The structures of **11** and **12** were determined by comparison of ir and nmr spectra with those of **9** and **10**.

11a: bp 83–85° (0.18 mm); nmr (CCl_4) τ 4.3–4.9 (m, 2 H, CH=C and CHOAc), 8.00 (s, 3 H, $OCOCH_3$), 8.08 (s, 3 H, $OCOCH_3$), 8.40 (broad s, 3 H, $CH_3C\leqslant$), 8.56 (s, 3 H, CH_3), 8.60 (s, 3 H, CH_3), 7.7–8.9 (m, 5 H, aliphatic); ir (film) 3025 (HC=), 1730 (C=O), 1450, 1435, 1240 (CO), 1020 (CO), 970, 925, 805 cm^{-1}. *Anal.* Calcd for $C_{14}H_{22}O_4$: C, 66.11; H, 8.72. Found: C, 66.28; H, 8.64.

12a: bp 83–85° (0.18 mm); nmr (CCl_4) τ 4.2–4.6 (m, 1 H, CH=C<), 4.7–5.0 (m, 1 H, CHOAc), 8.00 (s, 3 H, $OCOCH_3$), 8.10 (s, 3 H, $OCOCH_3$), 8.32 (broad s, 3 H, $CH_3C\leqslant$), 8.58 (broad s, 3 H, CH_3), 8.62 (broad s, 3 H, CH_3), 7.7–8.7 (m, 5 H, aliphatic); ir (film) 3020 (HC=), 1730 (C=O), 1440, 1240 (CO), 1020 (CO), 965, 950, 915, 805 cm^{-1}. *Anal.* Calcd for $C_{14}H_{22}O_4$: C, 66.11; H, 8.72. Found: C, 66.32; H, 8.53.

11b: bp 115–117° (17 mm); nmr (CCl_4) τ 4.5–4.7 (m, 1 H, CH=), 6.2–6.5 (m, 1 H, CHOMe), 6.69 (s, 3 H, OCH_3), 6.85 (s, 3 H, OCH_3), 8.35 (m, 3 H, $CH_3C\leqslant$), 8.91 (s, 6 H, CH_3), 7.7–9.0 (m, 5 H, aliphatic); ir (film) 2825 (OMe), 1380, 1365, 1105 (CO), 810 cm^{-1}. *Anal.* Calcd for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18. Found: C, 72.45; H, 11.33.

12b: bp 115–117° (17 mm); nmr (CCl_4) τ 4.4–4.6 (m, 1 H, CH=), 6.5–6.7 (m, 1 H, CHOMe), 6.67 (s, 3 H, OCH_3), 6.86 (s, 3 H, OCH_3), 8.28 (broad s, 3 H, $CH_3C\leqslant$), 8.91 (s, 6 H, CH_3), 7.7–9.0 (m, 5 H, aliphatic); ir (film) 2825 (OMe), 1360, 1380, 1080 (CO), 800 cm^{-1}. *Anal.* Calcd for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18. Found: C, 72.61; H, 10.89.

Electrooxidation of β-pinene (1.75 mol/l.) in methanol (1.45–1.55 V *vs.* sce, 23 mA/cm^2) yielded 2-methoxy-*p*-mentha-1(7),8-diene (**13**) (2.5%), 2,8-dimethoxy-*p*-menth-1(7)-ene (**14**) (7.4%), 7-methoxy-*p*-mentha-1,8-diene (**15**) (14.7%), and 7,8-dimethoxy-*p*-menth-1-ene (**16**) (23.4%).

13: bp 62–63° (5 mm); nmr (CCl_4) τ 5.1–5.3 (m, 2 H, C^1 methylene), 5.25–5.4 (m, 2 H, C^8 methylene), 6.39 (t, J = 3 Hz, 1 H, CHOMe), 6.87 (s, 3 H, OCH_3), 8.30 (t, J = 1 Hz, 3 H, CH_3), 7.5–9.0 (m, 7 H, aliphatic); ir (film) 3090 (=CH_2), 2825 (OMe), 1640 (C=C), 1110, 1090 (CO), 885 cm^{-1}. *Anal.* Calcd for $C_{11}H_{18}O$: C, 79.46; H, 10.92. Found: C, 79.27; H, 10.70.

