Ultraviolet Photoelectron Studies of the Ground-State Electronic Structure and Gas-Phase Tautomerism of Purine and Adenine

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Abstract: Ultraviolet photoelectron spectroscopy has been employed to study electronic structure and tautomerism in adenine and purine and in various methyl-substituted derivatives of these molecules. The assignment of the photoelectron spectra has been aided by results from HAM/3 molecular orbital calculations which are empirically parameterized to provide orbital ionization potentials. The calculations predict that the lone-pair orbitals in purine and adenine are similar in spacial distribution and have similar ionization potentials. This conclusion is supported by the spectroscopic data which indicate that in purine the three highest occupied lone-pair orbitals have ionization potentials of ~9.6, ~10.6, and ~11.7 eV. In adenine these orbitals have ionization potentials of ~9.6, ~10.5, and 11.39 eV. The planar exocyclic amino group in adenine causes the π structure of this molecule to be significantly different than that in purine. In adenine the ionization potentials of the five highest occupied π orbitals are 8.48, ~9.6, ~10.5, 12.10, and 13.21 eV. In purine the ionization potentials of the four highest occupied π orbitals are ~9.6, ~10.2, ~11.9, and 13.10 eV. The band arising from the fifth highest occupied π orbital in purine has an ionization potential greater than 13.5 eV and is not resolved. A comparison of the photoelectron spectra of purine, adenine, and their 7- and 9-methyl-substituted derivatives indicates that the spectra of purine and adenine are much more similar to the spectra of their 9-methyl derivatives than to the spectra of their 7-methyl derivatives. This observation supports the conclusion that in an isolated environment the N(9)H tautomers of both purine and adenine are more stable than the N(7)H tautomers.

Introduction

Prior to photoelectron studies most investigations of electronic structure in nucleotide bases were based primarily upon results from molecular orbital calculations.[1-5] As might be expected, predictions associated with the ordering and spacing of energy levels in these molecules vary greatly depending on the theoretical procedure employed. Previous spectroscopic investigations of these molecules have involved UV absorption measurements[6-9] as well as studies involving circular dichroism[10,11] and magnetic circular dichroism.[12,13] In these previous spectroscopic studies the fact that $\pi \rightarrow \pi^*$ transitions strongly overlap $n \rightarrow \pi^*$ transitions has often made assignments difficult to obtain.[12]

It has recently been demonstrated that UV photoelectron spectroscopy when used in conjunction with molecular orbital calculations can yield detailed information about the ordering and spacing of energy levels associated with valence electrons in nucleotide bases.[14-18] Most of the previous work in this area has focused on the biological pyrimidines: uracil,[14-17] thymine,[15-17] and cytosine.[18] The assignment of spectra and characterization of orbitals in uracil, thymine, and cytosine has been based upon the study of pyrimidine[19] and upon the study of several methyl derivatives of these nucleotide bases.

Because of the structural complexity of biological purines, UV photoelectron studies of these molecules have only begun. While preliminary photoelectron studies of biological purines have been undertaken,[20,21] assignments of these spectra were based upon data from only a limited sampling of related compounds. A major goal of the present study has been to carry out a more complete photoelectron investigation of adenine in order to better characterize its ground-state electronic structure. Molecules which have been studied include purine (I), 6-methylpurine (II), 7-methylpurine (III), 9-methylpurine (IV), adenine (V), 9-methyladenine (VI), 7-methyladenine (VII), N^6-methylaminopurine (VIII), N^6,N^6-dimethylaminopurine (IX), and N^6,9-dimethylaminopurine (X).

