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## **Influence of the Hydrogen on the $\pi$ Electrons for the Adenine-Thymine Pair**

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With 1 Figure and 3 Tables

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### **Abstract**

The influence of the hydrogen bonds on the orbital energies and the distribution of charges in the elements constituting those bonds, for  $\pi$  electrons is analysed. The distribution of charges in the  $A - T$  pair separately considered is calculated by means of Hückel's Extended Method. A simplified model to calculate the interaction between  $\pi$  electrons in one of the bases and  $\sigma$  and  $\pi$  electrons in the complementary one is also considered. We have used the theory of separated electronic groups to calculate that interaction.

The calculated variations are discussed.

### **1. Introduction**

Investigation of the electronic structure of the  $A - T$  and  $G - C$  bases of the DNA molecule is of marked interest in proving certain facets of very important biological processes, such as mutations, ageing, origin of tumors, etc. Of course, detailed comprehension of the whole physicochemical phenomena involved in the processes referred to will require many years of varied investigation in various branches of biology, physics, chemistry and mathematics, but amongst the many queries arising, the charge distribution in the pairs of bases is of special interest, since a reasonable approximation, or at least a semi-quantitative one, may be arrived at from the results obtainable through the utilisation of certain common ordinary working methods in quantum chemistry, such as those of Hückel, Pople, the separated electronic groups theory, etc.

In recent years various authors have undertaken this task of analysing the electronic structure of the constituents of DNA, amongst whom we may cite PULLMAN and PULLMAN [14], REIN and LADIK [16] and REIN and HARRIS [15]. The numerical results obtained can be used as adequate material to analyse the accuracy of the hypothesis developed by LÖWDIN [9, 10], as one of the more interesting possibilities.

The problem of determining the electronic structure of the  $A - T$  and  $G - C$  base pairs is very difficult because of the number of electrons involved; therefore, the methods used in this determination are of a semi-empirical character. If to this difficulty we add the fact that the electronic structure of the base pairs also depends on their location in the DNA molecule and of the surroundings in which the molecule is placed, it is evident that investigation of the electronic structure of the base pairs is far from exhausted, and this fact has led us to undertake our own studies in the manner described below, enabling us to arrive at the conclusions we have set down.

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## 2. Method Employed

Since the determination of the structure of DNA, considerable work has been done towards the calculation of its electronic structure. One approach has been to apply approximate molecular orbital calculations to the base pairs; in this paper the calculations are performed using the Hückel Theory. In this approximation each molecular orbital is given as a linear combination of atomic orbitals:

$$\phi_i = \sum_j c_{ij} \psi_j \quad (1)$$

Minimization of the total energy by the variational principle leads to the set of secular equations:

$$\sum [H_{ij} - ES_{ij}] c_{ij} = 0$$

The basis set is built up of 1s orbital of H, of one 2s and three 2p for each C, N and O atoms present. The diagonal matrix elements (Coulomb integrals of the type  $H_{ii} = \int \psi_i H \psi_i d\tau$ ) are usually chosen as valence state ionization potentials, and the particular values used are given by JORDAN and PULLMAN [7], the off-diagonal matrix elements are approximated by the use of the Wolsberg-Helmholtz formula:

$$H_{ij} = 0.5K(H_{ii} + H_{jj}) S_{ij} \quad (2)$$

where  $H_{ii}$ 's are the orbital valence state ionization potentials and  $S_{ij}$  is the overlap integral. A value of  $K = 1.75$  is generally used as suggested by HOFFMANN. Hoffmann's method will have to be thoroughly investigated before its advantages and disadvantages are fully revealed. The possibility of including  $\sigma$  electrons in the theory have been seriously criticized by DEWAR [5, 6]. Nevertheless, we have used this method for  $\pi$  electrons. The system was assumed to be planar for all calculations, then the orbitals of  $\pi$ -type symmetry can be separated since  $\sigma - \pi$  matrix elements vanish. The starting approximation was taken to be that of two separated bases and the off-diagonal matrix elements were assumed to be zero for distances larger than 4 Å.

