Note added in proof: The assignment of 1A2G as one of the glutamine triplets[1] has been made questionable by the finding that, in the presence of an excess of cold glutamic acid, there was little or no stimulation of the incorporation of glutamine by poly AG (5:1). This suggests that the previously observed stimulation may have been due to the occurrence of deamidation of glutamine to glutamic acid, the incorporation of which is stimulated by this polymer.

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*THE CRYSTAL STRUCTURE OF AN INTERMOLECULAR COMPLEX CONTAINING A GUANINE AND A CYTOSINE DERIVATIVE**

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The molecular basis for the specificity of the nucleic acids is believed to reside in the detailed system of hydrogen bonds formed between the purines and the pyrimidines. This bonding is specific in that adenine pairs to thymine and guanine to cytosine, thereby accounting for the complementarity which is found in the structure of DNA. X-ray diffraction studies have shown that DNA has a double-stranded helical structure in agreement with the hypothesis of Watson and Crick.[1] However, it is not possible to determine the precise manner in which the purine and pyrimidine bases come together solely from the X-ray fiber diffraction pattern. This is because the sequence of bases in DNA is irregular, and therefore they do not give rise to the coherent X-ray scattering which is necessary to determine atomic coordinates of individual bases. Most of our knowledge concerning the molecular structure of purines and pyrimidines comes from X-ray diffraction studies of model compounds.[2] These studies, however, have concentrated largely on the structure of individual molecules and this provides only indirect information regarding the assumptions concerning hydrogen bonding between different purines and pyrimidines. Here we present some results of a single crystal X-ray diffraction analysis in which we demonstrate that guanine and cytosine derivatives form a hydrogen-bonded complex in the crystalline state.

In Watson and Crick's original description of the DNA molecule, they postulated that the adenine-thymine pair, as well as the guanine-cytosine pair, were each held together by two hydrogen bonds. Three years later Pauling and Corey[3] extensively

reviewed the geometry of purines and pyrimidines and their potentialities for hydrogen bond formation, and concluded that the guanine-cytosine pair in DNA would probably be held together by three hydrogen bonds as shown in Figure 1. The potentialities for hydrogen bonding between purines and pyrimidines are numerous, however, and are not restricted to those which have been postulated for DNA.[4] It is for this reason that we have investigated the possibility of forming crystals of an intermolecular complex containing both purine and pyrimidine derivatives. In the present paper we describe the structure of a single crystal containing 9-ethyl-guanine and 1-methyl-5-bromocytosine. The bases chosen have either a methyl or ethyl group attached to the glycosidic nitrogen in order to prevent hydrogen bonding from occurring at that site and to increase the probability of having the same tautomeric form as would be found in the nucleotide itself. In this study a bromine derivative is used, since it greatly facilitates the X-ray diffraction analysis by making it possible to use the heavy atom method. A similar crystal structure involving 9-ethyl-guanine and 1-methyl-cytosine without the bromine atom has been determined recently.[5]

Methods.—Equimolar amounts of 9-ethyl-guanine and 1-methyl-5-bromocytosine were dissolved in dimethylsulfoxide and the solution was allowed to evaporate at room temperature. The crystals formed in this manner were clear, prismatic in shape, with well-developed faces. The derivatives were obtained from Cyclo Chemical Company. The use of this solvent was suggested to us by A. R. Katritzky, who also generously provided us with an initial sample of 9-ethyl-guanine. Paper chromatography and ultraviolet spectroscopy of aqueous solutions obtained from single crystals confirmed the presence of both base components. Following these initial observations, an X-ray analysis was undertaken.

Single crystals measuring approximately 0.7 by 0.7 mm and 0.3 mm thick were mounted and used for the structure determination. The cell dimensions and space group of the crystal was determined by 30° precession photographs. Intensity data was collected at room temperature on an equi-inclination integrating Weissenberg camera using copper Kα radiation and estimated visually using the multiple film technique. The intensities were corrected with the appropriate Lorentz polarization factor, but no absorption correction was made. Approximately 2,200 nonzero reflections were recorded from a total of 3,800 theoretically available in the copper sphere. The data was processed on an IBM 7090 computer using the crystallographic routines as described below.

