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*VAN DER WAALS-LONDON INTERACTIONS AND THE CONFIGURATION OF HYDROGEN-BONDED PURINE AND PYRIMIDINE PAIRS**

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The discovery by Hoogsteen[1, 2] that methylated derivatives of adenine and thymine (both carrying the substituent at their glycosidic nitrogen) cocrystallize as a hydrogen-bonded complex, whose configuration is, however, different from that which Watson and Crick observed in the nucleic acids, stimulated a large number of investigations on the cocrystallization of purine and pyrimidine bases. Among the most outstanding results in this field are (1) the observations that while the derivatives of adenine and thymine cocrystallize in at least two different complexes,[3–6] both different from that of Watson and Crick, the derivatives of guanine

and cytosine cocrystallize only following the Watson-Crick pairing scheme,[7, 8] as refined by Pauling and Corey[9] and as present in the nucleic acids, and (2) the observations that, as yet, cocrystallization of the nucleic acid bases could be obtained only with base pairs showing complementarity in the Watson-Crick sense, that is, no cocrystallization occurring between guanine and thymine or adenine and cytosine.[8]

Parallelly, the determination of the molecular and crystal structures of a number of biological purine and pyrimidine bases and the study of the properties of synthetic polynucleotides and of the their interactions, have again centered attention on the importance of hydrogen bonding and its significance for the over-all configuration.

The variety of results obtained in a number of cases raised, among others, the important problem of determining which of the hydrogen-bonded complexes correspond to intrinsic greater stability and which must, on the contrary, be attributed to the influence of environmental factors, such as those present in crystals or in the helical structures of polynucleotides.

Because of the difficulty of answering this question experimentally, it was thought to be useful to contribute to its solution by evaluating theoretically the relative stabilities of a series of fundamental configurations of hydrogen-bonded purine and pyrimidine base pairs. As the calculations refer to isolated base-pairs, they represent their intrinsic stabilities.

The Method of Calculation.—Among the different factors susceptible to account for the *relative stabilities* of hydrogen-bonded pairs, the most important one seems to be the van der Waals-London in-plane interactions between the linked partners.[10-13] These are usually evaluated, and have been so initially for the adeine-thymine and guanine-cytosine base-pairs of the nucleic acids,[10] in the "dipole" approximation which considers these forces (F_D) as the sum of three principal contributions:

$$F_D = F_{\mu\mu} + F_{\mu\alpha} + F_L,$$

where $F_{\mu\mu}$ are the dipole-dipole forces, $F_{\mu\alpha}$ the dipole-induced dipole forces, and F_L the London or dispersion forces. These are defined as follows:

$$F_{\mu\mu} = \frac{1}{R^3}\left[\mu_1\mu_2 - \frac{3}{R^2}(\mu_1\mathbf{R})(\mu_2\mathbf{R})\right], \tag{1}$$

where μ_1 and μ_2 are the respective dipole moments of molecules 1 and 2, $\mathbf{R}$ the distance between the points of location of these dipoles (the choice of the sense of $\mathbf{R}$ is irrelevant).

$$F_{\mu\alpha} = -\frac{1}{2}\frac{1}{R^3}\mu_1(\bar{\bar{T}}\bar{\bar{\alpha}}_2 T)\mu_1 - \frac{1}{2}\frac{1}{R^3}\mu_2(T\bar{\bar{\alpha}}_1 T)\mu_2, \tag{2}$$

where the first term is the interaction energy of dipole $\bar{\mu}_1$ with the dipole that it induces in molecule 2, and the second term the interaction of μ_2 with the dipole that it induces in 1. $\bar{\bar{\alpha}}_1$ and $\bar{\bar{\alpha}}_2$ are the respective polarizability tensors[14, 15] of the molecules and $\bar{\bar{T}}$ designs the tensor which appears in the expression of the field $\mathbf{E}$ created by a dipole μ at a point $\mathbf{R}$:

$$\mathbf{E} = \frac{1}{R^3}\left[3\frac{\mathbf{R}}{R}\left(\frac{\mathbf{R}}{R}\mu\right) - \mu\right] = \frac{1}{R^3}\left[3\frac{\mathbf{R}}{R}\otimes\frac{\mathbf{R}}{R} - \bar{\bar{1}}\right]\mu$$

