.



I



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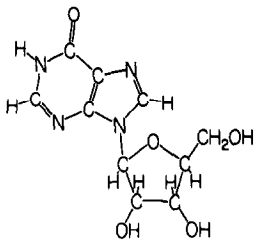

III

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METHODS

Crystals of guanosine were obtained by slowly cooling a hot, saturated aqueous solution; inosine was crystallized from water and guanine from a mixture of water and dimethylamine by slow evaporation at room temperature. Crystal data for the three compounds are listed in Table 1.

TABLE 1. CRYSTAL DATA

	GUANINE	GUANOSINE	INOSINE
Stoichiometry	$C_5H_5N_5O \cdot H_2O$	$C_{10}H_{13}N_5O_5 \cdot 2H_2O$	$C_{10}H_{12}N_4O_5 \cdot 2H_2O$
Space Group	$P2_1/n$	$P2_1$	$P2_1$
Z	4	4	4
a (Å)	16.510	17.518	17.573
b (Å)	11.277	11.502	11.278
c (Å)	3.645	6.658	6.654
β (degrees)	98.84	98.17	98.23

Intensity data were collected on a Datex-automated General Electric XRD-5 diffractometer using nickel-filtered copper radiation and a θ-2θ scan technique. For guanosine, each of the 2967 reflections in the range $7° \leq 2\theta \leq 154°$ was measured. Since the inosine crystals suffered x-ray damage which especially affected the high-angle reflections, measurements were confined to the 1431 reflections with $7° \leq 2\theta \leq 100°$. Our best guanine crystals were extremely small needles which produced very few, weak high-angle reflections; therefore only the 467 reflections with $7° \leq 2\theta \leq 90°$ were measured. In addition, multiple-equi-inclination Weissenberg photographs were taken of the layers $\ell = 0$, 1 and 2; the intensities were estimated visually and averaged with the diffractometer data.

The structure of guanosine was solved first. Trial coordinates for the atoms of the two guanine residues in the asymmetric unit were obtained

from a sharpened, three-dimensional Patterson map. Trial coordinates for the atoms of the sugar (ribose) groups were obtained by rotating reasonable models of the ribose ring around the glycosidic linkages and calculating the R index ($R = \Sigma ||Fo|-|Fc|| / \Sigma |Fo|$) for low angle hk0 data as a function of the rotation angle; the correct conformations resulted in minima in the R index. The water molecules were located on subsequent difference Fourier maps. Three-dimensional refinement was carried out mainly by the method of least squares; the hydrogen atoms were located on difference Fourier maps calculated during the final stages of refinement. Finally, all the positional parameters, along with anisotropic temperature factors for the heavy atoms and isotropic temperature factors for the hydrogen atoms, were adjusted by multiple-matrix least squares to give a final R index of 0.036.

The lattice constants (Table 1) of guanosine and inosine are very similar; also, the intensity patterns on Weissenberg photographs of the two compounds are similar. Therefore, we assumed that the two structures are approximately the same (except for the extra $-NH_2$ group in guanosine). Least squares refinement of inosine was initiated using the atomic parameters found for guanosine, and proceeded satisfactorily to a final R index of 0.032.

The structure of guanine was found by trial-and-error methods predicated on the presumption that the arrangement of hydrogen bonds along the two-fold screw axes was the same as in guanosine and inosine, as evidenced by the similarity in the lengths of the b axes. Least squares refinement proceeded to an R index of 0.101. The higher R index for this compound is a reflection of the very small crystal used to collect the data, which resulted in low intensities and poor counting statistics.

RESULTS AND DISCUSSION

Two prominent and common features of the three crystal structures are the arrangement of hydrogen bonds along two-fold screw axes and the stacking of parallel purine rings. The hydrogen bonding is shown in figure 1. In guanine and guanosine, adjacent bases are joined by two hydrogen bonds