In addition to providing detailed descriptions of ground-state electronic structure, photoelectron experiments also provide the opportunity to obtain information about the tautomerism of nucleotide bases.[18] These heterocyclic molecules contain labile hydrogen atoms which can reside at more than one ring position as well as on different exocyclic functional groups.[22,23] Furthermore, the stability of a given tautomeric structure can depend heavily on the environment within which the molecule resides.[24,25] In UV photoelectron studies, measurements are carried out in the gas phase, and the stable tautomeric form observed is associated with that of the free molecule. For purine and adenine an interesting question associated with tautomerism is whether an H atom is located at the N(7) position or at the N(9) position of the imidazole ring.[22,23] In the present study this question has been examined by comparing the photoelectron spectra of purine and adenine with those of their 7- and 9-methyl derivatives.

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Table I. Vertical Ionization of Upper Occupied Orbitals[a,b]

	orbitals							
	n_1	n_2	n_3	π_1	π_2	π_3	π_4	π_5
purine (117 °C)	9.6	10.6	11.7	9.6	10.2	11.9	13.10	
6-methylpurine (108 °C)	9.3	10.4	11.5	9.3	9.9	11.5	13.0	
9-methylpurine (87 °C)	9.4	10.4	11.5	9.4	9.7	11.5	12.73	
adenine (185 °C)	9.6	10.5	11.39	8.48	9.6	10.5	12.10	13.21
9-methyladenine (100 °C)	9.4	10.2	11.16	8.39	9.4	10.2	11.93	12.81
N^6-methylaminopurine (145 °C)	9.5	10.2	11.23	8.15	9.5	10.2	11.64	12.8
N^6,N^6-dimethylaminopurine (125 °C)	9.3	10.1	11.10	7.78	9.3	10.0	11.38	12.7
N^6,9-dimethylaminopurine (102 °C)	9.2	10.1	11.06	7.95	9.2	9.8	11.48	12.3
7-methylpurine (107 °C)	9.4	10.4	11.18	9.4	9.8	11.6	12.72	
7-methyladenine (200 °C)	9.4	10.5	10.96	8.64	9.4	10.5	12.04	13.05

[a] All ionization potentials given in eV. [b] Probe temperatures at which spectra were measured are given in parentheses.

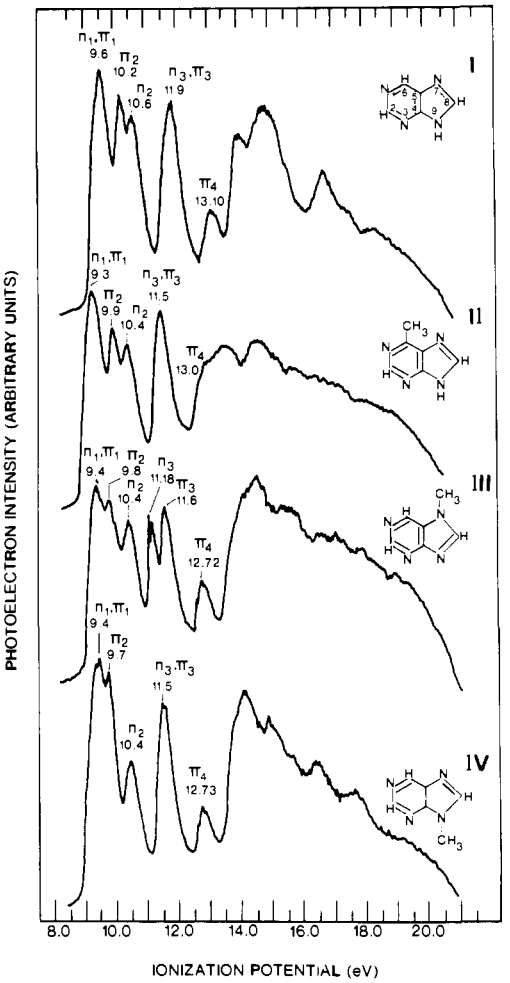

Figure 1. He I photoelectron spectra of purine, 6-methylpurine, 7-methylpurine, and 9-methylpurine. Assignments are given for the seven highest occupied molecular orbitals.