The interaction between the complementary bases was calculated using the theory of separated electronic groups and this theory proved clearly applicable for the following reasons:

Having calculated the orbital energies and their corresponding eigen-functions, we are faced with the problem of calculating the interaction effects between the  $\pi$  electrons of one of the bases and  $\sigma$  and  $\pi$  electrons of the complementary base. The applicability conditions of the theory (cf. BALLESTERO and SORARRAIN [1]), are very satisfactorily fulfilled since the overlapping integrals between the orbitals  $\sigma$  of one of the bases and the orbitals  $\pi$  of the other are eliminated by reasons of symmetry, while the overlapping between orbitals  $\pi$  belonging to the complementary bases is practically nil, since their inter-atomic distances exceed the 3 Å, that is, they fulfil validity conditions demanded in a very satisfactory way.

Prior to the application of the theory of separated electronic groups, we will briefly summarise the elements of the theory referred to which have been used:

a) The wave function for a system of  $N$  electrons is expressed as an anti-symmetrized product of partial wave functions, each one of which corresponds to a particular electronic group within the molecule:

$$A(1, 2 \dots N) = [A_1(1, 2 \dots N_1) A_2(N_1 + 1 \dots N_1 + N_2) \dots A_K \dots] \quad (3)$$

or else:

$$A(1, 2 \dots N) = (N!/N_1! N_2! \dots N_K! \dots) \sum_P (-1)^P P(A_1 A_2 \dots A_K \dots) \quad (4)$$

with

$$\sum_K N_K = N$$

where  $P$  is the permutation operator for electrons corresponding to different groups, and  $(-1)^P$  is their parity.

b) The wave functions corresponding to the different groups are normalised

$$\int |A_I(1, 2, \dots, N_I)|^2 d\tau_I = 1 \quad (5)$$

c) There is a set of molecular orbitals  $\{\phi_\alpha\}$  which can be assigned to different  $\{\phi_\alpha^I\}$  sub-groups, where  $I$  denotes the particular electronic groups, and  $\alpha$  is the corresponding molecular spin orbital. Therefore, the  $A_I$  wavefunction corresponding to the group  $I$  can be expressed as:

$$A_I(1, 2, \dots N) = \sum_r A_r^I \Phi_r^I(1, 2, \dots N_I) \quad (6)$$

where

$$\Phi_r^I(1, 2, \dots N_I) = (\phi_{r_1}^I(1) \phi_{r_2}^I(2) \dots \phi_{r_\alpha}^I(\alpha) \dots \phi_{r_{N_I}}^I(N_I)) \quad (7)$$

is the Slater determinant, built up with functions  $\{\phi_{r_\alpha}^I\}$ , where  $r$  indicates the configurational determinant,  $\alpha$  is the molecular spin orbital, and  $I$  is the corresponding electronic group.

Condition (c) is equivalent to the orthogonality condition between groups:

$$\int A_I^*(1, 2, \dots \gamma \dots N_I) A_J(1', 2', \dots \gamma, \dots N_J) d\tau_\gamma = 0 \quad (8)$$

From the above consideration we can infer that the total wave function is normalised:

$$\int |A(1, 2, \dots N)|^2 d\tau_1 d\tau_2 \dots d\tau_N = 1 \quad (9)$$

d) The orbital energies and their corresponding wave functions can be resolved regardless of the remaining groups.

When the interaction between groups is considered, a correction for the orbital energies becomes necessary. Therefore the following operator, characteristic of this interaction, appears:

$$H'_K(1) = -\sum_{\eta} (Z_{\eta}/r_{1\eta}) + \sum_{I \neq K} G_I(1) \quad (10)$$

where  $\eta$  denotes the set of nuclei belonging to the remaining groups,  $Z_{\eta}$  are their positive charges; 1 is the set of coordinates relative to the electron described by molecular spin

orbital  $\phi_{\mu\beta}$  within  $K$  group, when the coupling between the various groups is not considered.