14: bp 77–80° (5 mm); nmr (CCl_4) τ 5.15–5.35 (m, 2 H, =CH_2), 6.40 (t, J = 3 Hz, 1 H, CHOMe), 6.87 (s, 6 H, OCH_3), 8.97 (s, 6 H, CH_3), 7.6–8.9 (m, 5 H, aliphatic); ir (film) 3085 (=CH_2), 2825 (OMe), 1650 (C=C), 1380, 1365, 900 (=CH_2) cm^{-1}; mass spectrum *m/e* (rel intensity) 198 (0.3, P^+), 166 (4), 151 (8), 134 (7), 73 (100).

15: bp 66–68° (5 mm); nmr (CCl_4) τ 4.3–4.5 (m, 1 H, CH=), 5.30 (broad s, 2 H, =CH_2), 6.30 (broad s, 2 H, CH_2OMe), 6.82 (s, 3 H, OCH_3), 8.27 (broad s, 3 H, CH_3), 7.7–9.1 (m, 7 H, aliphatic); ir (film) 3095 (=CH_2), 3025 (HC=), 1670 (C=C), 1645 (C=C), 1100 (CO), 885 (H_2C=), 815 cm^{-1}; mass spectrum *m/e* (rel intensity) 166 (18, P^+), 134 (51), 93 (100).

16: bp 79–82° (5 mm); nmr (CCl_4) τ 4.3–4.5 (m, 1 H, CH=), 6.30 (broad s, 2 H, CH_2OMe), 6.81 (s, 3 H, OCH_3), 6.88 (s, 3 H, OCH_3), 8.93 (s, 6 H, CH_3), 7.8 ~ 8.6 (m, 7 H, aliphatic); ir (film) 2825 (OMe), 1380, 1360 (Me_2C), 1080 (CO), 810 cm^{-1}. *Anal.* Calcd for $C_{12}H_{22}O_2$: C, 72.68; H, 11.18. Found: C, 72.93; H, 11.18.

Anodic oxidation of cyclohexene in water–acetonitrile (1:1 mol/mol) gave 3-hydroxycyclohexene (**3**). This compound was identical with the authentic sample, which was prepared by hydrolysis of **1**.

Electrooxidation of cyclohexene in acetonitrile containing 0.23 mol/l. of water yielded 3-acetoaminocyclohexene (**4**): mp 78°; nmr (CCl_4) τ 2.5–3.1 (m, 1 H, NH), 4.0–4.7 (m, 2 H, CH=), 5.45, 4.9 (m, 1 H, CHN), 8.10 (s, 3 H, $COCH_3$), 7.8–8.7 (m, 6 H, aliphatic); ir (KBr) 3290 (NH), 3070, 3020 (HC=), 1640 (C=O), 1550 (NH), 1370 (CN), 730 cm^{-1}. *Anal.* Calcd for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 68.94; H, 9.55; N, 9.78.

Isotope Effect. Cyclohexene-*1,3,3-d*$_3$ (**20**) (purity is almost 100% by nmr) was prepared from cyclohexanone by the method of R. C. Fahey.[25] Anodic acetoxylation of **20** was carried out in a similar manner to cyclohexene. The content of deuterium at the C_3 position of the obtained 3-acetoxycyclohexene was determined by nmr to be 39% indicating the deuterium effect (k_H/k_D) of 1.6.

The current *vs.* anode potential curve was obtained by the following method. Anode potential was measured at constant temperature as a function of electrolysis current, which was supplied in both the ascending and descending directions. The results were independent of the sequence in which the current was varied.

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A Comparative Molecular Orbital Study of Protonated Adenine Tautomers and Their Intermolecular Interactions

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Abstract: Electronic structures of several neutral and protonated adenine tautomers were calculated using the CNDO/2 and MINDO methods. Substantial changes in net atomic charges and σ and π charge distributions and large increases in ionization potentials were noted upon protonation. The effect of protonation on base-base interactions was qualitatively evaluated taking into account monopole–monopole, monopole–induced dipole, and dispersion terms.