Solution of the structure: The space group, unit cell dimensions, and crystal density are shown in Table 1. There are four asymmetric units per unit cell,

TABLE 1

9-ethyl-guanine-5-bromo-1-methyl cytosine complex	9-ethyl-guanine	5-bromo-1-methyl cytosine
a = 8.38 ± 0.01 Å	a = 10.88 Å	a = 18.71 Å
b = 13.16 ± 0.01 Å	b = 29.37 Å	b = 5.54 Å
c = 14.51 ± 0.01 Å	c = 10.88 Å	c = 15.29 Å
β = 97° 30′	tetragonal	β = 100° 5′
monoclinic		monoclinic

Space group Pa_1/c
ζ obs. = 1.628 gm/cm^3
ζ calc. = 1.602 gm/cm^3

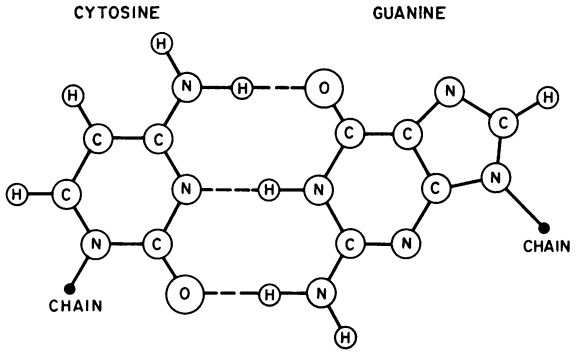

FIG. 1.—The hydrogen bonding which is believed to exist between guanine and cytosine in DNA.

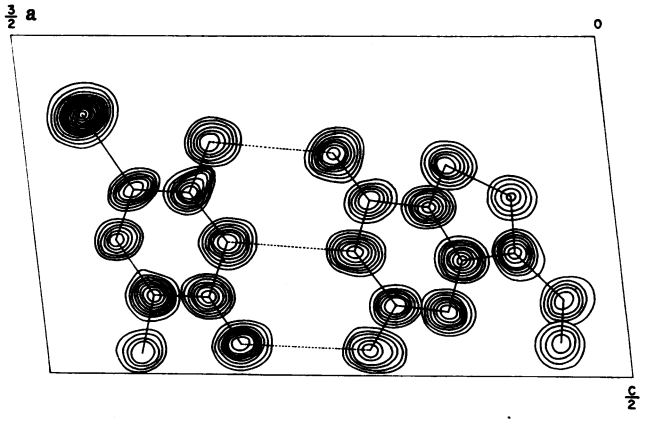

FIG. 2.—Three-dimensional contour map showing the electron density of a base pair as viewed down the *b* axis. The lowest contour is at 2 electrons/Å^3. The light atom peaks have contours at intervals of 1 electron/Å^3 while the very high bromine atom peak has contours at intervals of 5 electrons/Å^3. Solid lines indicate covalent bonds, dashed lines represent hydrogen bonds. The methyl group attached to cytosine and the ethyl group attached to guanine have somewhat lowered electron density peaks which may be associated with thermal vibration.

each asymmetric unit consisting of one guanine and one cytosine derivative. For comparison the cell dimensions of 9-ethyl-guanine and 1-methyl-5-bromocytosine are also listed in Table 1.

The structure was solved by the heavy atom method. A three-dimensional Patterson function was calculated which quickly revealed the bromine-bromine vector peaks and from this the position of the bromine atom was determined. A three-dimensional Fourier synthesis was then calculated using the bromine atom phases. This revealed the complete structure with the exception of the ethyl carbon atoms on 9-ethyl-guanine. A second Fourier synthesis was calculated using more complete phasing based on the initial structure and this revealed the remaining carbon atoms. The structure was then refined by the method of least squares using the refinement program devised by Busing.[6] Several cycles of least squares refine-

ment were calculated using individual light atom isotropic temperature factors. The unweighted residual factor at the present stage of refinement is 0.20. A new set of data is now being collected on a smaller crystal, and it is anticipated that this data will have smaller absorption error and will therefore be more reliable.

A three-dimensional Fourier synthesis was computed using the structure factor phases obtained at the present stage of refinement. Three-dimensional contour projections were made from this Fourier synthesis. No significant spurious peaks were observed in this electron density map and, in addition, a difference Fourier synthesis showed no large peaks.