The corresponding correction to the orbital energy  $\bar{\epsilon}_{\mu\beta}$  will be:

$$\bar{\epsilon}_{\mu\beta} = -\sum_{\eta} Z_{\eta} \int \phi_{\mu\beta}^K(1) (1/r_{1\eta}) \phi_{\mu\beta}^K(1) d\tau_1 + \sum_{I \neq K} \int \phi_{\mu\beta}^K(1) G_I(1) \phi_{\mu\beta}^K(1) d\tau_1 \quad (11)$$

where

$$G_I(\eta) = \sum_{\alpha(r,s)} A_r^{I*} A_s^I G_{\alpha}^{I,rs}(\eta)$$

and

$$\sum_{\alpha(r,s)} = \text{(I)} \sum_{\alpha=1}^{N_I} \\ = \text{(II)} \text{ (term with } \alpha = \nu)$$

(III) a factor 0x

I. when the determinants are identical, i.e.  $\Phi_r^I = \Phi_s^I$ ; II. when the determinants differ only in the molecular spin orbital,  $\nu$ , i.e., when only  $\phi_{r\nu}^I \neq \phi_{s\nu}^I$ , and III. in the remaining cases.

Further

$$G_{\alpha}^{I,rs}(1) = J_{\alpha}^{I,rs}(1) - K_{\alpha}^{I,rs}(1)$$

where

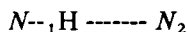
$$J_{\alpha}^{I,rs}(1) \phi(1) = \int \phi_{r\alpha}^{I*}(2) \phi_{s\alpha}^I(2) (1/r_{12}) \phi(1) d\tau_2 \quad (12)$$

$$K_{\alpha}^{I,rs}(1) \phi(1) = \int \phi_{r\alpha}^{I*}(2) \phi_{s\alpha}^I(1) (1/r_{12}) \phi(2) d\tau_2 \quad (13)$$

Operators (12) and (13) are usually called Coulomb and exchange operators.

In order to apply the theory of separated electronic groups, we shall consider, for each molecular orbital, as significant only the interaction between atomic orbitals belonging to the same hydrogen bond.

The interactions considered in these bonds involve the analysis of  $\sigma$  and  $\pi$  electrons. For instance, let us take a hydrogen bond having the following form:



as it is seen between the 8th. and 11th. atoms of the adenine-thymine pair. Thus if, for example, we wish to correct the orbital energies corresponding to the molecule to which  $N_2$  (adenine, in this case) belongs, the electronic and electron-nucleus interaction between both molecules must be considered. For the electronic interaction, the correcting terms for the orbital energies have the form:

$$\int \phi_i(1) \phi'_j(2) (1/r_{12}) \phi_i(1) \phi'_j(2) d\tau_{12} - \int \phi_i(1) \phi'_j(2) (1/r_{12}) \phi_i(2) \phi'_j(1) d\tau_{12} \quad (14)$$

If it is assumed that  $\phi_i$  is the molecular spin-orbital corresponding to level  $\epsilon_i$  in one of the pairs, adenine for instance,  $\phi'_j$  is the orbital corresponding to level  $\epsilon'_j$  in thymine. The first integral is independent of the spin states corresponding to orbitals  $\phi_i$  and  $\phi'_j$ ;

while the exchange integral will be zero if the spins corresponding to both orbitals are antiparallel. When correcting the orbital energies in one of the base pairs, we shall consider significant only the interaction with the occupied levels of the complementary base; this renders all coefficients  $A_r^1$  corresponding to "excited configurations" negligible.

If we consider the type of interaction  $\pi - \pi$  between both molecules, the integrals taken into account are reduced to Coulomb and exchange integrals between the orbitals  $\pi$  of the nitrogens  $N_1$  and  $N_2$  multiplied by the coefficients corresponding to them in their respective molecular orbitals. The  $\sigma - \pi$  interactions are more complex and require a more detailed analysis. For instance, if we wish to correct level  $\varepsilon_i$  in adenine, in a general way, valid for  $\pi - \pi$  or  $\sigma - \pi$  Coulomb interactions, we can write:

