

# Ultraviolet photoelectron studies of biological purines: The valence electronic structure of adenine

(methyl substituted adenines/base stacking)

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**ABSTRACT** The UV photoelectron spectra of adenine, 9-methyladenine, and 6-methylaminopurine contain highly resolved bands arising from the six highest occupied molecular orbitals. The spectra have been analyzed using UV absorption data, photoelectron data from previous studies of heterocyclic compounds, and results from both semi-empirical and *ab initio* molecular orbital calculations. The analysis indicates that the first, third, and fifth photoelectron bands in adenine and the two methyl substituted derivatives arise from  $\pi$  orbitals. The second, fourth, and sixth bands arise from nitrogen atom lone-pair orbitals. Compared to adenine, the six uppermost orbitals of 9-methyladenine and 6-methylaminopurine have lower ionization potentials. This destabilization of the valence electrons is expected to play an important role in causing the increase in base stacking forces observed in methyl substituted adenines.

The binding characteristics of biological purines are thought to be strongly influenced by charge transfer interactions (1-3) and polarization forces (3, 4) involving these molecules. Because electrons residing in the highest occupied and lone-pair orbitals of purines are easily removed, such electrons play an important role in determining binding characteristics. The energy levels of valence electrons in biological purines have been extensively investigated in both semi-empirical and *ab initio* molecular orbital calculations (5-8). To date, UV absorption measurements have provided virtually all of the experimental information available about these molecules (6, 7, 9, 10). It is found, however, that the absorption spectra of purines contain strongly overlapping bands which preclude a simple interpretation. More highly resolved experimental information about the occupied valence orbitals of biological purines will greatly facilitate an understanding of their ground-state electronic structure. In recent studies of biological pyrimidines it has been found that UV photoelectron spectroscopy can provide this information (11-13). The present study of adenine, 9-methyladenine, and 6-methylaminopurine is a detailed photoelectron examination of biological purines.

## EXPERIMENTAL

Photoelectron spectra were measured with a Perkin Elmer PS-18 spectrometer equipped with a heated probe. Adenine and 6-methylaminopurine were obtained from Sigma Chemical Co. 9-Methyladenine was obtained from Fox Chemical Co. All compounds were used without further purification. The spectra were run in the temperature range 126-185°. Ionization potentials were calibrated using the  $^2P_{3/2}$  and  $^2P_{1/2}$  bands of Xe and Ar. For all three molecules, spectra measured from the same sample over a 1- to 2-hr period were identical, indicating that no decomposition occurred.

## RESULTS AND DISCUSSION

Fig. 1 shows He I photoelectron spectra of the molecules studied. An examination of the spectra indicates that adenine ex-

hibits six widely separated bands in the energy region 8.5-13.2 eV and that the ordering of these bands is identical in all three of the molecules investigated.

The assignment of the first six bands of the adenine spectrum is based upon the following observations:

(i) In all recent molecular orbital calculations of adenine (5-8) it is found that, of the six upper orbitals, three are  $\pi$  orbitals and three are lone-pair orbitals associated with nitrogen atoms in the purine moiety.

(ii) *Ab initio* calculations indicate that the ordering of the upper six orbitals in adenine is  $\pi_1, n_1, \pi_2, n_2, \pi_3,$  and  $n_3$  (8).

(iii) CNDO/2 (14), CNDO/S (15), and INDO (16) calculations indicate that the  $n_1$  orbital has an electron distribution similar to the  $b_2(\sigma, n_-)$  orbital of pyrimidine. In pyrimidine this orbital has a vertical ionization potential of 9.7 eV (17, 18). In adenine the band assigned to the  $n_1$  orbital appears at 9.6 eV.

(iv) The semi-empirical calculations also indicate that the  $n_2$  orbital of adenine, with an ionization potential of 11.4 eV, has some weak bonding properties. The orbital is characterized by bonding between the  $N_1-C_6$  atoms and between the  $N_7-C_5$  atoms. The character of this orbital is similar to the  $a_1(\sigma, n_+)$  orbital of pyrimidine (17, 18) and the  $b_{2u}(\sigma, n_-)$  orbital of pyrazine (17). These latter two orbitals have ionization potentials of 11.3 and 11.4 eV, respectively.