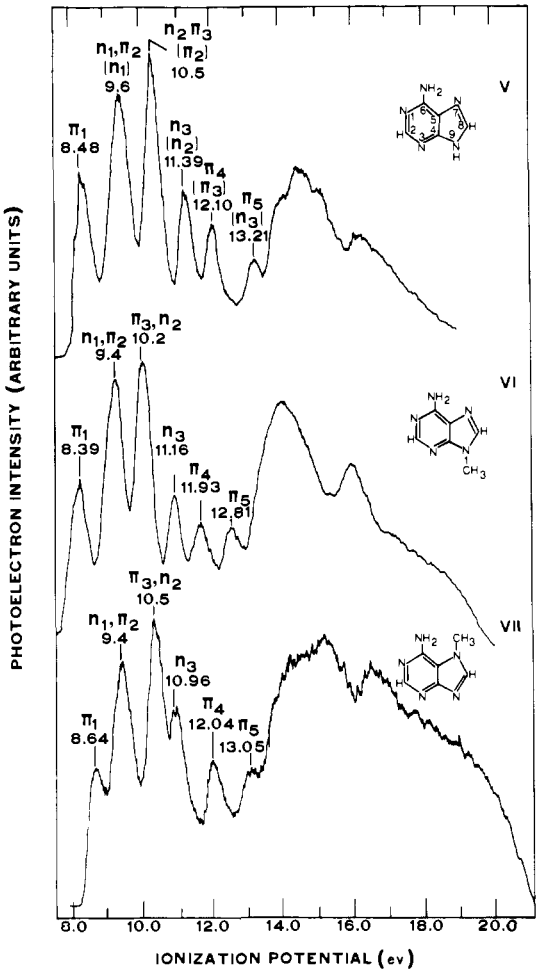

Figure 2. He I photoelectron spectra of adenine, 9-methyladenine, and 7-methyladenine. Assisgnments are given for the eight highest occupied molecular orbitals. Where they differ, previous assignments for adenine taken from ref 20 are shown in parentheses.

Experimental Section

Gas-phase UV photoelectron spectra were measured with a Perkin-Elmer PS-18 spectrometer equipped with a heated probe and a He I lamp. Ionization potentials were calibrated by using the $^2P_{3/2}$ and $^2P_{1/2}$ bands of Xe and Ar. Samples of compounds I, II, V, VII, and VIII were purchased from Sigma Chemical Co. Samples of compounds III, IV, VI, IX, and X were obtained from Vega-Fox Biochemical Co.

Table I gives probe temperatures at which the photoelectron spectra were measured. As each spectrum was measured, the probe temperature was kept constant to within ±1 °C. None of the compounds showed signs of decomposition during the time in which their spectra were measured.

Results and Discussion

Figure 1 shows He I photoelectron spectra along with assignments and vertical ionization potentials for the low-energy bands of purine, 6-methylpurine, 7-methylpurine, and 9-methylpurine. Figure 2 shows similar results for adenine, 9-methyladenine, and 7-methyladenine. Where they differ from the present assignments, preleminary assignments[20] of the spectrum of adenine are given in parentheses in Figure 2. Table I lists vertical ionization potentials for all of the molecules studied here. Where there is an overlapping of spectral bands, the vertical ionization potentials given in Figures 1 and 2 and in Table I are approximate.

In the spectrum of purine, bands arising from four orbitals are assigned to the energy region 9.6–10.6 eV. Two of these orbitals, the second highest occupied π orbital (π_2) and the second highest occupied lone-pair orbital (n_2), are resolved and have ionization potentials of 10.2 and 10.6 eV, respectively. The highest occupied π and lone-pair orbitals have ionization potentials of ~9.6 eV. The bands arising from the π_1 and n_1 orbitals are not resolved in the spectra of any of the purines which have been studied here.

In purine the bands arising from the π_3 and n_3 orbitals have ionization potentials of ~11.9 eV and are resolved only in the spectrum of 7-methylpurine. The band occurring at 13.10 eV in the spectrum of purine arises from the π_4 orbital. The band arising from the π_5 has an ionization potential greater than 13.5 eV and is not resolved in the spectra of any of the purines studied here.