$$\int c_1 \psi_\pi(1) [c'_1 \psi'_\sigma(2) + c'_2 \psi'_{\pi'}(2) + c'_3 \psi'_{\pi''}(2) + c'_4 \psi'_H(2) + c'_5 \psi'_s(2)] (1/r_{12}) \\ c_1 \psi_\pi(1) [c'_1 \psi'_\sigma(2) + c'_2 \psi'_{\pi'}(2) + c'_3 \psi'_{\pi''}(2) + c'_4 \psi'_H(2) + c'_5 \psi'_s(2)] d\tau_{12} \quad (15)$$

where orbitals  $\psi'_\sigma, \psi'_{\pi'}, \psi'_{\pi''}, \psi'_s$  are supposed to be located in  $N_1$  belonging to thymine and  $\psi'_H$  is in the hydrogen of the bond. Orbital  $\psi'_\sigma$  is pointed in the  $N_1 - N_2$  direction, while  $\psi'_{\pi'}, \psi'_{\pi''}$  are supposed to be forming orthogonal triad with  $\psi'_\sigma$ , that is to say, Kotani's notation has been used; (KOTANI, AMEMIYA and SIMOSE [8]) where  $\psi'_s$  indicates the atomic orbital (2s).

Thus, in applying the approximation proposed by MÜLLIKEN for the integrals located in three centres, we have:

$$c_1^2 [c_1'^2 D_{\pi\sigma\pi\sigma} + c_1' c_4' S_{\sigma h} D_{\pi\sigma\pi\sigma} + c_2'^2 D_{\pi\pi'\pi\pi'} + c_3'^2 D_{\pi\pi''\pi\pi''} + c_4'^2 M_{\pi\pi\pi} + c_4' c_5' S_{s_h} D_{\pi s \pi s} + c_5'^2 D_{\pi s \pi s}] \quad (16)$$

Proceeding in the same way with the exchange terms, the result is:

$$c_1^2 [c_1'^2 C_{\pi\sigma\sigma\pi} + 2c_1' c_5' C_{\pi\sigma s \pi} + c_2'^2 C_{\pi\pi'\pi'\pi'} + c_4'^2 N_{\pi\pi\pi} + c_5'^2 C_{\pi s \pi s}] \quad (17)$$

For the hydrogen bond  $N-H\cdots N$  between the 8th. and 11th. atoms of the adenine-thymine pair, for the adenine correction, integrals  $D$  and  $C$  have been taken for a distance  $R = 3 \text{ \AA}$ , while  $S_{\sigma h}, S_{s_h}$  must be taken with  $R = 1 \text{ \AA}$ , and  $M_{\pi\pi\pi}, N_{\pi\pi\pi}$  should be taken for  $R = 2 \text{ \AA}$ . The notation used in formulas (16) and (17) is also the same as given by KOTANI, AMEMIYA and SIMOSE [8].

Formulas (16) and (17) are of a general character, and are valid for  $\sigma - \pi$  and  $\pi - \pi$  interactions. Thus, for the  $\sigma - \pi$  interactions,  $C_2' = C_3' = 0$ , but if, to the contrary, the interaction is of the  $\pi - \pi$  type,  $C_1' = C_2' = C_4' = C_5' = 0$ .

The coefficient  $C_1$  is equal to  $C(i, 8)$  for the adenine, where  $i$  is the  $i$ -th level of this base. The coefficient  $C_3 = C(j, 11)$  has the same meaning for thymine, where we must sum over all the occupied levels, multiplying by two in the Coulomb terms, while in the exchange terms only a simple addition will be necessary.

The determining of the coefficients corresponding to the  $\sigma$  pairs of electrons requires a more detailed example. Thus, for instance, considering  $N_1-H\cdots N_2$ , there are two pairs of electrons establishing the hydrogen bond. If the correction of the orbital

energies and the eigenfunctions corresponding to adenine are sought, only the interaction of this base over the localised  $N_1$ : H pair will be considered, as pair H:  $N_2$  would be included within the first approximation established when calculating the bases separately by means of the above mentioned method given by JORDAN and PULLMAN [7].