There are numerous calculations in the literature on the electronic structure of adenine.[1] No such calculations are to be found on protonated DNA bases other than one on cytosine.[2] As part of our systematic study of the electronic structures of protonated DNA bases, nucleosides, and nucleotides we have performed calculations on N-7–H and N-9–H tautomers and their N-1 protonated analogs—the N_7-methyl and N_9-methyladenines and corresponding N-1 protonated structures—always maintaining the amino configuration at N-10.

For these calculations we have employed the well-documented CNDO/2[3] and MINDO[4] methods using

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Table I. Energy, Dipole Moments, and Net Atomic Charges of Adenines

Adenine tautomer or ion[a]	Method[b]	Total energy[c] or H_f, kcal/mol	Dipole[d] moment, D	IP[e]	Geometry[f] employed	Net atomic charges N-1	N-7	N-9	N-1–H	N-10–H[h]	C-8–H
N-9–H	C	−60964.1	2.99	10.3	P-H, I-C	−0.296	−0.261	−0.084		+0.120	+0.010
N-9–H	C	−61037.9	2.50	10.5	P-H, I-H[g]	−0.285	−0.205	−0.145		+0.121	+0.000
N-9–H	M	−101.13	2.30	9.0	P-H, I-C	−0.703	−0.490	−0.605		+0.268	−0.045
N-7–H	C	−60956.8	8.43	10.7	P-H, I-C	−0.277	−0.060	−0.290		+0.118	+0.007
N-7–H	C	−61036.4	7.61	10.8	P-H, I-HI[g]	−0.277	−0.109	−0.254		+0.120	+0.000
N-7–H	M	−103.73	6.59	9.2	P-H, I-CI	−0.690	−0.507	−0.566		+0.262	−0.049
N-9–CH_3	C	−66416.3	2.40	10.3	P-H, I-H	−0.273	−0.143	−0.162		+0.120	−0.027
N-9–CH_3	M	−121.00	2.45	9.0	P-H, I-H	−0.699	−0.496	−0.666		+0.266	−0.053
N-7–CH_3	C	−66406.0	7.05	10.7	P-H, I-HI	−0.284	−0.101	−0.218		+0.121	+0.008
N-7–CH_3	M	−117.94	6.25	9.2	P-H, I-HI	−0.688	−0.580	−0.569		0.266	−0.055
N-9–H·H^+	C	−61171.1		16.2	P-C, I-C	−0.138	−0.138	−0.118	+0.171	+0.185	+0.056
N-9–H·H^+	M	−14.87		13.9	P-C, I-C	−0.743	−0.421	−0.591	+0.319	+0.337	+0.029
N-7–H·H^+	C	−61162.9		17.3	P-C, I-CI	−0.144	−0.123	−0.146	+0.174	+0.178	+0.046
N-7–H·H^+	M	−9.70		14.2	P-C, I-CI	−0.724	−0.511	−0.471	+0.320	+0.331	+0.030
N-9–CH_3·H^+	C	−66574.9		15.9	P-C, I-C+M	−0.133	−0.120	−0.094	+0.169	+0.180	+0.044
N-9–CH_3·H^+	M	−71.20		13.6	P-C, I-C+M	−0.739	−0.431	−0.620	+0.316	+0.335	+0.019
N-7–CH_3·H^+	C	−66555.7		16.4	P-C, I-CI + M	−0.117	+0.049	−0.269	+0.168	+0.175	+0.045
N-9–H·$2H^+$ [i]	C	−61346.4		22.9	BI	−0.088	−0.016	−0.097	+0.211	+0.205	+0.119
N-9–H·H^+ [j]	C	−61260.5		16.5	P-H, I-BT	−0.229	−0.026	−0.064		+0.150	+0.088