which leads to define $\bar{\bar{T}} = 3\left(\frac{\mathbf{R}}{R}\otimes\frac{\mathbf{R}}{R}\right) - \bar{\bar{1}}$; $\bar{\bar{1}}$ designs the unit tensor. In an orthogonal basis O $x\ y\ z$ the matrix representing $\frac{\mathbf{R}}{R}\otimes\frac{\mathbf{R}}{R}$ is extremely easy to obtain: $\left[\frac{\mathbf{R}}{R}\otimes\frac{\mathbf{R}}{R}\right]_{xy} = \frac{R_x}{R}\frac{R_y}{R}$, etc., and hence the matrix representing $\bar{\bar{T}}$ can be immediately constructed.

Note that the general matrix form used here avoids the preliminary research of the principal polarization axis and the corresponding principal polarizabilities, which are necessary in the developed formulas used by de Voe and Tinoco.[10]

$$F_L = -\frac{1}{4}\frac{I_1 I_2}{I_1 + I_2}\frac{1}{R^6} Tr\,(T\bar{\bar{\alpha}}_1 T\bar{\bar{\alpha}}_2), \tag{3}$$

where Tr designs the trace (sum of the diagonal elements). I_1 and I_2 are the respective ionization potentials of the molecules and $\bar{\bar{\alpha}}_1$, $\bar{\bar{\alpha}}_2$, and $\bar{\bar{T}}$ are the tensors defined above.

For $F_{\mu\alpha}$, F_L, and $F_{\rho\alpha}$ we have used the anisotropic polarizabilities as well as the isotropic ones and compared the two results. The differences were always small.

It may, however, be observed that because of the shortage of the intermolecular distances, with respect to the molecular dimensions the "dipole" approximation may be rather inaccurate in this particular case and that it may be preferable to treat the problem in the "monopole" approximation, i.e., by considering all the negative and positive charges in the system as interacting in a simple coulombic fashion. In this "monopole" approximation the total force (F_M) may then be considered as the sum of three main contributions:

$$F_M = F_{\rho\rho} + F_{\rho\alpha} + F_L,$$

where $F_{\rho\rho}$ are the monopole-monopole forces, $F_{\rho\alpha}$ the monopole-induced dipole forces, and F_L the dispersion forces.

$$F_{\rho\rho} = \sum_{i_1}\sum_{i_2}\frac{\rho_{i_1}\rho_{i_2}}{R_{i_1 i_2}},$$

where index i_1 designs the atoms of molecule 1, and i_2 those of molecule 2, ρ_{i_1} and ρ_{i_2} are the net charges of atoms i_1 and i_2, respectively, and $R_{i_1 i_2}$ is their distance; and

$$F_{\rho\alpha} = -\frac{1}{2}\mathbf{E}_2\bar{\bar{\alpha}}_2\mathbf{E}_2 - \frac{1}{2}\mathbf{E}_1\bar{\bar{\alpha}}_1\mathbf{E}_1,$$

where $\mathbf{E}_2 = \sum_{i_1}\frac{\rho_{i_1}}{(R_{i_1,2})}\mathbf{R}_{i_1,2}$ is the field created by the net charges of molecule 1 at the point of location of the (induced) dipole of the molecule 2 ($\mathbf{R}_{i_1,2}$ designing the vector from the atom i_1 to this point); with a similar definition for $\mathbf{E}_1$.

Of course, all the formulas for $F_{\mu\mu}$, $F_{\mu\alpha}$, F_L, $F_{\rho\rho}$, and $F_{\rho\alpha}$ involve a numerical factor, not written here, and depending on the chosen units.

Explicit calculations[11, 13] carried out with the two approximations for the complementary Watson-Crick base-pairs of the nucleic acids show that, in fact, the two sets of results, although indicating both stronger interactions inside the guanine-cytosine pair than inside the adenine-thymine pair, lead to quite different absolute values for the interactions (Table 1).

In the present paper, we have carried out a series of similar calculations on the van der Waals-London interactions inside a number of purine and/or pyrimidine hydrogen-bonded pairs corresponding to different problems raised by recent experimentation in this field. The calculations have been carried out in both the "monopole" and the "dipole" approximation. Because of the *a priori* higher value of the "monopole" approximation, the discussion of the results will be carried out essentially for this approximation.