(v) Previous molecular orbital studies of purine and adenine (6) indicate that the three uppermost lone-pair orbitals of these two molecules are very similar. A measurement of the photoelectron spectrum of purine indicated that bands corresponding to the  $n_1, n_2,$  and  $n_3$  bands of adenine occur at 9.6, 11.9, and 13.1 eV, respectively.

(vi) Results of UV absorption studies of adenine indicate that the transition from the highest occupied to the lowest empty molecular orbital is a  $\pi \rightarrow \pi^*$  transition (7, 9).

(vii) The semi-empirical calculations indicate that the lone-pair electrons of the amino group in adenine contribute to the  $\pi$  system and are most strongly coupled to the  $\pi_1$  and  $\pi_3$  orbitals. This description is consistent with the large shifts of 0.33 and 0.46 eV occurring in the first and fifth bands, respectively, when the spectra of adenine and 6-methylaminopurine are compared.

When results of CNDO/2, CNDO/S, INDO, and *ab initio* (8) calculations on adenine were used in conjunction with Koopmans' Theorem (19) to predict the *spacing* of photoelectron bands in adenine, 9-methyladenine, and 6-methylaminopurine, it was found that the CNDO/2 results agreed best with experiment† (20, 21). Fig. 2 shows a comparison of experimental ionization potentials and energy levels obtained from CNDO/2 calculations. The energy level diagram indicates

† Crystallographic data used in the semi-empirical calculations were obtained from refs. 20 and 21.

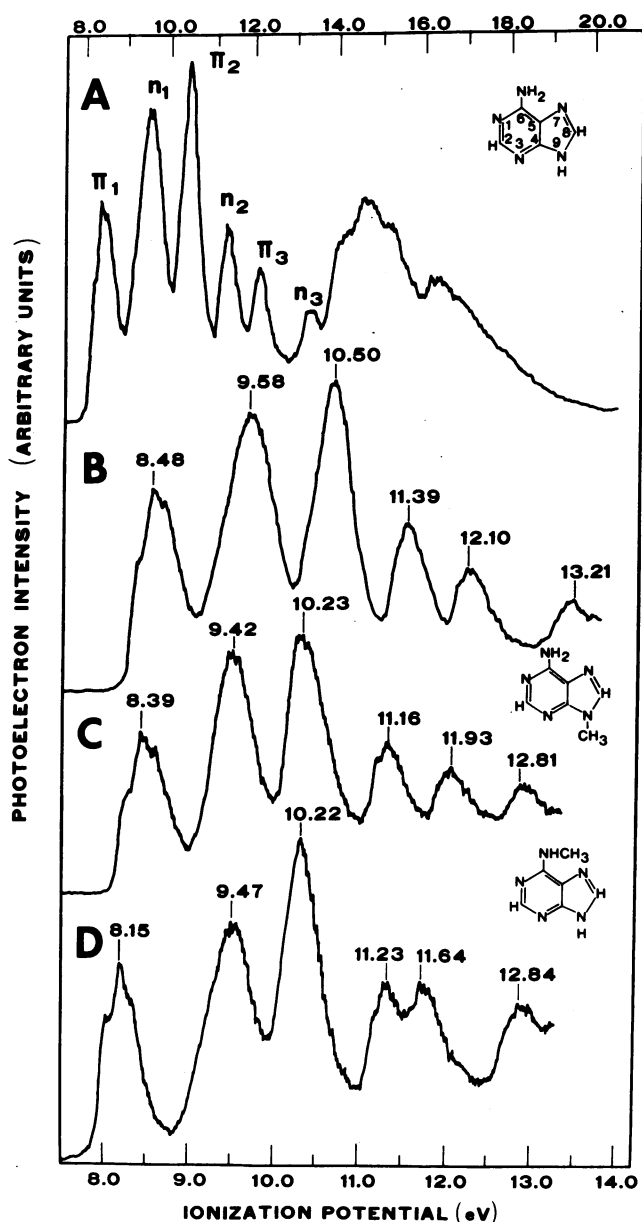


FIG. 1. Helium I photoelectron spectra of adenine, 9-methyladenine, and 6-methylaminopurine. Panel A shows a low resolution adenine spectrum in the energy region 8–21 eV. Panels B, C, and D show high resolution spectra of adenine, 9-methyladenine, and 6-methylaminopurine, respectively, in the energy region 7.5–13.5 eV. The energy scale at the top refers to panel A; the energy scale at the bottom refers to panels B, C, and D.