[a] Protonation always on N-1, unless otherwise indicated. [b] C, CNDO/2 or ref 3; M, MINDO of ref 4 employing original parameters. [c] Total molecular energies, including nuclear repulsion as given by CNDO/2 and heats of formations given by MINDO. [d] Both methods include hybrid moments as described in ref 3. [e] Negative of the energy of the highest occupied molecular orbital following Koopmans' theorem. [f] P, pyrimidine ring; I, imidazole ring; C, W. Cochran, *Acta Crystallogr.*, **4**, 81 (1951); H, K. Hoogsteen, *ibid.*, **16**, 907 (1963); BT, R. F. Bryan and K. Tomita, *ibid.*, **15**, 1179 (1962); I-HI, Hoogsteen's imidazole inverted; I-CI, Cochran's imidazole inverted; M, methyl constructed with Hoogsteen's parameter. [g] Hoogsteen's adenine assumed except C-8–H = 1.01 Å, N-9–H = 1.00 Å; closer to average geometry found in neutral adenine nucleosides, *e.g.*, S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4963 (1970). [h] Average values for the two hydrogens on N-10. [i] Protons on N-1 and N-7. [j] Proton on N-7.

parametrization suggested in the original articles. The CNDO/2 method has recently been shown to lead to theoretical dipole moments in satisfactory agreement with experimental values for several adenine tautomers.[5] Newton and Ehrenson reported that *ab initio* studies on hydrated proton structures give results in good agreement with those obtained by CNDO/2,[6] supporting the use of the latter method in this study. MINDO was chosen because it is parametrized to give "chemical quality" heat of formation values for a variety of structures[4] and it was thought that confidence in the results would be greatly increased if agreement were found between the results of the two different approaches.

Results of the Molecular Orbital Calculations

The essential results are summarized in Table I. Relative energies of the neutral N-7–H and N-9–H tautomeric forms confirm the results of Pullman, *et al.* (as quoted in ref 5), but are shown to depend on the assumptions made about the geometry and on the parametrization. For the same geometries MINDO predicts more stability for N-7–H and CNDO/2 for N-9–H; both methods predict very similar energies for the two tautomers. For all other tautomeric pairs, including N-1 protonated ones, N-9 forms are always preferred by both methods. Our calculations, as all those before ours, suffer from a lack of crystallographic data on N-7 substituted adenines.

In contrast to the relative stabilities of tautomers, the electronic structures, dipole moments, and ionization potentials[7] appear to be essentially independent of modest geometric modifications; here the two methods are in good accord. Perhaps most impressive is the charge reorganization and increased ionization potential upon protonation.

Table I summarizes these results as well as the net charges on atoms thought to be important in hydrogen bonding schemes. Figure 1 gives a detailed account of the net atomic charges and the π charges present in the neutral and protonated tautomers according to both methods of calculation.

The substantial delocalization of the positive charge throughout the purine system is noteworthy. The attached proton retains only a fraction of its initial full positive charge. That protonation profoundly affects the π system as well as the σ is reflected by the change in π moment upon protonation.[8] For example, the CNDO π moment of N-9–H adenine is 1.25 D, while that of the N-1–H^+ analog is 3.58 D. The direction of the π moment is not significantly altered by protonation. The center of charge in the protonated form resides in the pyrimidine ring near the center of the dipole in the neutral molecule. It should be noted that CNDO and MINDO give very different net atomic charges but relatively similar dipole moments. For adenine, CNDO gives 2.5 and 3.0 D (depending on geometry used); MINDO gives 2.3 D.[9,10] The ionization potential of adenine is greatly increased upon protonation (10.9 and 9.0 eV for neutral and 16.2 and 13.9 eV for protonated adenine according to CNDO and

(5) E. D. Bergmann, H. Weiler-Feilchenfeld, and Z. Neiman, *J. Chem. Soc. B*, 1334 (1970).

(6) M. D. Newton and S. Ehrenson, *J. Amer. Chem. Soc.*, **93**, 4971 (1971).

(7) Ionization potential is given by the negative of the energy of the highest occupied molecular orbital, according to Koopmans' theorem.

(8) Although the dipole moment of the entire protonated system is origin dependent, the total π charge remains zero upon protonation, thus permitting a π moment calculation for both protonated and neutral bases. π moment changes thus represent perturbations in the π distribution upon protonation.