In the same way, if the corrections corresponding to thymine are considered, the same preceding arguments are valid when  $N_1$  interacts with the localised H:  $N_2$  pair, but the integrals  $S_{sh}$ ,  $S_{oh}$  must be taken with  $R = 2 \text{ \AA}$ , and  $M_{\pi\pi}$ ,  $N_{\pi\pi}$  with  $R = 1 \text{ \AA}$ .

The interatomic distances have been taken as equal to  $3 \text{ \AA}$  in all hydrogen bonds.

To calculate the coefficients corresponding to the atomic orbitals used, let us proceed as follows:

Assuming a hydrogen bond in the form  $X\text{--}H\text{-----}Y$ , the atomic orbitals will be:

$$\omega_1 = (1/\sqrt{3})(S_x + \sqrt{2} P_{x\sigma}) \quad (18)$$

$$\omega_2 = (1/\sqrt{3})(S_y + \sqrt{2} P_{y\sigma}) \quad (19)$$

The atomic orbitals  $S_x$ ,  $P_{x\sigma}$  are presumed to be located in the  $X$  atom, whilst  $S_y$ ,  $P_{y\sigma}$  will be located in the  $Y$  atom.

It is easy to associate either orbitals ( $S_x = \psi'_s$ ;  $P_{x\sigma} = \psi'_\sigma$ ) or ( $S_y = \psi'_s$ ;  $P_{y\sigma} = \psi'_\sigma$ ) with the orbitals used in the preceding discussion, depending upon from which base the interaction is considered.

We then have, following a somewhat similar treatment to the one given by PAOLONI [11],

$$\int \omega_1 \omega_1 d\tau_1 = 0 \quad (\text{only approximately}) \quad (20)$$

$$\int \omega_1 \theta_1 d\tau_1 = \delta_1 = (1/\sqrt{3})(S_{xh} + \sqrt{2} S_{x\sigma h}) \quad (21)$$

$$\int \omega_2 \theta_1 d\tau_1 = \delta_2 = (1/\sqrt{3})(S_y + \sqrt{2} S_{y\sigma h}) \quad (22)$$

$$\int \theta_1 d\tau_1 = 1 \quad (23)$$

where  $\delta$  are the overlapping integrals between the orbitals located in H and the  $X$  and  $Y$  elements.

The orbital ( $1s$ ) pertaining to the element H has been taken as orbital  $\theta_1$  located in H.

Let us consider the molecular orbitals:

$$\phi_1 = N_1(\omega_1 + \lambda\theta_1)$$

$$\phi_2 = N_2(\omega_2 + \eta\theta_1)$$

where

$$N_1 = (1 + \lambda + 2\lambda\delta_1)^{-1/2}, \quad N_2 = (1 + \eta^2 + 2\eta\delta)^{-1/2}$$

If  $\phi_1$  and  $\phi_2$  are assumed to be orthogonalised, the result is:

$$\eta = -\frac{\lambda\delta_2}{\delta_1 + \lambda}; \quad (24)$$

with these two spatial orbitals four molecular spin-orbitals can be built up, having the form:

$$\bar{\phi}_1\phi_1\bar{\phi}_2\phi_2$$

which allow us, within (16) and (17), to identify the coefficients corresponding to atomic orbitals  $\psi'_s, \psi'_\sigma, \psi'_\pi, \psi'_h$ , depending upon whether we deal with the interaction  $N_2$  with  $N_1 : H$  or  $N_1$  with  $H : N_2$ . The stated model is not in fact rigorous but it seems to us a plausible approach, particularly if it is taken into consideration that the fundamentally unknown coefficient  $\lambda$  can be varied within reasonable limits.

The matrix elements between the eigen-functions corresponding to different states, necessary to calculate the disturbed terms introduced by operator (10), resolve themselves immediately by analogous considerations to those given in the preceding remarks. For example, for the matrix elements between  $\phi_i$  and  $\phi_l$ , in adenine,  $c_1^2$  must be replaced by  $c_1c_2$ , where  $C_1 = C(i, 8)$ ,  $C_2 = C(l, 8)$  in formulas (16) and (17).

