plete series are now available. Examination of the trends of the various hyperfine splittings shows that the overall spin density on the ring is increasing in going down the series. Thus, the epr data on both the parent radicals and their monofluoro derivatives support the hypothesis that an ionic resonance form with a symmetric aromatic ring is important in explaining the structure of these radicals.

We have confirmed both experimentally and theoretically the existence of the "ortho" effect in benzyl and anilino radicals, whereby the fluorine hfs in o-fluorinated radicals is smaller than would normally be expected. Our results suggest that when a proton is present on the exo group this effect is due to hydrogen bonding between the proton and the fluorine.

The Electronic Spectra of Nucleic Acid Bases.
II.   Out-of-Plane Transitions and the
Structure of the Nonbonding Orbitals

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Abstract: The energies of the lower out-of-plane transitions of nucleic acid bases and their tautomers and ions are calculated with the CNDO-CI method, which includes all valence electrons. Precise values are obtained by using the energy differences between the observable $\pi\pi^*$ transitions and the $n\pi^*$ transitions, instead of calculating absolute values, and by correcting them empirically for systematic deficiencies of the MO-CI model applied. The $n\pi^*$ transitions of the various bases are characterized and correlated by their transition densities. This reveals that the lowest $n\pi^*$ state bears a close relation to the structure and energy of the highest nonbonding orbital. The structure of the nonbonding orbitals of interest can also be rationalized in simple terms. From the structure of the lowest $n\pi^*$ state and the highest nonbonding orbital it is shown that the bases naturally fall into three classes with respect to their low-energy out-of-plane spectra. The first, which we call the pyrimidine class, comprises the bases without a carbonyl group. The lower $n\pi^*$ transitions are located on nitrogen atoms of the six-membered ring of the bases and have a structure similar to those of pyrimidine. The second, or carbonyl class, consists of the bases containing a carbonyl group with no heteroatom with a lone pair of σ electrons next to it. The lowest $n\pi^*$ transitions are confined largely to the carbonyl oxygen and strongly resemble that of cyclohexanone. The third class is intermediate to the other two, and the bases belonging to it have a nitrogen atom with a lone pair of σ electrons next to a carbonyl group. The two lowest $n\pi^*$ states are mainly spread out over this fragment of the molecule. Their energy is lower than for the corresponding tautomers belonging to the carbonyl class. From the structure of the $n\pi^*$ transitions the energetic shifts occurring upon structural alterations and the formation of ions can readily be understood. The introduction of an amino or hydroxyl group in α position to the location of a transition leads to a strong blue shift. For this reason, the lowest $n\pi^*$ transition in adenine is shifted in energy above the lowest $\pi\pi^*$ transition, whereas in purine the $n\pi^*$ transition is lowest in energy. For no neutral nucleic acid base is the $n\pi^*$ transition calculated to be lower in energy than the first $\pi\pi^*$ transition. Protonation of the nucleic acid bases often does not affect the structure of a $n\pi^*$ transition. In such a case a *red shift* rather than a blue shift occurs. For deprotonation the opposite is true. Both findings show that the generally accepted conclusions drawn from small chromophores are not necessarily valid for larger ones containing several heteroatoms.

In the preceding paper I[1] we have interpreted the in-plane spectra of nucleic acid bases and their tautomers and ions with the aid of all valence electron MO-CI calculations. These spectra comprise the $\pi\pi^*$ and $\sigma\sigma^*$ transitions, the latter mainly of $n\sigma^*$ character. They are in general intense, except where forbidden by reasons of molecular or local symmetry, and determine therefore the gross appearance of the uv spectra of the bases. The high intensity of many in-plane transitions is a natural consequence of the large dimension of the chromophore in the π plane. Perpendicular to this plane the dimension is much smaller, and the out-of-plane transitions, particularly the $n\pi^*$

transitions dealt with in this paper, therefore, are of much lower intensity. Where out-of-plane transitions are not well separated energetically from intense in-plane transitions, they cannot be observed by simple absorption spectroscopy. Some out-of-plane transitions appearing at higher energy and probably of $\pi\sigma^*$ type have been inferred from single-crystal absorption and reflection spectra.[2,3] Fluorescence measurements[4] and the complicated nature of the CD spectra of mononucleosides[5] as well as perturbations observed in elec-

(1) W. Hug and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **95**, 2803 (1973).

(2) W. A. Eaton and T. P. Lewis, *J. Chem. Phys.*, **53**, 2164 (1970).
(3) L. B. Clark, private communication.
(4) P. R. Callis, E. J. Rosa, and W. T. Simpson, *J. Amer. Chem. Soc.*, **86**, 2292 (1964).
(5) W. Voelter, R. Records, E. Bunnenberg, and C. Djerassi, *J. Amer. Chem. Soc.*, **90**, 6163 (1968).

trochromism measurements[6] provide evidence also for lower lying out-of-plane transitions. Yet, up to now no conclusive arguments even for the relative position of the lowest $n\pi^*$ and the lowest $\pi\pi^*$ transitions in most nucleic acid bases have been given. One of the goals of the present paper therefore is to establish the relative positions of these two types of transitions within limits of useful accuracy, i.e., 1–2 kK.

The energetic splittings between different $n\pi^*$ transitions are also of interest. Evidence has recently accumulated that similar $n\pi^*$ transitions in multifunctional chromophores are usually not degenerate, and a systematic treatment for the case of two interacting carbonyl groups has been given.[7,8] The situation is more complex for nucleic acid bases, but it will be shown that many of the earlier conclusions on simpler systems remain valid.

Protonation and deprotonation often leads to extensive changes in observed spectra. The general assumption is, that upon protonation of the σ system, the n orbitals are lowered more in energy than the π^* orbitals. The $n\pi^*$ transitions therefore are expected to undergo a strong blue shift. In paper I we have shown that the individual $\pi\pi^*$ transitions undergo large changes, too, due to the charge reorganization in the whole molecule. Here we will show that the position of the $n\pi^*$ transition lowest in energy is often not affected to the degree expected from simple arguments.

The method of calculation is the CNDO–CI procedure as used in paper I. Some of the limitations of the method as applied to electronic spectra are discussed in that paper. The parameters used in the present calculation are given in the Appendix of paper I.