An examination of the spectrum of adenine in Figure 2 indicates that at low energies between 8.0 and 13.5 eV there are six regions of high photoelectron intensity. In the present interpretation resolved bands appearing at 8.48, 11.39, 12.10, and 13.21 eV arise from the π_1, n_3, π_4, and π_5 orbitals, respectively. The regions of highest photoelectron intensity which occur at ~9.6 and ~10.5 eV are each associated with two bands. Bands arising from the n_1 and π_2 orbitals give rise to emission in the 9.6-eV energy region; bands arising from the n_2 and π_3 orbitals give rise to the emission in the 10.5-eV region.

The present interpretation of the adenine spectrum is consistent with ab initio molecular orbital calculations on adenine which predict that five of the eight highest occupied orbitals are π orbitals and three are lone-pair orbitals.[4] The ab initio calculations also predict that the energies of the n_1 and π_2 orbitals in adenine differ by only a small amount (~0.2eV) and that the n_2 and π_3 orbitals are separated by a similarly small energy difference.[4,5]

In a preliminary interpetation of the adenine spectrum[20] each of the six regions of high photoelectron intensity between 8.0 and 13.5 eV was assigned to a band arising from a single orbital. Two considerations point out the need to revise these assignments. The first is indicated by the spectrum of 7-methylpurine which has not been previously reported. Between 9.0 and 13.0 eV the spectrum of this molecule exhibits six distinct maxima, indicating that in this energy region the spectrum arises from bands associated with six or more orbitals. Such an observation indicates that in the energy region 8.0–13.5 eV the spectrum of adenine contains bands arising from seven or more orbitals. The additional orbital in adenine arises from the mixing of the π system of the purine moeity with what is normally considered to be a nitrogen atom lone-pair orbital associated with the coplanar exocyclic amino group at the 6-position of adenine.[20]

The second consideration which points out the need to revise the early adenine assignments involves the difference in ionization potentials of the lone-pair orbitals in adenine. According to the early assignments the energy difference between the ionization potentials of the n_1 and n_3 orbitals is 3.6 eV. This energy difference is found to be too large when compared to differences in lone-pair ionization potentials observed in other nitrogen-containing heterocyclic molecules in which lone-pair interactions are expected to be stronger than those in adenine. For example in *s*-triazine, which consists of a six-membered ring containing three alternating nitrogen atoms, the ionization potentials of the n_1 and n_3 orbitals differ by only 3.0 eV.[26] Similarly in *s*-tetrazine, which consists of a six membered ring containing four nitrogen atoms, the difference in ionization potential between the n_1 and n_4 orbitals is only 3.6 eV.[26] In the present assignments of the spectra of purine and adenine the differences between the ionization potentials of the n_1 and n_3 orbitals have reasonable values of 2.1 and 1.8 eV, respectively.

Molecular Orbital Calculations. The present assignments of the spectra of purine and adenine have been aided by the results of HAM/3 molecular orbital calculations,[27] which have been carried out on all the molecules studied here. These calculations were found to yield useful descriptions of molecular orbital structure and photoelectron data from other heterocyclic aromatic molecules such as pyridine and uracil.[27]

For the calculations the molecular geometry of the N(7)H tautomer of purine[28] and the geometry of the N(9)H tautomer of N^6-methylaminopurine[29] were obtained from crystallographic data. The geometry of the N(9)H tautomeric form of purine was obtained by inverting the imidazole ring of the N(7)H tautomer. This procedure has been adopted in previous theoretical calculations.[30] To obtain the computational results summarized in Figures 3, 4, and 5, we derived the geometries of molecules II and IV from the geometry of the N(9)H tautomer of purine. The gometries of molecules V, VI, IX, and X were derived from the geometry of N^6-methylaminopurine (VIII). The geometry of 7-methylpurine (III) was derived from the geometry of the N(7)H tautomer of purine. The geometry of 7-methyladenine (VII) was obtained by inverting the imidazole ring of 9-methyladenine (VI). When not provided by crystallographic data, bond lengths and bond angles which were used in the calculations are as follows: r_{H_3C-C} = 1.50 Å, r_{H_3C-N} = 1.48 Å, r_{C-H} = 1.1 Å, r_{N-H} = 1.0 Å, $\angle$H–C–H = 109.5°.[31]