The practical basis for the evaluation of the electrostatic interaction are the results of calculations of the distribution of electronic charges, both σ and π, in the purine and pyrimidine bases, carried out by the molecular orbital method[16] in the Hückel approximation, specifically calibrated for the good reproduction of experimental dipole moments.[17, 18] The results are, as far as the π electronic distribution is concerned, in satisfactory agreement with recent self-consistent field molecular orbital calculations.[19, 13] The polarizabilities are obtained with the help of the usual additivity rules.[14, 15] The knowledge of the transversal and longitudinal polarizabilities gives immediately the polarizability tensor in a coordinate system with z axis along the bond.[15] A matrix transformation gives the new matrix in the coordinate system chosen for the whole molecule, and the total polarizability tensor is represented by the matrix sum of these bond polariza-

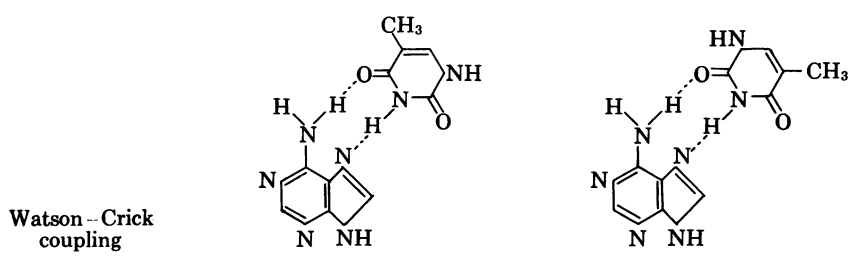
TABLE 1

Base-pair	$F_{\mu\mu}$	$F_{\mu\alpha}$	F_L	F_D	$F_{\rho\rho}$	$F_{\rho\alpha}$	F_L	F_M
A-T I	+1.6	−0.3	−0.7	+0.6	−4.6	−0.2	−0.7	−5.5
A-T II	−0.8	−0.3	−0.9	−2.0	−5.9	−0.2	−0.9	−7.0
A-T III	−1.2	−0.2	−0.9	−2.3	−5.6	−0.15	−0.9	−6.65
G-C IV	−3.14	−1.10	−1.25	−5.49	−15.91	−2.02	−1.25	−19.18
G-C V	+3.24	−0.46	−0.44	+2.34	−3.98	−1.33	−0.44	−5.75
A-A VI	+0.82	−0.12	−0.45	+0.25	−5.23	−0.11	−0.45	−5.79
A-A VII	−0.01	−0.09	−0.54	−0.64	−4.69	−0.13	−0.54	−5.36
A-A VIII	−0.38	−0.10	−0.82	−1.30	−1.20	−0.18	−0.82	−2.20
G-G IX	−1.87	−0.19	−0.53	−2.53	−13.37	−0.62	−0.53	−14.52
G-G X	−0.74	−0.16	−0.17	−1.07	−5.50	−0.55	−0.17	−6.22
G-G XI	+5.86	−0.85	−0.63	+4.38	−5.79	−0.73	−0.63	−7.15
T-T XII	+2.39	−0.47	−1.19	+0.73	−3.62	−0.38	−1.19	−5.19
T-T XIII	+0.61	−0.28	−1.10	−0.77	−2.61	−0.15	−1.10	−3.86
C-C XIV	−1.26	−1.00	−1.23	−3.49	−10.65	−1.09	−1.23	−12.97
C-C XV	−1.85	−0.52	−0.58	−2.95	−11.23	−1.04	−0.58	−12.85
A-C XVI	+1.38	−0.57	−0.96	−0.15	−6.20	−0.59	−0.96	−7.75
G-T XVII	+1.82	−0.20	−0.58	+1.04	−4.41	−0.50	−0.58	−5.49
G-T XVIII	+1.14	−0.26	−0.58	+0.30	−6.24	−0.58	−0.58	−7.40

bility matrices. The values of the ionization potentials are deduced from a reference curve, connecting these values with the coefficient of the highest filled molecular orbitals.[16, 19] The values thus obtained are in satisfactory agreement with the results of more refined self-consistent field calculations[20] and, in the only case in which an experimental value is available, namely, that of adenine,[21] are in very good agreement with it, too. The geometrical configurations of the pairs are those indicated by X-ray crystallography, in the case where these are known. In the other cases the most probable bond distances and angles have been adopted.