that while the absolute ionization potentials predicted by the calculation are not accurate, the overall pattern of energy levels agrees with experiment. An examination of the diagram also points out that energy shifts associated with the methyl substituted adenine derivatives are much more accurately predicted for the  $\pi_1$ ,  $n_1$ ,  $n_2$ , and  $n_3$  bands than for the  $\pi_2$  and  $\pi_3$  bands.

The possibility has been considered that both the most stable 9-H tautomer and the second most stable 7-H tautomer of adenine contribute to the observed adenine spectrum (22–24). However, the wide separation of valence bands observed in the adenine spectrum indicates that only one tautomeric form of the molecule occurs under the present experimental conditions. Furthermore, when CNDO/2, CNDO/S, and INDO calcula-

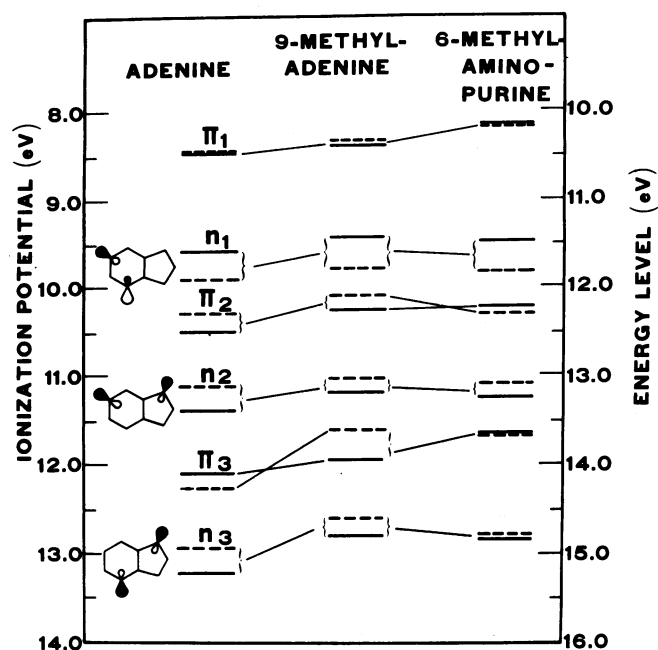


FIG. 2. Energy level diagram comparing experimental ionization potentials with energy levels predicted by CNDO/2 calculations. The solid lines and the energy scale on the left refer to experimental results. The dashed lines and the energy scale on the right refer to theoretical energy levels. The two energy scales were normalized to the first ionization potential of adenine. Orbital diagrams show approximate nitrogen atom lone-pair contributions to the  $n_1$ ,  $n_2$ , and  $n_3$  orbitals as predicted by CNDO/2 calculations.

tions were performed on the two tautomers and on 7-methyladenine, it was found that for each of the calculations the ordering of the first ionization potentials is 7-H adenine > 7-methyladenine > 9-H adenine. A measurement of the photoelectron spectrum of 7-methyladenine indicated that its ionization potential is 0.23 eV higher than that observed in the adenine spectrum. These results indicate that the adenine spectrum arises from the 9-H tautomer.

There is much current evidence indicating that base stacking forces involving purines and pyrimidines are dependent upon the polarizabilities of these molecules (4, 25–27). In studies of monomeric nucleosides in aqueous solutions it is found that the association constants increase as the polarizability of the base increases. It is also found that methyl substitution generally enhances the ability of a base to participate in stacking interactions. For example, the association of 6-methylaminopurine is greater than that of adenine (4). This result, as well as the overall view that polarizability influences base stacking, is consistent with the observation that the binding energies of the upper orbitals of 9-methyladenine and 6-methylaminopurine are lower than those in adenine. The destabilization of the valence manifold may be expected to increase the polarizability of the methyl substituted adenines.

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