Wave Functions and Energies of Excited States

Characterization of $n\pi^*$ States. To be able to compare and correlate the $n\pi^*$ transitions of the various nucleic acid bases it is necessary to characterize them in terms useful in chemistry. As we deal with multideterminantal wave functions, and as the bases are devoid of symmetry higher than C_s, this is not straightforward. For nucleic acid bases configuration interaction in our calculations is often extensive. In paper I we have therefore used the nodal pattern of the transition monopoles to characterize the in-plane transitions. A generalization of this procedure leads to the use of transition densities.[9] A more general discussion of this approach is given elsewhere.[10] For the $n\pi^*$ states of the planar nucleic acid bases discussed here, and within the frame of the zero-differential overlap approximation, this means that we have to visualize spatial distributions of the form

$$\sum_{\mu,\nu} B_{\mu\nu} \sum_A (c_{s\mu}{}^A 2s^A + c_{x\mu}{}^A 2p_z{}^A + c_{y\mu}{}^A 2p_y{}^A)c_{z\nu}{}^A 2p_z{}^A =$$

$$\sum_A (d_s{}^A 2s^A + d_z{}^A 2p_z{}^A + d_y{}^A 2p_y{}^A)2p_z{}^A$$

Σ_A designates a sum over all atoms of the first row. $c_{s\mu}{}^A$, $c_{x\mu}{}^A$, etc., are the coefficients of MO μ of the $2s^A$, $2p_z{}^A$, etc., basis function on atom A. $\Sigma_{\mu,\nu}$ designates

(6) K. Seibold and H. Labhart, *Biopolymers*, **10**, 2063 (1971).

(7) W. Hug, J. Kuhn, K. J. Seibold, H. Labhart, and G. Wagnière, *Helv. Chim. Acta*, **54**, 1451 (1971).

(8) T.-K. Ha and W. Hug, *Helv. Chim. Acta*, **54**, 2278 (1971).

(9) E. F. Haugh and J. O. Hirschfelder, *J. Chem. Phys.*, **23**, 1778 (1955).

(10) W. Hug, to be submitted for publication.

a sum over the singly excited configurations $\mu \rightarrow \nu$ contained with the coefficients $B_{\mu\nu}$ in the CI wave function (see paper I). It is assumed that the π plane of the molecule coincides with the x,y plane and that therefore $2p_z \equiv 2p_\pi$. As is obvious from the right side of the above equation, the distribution on each atom A can be visualized as that of an atomic hybrid function $d_s{}^A 2s^A + d_z{}^A 2p_z{}^A + d_y{}^A 2p_y{}^A$ times that of the $2p_z{}^A$ function. This product is, of course, antisymmetric with respect to the π plane. In planes parallel to the π plane it has a form qualitatively similar to the familiar atomic hybrid functions. For our gross qualitative purpose we will indicate the shape of the hybrid function on each atom by depicting its 2s and 2p part with sizes proportional to the coefficients d_s and $(d_z{}^2 + d_y{}^2)^{1/2}$, respectively. The phases are indicated by the contour lines being filled or not.

Excitation Energies in the Single Transition Approximation and the Shape of Orbitals. To understand the out-of-plane spectra of the nucleic acid bases the relative energies of $n\pi^*$ and $\pi\pi^*$ transitions are of prime importance. We use the single-transition approximation in our qualitative discussion here, but the results remain valid if configuration interaction is introduced. The energies of the singlet states are the eigenvalues of the CI matrix, the diagonal elements of which are given by the energy of the singly excited configurations. A change of all the energies of the single transitions of a given symmetry by the same amount will cause an identical change in the energies of the corresponding singlet states.

The single-transition energies for a $\pi\pi^*$ and a $n\pi^*$ transition are, if π^* is a virtual orbital

$$E_{\pi\pi^*} = \epsilon_{\pi^*} - \epsilon_\pi - (J_{\pi\pi^*} - 2K_{\pi\pi^*})$$

$$E_{n\pi^*} = \epsilon_{\pi^*} - \epsilon_n - (J_{n\pi^*} - 2K_{n\pi^*})$$

ϵ are the MO energies, J the coulomb, and K the exchange integrals, both of which are positive. The differences $(\epsilon_{\pi^*} - \epsilon_\pi)$ and $(\epsilon_{\pi^*} - \epsilon_n)$ of the orbital energies are of the order of 10 eV in our calculation for the lower transitions. The transition energy is typically about 4 eV. The delocalization of the occupied (π, n) and empty (π^*) orbitals is one of the important factors determining the energetic gap between them. An increase in delocalization in general is accompanied by a reduction of the energy differences $(\epsilon_{\pi^*} - \epsilon_\pi)$ and $(\epsilon_{\pi^*} - \epsilon_n)$. The term $(J - 2K)$ on the other hand is determined by the *relative* location of the filled and empty orbitals. This term will be small when they are located on different parts of the molecule and large when both the occupied and empty orbitals have large coefficients on the same atoms.

The balancing effect of these two terms can be illustrated by the experimentally well-known phenomenon that in complex chromophores like conjugated ketones[11] or aromatic quinones[12] the $\pi\pi^*$ transitions tend to "pass" the $n\pi^*$ transitions. For both types of transitions the gap between filled and empty orbitals decreases with increased delocalization of the electrons. Yet, for the $n\pi^*$ transition the decrease of $(\epsilon_{\pi^*} - \epsilon_n)$ is in part canceled by the decrease of the term $(J_{n\pi^*} - 2K_{n\pi^*})$, due to the fact that the π^* orbital gets "diluted" at the site of the n orbital. The phenomenon will be of importance

(11) E. R. Blout and M. Fields, *J. Amer. Chem. Soc.*, **70**, 189 (1948).

(12) H. Hartman and E. Lorentz, *Z. Naturforsch. A*, **7**, 360 (1952); S. Nagakura and A. Kuboyama, *J. Amer. Chem. Soc.*, **76**, 1003 (1954).
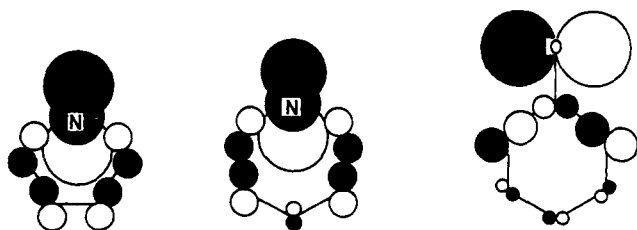
Figure 1. The structure of the nonbonding orbitals in pyrrole, pyridine, and cyclohexanone. Nodes and delocalization are characteristic for that found on corresponding fragments of nucleic acid bases.

in later discussions for a series of chromophores like hydantoin–uracil–xanthine or pyrimidine–purine. An increase of the delocalization of the n orbital, by involving more than one atom with lone pair electrons, often leads to a spectacular decrease of $n\pi^*$ transition energies. This is in part due to a decrease of the n ionization potential and a concomitant decrease of the gap between filled and empty orbitals. At least equally important is an increase of $(J_{n\pi^*} - 2K_{n\pi^*})$, however, due to the fact that the n orbital extends over more of the π system. An example is the series pyridine–pyrimidine–tetrazine, where the lowest $n\pi^*$ transition is shifted from 41.5 kK (240 nm) to 34 kK (295 nm) to 18.5 kK (540 nm). The $\pi\pi^*$ transition remains at around 39 kK (255 nm).