Results and Discussion.—The results are summed up in Table 1. The main conclusions which may be drawn from their examination, in the "monopole" approximation, are the following.

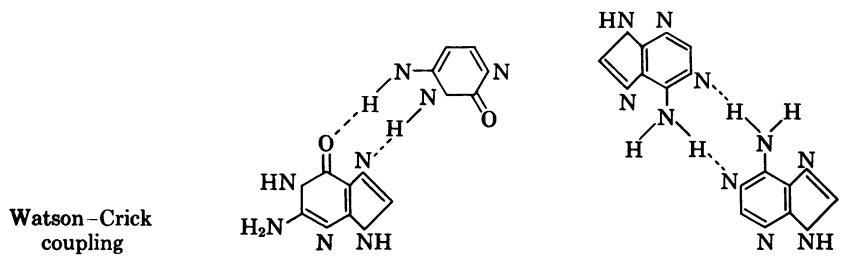
(1) Among the three configurations considered for the adenine-thymine pairing, I, II, and III, the most stable one *per se* is configuration II corresponding to the structure of the cocrystallization product observed by Hoogsteen.[1, 2]



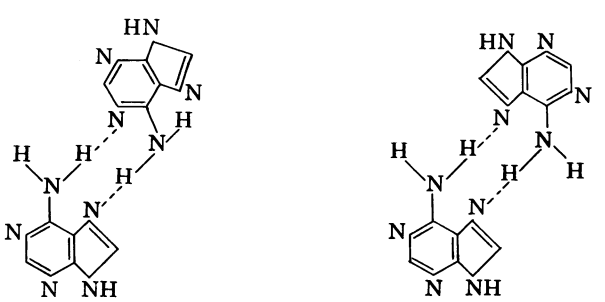
It is followed in the order of decreasing stability by configuration III, in which thymine is still linked to N_7 of adenine but in which the hydrogen bonding with the amine group of adenine is through O_2 rather than O_4 of the pyrimidine. This configuration seems to be the predominant one in complexes formed between adenine and 5-bromouracil derivatives[22, 6] and seems also to be found in the structure of the three-stranded helix poly (A + 2U).[23] The Watson-Crick arrangement of the bases corresponds to the smallest energy of interaction and is thus probably imposed in the nucleic acids by the exigencies produced for a regular double-stranded helix with the guanine-cytosine pair.

(2) In the guanine-cytosine pairing, the Watson-Crick arrangement IV is much more stable than the hypothetical arrangement V, never observed yet, in which

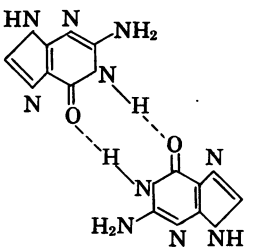
cytosine would be "on the other side" of guanine. It is also much more stable than any of the adenine-thymine arrangements. It is therefore understandable that this Watson-Crick pair should impose the general features of the configuration of the base-pairs in the nucleic acids. It may be added that the greater stability of the guanine-cytosine pair over the adenine-thymine pair, in the Watson-Crick configurations, was also deduced from calculations of resonance energy stabilization through electronic delocalization[24] and that such calculations have also led to the explicit prediction that configuration V, which involves a rare, less stable tautomeric form of cytosine, should be much less stable than configuration IV and that consequently, "it seems improbable that this alternative pairing may occur, even in a crystal."[16]