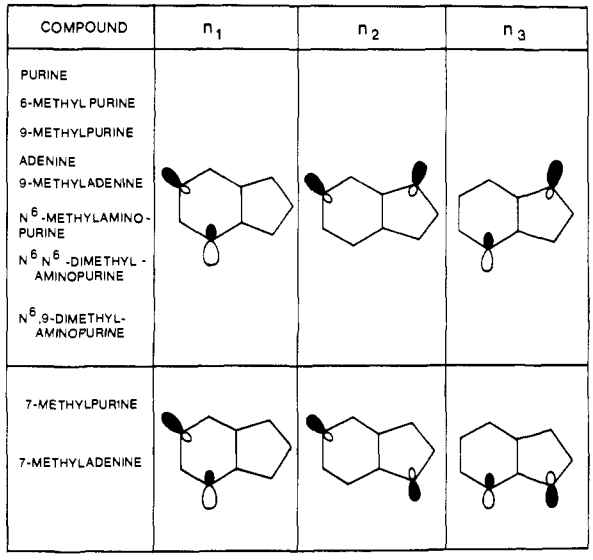

Figure 3. Location and phase of the nonbonding orbitals of purine, 6-methylpurine, 7-methylpurine, 9-methylpurine, adenine, 9-methyladenine, N^6-methylaminopurine, N^6,N^6-dimethylaminopurine, N^6,9-dimethylaminopurine, and 7-methyladenine.

Figure 3 shows diagrams of lone-pair orbital structure in molecules I–X which are predicted by the calculations. While the calculations predict that all of the lone-pair orbitals exhibit some σ-bonding character, the diagrams in Figure 3 show only the major atomic orbital contributions to each lone-pair orbital. For purine and adenine, diagrams of lone-pair orbitals which agree with those presented in Figure 3 have been previously constructed from CNDO calculations.[2]

As Figure 3 indicates, the n_1 orbital structure is identical in molecules I–X. Figure 3 also indicates that the n_2 and n_3 orbitals in 7-methylpurine (III) and 7-methyladenine (VII) have a different structure than those in the other molecules. However, the distances between the interacting nitrogen atom lone-pair atomic orbitals which contribute to the n_2 and n_3 molecular orbitals in molecules III and VII are similar to the distances in the other molecules.

These considerations suggest that ionization potentials associated with analogous lone-pair orbitals in molecules I–X will lie in the same energy region. As pointed out by the data in Table I, the present assignments of the lone-pair bands for the molecules studied here are consistent with these observations. For all of the molecules studied, the n_1 ionization potentials lie between 9.2 and 9.6 eV, the n_2 ionization potentials lie between 10.1 and 10.6 eV, and the n_3 ionization potentials lie between 11.0 and 11.7 eV. It is also interesting to note that the n_1 orbital in molecules I–X is