For one electron operators the matrix elements follow at once. The effective nuclear charges for the elements in each hydrogen bond have been estimated by means of the expression  $Z - s$ ,  $s$  being the corresponding value, according to Slater's rules for the electrons which had not been considered.

For the calculation of the disturbed eigen-functions, the theory of perturbations has been used up to the first order. For each perturbed state its interaction with all other available orbital levels was considered.

The charge density on atom  $r$  can be expected to be given by

$$q_r = \sum q_{rj} \quad \phi_j = \sum c_{jr}\psi_r$$

where

$$q_{rj} = n_j \int \phi_j^* \phi_j dv$$

is the fractional part of the  $n_j$  electrons occupying the  $j$ th MO associated with a given atom  $r$ , ( $q_{rj}$  is given in units of electron charge  $e$ ).

The integration is limited to a space in the immediate neighbourhood of atom  $r$ . Expressing the above quantity in the basis  $\psi_r$  and assuming that for integration in the neighbourhood of atom  $r$

$$\int \psi_r^* \psi_s dv = S_{sr}$$

$$\int \psi_r^* \psi_r dv = 1$$

$$\int \psi_s^* \psi_s dv = 0,$$

we have for  $q_r$  the same formulas given by CHIRGWIN [4], or PILAR [12]. These formulas were used with the coefficients  $\{c_{jr}\}$  obtained before and after the application of the theory of separated electronic groups.

### 3. Numerical Results and Discussion

Taking into account that the initial values corresponding to the separately considered bases depend upon semi-empiric constants obtained from other molecules, we have principally analysed the variation order introduced by the hydrogen bonds.

The resultant values for the elements involved in the hydrogen bonds are as follows:

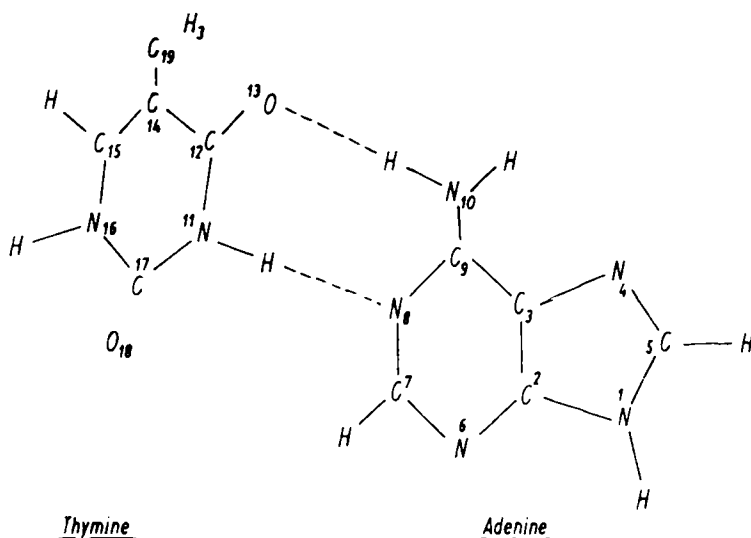


Fig. 1.

Table 1. Electronic distribution

Adenine-Thymine Separated	
Extended Hückel Method	
8	1.314
10	1.866
11	1.783
13	1.502

For the calculation of Table 1, the elements  $H_{ij}$  given by (2) have been considered as non-zero for each pair of  $i, j$  elements belonging to the same ring, or in general for distances larger than 4 Å.

The attainable approximations, applying the above method, is based upon the transferability of semi-empirical parameters in molecules, assuming also that they are characteristic for each union type, independent of their location within the molecule. These circumstances can lead to considerable differences with the actual values; thus, we consider it more adequate to analyse the variations arising from the distribution of charges corresponding to  $\pi$  electrons as a consequence of the formation of hydrogen



bonds connecting the complementary bases in the DNA, than to presume these variations to be less sensitive to the above mentioned factors. Considering that the previously defined  $\lambda$  does not have a known value, we take into our consideration the interaction effects corresponding to several values of  $\lambda$ . The  $\lambda$  values been taken as having the same value for all hydrogen bonds belonging to the same type. That is why the parameters  $\lambda$  for N--H---N and  $\lambda'$  for N--H---O have been used. The combinations involving different values of the parameters corresponding to the same type hydrogen bonds are, in general, comprised within the range of tabulated values.