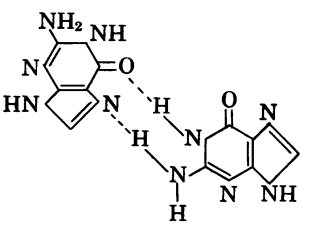
(3) Among the three configurations, VI, VII, and VIII, considered for the autoassociation of adenine, VI and VII are of equal predicted stability. Configuration VII seems to be the one observed in the crystal structure of 9-methyladenine,[25] although it was believed until recently that this structure corresponded to model VI.[26] The predominance of VII must be attributed to environmental factors, such as the influence of the extended hydrogen-bonded network, or even possibly to the role of the methyl group. Configuration VIII corresponds to relatively low stability. This mutual arrangement of adenines is nevertheless observed in the helical form of polyadenylic acid.[27] It must, however, be realized that the calculations refer to the association of two neutral adenine molecules, while in poly A at low pH adenine is protonated at N_1, and moreover, that the experimental results refer to a solution and to a polymer (*vide infra*). This state of affairs confirms the view following which the stability of the double helix of polyadenylic acid at acid pH is due to a large extent to the electrostatic interactions between the extra positive charges on the bases and the negative charges of the phosphate groups.



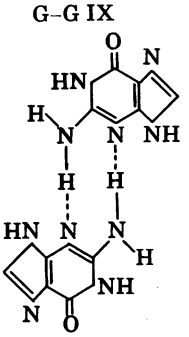
(4) Three configurations have been considered for the guanine-guanine autoassociation: configuration IX suggested to occur in polyguanylic acid,[28] configuration X suggested to be involved in gels of guanylic acid,[29] and the curious configuration XI, observed in the crystal structure of guanine hydrochloride dihydrate.[30] Of these three structures, IX is much more stable—in fact very stable on an absolute scale, a situation perhaps not without significance, in the limits of the indicated restrictions, for the relatively great stability of polyguanylic acid.[28] The existence of structure XI in the crystal of guanine hydrochloride dihydrate must therefore be attributed to strong environmental effects.



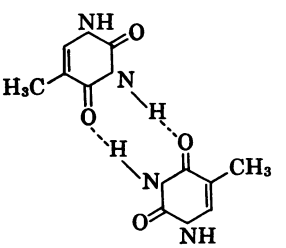
G-G IX



G-G X

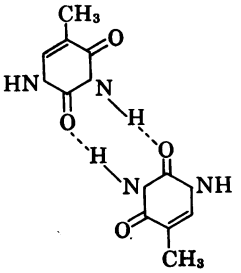


G-G XI

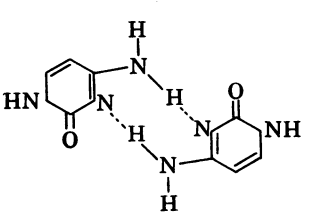


T-T XII

(5) Two possible configurations, XII and XIII, have been considered in connection with the crystal structure of 1-methyl thymine.[31] Of these two structures, XII, which is actually observed (with a similar configuration observed also in the crystal structure of N-methyl uracil[32]), is theoretically the most stable one.



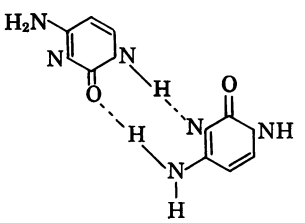
T-T XIII



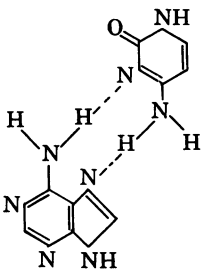
C-C XIV

(6) Two configurations have also been considered for the autoassociation of cytosine: configuration XIV observed in the crystal of N-methyl cytosine[33] (the structure being, however, somewhat unusual, the two bases not lying in the same

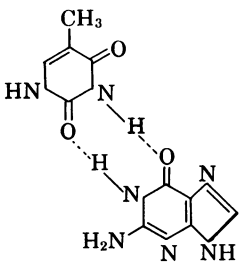
plane) and configuration XV observed in the crystal of cytosine.[34] The outstanding feature of these two configurations is the high value of the predicted stabilities.