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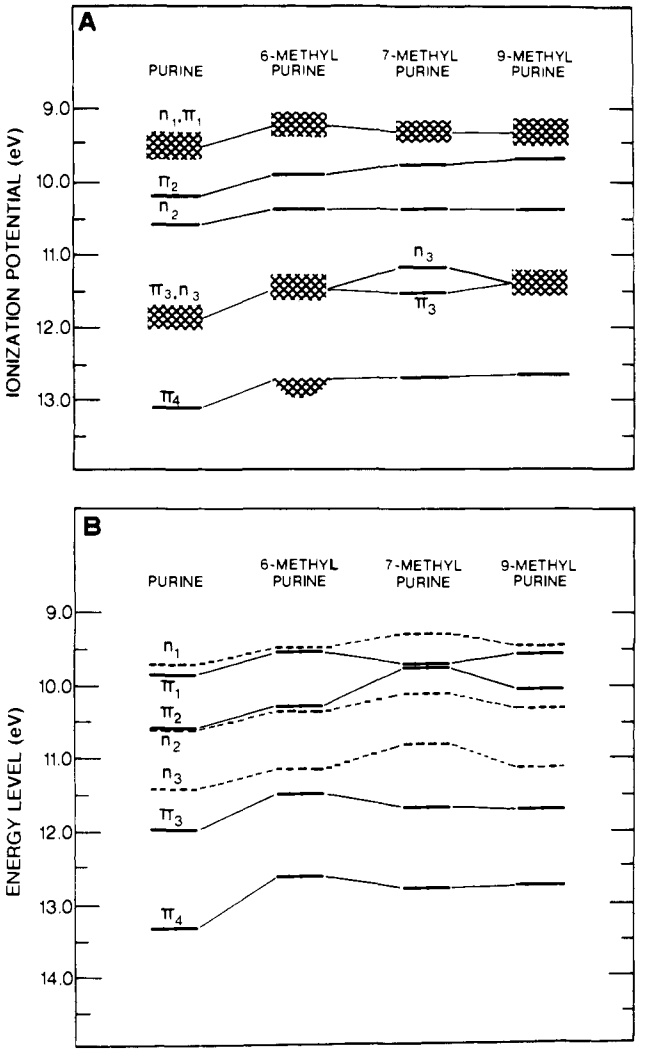

Figure 4. Energy-level diagram showing the upper occupied molecular orbitals in purine, 6-methylpurine, 7-methylpurine, and 9-methylpurine. Panel A shows the experimentally measured vertical ionization potentials. Panel B shows theoretical ionization potentials obtained from HAM/3 calculations. In Panel A hatched areas show regions in which there is an overlap of photoelectron bands and for which the precise ordering of bands is uncertain. In Panel B solid lines give π ionization potential levels; dashed lines give lone-pair ionization potentials.

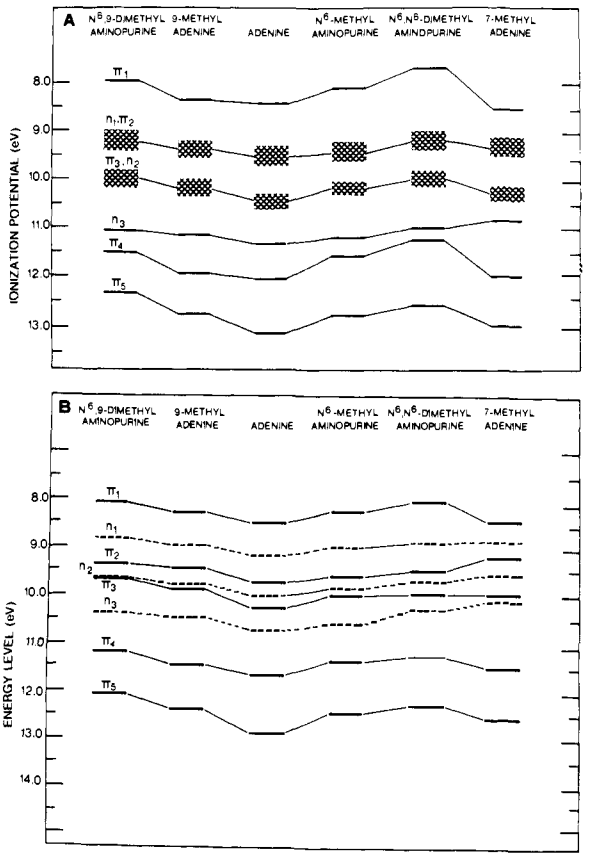

Figure 5. Energy-level diagram showing the eight upper molecular orbitals in N^6,9-dimethyladenine, 9-methyladenine, adenine, N^6-methylaminopurine, N^6,N^6-dimethylaminopurine, and 7-methyladenine. Panel A shows experimentally measured vertical ionization potentials. Panel B shows ionization potentials obtained from HAM/3 calculations.