The following values have been obtained:

Table 2. Electronic distribution

	$\lambda/\lambda'$	0.8	0.9	0.95
8	0.8	1.355	1.357	1.358
	0.9	1.362	1.368	1.369
10	0.8	1.821	1.819	1.817
	0.9	1.819	1.817	1.816
11	0.8	1.725	1.724	1.723
	0.9	1.721	1.721	1.721
13	0.8	1.546	1.547	1.547
	0.9	1.542	1.544	1.545

The results obtained show the variations of charges and orbital energies introduced by the hydrogen bonds. There are only slight variations in the electronic levels (s. Tables) and the wave functions have only a little change for each base. (We have considered all levels up to the second excited one on each of the bases.)

Table 3

Adenine	A - T	Thymine	A - T
-13.8753	-13.6125	-16.3063	-16.0824
-12.5845	-12.2542	-13.5104	-13.5501
-6.8654	-6.5453	-6.4956	-6.3148
-6.0550	-6.2561	-3.1939	-3.2197

Two highest filled and two lowest empty orbitals in both bases (ev).

In any event, the most attainable conclusion, from the numerical results given in Table 2, seems to be that the electronic charges corresponding to the elements under consideration show a small variation in terms of  $\lambda$  and  $\lambda'$ . The range of obtained values implies that the variation produced in the elements under consideration remains fairly independent within the variation interval of  $\lambda$  and  $\lambda'$ . (This range has been selected in view of the relation between, and the dipolar moment for, N--H given by COULSON [3].)

#### 4. Last Comments and Final Conclusions

The theory of separated electronic groups proves to be adequate for the treatment of the influence of hydrogen bonds in the  $A - T$  pair. The introduction of the theory of perturbations by use of the perturbation operator defined by means of formula (10) and (11) is particularly rigorous, since the  $\sigma$  and  $\pi$  electronic configurations fulfill the orthogonality condition (8). Furthermore, the started treatment has other advantages:

a) The treatment is valid for any starting approximation in calculating the separately considered bases, with no restriction other than the expression of the corresponding molecular orbitals through linear combinations of atomic orbitals. It can be used, therefore, with different methods, and not only with this given herein.

b) It allows of considerable improvement with very slight modification, or none at all, in its essential delineation. For instance:

- I. The integrals located in more than two centres can be calculated in a more satisfactory manner than through Mulliken's approximation.
- II. It easily allows the analysis of dependence of the perturbations obtained by interaction between both bases in terms of the semi-empirical constants used in the calculation of the separately considered bases.
- III. Hückel's method applied to  $\sigma'$  electrons still presents very serious doubts in our mind, and we have, therefore, given up using it. However, if this method should succeed in proving its validity, the formalism here used permits "ab-initio" calculations with no necessity to estimate the coefficients corresponding to  $\sigma$  orbitals by means of plausible "ad-hoc" hypotheses. The authors consider this possibility of improvement to be by far the most important one.

c) The theory of perturbations allows a faster determination of the orbital energies than the distribution of charges or the bond orders, particularly if all electrons ( $\sigma$  and  $\pi$ ) are included.

The orbital energies are very important because of the formula

$$E^k = \sum n_i \epsilon_i + G^k$$

for the total energy of the state  $k$  it is possible to analyse the variation of  $E^k$  from the position of the hydrogens in each one of the hydrogen bonds, if it is assumed that  $G^k$  is independent of  $k$  and the hydrogens position. The change of  $E^k$  as a function of the hydrogens position is closely linked with the possibility of the proton transfer in the considered state. Specifically, it is very interesting to determine the conditions under which protons may transfer from one member of the base pair to the other, thus altering the genetic code. These results should be compared with those obtained from other authors, e.g., BLIZZARD and SANTRY [2] whose calculations were performed using CNDO/2 method described by POPLE and SEGAL [13].

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