(9) Experimental value given in ref 5 is 3.25 D.

(10) The better dipole moments obtained with CNDO confirm Klopman's suggestion that CNDO is more reliable in this respect than MINDO; see G. Klopman, "Topics in Current Chemistry," Vol. 15, No. 4, Springer Verlag, Berlin, 1970.

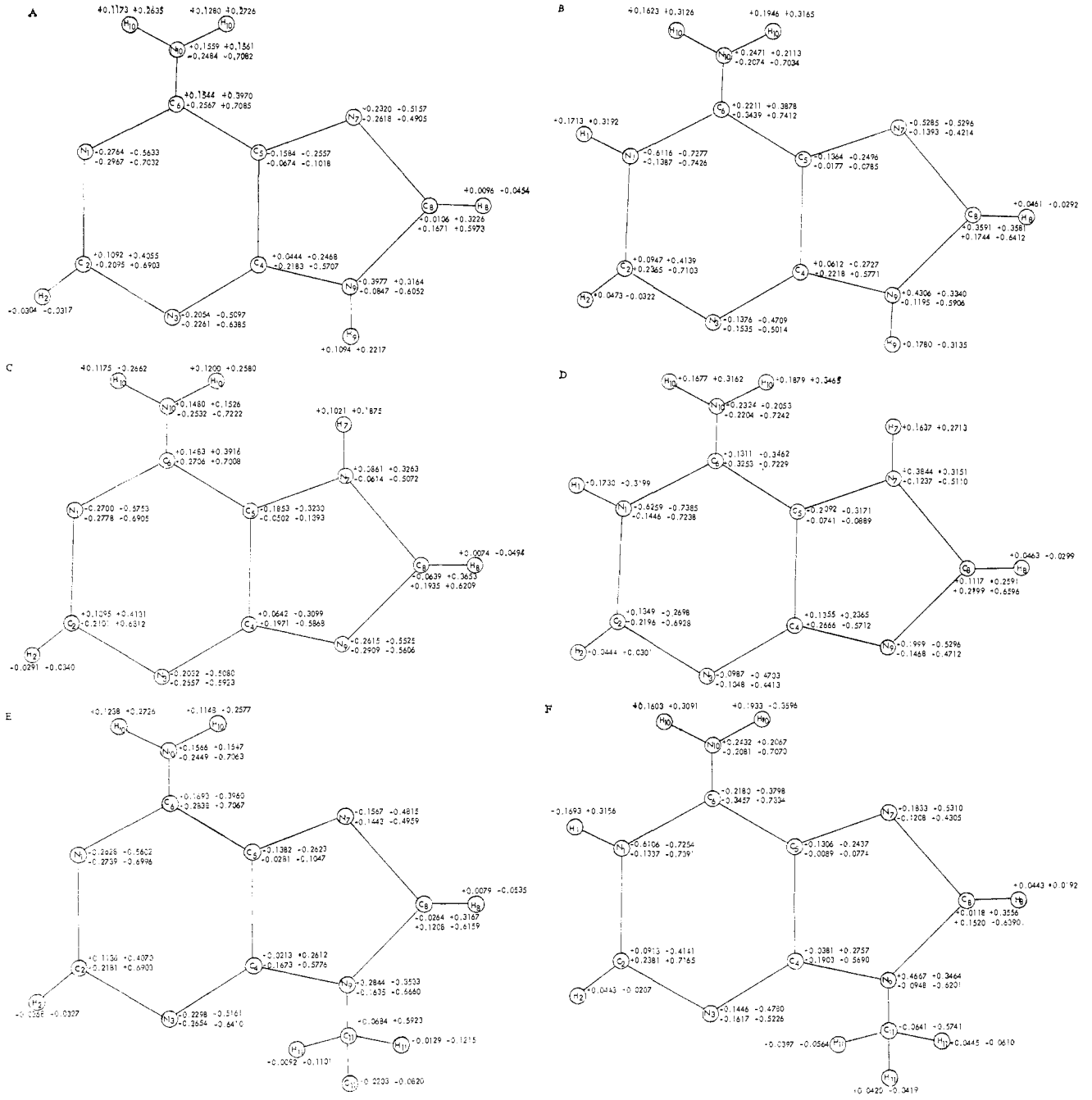

Figure 1. Net atomic and π charges of neutral and protonated adenines. Left-hand numbers obtained by CNDO/2; right-hand numbers obtained by MINDO; upper rows represent π charges; lower rows represent net atomic charges: A, 9*H*-adenine; B, 9*H*-adenine·H^+; C, 7*H*-adenine; D, 7*H*-adenine·H^+; E, N^9-methyladenine; F, N^9-methyladenine·H^+.

MINDO, respectively). The experimental value for a neutral adenine derivative is 8.9 eV.[11] Such a large increase may appear unusual, and experimental verification is not available. However, *ab initio* calculations on H_2O and H_3O^+ show a sizable increase from 13.7 to 25 eV upon protonation in this (admittedly smaller) species.[12]

Finally, it is of interest that replacement of the N-9 proton by a methyl group increases the acidity of the proton attached to C-8 according to the larger s character in the C-8–H bond in the methylated case. This has been experimentally confirmed recently in a study on the mechanism of hydrogen–deuterium exchange in adenine and 9-methyladenine, the rate for the latter being much faster.[13]

Intermolecular Force Calculations

Our intent was to obtain information on the electronic consequences of protonation in adenine and to obtain the ground-state wave functions for approximate calculations of the intermolecular interactions in these bases. Accordingly, a brief discussion of the background of this aspect of our work is in order.

(11) C. Lifschitz, E. D. Bergmann, and B. Pullman, *Tetrahedron Lett.*, 4583 (1967).

(12) P. A. Kollman and L. C. Allen, *J. Amer. Chem. Soc.*, **92**, 6104 (1970).

(13) M. Maeda, M. Saneyoshi, and Y. Kawazoe, *Chem. Pharm. Bull.*, **19**, 1641 (1971).