Numerical Values for $n\pi^*$ Transition Energies. To assign the intense and directly observable $\pi\pi^*$ transitions, a calculation accuracy of 5–10% is in general sufficient, provided that the energetic sequence is calculated correctly. With the $n\pi^*$ transitions the situation is more difficult, because in most cases we cannot simply assign a directly observable transition. It seems more feasible to calculate the energy difference between the lowest $n\pi^*$ and $\pi\pi^*$ transition (or, more precisely, the $\pi\pi^*$ transition involving the same π^* orbital) with the required accuracy than to calculate the $n\pi^*$ energy on an absolute scale. In the single-transition approximation this energy difference ΔE is

$$\Delta E = E_{n\pi^*(\text{calcd})} - E_{\pi\pi^*(\text{calcd})} =$$

$$(\epsilon_\pi - \epsilon_n) + (J_{\pi\pi^*} - J_{n\pi^*}) - 2(K_{\pi\pi^*} - K_{n\pi^*})$$

On the right side there appear only differences of similar terms, and we can expect that some of the errors due to the CNDO method and the choice of parameters cancel. Some inaccuracies will not cancel, however, because the CNDO treatment, in particular the invariance requirements,[13] leads to systematic differences in the errors of integrals involving different atomic orbitals. In writing the expression for the $n\pi^*$ energy $(E_{n\pi^*})$ we therefore allow for a corrective constant $\Delta E'$

$$E_{n\pi^*} = E_{\pi\pi^*(\text{exptl})} + \Delta E + \Delta E'$$

We can expect that for "similar" $n\pi^*$ transitions $\Delta E'$ will be nearly constant and therefore can be calibrated on molecules where the energies of the $n\pi^*$ and $\pi\pi^*$ transitions are known. It should be emphasized that our method of calibration will not only take care of errors in the calculation of ΔE but also contain corrections due to differences in Franck–Condon factors of $n\pi^*$ and $\pi\pi^*$ transitions and the influence of solvents.

(13) J. A. Pople, D. P. Santry, and G. A. Segal, *J. Chem. Phys.*, **43**, S129 (1965).
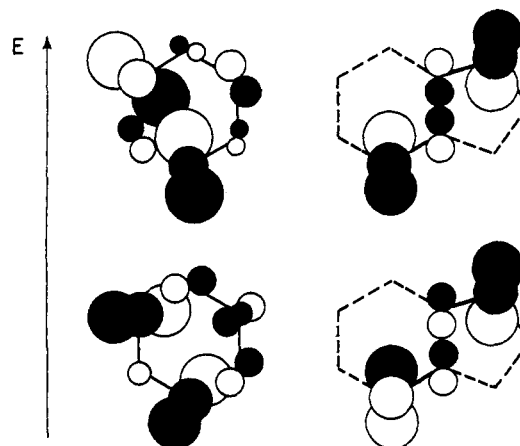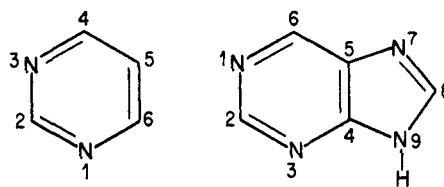

Figure 2. Phase and energetic sequence of n orbitals in the case of two lone pairs of electrons of similar energy. Pyrimidine and the purine fragment N3–C4–C5–N7 are depicted, the numbering and the arrangement of the formulas being the same throughout the paper.

The Nonbonding Orbitals

The understanding of the structure of the n orbitals of the σ type is necessary to understand the data obtained from the use of transition densities. The knowledge of their structure is also of interest with respect to the site of protonation, hydrogen bonding, and the photochemistry of nucleic acid bases and concerted chemical reactions.[14] The orbitals relevant for a discussion of electronic transitions within an SCF–MO scheme are the *canonical* orbitals, however. Strictly speaking it is impossible to distinguish between "nonbonding" and "ordinary" σ orbitals.[15] It is a matter of convenience to adhere to these terms. In practice, it is easy to identify the "n" orbitals in a MO calculation by their preponderate localization on the atoms with the lone pair electrons, typically 60–80%, and by their energetic position at the top of the σ orbitals. A nitrogen atom with only two σ bonds therefore gives one molecular n orbital in the planar nucleic acid bases. A carbonyl oxygen would correspondingly lead to two n orbitals of σ type. Yet, the 2s lone pair is so low in energy[16] that the SCF calculations lead to a thorough mixing with low-lying σ orbitals, and we will therefore consider only the $2p_n$ orbital perpendicular to the bond. In the enol form of the bases the resultant hydroxyl oxygen no longer has a high-lying n orbital.

(14) R. B. Woodward and R. Hoffmann, *Angew. Chem.*, **81**, 797 (1969).

(15) A description in better accord with the chemist's picture can be obtained from SCF calculations by a localizing procedure using a unitary transformation. The orbitals obtained are, however, not suited for a discussion of spectroscopic properties; see, *e.g.*, C. Edmiston and K. Ruedenberg, *Rev. Mod. Phys.*, **35**, 457 (1963). Another possibility is the decomposition of atomic orbital populations in components along distinguished directions on the heteroatoms, as done by A. Pullman, *Int. J. Quantum Chem. Symp.*, **2**, 187 (1968).

(16) J. N. Murrell, "The Theory of the Electronic Spectra of Organic Molecules," Methuen and Co., Ltd., London, 1963, p 158.

Important n-Orbital Fragments. The structure of n orbitals shows a considerable constancy in similar molecular fragments, thus simplifying their treatment and understanding. In our schematic representation of the n orbitals of the bases only the preponderant contributions on each heteroatom are given. The rest of their structure, corresponding roughly to the representative fragments, is depicted qualitatively in Figures 1 and 2.

In pyrrole and pyridine the heteroatom is contained in the ring, and in cyclohexanone it is adjacent to it. In both cases the n orbital spreads out manly over the heteroatom and the ring, the nodes within the ring being fairly constant, even if it is part of a larger system. The atoms adjacent to the carbonyl group exhibit the largest contribution of the ring atoms in the ketone (Figure 1).

For the case of two heteroatoms with a lone pair of σ electrons, the two n orbitals obtained correspond to the (+) or in-phase and the (−) or out-of-phase combination of the two localized nonbonding orbitals (Figure 2). Their energetic splitting is in most cases considerable and their sequence is important for the electronic spectra.[7] As only C_s symmetry is present in nucleic acid bases, the contribution of the n orbitals on the two heteroatoms is in general not equal. One of the n orbitals may appear associated mainly with one heteroatom, the other with the second. They are characterized in this way in later sections. The phase relations remain, however, as given in Figure 2. A corresponding situation is frequent, if three or more heteroatoms with lone pairs of σ electrons are present. It is then often the case that each of the n orbitals involves mainly *two* of the heteroatoms.