Description of the structure: The asymmetric unit in the crystallographic unit cell consists of a guanine and cytosine derivative lying roughly parallel to each other approximately 3.4 Å apart, tilted by about 30° to $a - c$ (010) plane. These residues are hydrogen-bonded with cytosine and guanine derivatives in adjacent unit cells along the a axis. Figure 2 shows the electron density map of a three-dimensional contour projection looking down the b axis. It can be seen that the guanine derivative is hydrogen-bonded to a cytosine derivative in an adjoining unit cell by three hydrogen bonds in a manner completely analogous to that described by Pauling and Corey.[3] This is shown again in Figure 3 which is a similar contour

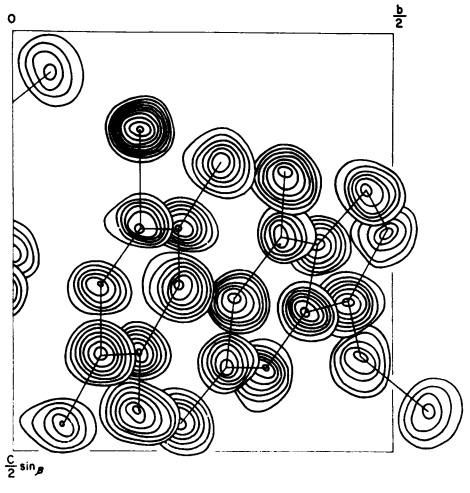

FIG. 3.—Three-dimensional contour map showing the electron density of a base pair as viewed down the a axis. The contours are drawn in the same manner as in Fig. 2.

projection viewed down the a axis in which the purine-pyrimidine pair lies somewhat more obliquely. A least squares analysis shows that, to the limit of our experimental accuracy, the guanine and cytosine molecules are planar, no atom deviating more than 0.04 Å from the molecular least squares plane. However, the complex itself is not exactly coplanar. The two molecules have a dihedral angle of approximately 6.5° between them. This involves a slight propeller-like twist between the bases with the carbonyl oxygen of guanine in the same plane as the cytosine derivative, and the cytosine amino group in the same plane as the guanine derivative.

Figure 4 shows two adjacent base pairs related by glide plane symmetry and projected onto the $a - c$ (010) plane. The heavy circles indicate atoms closest to the reader, the lighter circles indicate atoms further away. Each base pair is

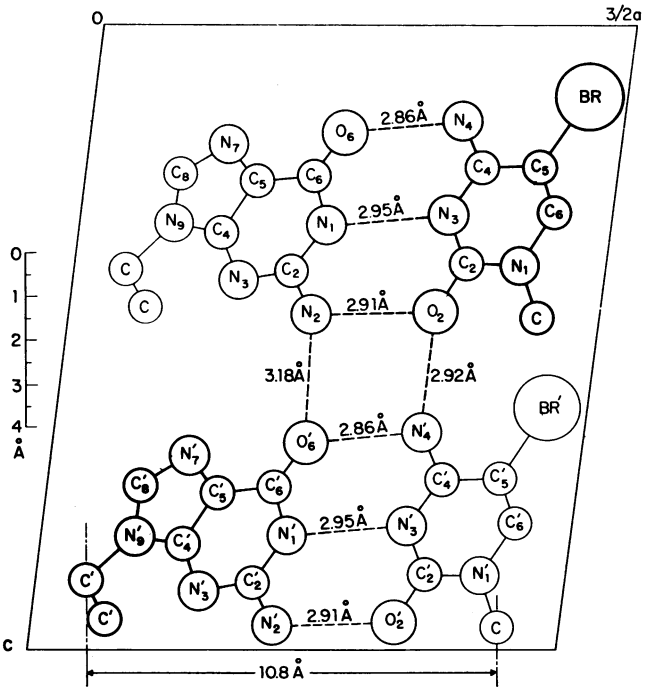

FIG. 4.—Diagram showing the position of two sets of base pairs as viewed down the *b* axis. The base pairs are tipped relative to each other; the heavy lines indicate atoms which are closer to the reader, the light lines indicate atoms further away. The distance separating the carbon atoms attached to the ring nitrogen atoms is 10.8 Å.

tilted 30° from the $a - c$ (010) plane, and they are therefore related to one another by an angle of about 60°. The hydrogen-bonding distances are in close agreement with those proposed by Pauling and Corey.[3] In addition, the distance of 10.8 Å between the carbon atoms attached to the glycosidic nitrogens of the purine and pyrimidine is the same as that proposed by Pauling and Corey and close to the distance suggested by Spencer.[7] This distance is of great importance, since it corresponds to the separation between the two polynucleotide chains in a DNA molecule.