Experimental evidence indicates that protonation of adenine at N-1 leads to maximal double strand formation near the N-1 pK_a of oligoriboadenylic acids.[14] Furthermore, polyriboadenylic acids and polydeoxyadenylic acids have two distinct double-strand forms, one predominating at the N-1 pK_a, and the other at higher acidities, thought to be a fully protonated double strand.[15]

The theoretical treatments of nucleic acid interactions have not, to date, included the interactions with protonated bases.[16] While the salt bridge of the N-1 proton with the phosphate charge is undoubtedly important, the stacking and hydrogen bonding effects should also be taken into account. Stacking interactions have been interpreted in terms of dipole–induced dipole and London dispersion forces on the basis of solid state[17a] and solution[17b] data.

The level of approximation employed to study the intermolecular interactions is based on the theoretical approach outlined elsewhere[18a] and detailed recently by Rendell, *et al.*[18b] Since we were dealing with charged species, it was thought advisable to use the sum of the following three terms in determining intermolecular energy values between two groups of charges with N-1 and N-2 atoms and N-11 and N-22 bonds, respectively. (1) Monopole–monopole interactions

$$\rho\rho = \sum_{i=1}^{N_1}\sum_{j=1}^{N_2}\frac{q_i q_j}{R_{ij}} \qquad (1)$$

with q_i and q_j the net atomic charges on atoms in groups 1 and 2, respectively, and R_{ij} the distance separating the atoms i and j. (2) Polarization (monopole–induced dipole) interactions are shown by

$$\rho\alpha(\text{induced in group 1}) = -0.5\sum_{i=1}^{N_{11}}\alpha_i\left(\sum_{j=1}^{N_2}\frac{q_j\bar{R}_{ij}}{R_{ij}^3}\right)\left(\sum_{j=1}^{N_2}\frac{q_j\bar{R}_{ij}}{R_{ij}^3}\right) \qquad (2)$$

where α_i is the isotropic polarizability of bond i in group 1, $\bar{R}_{ij}$ is a vector pointing from charge j (in group 2) to the midpoint of bond i (in group 1), and R_{ij} is the magnitude of this vector. There is a symmetrical term for the energy induced in group 2 by the charge distribution of group 1

$$\rho\alpha(\text{induced in group 2}) = -0.5\sum_{j=1}^{N_{22}}\alpha_j\left(\sum_{i=1}^{N_1}\frac{q_i\bar{R}_{ij}}{R_{ij}^3}\right)\left(\sum_{i=1}^{N}\frac{q_i\bar{R}_{ij}}{R_{ij}^3}\right) \qquad (3)$$