The n Orbitals of Pyrimidine Bases. The neutral pyrimidine bases have two heteroatoms with lone pairs of σ electrons, cations have one, and anions have three. Correspondingly, two nonbonding orbitals for neutral, one for cations, and three for anions are found. Their structural characteristics are collected in Table I, where

Table I. Location and Phase of the Nonbonding Orbitals of the Pyrimidine Bases[a]

Compound	MO - energy	n_I	n_{II}	n_{III}
cytosine	π : -12.04 n_I : -12.47 π : -12.84 n_{II} : -13.82 π : -15.10			—
uracil enol		identical	identical	—
cytosine cation	π : -17.66 n_I : -18.37 π : -18.70 π : -20.93		—	—
uracil	π : -12.64 n_I : -13.17 π : -13.57 n_{II} : -13.97 π : -16.09			—
cytosine imine		(identical)	identical	—
uracil anion	n_I : - 6.91 π : - 6.96 π : - 7.30 n_{II} : - 7.77 n_{III}: - 9.16 π : -10.27			

[a] It is assumed that all tautomers and ions carry a proton at N1. Energies in electron volts.

also the energies and the sequence of the other higher MO's according to our calculation are given.

Most of the regularities found in nucleic acid bases

can be illustrated by the n orbitals of uracil and cytosine and their tautomers. The highest n orbital, on which we will concentrate most of our attention, is preponderantly located on carbonyl oxygen or imino nitrogen and not on ring nitrogen atoms. This is in agreement with the sequence of the ionization potentials for the hybrid functions contained in the n orbitals on these heteroatoms and the stabilization expected from neighboring atoms without lone σ pairs. *Cytosine* and the *enol of uracil* illustrate this with a preponderant localization of the highest n orbital on carbonyl oxygen O2. The admixing of the sp^2 function of N3 is large in these cases, however, because the nitrogen is adjacent to the carbonyl group and carries a lone σ pair (see Figure 1 where a strong contribution is already found for the α-carbon atoms in cyclohexanone). When there is a choice between two carbonyls or a carbonyl and an imine, the group with more heteroatoms without lone σ pairs around it has the lower n orbital. *Uracil* and the *imine of cytosine* are examples of this. Of the two carbonyl groups in uracil, O2 has the more stable n orbital. Similarly, in the imine of cytosine the carbonyl O2 carries most of the lower n orbital.

The formation of ions has a large effect on the energy and, depending on the site of protonation or dissociation, the shape of the orbitals of a molecule. *Protonation* raises the core charge by $+1$ and eliminates one of the n orbitals. In our calculations, this leads to an increase of the ionization potentials by 5 to 6 eV, as can be seen from the values of the orbital energies of *cytosine* and its *cation*.[17] *Deprotonation* has the opposite effect and lowers the ionization potentials by 5–6 eV, as illustrated by *uracil* and its *anion*.

Purine Bases without Carbonyl Groups. Neutral purine bases with or without carbonyl groups have three n orbitals. Protonation reduces this number to two and deprotonation increases it to four. In the absence of carbonyl or imino groups these oribtals are mainly located on the ring nitrogen atoms without a proton, *i.e.*, on N1, N3, and N7 in neutral species. They are qualitatively identical in *purine*, *adenine*, and the *enols of hypoxanthine and guanine* (Table II). The highest one, n_I, shows its main contributions on N1 and N3 and corresponds closely to the higher n orbital of pyrimidine (Figure 2). The lowest one, n_{III}, is located on N3 and N7 and corresponds to the more stable of the two possible combinations on a chain of four atoms (Figure 2). n_{II} is intermediate in energy and classification. The 2p part corresponds to the more stable and the 2s part to the less stable combination in a hypothetical symmetric arrangement. Between purine and the other bases of this group there is a considerable difference between the relative position of the highest n and π orbital. $\epsilon_n - \epsilon_\pi$ is -0.80 eV in purine and -1.33 eV in adenine. The presence of the planar exocyclic amino group in the α position to N1 in adenine therefore leads to a relative lowering of the highest n orbital by 0.53 eV, suggesting a blue shift of the lowest $n\pi^*$ transition relative to the $\pi\pi^*$ transition. Again, as for uracil and cytosine, the n orbitals are by no means degenerate, and the energy gaps between successive n orbitals amount to more than 1 eV in all the examples.

Table II. Location and Phase of the Nonbonding Orbitals of the Purine Bases without a Carbonyl Group[a]

Compound	MO - energy	n_I	n_{II}	n_{III}
adenine	π : -10.89 n_I : -12.22 π : -12.77 π : -13.40 n_{II} : -13.63 n_{III} : -14.92 π : -15.22			
purine	π : -11.51 n_I : -12.31 π : -13.05 n_{II} : -13.79 π : -14.67 n_{III} : -15.12 π : -16.53	identical	identical	identical
hypoxanthine enol		identical	identical	identical
guanine enol		identical	identical	identical
adenine cation	a) π : -16.29 n_I : -17.09 π : -17.64 n_{II} : -18.88 π : -18.95 π : -20.50			—
	c) π : -15.92 n_I : -17.74 π : -17.97 π : -18.60 n_{II} : -19.60 π : -20.59			—

[a] Energies in electron volts.

Table III. Location and Phase of the Nonbonding Orbitals of the Purine Bases with a Carbonyl Group[a]

Compound	MO - energy	n_I	n_{II}	n_{III}	n_{IV}
guanine	a) π : -10.57 n_I : -12.36 π : -13.08 n_{II} : -13.12 π : -13.53 π : -14.27 n_{III} : -14.81				—
	b) π : -10.99 n_I : -11.47 π : -12.40 π : -13.39 n_{II} : -13.77 n_{III} : -13.93 π : -14.93				
hypoxanthine		identical with guanine	identical with guanine	identical with guanine	—
adenine imine		identical	identical	identical	—
xanthine	π : -11.48 n_I : -12.85 π : -13.38 n_{II} : -13.84 π : -14.01 π : -14.56 n_{III} : -15.05				—
guanine cation	a) π : -15.92 n_I : -17.12 π : -17.62 n_{II} : -18.38 π : -18.69 π : -19.31			—	—
	b) π : -15.74 n_I : -17.09 π : -17.67 n_{II} : -18.37			—	—
	c) π : -18.46 π : -19.82			—	—
hypoxanthine cation		identical	identical	—	—
guanine anion	π : -5.65 n_I : -6.51 n_{II} : -7.38 π : -7.89 π : -8.56 n_{III} : -8.86 π : -9.40 n_{IV} : -10.50 π : -10.66				
hypoxanthine anion		identical	identical	identical	identical

[a] N9 is always assumed to carry a proton. Energies in electron volts.