There are several other close contacts of interest. Oxygen 2 of cytosine is 2.92 Å from the cytosine nitrogen 4′ in the residue below it. Although this corresponds to a hydrogen bond distance, the C'_4–N'_4–O_2 angle is 149°. This is an unfavorable angle for optimal hydrogen bonding, and the hydrogen bond formed is probably a weak one. Another close contact involves the nitrogen 2 of guanine and oxygen 6′ of the adjacent guanine molecule. This distance is 3.18 Å which is long for a hydrogen-bonding contact. In addition, the angle C_2–N_2–O'_6 is nearly 150° which is unfavorable for hydrogen bonding, suggesting that the interaction between these groups is very weak, if any. The bromine atom does not participate in hydrogen bonding; its closest contact is 3.4 Å to a neighboring guanine nitrogen 3. It is also in van der Waals contact with the carbon atoms of the ethyl group in the same guanine derivative.

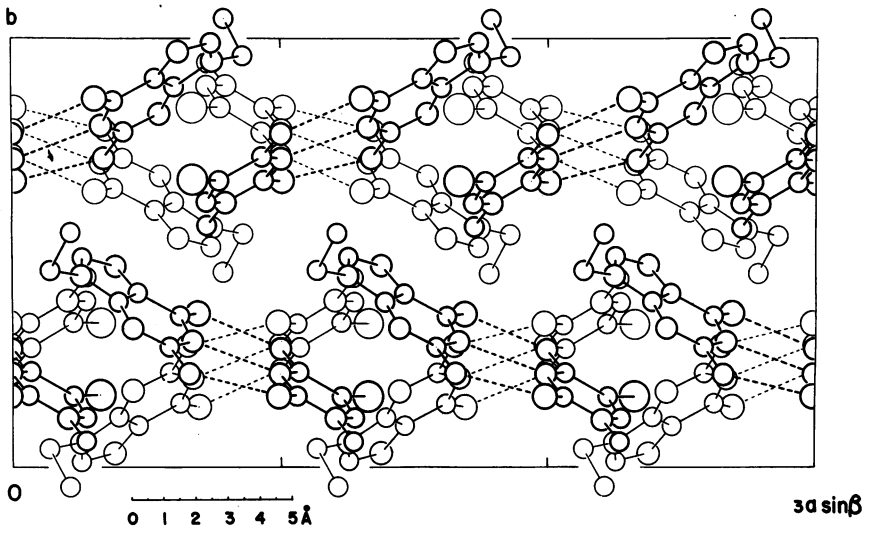

FIG. 5.—A diagram showing the molecular packing in the crystal as viewed down the *c* axis. The three hydrogen bonds between base pairs are shown by the dashed lines. The darker atoms are closer to the reader, while those drawn with light lines are behind. The slight propeller-like twist between the two paired bases can be seen.

The general packing in the crystal is shown in Figure 5, which is a projection down the *c* axis. Three unit cells are shown along the *a* axis in order to show the hydrogen bonding between residues in adjoining unit cells. The darker molecules are in front, while those sketched more lightly are lying behind. The almost planar guanine-cytosine pairs can be seen, as well as the oblique orientation of the base pair lying behind them. It can be seen that, in the same unit cell, the guanine and cytosine residues are approximately parallel to each other, 3.4 Å apart.

The crystal is held together predominantly by van der Waals forces along the *b* axis, while van der Waals forces and hydrogen bonds between base pairs hold the crystal together along the *a* axis. Along the *c* axis, there is a twisted ribbon of base pairs, each tilted 60° to its neighbor, held together by the weaker hydrogen bonds as shown in Figure 4.

Discussion.—Considerable interest was attached to the question of the hydrogen-bonding potentialities of the purines and pyrimidines by the discovery that it is possible to obtain a specific three-stranded polynucleotide complex in which one adenine base is hydrogen-bonded to two uracil residues.[8] It was postulated that one uracil residue was attached by the Watson-Crick pairing, while the second uracil residue was hydrogen-bonded to the amino group and the imidazole nitrogen 7 of the adenine molecule. In a single crystal analysis, Hoogsteen[4] has shown that 9-methyl-adenine and 1-methyl-thymine form an intermolecular complex containing this second type of hydrogen bonding involving the amino group and nitrogen 7 of adenine. This differs significantly from the type of adenine-thymine hydrogen bonding which was postulated in DNA itself. This suggested an alternative formulation for hydrogen bonding between the two polynucleotide strands of DNA in which the guanine and cytosine residues are held together in a similar fashion, the guanine carbonyl oxygen 6 and nitrogen 7 hydrogen bonding to a tautomeric form

of the cytosine base.[9] However, such a tautomeric form for cytosine has been shown to be unlikely by infrared studies.[10] It is of considerable interest that in the present work we have found, in the crystalline state, a complex containing derivatives of guanine and cytosine which are held together by three hydrogen bonds, suggesting that this is a particularly stable hydrogen-bonding configuration. This lends support to the type of molecular organization which is believed to exist in the structure of DNA. Furthermore, since the individual bases crystallize as a complex rather than individually, one might suspect that the hydrogen-bonded base pair may even exist in solution to a significant degree prior to crystallization. We are investigating this currently.