(3) Finally the London dispersion energy is shown by

$$\alpha\alpha = -1.5\frac{\text{IP(1)IP(2)}}{\text{IP(1)} + \text{IP(2)}}\sum_{i=1}^{N_{11}}\sum_{j=1}^{N_{22}}\frac{\alpha_i\alpha_j}{R_{ij}^6} \qquad (4)$$

where α_i and α_j are the isotropic bond polarizabilities in groups 1 and 2, respectively, R_{ij} is the distance between the midpoints of bonds i and j, and IP(1) and IP(2) are the ionization potentials of groups 1 and 2, respectively.

These formulas neglect the dielectric constant in each denominator (set it equal to 1.0) and do not include the numerical factors needed to convert to desired energy units. Use of isotropic bond polarizabilities is not thought to introduce serious errors at this level of approximation.

In our calculations we employed both CNDO/2 and MINDO net atomic charges for the q values, MINDO ionization potentials (more closely resembling the experimental value in neutral species), and literature values of the isotropic bond polarizabilities.[19] A computer program written for the IBM 360/67 performs both the desired geometric variation of the interacting species as well as the actual intermolecular energy calculations.[20] It is of some interest to note that the approximation here employed in the intermolecular force calculations is much more costly than the one using group polarizabilities and induced moments in entire groups of charges as in ref 16a and 16d.

The essential results obtained with the above approximations are quoted below.

It should be pointed out first, however, that at this level of approximation one cannot expect to accomplish absolute minimization of electrostatic interaction energies with respect to all geometric coordinates without the inclusion of at least an empirical repulsion function. What one can do is to calculate interaction energies at experimentally established hydrogen bonding and stacking distances and do partial minimization at such distances with respect to relative orientation of interacting species. The absolute magnitudes of the interaction energies are considered to be in some doubt. The relative contributions of the three terms to the total interaction energy are of great interest. It is of importance to recall that while monopole–monopole interactions can be stabilizing or destabilizing, the monopole–induced dipole and dispersion terms are by definition stabilizing ones (*i.e.*, with a negative contribution to the overall energy).

Hydrogen Bonding Interactions

The only geometric arrangement was a symmetrical one in which both members donate the N-10 proton and accept at N-7. This is the arrangement found in N-1 protonated polyadenylic acid.[21] The choice of this arrangement is dictated by the fact that this is the only one allowing a direct comparison of interaction energies as a function of the state of protonation of bases. It was found that for a given hydrogen bonding distance translation of one of the species over small angles does not have a significant influence on the interaction energies. Rotation of one of the species even over small angles (by necessity leading to variations between the two hydrogen bonding distances) leads to significant increases in energy (less stable) with the gen-

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(20) A FORTRAN program to perform the geometric variations and the intermolecular force calculations as well as any further details are available from the authors upon request.
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eral prediction that as nearly as possible equal hydrogen bond lengths are preferred when two nearly equal or identical sites are available.