Changes upon *protonation* at different sites are easily understood from the structure of the n orbitals in the neutral bases. The two surviving n orbitals are mainly located on the nonprotonated nitrogen orbitals, with a structure and energetic sequence as discussed in Figure 2. Protonation at N7 in the five-membered ring does not alter the structure of the highest n orbital, which is located in the six-membered ring (case a in Table II). Its energy is lowered less than that of the π orbitals. Reasons for this unexpected behavior are given in the next section. In cases b and c, namely, protonation of N1 and N3, respectively, the shape of the highest n orbital changes upon protonation and its energy shift is larger than that of the highest π orbital.

Purine Bases with a Carbonyl Group. If a carbonyl group is present in purine bases, the highest n orbital is, as discussed in the section on pyrimidine bases, preponderantly located on oxygen. In neutral bases the former situation is characteristic for the *guanine* and *hypoxanthine* tautomers with a hydrogen atom at N1 and for *xanthine* (Table III). In guanine and hypoxanthine the two nitrogen contributions of N3 and N7 combine separately to form the two lower n orbitals, identical with those of adenine protonated at N1. In xanthine the two oxygen contributions on O2 and O6 combine separately to form the two higher n orbitals identical with those of uracil.

Large contributions from nitrogen to the highest n orbital are found, similar to the pyrimidine bases, if the nitrogen atom N1, which is adjacent to the carbonyl group, bears a lone σ pair. In general, the situation is best described as the two localized n orbitals of O6 and N1 combining separately to form the two highest molecular n orbitals, the third one being associated with the remaining nitrogen atom N7 carrying the third lone σ pair. Energetically, this leads to an increased interaction and larger gap for the two higher n orbitals. The energy of the highest n orbital appears raised relative to that of the highest π orbital.

The effect of protonation of purine bases with a carbonyl group is illustrated in Table III by the three cations of guanine. Contrary to the general assumption the energy of the highest n orbital appears *raised* in the cations rather than lowered *relative* to that of the highest π orbital. The reason is that the highest n orbital, unaltered in the cations, spreads out less over the parts of the molecule affected most by the attachment of a proton than the more delocalized π orbital, and the shift toward lower energy upon protonation therefore is larger for the π than for the n electrons.

The *anion of guanine* stands as the example for the n orbitals of deprotonated bases. They can be understood best if we first assume the localized nonbonding orbitals of the three nitrogen atoms N1, N3, and N7 to form the same molecular n orbitals as in adenine or purine. The highest, pyrimidine-like of these orbitals is of similar energy as the oxygen n orbital on O6, combination with which then forms the two highest molecular n orbitals of the anion of guanine.

The Out-of-Plane Spectra

Classification of the Bases. The empirical correction $\Delta E'$ for the calculated energy difference between the lowest nπ* and ππ* transitions is likely to have a constant value only for similar transitions. With respect to their transition densities the lower ππ* transitions of the nucleic acid bases all resemble those of conjugated polyenes of various length.[1] The nπ* transitions are more different from each other, as already hinted by the large variations found for the structure of the higher n orbitals. Using transition densities for our CI calcula-

Table IV. The Calculated Transition Energies of the Lower $\pi\pi^*$, $n\pi^*$, and $\pi\sigma^*$ Transitions and the Corrected Energies of the Lowest $n\pi^*$ and the Lowest and Two Lowest $\pi\pi^*$ Transitions in Pyrimidine and Purine Bases, Respectively (See Text)[a]

Compd (site of protons)	Calculated values, kK $\pi\pi^*$	$\pi\sigma^*$	$n\pi^*$ (location)		$\Delta E'$	Corrected values, kK $\pi\pi^*$	Lowest $n\pi^*$
Pyrimidine	44.0, 45.1	68.7	40.2 (N1, N3),	44.5 (N1, N3)	−3.5	41.3*	34.0
Purine	38.7, 41.8	53.8	39.0 (N1, *N3*),	44.3 (*N1*, N3)	−3.5	37.9,* 41.0	34.8
Guanine enol	37.2, 40.2	46.2	42.9 (N1, *N3*),	49.8 (*N1*, N3)	−3.5		
Adenine	38.7, 40.7	50.8	43.8 (N3),	45.8 (N1)	−3.5	38.0,* 40.0	39.6
Hypoxanthine enol	38.5, 40.4	49.6	42.2 (N3),	44.6 (N1)	−3.5	39.8,* 41.7	40.0
Adenine (+) (N7)	36.2, 43.4	49.4	42.3 (N3),	45.0 (N1)	−3.5		
Adenine (+) (N1)	36.0, 38.1	49.1	43.4 (N3),	45.5 (N7)	−3.5	36.9, 39.0*	40.8
Adenine (+) (N3)	37.7, 38.7	46.3	46.4 (N1),	50.8 (N7)	−3.5	38.0, 39.0*	43.2
Adenine imine	32.2, 38.6	46.1	42.2 (N3),	43.8 (N6)	−3.5		
Uracil	39.9, 51.3	53.0	39.5 (O4),	46.5 (O2)	1.5	38.8*	39.9
Cytosine (+)	38.7, 51.3	52.3	43.2 (O2)		1.5	36.3*	42.3
Cytosine imine	38.1, 48.4	52.4	42.0 (N4),	47.0 (O2)	1.5		
Xanthine	37.4, 40.0	42.3	39.9 (O6),	47.9 (O2)	1.5	39.0, 41.6*	43.0
Hypoxanthine	34.0, 39.7	47.3	41.6 (O6),	44.7 (N3)	1.5	35.1, 40.8*	44.2
Guanine (+) (N1, N7)	34.9, 39.9	45.9	40.2 (O6),	53.1 (N3)	1.5	36.0,* 41.0	42.8
Guanine (N1)	36.6, 39.0	43.3	41.9 (O6),	50.0 (N7)	1.5	36.8, 39.2*	43.6
Guanine (+) (N1, N3)	34.6, 39.9	39.5	37.2 (O6),	50.0 (N7)	1.5		
Uracil (−)	39.8, 43.8	54.2	36.5 (O4, N3),	47.5 (O4, N3)	0	38.3*	35.0
Guanine (−)	34.6, 40.2	44.8	41.7 (O6, N1),	46.7 (O6, N1)	0	37.0,* 42.6	44.1
Guanine (N3)	37.2, 40.7	41.5	37.7 (O6, N1),	44.6 (O6, N1)	0	37.8, 41.3*	38.3
Guanine (+) (N3, N7)	35.1, 38.6	44.1	34.5 (O6, N1),	44.7 (O6, N1)	0		
Hypoxanthine (N3)	35.4, 40.5	43.1	36.2 (O6, N1),	39.0 (O6, N1)	0	34.1, 39.2*	34.9
Cytosine	38.3, 47.4	55.2	43.1 (N3),	47.5 (O2)	−2.0	36.2*	39.0
Uracil enol	36.3, 48.4	51.0	38.5 (N3),	47.3 (O2)	−2.0		

[a] Figures corresponding to observed values are marked by an asterisk; the solvent is trimethyl phosphate except for the ions, xanthine, and the cyclic purine nucleosides from which the values for the tautomers with a proton at N3 were taken (D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, **57**, 1138 (1967)).

tions, it is possible to divide all the bases including their tautomers and ions into three classes according to the structure of their lowest $n\pi^*$ state (Table IV): (i) the pyrimidine class—location and structure on nitrogen and ring carbon atoms similar to that found for pyrimidine; (ii) the carbonyl class—location mainly on carbonyl oxygen as in a cyclic ketone; (iii) an intermediate class—location preponderantly on carbonyl oxygen and/or the adjacent nitrogen atom bearing no proton.