similar in structure to the n_1 orbital in pyrimidine which has an ionization potential of 9.7 eV.[19,26]

In addition to employing the calculations to examine electron distribution in lone-pair orbitals, the ordering and spacing of energy levels predicted by the calculations have also been compared with the photoelectron data. Figures 4 and 5 show a comparison of spectroscopic data and theoretical results for the purines and adenines studied here. The top panels of Figures 4 and 5 show vertical ionization potentials obtained from photoelectron data; the lower panels show ionization potentials predicted by the calculations.

A comparison of the experimental and theoretical results for the purines shown in Figure 4 indicates that the agreement is generally good. The calculations accurately predict that for all purines studied the first ionization potentials have values of approximately 9.5 eV. In agreement with the present assignments, the calculations predict that in each of the purines studied the spectrum contains seven bands in the energy region 9.0–13.5 eV and that four of these bands arise from π orbitals and three arise from lone-pair orbitals. Also in agreement with the present assignments the calculations predict that for the four purines studied the bands arising from the three lone-pair orbitals have energies similar to bands arising from the three highest occupied π orbitals. For example in the spectrum of purine the n_1 and π_1 bands are unresolved, the n_3 and π_3 bands are unresolved and the energy difference between the n_2 and π_2 bands is less than 0.5 eV.

An examination of the adenine results shown in Figure 5 indicates again that the agreement between the photoelectron data and the computational results is good. For all the adenines studied the calculations accurately predict that the first ionization potential has a value between 7.8 and 8.5 eV. The calculations also indicate that in the energy region between 8.0 and 13.2 eV the spectrum of adenine contains bands arising from eight orbitals, five of which are π orbitals and three of which are lone-pair orbitals. This is in agreement with the present assignments.

The calculations predict that for all the adenines studied the ionization potentials associated with the n_1 and π_2 orbitals differ by no more than 0.5 eV and that the ionization potentials associated with the n_2 and π_3 orbitals differ by no more than 0.4 eV. In all of the spectra for the adenines the bands arising form the n_1 and π_2 orbitals are unresolved and the bands arising from the π_3 and n_2 orbitals are unresolved.

In previous photoelectron studies of nucleotide bases[15,18] it was found that semiempirical calculations used in conjunction with Koopmans Theorem[32] often accurately predict spectroscopically observed perturbation patterns which arise from methyl substitution at various positions. Because of the generally poor resolution of the spectra of purine and molecules II–IV in the energy region 9.0–10.9 eV, highly reliable vertical ionization potentials in this energy region cannot be obtained. This makes a comparison of perturbation patterns predicted by calculations for the n_1, n_2, π_1, and π_2 orbitals with experimental ionization potentials difficult to carry out.

For the other orbitals it is easier to make such a comparison. For example the calculations do accurately predict the stabilization of the n_3 orbital in going from 7-methylpurine to 9-methylpurine.

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The calculations also yield π_3 and π_4 perturbation patterns which are consistent with the present assignments.

For the adenines the perturbation pattern of the π_1, π_4, π_5, and n_3 ionization potentials which arises from methyl substitution at various positions on adenine is remarkably well reproduced by the calculations. Furthermore the similar perturbation of the n_1 and π_2 orbitals associated with all the methyl-substituted adenines studied here is consistent with the observation that the bands arising from these two orbitals are not resolved in any of the spectra.

For the adenines the calculations also predict that the energy of the π_3 orbital is more sensitive to methyl substitution, especially at the amino group and at the 9-position, than is the energy of the n_2 orbital. This observation suggests that evidence of the occurrence of two bands associated with the photoelectron emission in the 10.5-eV region of the spectrum of adenine might occur in the spectra of N^6- and 9-methyl-substituted adenines. The spectrum of N^6,9-dimethylaminopurine does indeed provide this evidence. In the spectrum of this molecule the intense emission occurring at approximately 10.0 eV exhibits a bandlike structure which is broader and much more asymmetric than the emission which is observed in the same energy region of the spectrum of adenine.