Table II indicates the magnitude of the terms at the

Table II. Intermolecular Interaction Terms between Two Hydrogen-Bonded Adenines (kcal/mol)[a]

Pair[b]	$\rho\rho$	$\rho\alpha(1)$[c]	$\rho\alpha(2)$[d]	$\alpha\alpha$	E_{total}
Neutral–					
neutral (M)	−2.69	−0.20	−0.20	−1.86	−4.94
(C)	−1.15	−0.09	−0.09	−1.86	−3.19
Protonated–					
neutral (M)	−2.62	−0.18	−1.12	−2.28	−6.19
(C)	−2.17	−0.09	−1.73	−2.28	−6.27
Protonated–					
protonated (M)	39.51	−1.21	−1.21	−2.92	34.16
(C)	48.89	−1.86	−1.86	−2.92	42.25

[a] 3.0-Å symmetrical hydrogen bonding arrangement described in text. [b] In parentheses the method employed in obtaining the net atomic charges: C, CNDO/2; M, MINDO. [c] Induced energy in first partner. [d] Induced energy in second partner.

cate that in nucleotides in which the base is protonated on N-1 there is a salt bridge between the phosphate charge of another molecule and this N-1 proton.[23]

Stacking Interactions

The vertical (stacking) interactions were again calculated only at fixed interplanar distances to allow direct observation of the effect of the state of protonation on such interactions. Extensive geometric variation at fixed interplanar distances was performed for all three combinations indicating that there are several regions of similar energies for each. In Table III a representative sampling of results is given for 3.22-Å interplanar separation of parallel bases, the C-4 of one base under C-5 of the other and C-5 of the first directly under C-4 of the other. The results again indicate that a half-protonated pair is more stable than a neutral pair and that the doubly protonated pair is destabilized.

The results help to explain why the dinucleoside monophosphate ApA stacks at pH 7 but does not

Table III. Intermolecular Interaction Terms between Two Stacked Adenines (kcal/mol)

Pair[c]		$\rho\rho$	$\rho\alpha(1)$[d]	$\rho\alpha(2)$[e]	$\alpha\alpha$	E_{total}
Neutral–neutral	(M)[a]	0.19	−0.10	−0.10	−9.02	−9.05
	(C)	−0.29	−0.12	−0.12	−9.02	−9.56
Protonated–neutral	(M)[a]	0.95	−0.11	−4.40	−11.25	−14.80
	(C)	1.24	−0.13	−4.80	−11.25	−14.95
Protonated–protonated	(M)[b]	64.24	−4.59	−4.59	−14.69	40.36
	(C)	69.28	−4.92	−4.92	−14.69	44.74
Protonated–neutral	(M)[b]	−0.91	−0.12	−4.24	−11.20	−16.47
	(C)	−1.72	−0.12	−5.09	−11.20	−18.12

[a] 3.22-Å interplanar separation, configuration described in text. [b] Most stable configuration at 3.22 Å interplanar separation with two adenines superimposed but rotated 45° from each other. [c] Method employed to calculate net charges: C, CNDO/2; M, MINDO, indicated in parentheses. [d] Induced in partner 1. [e] Induced in partner 2.

above described arrangement of the two interacting species separated by an N···N hydrogen bonding distance of 3.0 Å, near the value quoted in ref 21. Obviously, the effect of protonation is apparent in all three contributions, entering the dispersion ($\alpha\alpha$) term through the increased ionization potential predicted to result from protonation. It appears that for the same geometric arrangement the half-protonated pair is more stable than the neutral one and the totally (doubly) protonated pair is very destabilized due to monopole–monopole interactions. These results on fully protonated pairs can only be reconciled with experiment[21] if one assumes that the phosphate–base (especially N-1–H) salt bridge can overcome such destabilization. That such a salt bridge is a very important stabilizing factor is supported by recent extensive calculations on nucleotides[22] indicating that in 2′-, 3′-, and 5′-AMP nearly integral positive charge remains on the base. This finding supports the present work's assumption of a full positive charge on the base. X-Ray studies indi-

(22) F. Jordan, unpublished observations.

stack at pH 1.[24] There is also some evidence indicating the special properties and enhanced stacking[15,25] and hydrogen bonding[15] in monoprotonated pairs.

Finally, it is of theoretical interest to note that CNDO/2 and MINDO do not differ greatly so far as total energy predictions are concerned. It is to be noted further that the stabilization of monoprotonated pairs over neutral ones is not due to the increased ionization potential of protonated adenine alone, but also has a major contribution from the induced energy terms. Calculations on the electronic structures of other protonated DNA bases and of their interactions are currently under way.

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