For bases of the pyrimidine class, $\Delta E'$ can be easily obtained as the lowest $n\pi^*$, and the lowest $\pi\pi^*$ state can be observed for pyrimidine as well as purine. The values for pyrimidine are 34 kK (294 nm) for the $n\pi^*$ and 41.3 kK (242 nm) for the $\pi\pi^*$ in hydrocarbon solvents and for purine 34.5 kK (290 nm) and 37.9 kK (263 nm), respectively, in trimethyl phosphate.[18] From the calculated values in Table IV, we see that in both molecules the $n\pi^*$ state is calculated too high in energy relative to the $\pi\pi^*$ state by about 3.5 kK. The value of $\Delta E'$ is therefore −3.5 kK for bases of the pyrimidine class in nonpolar or slightly polar solvents like trimethyl phosphate.

For the carbonyl class the lowest $n\pi^*$ state is not directly observable in any representative bases. From electrochromism measurements on uracil in dioxane it must be concluded that it lies above an energy of 39 kK (255 nm).[6] An upper limit follows from a comparison of the uracil chromophore with the chromophoric system of spirobihydantoin. In the CD spectrum of the latter, taken in tetrahydrofuran, the doublet stemming from the two lowest $n\pi^*$ states is centered around 41 kK (243 nm).[19] The hydantoin and the uracil chromo-

(18) L. B. Clark and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **87**, 11 (1965).
(19) S. Feinleib, F. A. Bovey, and J. W. Longworth, *Chem. Commun.*, 238 (1968).

phore are identical except for the additional double bond in uracil. It seems certain that the lowest $n\pi^*$ transition will be lower in uracil than in hydantoin; we therefore place the lowest $n\pi^*$ transition in uracil at about 40 kK (250 nm) in a solvent like trimethyl phosphate. This means that $\Delta E'$ for the carbonyl class is +1.5 kK.

For the intermediate class the value of $\Delta E'$ is more difficult to assess. We know, however, that it will be somewhere between −3.5 and 1.5 kK. In all molecules resembling the anion of uracil, where the lowest $n\pi^*$ state is spread out over the ring atoms and in particular the nitrogen atom adjacent to the carbonyl group, but still shows the main contribution on oxygen, we have set $\Delta E'$ equal to 0. For cytosine, where the lowest $n\pi^*$ state is mainly located on the nitrogen atom N3, with contributions from other ring atoms as in pyrimidine, we choose $\Delta E' = -2.0$ kK.

The value obtained in this way for the energies of the lowest $n\pi^*$ states are compiled in Table IV, together with the energies of the observed maxima of the lowest $\pi\pi^*$ states used to calculate them. In the purine bases the figures for the *two* lowest $\pi\pi^*$ transitions are given. We have always used transition I (marked by an asterisk in Table IV; see paper I) to calculate the position of the $n\pi^*$ states, even if transition II was lying lower. The reasons are usually higher dipole strength and therefore better observability and the fact that I rather than II involves the same π^* orbitals as the lowest $n\pi^*$ state. The calculated dipole strengths are not included in Table IV because they are small, in general, and nowhere exceed 0.5 D².

Discussion of the Lower $n\pi^*$ States. The structure and energy of the two lowest $n\pi^*$ states of the bases of the *pyrimidine class* are understood best by comparing them with those of pyrimidine itself. *Pyrimidine*
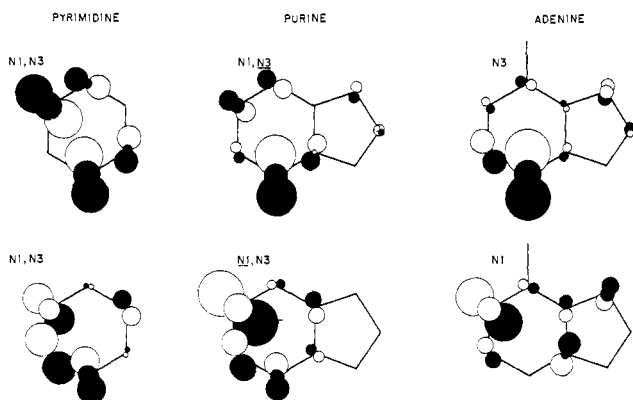
Figure 3. The transition density of the lowest (top) and second to lowest (bottom) nπ* transition of the bases of the pyrimidine class in a plane parallel to the π plane.

Figure 4. The transition density of the lowest (top) and second to lowest (bottom) nπ* transition of the bases of the carbonyl class.

belongs to the point group C_{2v}. From Figure 3 it can be seen that its lowest nπ* state is antisymmetric with respect to the C_2 axis, transforming according to the representation B_2, the next higher symmetric transforming according to A_2. (It should be observed that the drawings represent a section parallel to the molecular plane, the figures being antisymmetric with respect to it.) This result could have been obtained simply by looking at the symmetry of the molecular orbitals of the excited configurations of the CI wave functions. In *purine*, however, the structure of the orbitals of the excited configurations is of very limited help, and the use of group theoretical considerations is no longer possible. Yet, from the nodes of the transition densities depicted in Figure 3, we can easily correlate the two lowest excited nπ* states of purine with those of pyrimidine. The nodes are qualitatively the same in both molecules, and the lower state in purine corresponds to the B_2 state of pyrimidine and the higher to the A_2 state. The inequality of the contributions on N1 and N3 in purine reflects the lack of symmetry. The lower nπ* state is shifted toward N3 and the higher toward N1. This means that in purine an nπ* state on N1 is less stable than on N3. In *adenine* an additional amino group is present at C6. It can be seen from Figure 3 that this furthers the trend and that the lowest nπ* state appears associated mainly with N3 and the next with N1. The consequence is a *blue shift* of the lowest nπ* transition by about 5 kK, which clearly reveals the destabilizing effect of the amino group on the nπ* states, as already conjectured from the n-orbital energies. There has been considerable experimental effort to locate the lowest nπ* transition in adenine, but so far no convincing evidence emerged. From our results we can conclude that it must appear at about 40 kK, *i.e.*, *between* the two narrowly spaced ππ* transitions lowest in energy, and this explains the experimental difficulties in locating this weak transition.