In the crystal structure of cytosine-5-acetic acid, Marsh, Bierstedt, and Eichhorn[11] have shown that pairs of cytosine rings are linked together by the same set of three hydrogen bonds which are linking the guanine and cytosine residues in this structure. This is possible since one of the two cytosine rings is protonated on nitrogen 3, a situation which also exists in the helical form of polycytidylic acid.[12] The cytosine rings in cytosine-5-acetic acid are also related by a glide plane and are connected by a similar system of hydrogen bonds to that shown in Figure 4, with the amino acid group of one cytosine linked to the oxygen atom of the neighboring ring.

Since the guanine and cytosine derivatives are almost coplanar, we have looked carefully at the structure to see what keeps them from being completely coplanar. The close van der Waals contact between a neighboring bromine atom and the guanine nitrogen 3, as well as the ethyl group carbon atoms, appears to prevent the two bases from being completely coplanar. A compromise position is established in which the hydrogen bonds between the base pairs are nearly optimal, and no short van der Waals contacts are formed.

Although the present investigation demonstrates that it is physically possible for guanine and cytosine to form a hydrogen-bonded base pair in a manner analogous to that which we believe exists in the DNA molecule, it does not, of course, prove that this type of arrangement is actually present in DNA. It does, however, suggest that this may be a stable form in which these molecules associate and thereby lends considerable support to the type of hydrogen-bonding specificity which we believe occurs in the polymer itself.

Summary.—9-ethyl-guanine and 1-methyl-5-bromocytosine form an intermolecular complex in a crystal in which they are held together in pairs by three hydrogen bonds. These hydrogen-bonded pairs are linked in long chains by weaker hydrogen bonds which connect adjacent base pairs to form a twisted ribbon-like network. The geometry of the hydrogen-bonded guanine-cytosine pair is similar to that which has been postulated as existing in the DNA molecule.

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† Helen Hay Whitney Postdoctoral Fellow.

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*SYNTHESIS OF VALINE AND ISOLEUCINE IN THE PRESENCE OF A PARTICULATE CELL FRACTION OF NEUROSPORA**

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It has been established that valine is synthesized from pyruvate in a series of at least four steps, and that isoleucine is synthesized in a series of four similar steps from pyruvate and α-ketobutyrate. The four enzymes involved are apparently common to both pathways, but this has not been definitely proved in any one case. The enzymes will be described here as the *condensing enzyme*, which produces α-acetolactate and α-acetohydroxybutyrate from pyruvate, and pyruvate + α-ketobutyrate, respectively; the *reductoisomerase*, which converts the α-aceto-acids to the dihydroxy acids; the *dehydrase* which causes a dehydration of the dihydroxy acids with the formation of the α-keto acids; and the *transaminase* which transaminates between phenylalanine and the respective α-keto acids to form valine and isoleucine.

In at least two organisms, Salmonella and Neurospora, it has been shown that two or more of the genes affecting these enzymes are closely linked.[1-3] In *Neurospora crassa* at least two of the enzymes, reductoisomerase and dehydrase, are apparently controlled by two genes which are estimated to be about four map units apart.[4] Between them lies a region in which mutations have been detected only within a small segment. These mutations cause an absolute requirement for both isoleucine and valine, but the mutant strains do not show a deficiency for any of the four enzymes. That the blocks in these are *not* prior to pyruvate and α-acetobutyrate has been established by showing that they accumulate either the α-aceto-acids or dihydroxy acids or both. It was postulated that the defect in these mutants, the group II mutants, lies not in their inability to produce the enzymes, but in their inability to organize them properly to be active *in vivo*.[3] The experiments described below were designed to explore this possibility by first determining whether there exists an isoleucine-valine forming system in the particulate fraction of the wild-type mycelial homogenate.

Methods and Materials.—The assay procedures for the individual enzymes, the condensing enzyme, reductoisomerase, dehydrase, and transaminase have been described in detail previously.[5-8] Succinic cytochrome c reductase activity was determined by the method of Crane *et al.*[9] Protein was determined by the method of Lowry *et al.*[10]

The wild-type strain employed here, *KJT1960a*, is a segregant from a cross between the Emer-