Gas-Phase Tautomerism of Purine and Adenine. Previous studies of cytosine[18] indicate that photoelectron spectra can be useful for determining the most stable tautomeric forms of nucleotide bases in the gas phase. The tautomerism of purine and adenine has been previously examined in both theoretical[23,30,33,34] and experimental[13,25,35-39] studies. Several molecular orbital calculations indicate that the N(7)H and N(9)H amino tautomeric forms of adenine are considerably more stable than all imino tautomeric forms.[33] In one study the difference in energy between amino and imino tautomers of adenine is estimated to be as great as 27 kcal/mol.[33]

It is upon the more difficult question regarding the relative stability of N(7)H and N(9)H tautomeric forms of purines that many recent theoretical and experimental studies have focused. A comparison of the experimental dipole moments of methyl and benzyl adenine derivatives, measured in dioxane, with calculated dipole moments indicates that the most stable tautomer of adenine is the N(9)H form.[35] In NMR studies of adenine in Me_2SO it is found that adenine exists in an amino N(9)H structure.[36] Results of magnetic circular dichromism studies of adenine,[12] 7-methyl-adenine,[13] and 9-methyladenine[13] in aqueous solution also indicate that the N(9)H tautomeric form is most stable.

For puine, previous studies of the relative stability of the N(7)H vs. N(9)H tautomeric forms are less conclusive. For example, in crystals purine assumes the N(7)H tautomeric form;[28] however, UV absorption studies indicate that the N(9)H form is more stable in aqueous solution.[37] Results from NMR studies[38] in Me_2SO and from reactivity studies involving acetylation[39] indicate that the N(9)H and N(7)H tautomers occur in approximately equal quantities. Theoretical studies[33,34] of the N(7)H and N(9)H tautomers of purine indicate that the N(9)H tautomer is more stable by ~0.3 eV.

The present results indicate that in the gas phase the N(9)H tautomers of both purine and adenine are more stable than the N(7)H tautomers. For purine the occurrence of the N(9)H tautomer is demonstrated by examining the results of Figure 1. The spectra shown in Figure 1 indicate that in the energy region 11.0–12.0 eV the spectrum measured for purine is much more similar to that obtained for 9-methylpurine than that obtained for 7-methylpurine.

As pointed out above, 7-methylpurine has different n_2 and n_3 lone-pair structure than 9-methylpurine, and the n_3 orbital is destabilized in going from 9-methylpurine to 7-methylpurine. In going from purine to 7-methylpurine the photoelectron data indicate that this destabilization is approximately 0.5 eV. In the photoelectron spectrum of 7-methylpurine this destabilization gives rise to an n_3 band which is well separated from the π_3 band.

Calculations employing the HAM/3 method were also carried out on the N(7)H tautomer of purine. The calculations predict that the destabilization of the n_3 orbital is going from the N(7)H tautomer to 7-methylpurine is much smaller (0.25 eV) than the spectroscopically observed destabilization. This observation supports the conclusion that the difference in the purine and 7-methylpurine n_3 ionization potentials arises primarily from a change in tautomeric structure, and that in an isolated environment the N(9)H tautomer of purine is more stable than the N(7)H tautomer.

In adenine the greater stability of the N(9)H tautomer relative to the N(7)H tautomer is indicated by the results of Figure 2. Here the photoelectron spectrum of adenine in the energy region 11.0–12.0 eV is observed to be much more similar to that of 9-methyladenine than to that of 7-methyladenine. As in the case of the purines the spectra of adenine and 9-methyladenine differ from that of 7-methyladenine largely because of a destabilization of the n_3 orbital which occurs in going from an N(9)H-like structure to an N(7)H-like structure. This conclusion is again supported by the results of the calculations.

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