As with adenine, the other bases of the pyrimidine class all should have their lowest nπ* state at higher energy than the lowest ππ* state. This is in agreement with the fact that in none of the bases has an nπ* state been observed at lower energy. The *enol of hypoxanthine* closely corresponds to adenine itself. In the *enol of guanine* we see that the amino group at C2 mainly decreases the stability of the nπ* states on N3. It is of interest to compare these blue shifts of the nπ*
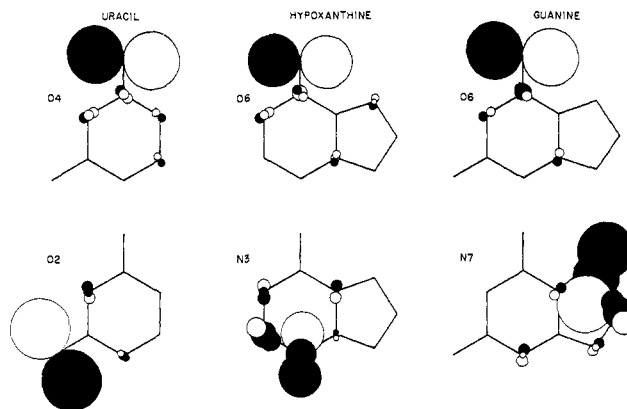
transitions with the well-known energy difference of about 10 kK of the nπ* transitions in formaldehyde and formamide. This is about twice as much as found here. In the light of the delocalization of the nπ* states in purine over two heteroatoms, the calculated figures therefore seem reasonable. The different possible *cations of adenine* are examples of the effect of protonation. For the most stable one, which presumably carries the additional proton at N1, the energy and structure of the lowest nπ* transition is almost the same as in neutral adenine. This is a reasonable result, as the lowest nπ* transition is localized on N3. The energy of the ππ* transitions on the other side appears lowered in the cation. For N7 as the site of protonation the calculations predict a *red shift* of the lowest nπ* transition, even relative to the ππ* transitions. The reasons for this at a first glance unexpected behavior have been discussed in the sections on nonbonding orbitals. Protonation at N3 leads to a blue shift, of course.

The lowest nπ* state of the bases of the *carbonyl class* can all be correlated with one or both of the two nπ* states of *uracil* (Figure 4). The more stable excited nπ* state of uracil is located on O4; the next higher nπ* state is on O2. The structures of the first two nπ* states in *xanthine* are virtually identical with that found for uracil. They are calculated to be shifted slightly toward *higher* energy. At first glance, this seems to be in disagreement with an intuitive guess, as the ππ* transitions are found to be red shifted in the observed as well as calculated spectrum. This is, however, only an extreme case for the effect discussed earlier that ππ* transitions tend to pass the nπ* transitions. The shift of the ππ* transitions is small, and the additional extension of the π system in xanthine is confined to the five-membered ring, over which the two higher n orbitals are not spread out. The situation is very similar for the pair pyrimidine and purine. As the nπ* transitions can be observed in this latter case, our analysis is experimentally confirmed (Table IV).

The *cation of cytosine* is a molecule where the excited nπ* state is located on O2 and therefore corresponds to the second nπ* state of uracil. In addition to its structure, this also is reflected in the large energetic separation of 6 kK from the lowest ππ* state. The *imine of cytosine* has been placed in the carbonyl class because of its resemblance with uracil itself, though the lowest nπ* state is located on a nitrogen atom. All the
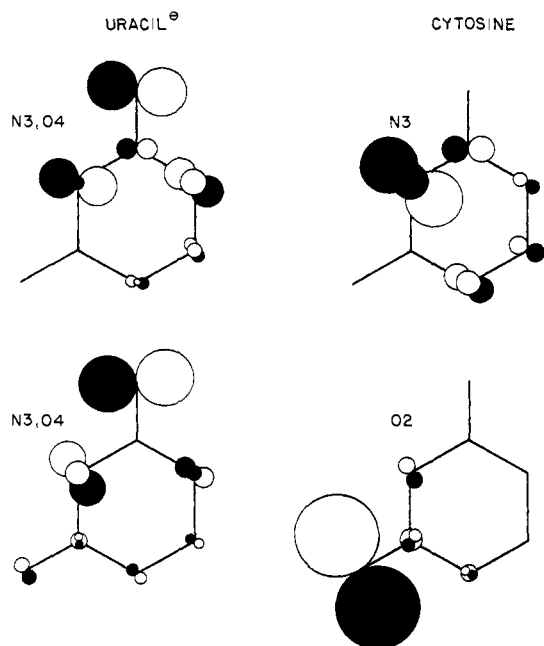
Figure 5. The transition density of the lowest (top) and second to lowest (bottom) nπ* transition of the bases of the intermediate class.

other bases of the carbonyl class are purine bases, and the lowest excited state is identical with that of xanthine, the higher ones located on the ring nitrogen atoms. In *hypoxanthine* the second is found on N3 in the six-membered ring and in *guanine* on N7 of the five-membered ring (Figure 4). The reason, again, is the destabilization of an nπ* state on N3 in guanine by the amino group at C2. In agreement with this view the gap between the two lowest nπ* states rises from 3 kK in hypoxanthine to 8 kK in guanine.

The *cations of guanine* form an instructive set. As a rule, our calculations give a *red shift*, if the neutral molecule and the cation both belong to the same class. This simply means that the proton gets attached to a site over which the lowest nπ* state does not extend, and we have already discussed the physical reasons for this result. *The most stable cation* in solution is thought to carry the additional proton at N7 of the five-membered ring, and it should therefore have its first nπ* transition at lower energy than the most stable tautomer of neutral guanine. If the attachment of a proton leads to a change of class, a blue shift is calculated. This can be seen from a comparison of the guanine tautomer of the intermediate class with the hydrogen atom on N3 and the corresponding cationic tautomer of the carbonyl class with the additional proton at N1.

The *intermediate class* comprises the remaining bases. Of *hypoxanthine* and *guanine* there exist tautomeric forms which belong to the carbonyl class and others which belong to the intermediate class. As can be seen from Table IV, the tautomers of the intermediate class, the nπ* states of which extend over a larger fragment of the molecule, should have their first nπ* state at an energy more than 4 kK lower than those belonging to the carbonyl class. This parallels the findings for the energy of the nonbonding orbitals in previous sections. It is also in agreement with our general arguments given on the dependence of the nπ* transition energy on the extension of the n orbitals.

Dissociation of bases of the carbonyl class at high pH values involves the loss of a proton at a nitrogen atom adjacent to the carbonyl group, and the anions therefore fall into the intermediate class. From the change of the structure of the nπ* state we would therefore expect a *red shift* upon dissociation. This is true for the *anion of uracil*. For the *anion of guanine*, a small *blue shift* is obtained from the calculation. The reason for this behavior is that here the change of the core charge by −1 affects the π system, which is larger than in uracil, more than the nonbonding orbitals. The effect exactly parallels the red shifts found earlier upon protonation, only in the opposite direction.