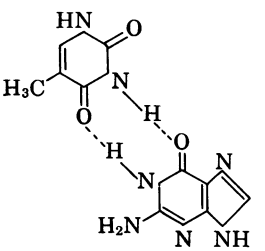
C-C XV



A-C XVI



G-T XVII



G-T XVIII

(7) The last three configurations studied, XVI, XVII, and XVIII, correspond to hypothetical associations between bases noncomplementary in the Watson-Crick sense. The most plausible associations have been considered, and it can be seen that they involve, in principle, considerable stabilization energies. As mentioned, however, these associations have not as yet been observed and, in particular, could not be produced in cocrystallization reactions. As a possible explanation for this failure, we would like to suggest the relatively very strong interactions predicted to exist in the hydrogen-bonded autoassociations of guanine and cytosine. Also, it may be observed that the stabilization of the most stable form (II) of the adenine-thymine association, although relatively moderate, is nevertheless greater than that of the adenine-adenine or thymine-thymine autoassociations. Similarly, although the stabilization of the guanine and cytosine autoassociations is relatively very strong, that of the guanine-cytosine association is still stronger. On the contrary, the calculated associations of adenine-cytosine or guanine-thymine correspond to predicted stabilities smaller than those calculated for the autoassociations of guanine or cytosine. It may therefore be imagined that these autoassociations will have a strong tendency to be formed preferentially to any association between the non-complementary bases, preventing therefore the corresponding cocrystallization phenomena.

Conclusions.—By evaluating the intrinsic stabilities of hydrogen-bonded purine and pyrimidine pairs as resulting from the van der Waals-London interactions among the linked bases, the recorded calculations permit us to decide whether the different observed configurations are the result of these preferential stabilities or whether supplementary factors, due to substitutents, the extension of hydrogen network beyond the base-pairs, or other environmental factors present in crystals

and in polynucleotides, play an essential role in their elaboration. In practice the calculations seem to account satisfactorily for the essential features of the H-bonding in crystals. It must, on the other hand, be pointed out that results obtained for the interaction between two isolated molecules should not be generalized without caution to the case of the interaction in a solvent, especially a polar one. In such a case, the solvent effect may be represented by a more or less "effective" dielectric constant ϵ (of the order of 10–15 for water,[35] $F_{\mu\mu}$ and $F_{\rho\rho}$ being divided by ϵ, $F_{\mu\alpha}$ and $F_{\rho\alpha}$ by ϵ^2), and by a decrease of F_L (about 30% for water).[36, 37] In such conditions the contribution F_L becomes relatively more important, and the differences between the interacting configurations may tend to be somewhat smoothed out. This does not preclude, of course, the fact that the order of interactions evaluated in vacuum will generally be conserved for a series of related associations produced in the same solvent in which H-bonding occurs. Thus, for example, the recent results of Hamlin *et al.*,[38] showing that 9-ethyladenine and 1-cyclohexyluracil are hydrogen-bonded in solution in deuterochloroform more strongly than they are with themselves, is in agreement with our findings on the relative stabilities of these types of associations. (See Table 1, pairs VI or VII, XII, and II, where thymine may be considered as representing uracil.) In associations concerning polynucleotides, attention must also be paid, besides the effect of the solvent, to the "stacking" component of the stabilization energy and to protonation effects, such as the one quoted above in connection with polyadenylic acid or the one manifesting itself in polycytidylic acid where the stable helical form is hemiprotonated, with the additional proton involved in the hydrogen bonding itself.[39]

Finally, as announced in the introduction, the discussion was based essentially on the "monopole" approximation, *a priori* more reliable for these calculations. It may be remarked, however, that the "dipole" approximation, although leading to absolute values of the energies of interaction generally different from those of the "monopole" approximation, frequently leads to the same general qualitative conclusions as to the relative stabilities of isomeric or even different pairs. In the few cases in which a disagreement appears between the two approximations from the last point of view, the comparison between the calculation and experiment nearly always favors the indications of the "monopole" approximation.

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RNA CODONS AND PROTEIN SYNTHESIS, IX. SYNONYM CODON RECOGNITION BY MULTIPLE SPECIES OF VALINE-, ALANINE-, AND METHIONINE-SRNA

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By studying the response of AA-sRNA to trinucleotide templates, nucleotide sequences of RNA codons and general patterns of degeneracy have been defined.[1–8] Most synonym codons differ only by one base, usually at the 3′-terminal position. During the course of these investigations, evidence was obtained that one Phe-sRNA could recognize two Phe-sRNA codons, UUU and UUC,[4] and that a highly purified species of yeast Ala-sRNA could recognize at least three Ala-codons, GCU, GCC, and GCA.[9, 10] A detailed mechanism for alternate acceptable base pairing has been proposed by Crick.[11]

Fractionation of sRNA has often revealed multiple species accepting the same