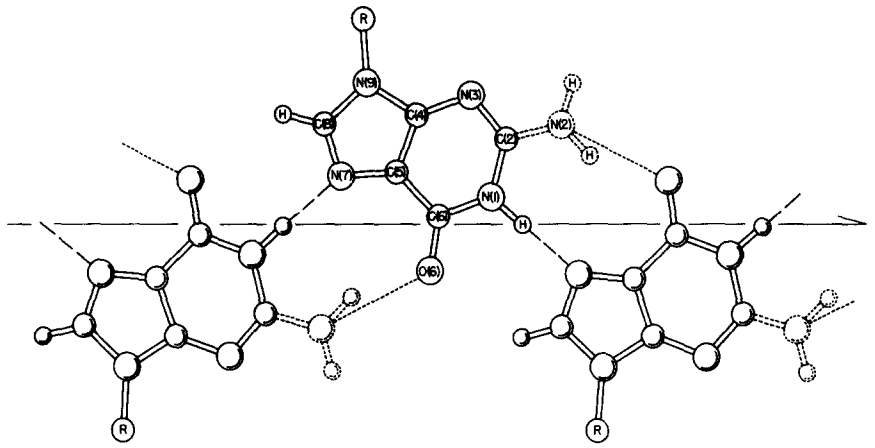

Figure 1: Hydrogen bonding between purine bases in guanine, guanosine and inosine (inosine lacks the $-NH_2$ group).

(N_1--N_7 and N_2---O_6); in inosine, which lacks the $-NH_2$ group, a single N_1---N_7 hydrogen bond joins adjacent bases. The N_1---N_7 distances range from 2.80 Å to 2.88 Å, and the N_2---O_6 distances range from 2.92 Å to 2.99 Å.

The major interaction between the crystallographically independent nucleosides in guanosine and inosine involves parallel stacking of the purines; this stacking is shown in figure 2 (b). The planes of the structurally independent purines are practically parallel to the (001) crystallographic plane and are situated at approximately z=0 and z=0.5; the separation between successive purine planes is 3.3 Å. The stacking in guanine is shown in figure 2 (a); once again, the spacing between successive purine planes is 3.3 Å. It is interesting to note that the overlapping of bases is accomplished so that, for the most part, atoms of differing electronegativities lie above one another.

It is especially interesting that inosine forms the same crystal structure as guanosine in spite of the fact that it lacks the amino group which is an integral part of the hydrogen bonding between bases. This suggests that the stacking of the bases is of preponderant importance in determining the structure, and may be a reasonable model for the nucleoside interactions in solution.

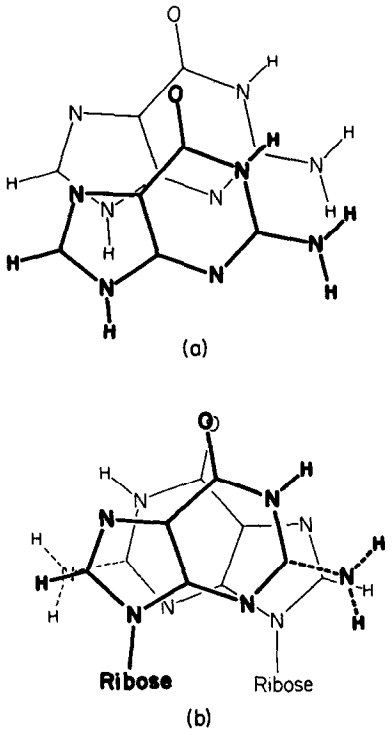

Figure 2: (a) Stacking of guanine as viewed perpendicular to the guanine planes. (b) c axis projection showing the purine stacking in guanosine and inosine (inosine lacks the $-NH_2$ group).

The detailed crystal structures of these compounds will be published elsewhere.

REFERENCES

Chan, S. I., Bangerter, B. W., and Peter, H. H., Proc. Nat. Acad. Sci., Wash., 55, 720 (1966).

Chan, S. I., Schweizer, M. P., Ts'o, P. O. P., and Helmkamp, G. K., J. Amer. Chem. Soc., 86, 4182 (1964).

Solie, T. N., and Schellman, J. A., J. Mol. Biol., 33, 61 (1968).

Ts'o, P. O. P., and Chan, S. I., J. Amer. Chem. Soc., 86, 4176 (1964).

Ts'o, P. O. P., Melvin, I. S., and Olsen, A. C., J. Amer. Chem. Soc., 85, 1289 (1963).

Watson, J. D., and Crick, F. H. C., Nature, 171, 737 (1963).