Cytosine (Figure 5) and the *enol of uracil* are two examples of the intermediate class, where the lowest nπ* state is found mainly on the nitrogen atom adjacent to a carbonyl group and the second is found on the carbonyl group. The reason for this energetic sequence obviously is that the nπ* state on the carbonyl oxygen gets strongly destabilized by the two nitrogen atoms adjacent to the carbonyl group. This result is obtained only after configuration interaction, and it cannot be inferred in a simple way from the structure and sequence of only the nonbonding orbitals of these molecules.

πσ* **Transitions.** Reliable experimental data on πσ* transitions, even for simple chromophores, are scarce and for nucleic acid bases nonexistent. In view of this fact, the inherent deficiency of the CNDO–CI method to properly describe πσ* and σπ* mixing as pointed out in I, and the limitation of the basis set to pure valence orbitals, it does not seem possible at present to quantitatively assess the calculated data for this type of transition. We have not performed an analysis of the structure of the πσ* states in the same way as for the nπ* states. Yet, our set of parameters has been chosen to at least partly simulate excited πσ* states of more diffuse character, and we think that some qualitative conclusions are possible with respect to calculated shifts.

From Table IV we see that the energy of the πσ* states decreases when the chromophore increases in size, as is obvious from the pair pyrimidine and purine. Exocyclic substituents like NH₂ or OH also stabilize πσ* states, in contrast to what we found for nπ* states. This is apparent from a comparison of purine with adenine and the enol of hypoxanthine or all the tautomers and cations of hypoxanthine with the corresponding ones of guanine. The energy decreases most if a carbonyl or imino group is introduced. Part of the change in these cases depends on the additional proton on a nitrogen atom of the six-membered ring. The πσ* energies depend on the nitrogen atom on which the proton gets attached, N3 leading to the lowest energy. From these rules we would predict that the cation of guanine, with protons at N1 and N3, should have the lowest calculated πσ* energy of all the tautomers of the nucleic acid bases. From Table IV this is found to be correct. It seems, however, that the magnitude of the calculated shifts is too large and that the energy of the πσ* state therefore is too low for this molecule.

Conclusion

In this and the preceding paper I we give a rationalization of the electronic spectra of the complex multi-

functional chromophores of the nucleic acid bases in physical terms. Here, in particular, we show that a close relationship of the out-of-plane transitions with the structure of the nonbonding orbitals exists, *i.e.*, with a property of the electronic structure of the ground state. The transition densities of the $n\pi^*$ states suggest that configuration interaction in general tends to increase the localization of these transitions on certain atoms or groups of atoms, as compared to the nonbonding orbitals themselves or results obtained without configuration interaction.[20] The structure of the transition density on such fragments often closely resembles that of small, well-known chromophores. The first $n\pi^*$ transition of the molecules of the carbonyl class is an example of this, having a transition density qualitatively identical with that of the $n\pi^*$ transition in acetone or formaldehyde. This means that the expectation values of many operators will be similar;[10] *i.e.*, the first $n\pi^*$ transition in uracil or guanine will be essentially electric dipole forbidden but will have a large magnetic moment along the C=O axis. Due to the fact that the out-of-plane transitions of nucleic acid bases cannot be observed in the absorp-

(20) Similar observations on the localizing effect of CI on the hole left by an electron upon excitation from a nonbonding orbital have been made for thymine; see L. C. Snyder, R. G. Shulman, and D. B. Neuman, *J. Chem. Phys.*, **53**, 256 (1970).

tion spectra, many of our results cannot be checked directly against experimental data. In a paper[21] on the CD and MCD spectra of the mononucleosides, it will be shown, however, that the calculations are in good agreement with the indirect evidence provided by these spectra on out-of-plane transitions and that they provide a basis for their correct interpretation.

Many of the conclusions reached in this work are not limited to nucleic acid bases themselves. These chromophores represent model systems for and contain structural elements of a variety of compounds of chemical and biological interest, like pterins, flavins, or urobilins, and some of our results are useful in understanding the electronic structure and spectra of such compounds as well. The approach itself in dealing with complex low-symmetry chromophores in particular is also suited to rationalize data on excited states obtained by other than the CNDO–CI method, including as simple models as the Hückel method up to elaborate *ab initio* calculations.

(21) W. Hug and I. Tinoco, Jr., manuscript in preparation.
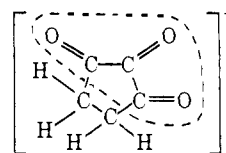
Electron Spin Resonance Spectra of Radicals Related to the Intermediates in the Oxidation of Ascorbic Acid. Substituted 2,3,4-Trioxopyrrolidine Radical Anions[1]

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Abstract: Four substituted 2,3,4-trioxopyrrolidine radical anions have been prepared by the oxidative debromination and denitration of the corresponding 4-bromo- and 4-nitro-3-hydroxy-3-pyrrolin-2-ones. *In situ* radiolysis–esr methods were used to observe the radicals from the nitro derivatives whereas the bromo derivatives readily give the radicals chemically either in aqueous solution or in basic DMSO. The radicals substituted on the nitrogen are extremely unreactive and are observable for a period of at least 24 hr after sample preparation. All four radicals are very similar in that they have nitrogen hyperfine constants of $\sim$2 G. A methyl proton hyperfine constant of 3.35 G is observed for the *N*-methyl derivative showing that there is an electron spin density of $\sim$0.11 on the nitrogen atom. The very small hyperfine constants for the protons at the C_5 position (0.2–0.5 G) are explained as the result of almost complete cancelation of the effects of spin density on the nitrogen and C_4 carbon atoms. Comparison of the parameters observed in water and in DMSO shows that in the latter case an appreciably greater amount of spin density has been transferred to the carbonyl oxygen atoms. These present results substantiate the previous suggestion that in the similar radicals derived from α-hydroxytetronic and ascorbic acids there is a spin density of 0.03 on the heterocyclic oxygen atom.

The radical intermediate in the oxidation of reductic acid has long been known from its esr spectrum to be a tricarbonyl anion (radical I) related to the intermediate present in the oxidation of ascorbic acid.[2]

(1) Supported in part by the U. S. Atomic Energy Commission.
(2) L. H. Piette, I. Yamazaki, and H. S. Mason, "Free Radicals in Biological Systems," M. S. Blois, Jr., H. W. Brown, R. W. Lemmon, R. O. Hindblom, and M. Weissbluth, Ed., Academic Press, New York, N. Y., 1961, Chapter 14, p 195.

radical I

More recently the esr spectrum of the isoelectronic radical produced in the oxidation of α-hydroxytetronic