Purine Carbon-8 Substituent as Probe of the Electronic Structures of Adenine and Guanine. A Computational Study

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Abstract: The results of CNDO/2 calculations are reported on neutral and protonated 9-methyladenines and 9-methylguanines in which the C-8 substituent is varied through H, Cl, and NH_2. Correlation of the theoretical results with the available experimental evidence suggests that substitution in this position should be useful in delineating a number of intra- and intermolecular physical and chemical properties of the purine nucleosides. A novel correlation is suggested for predicting preferred sites of protonation of the title compounds and is shown to be quite effective in all DNA bases. Ground-state experimental results can be better rationalized than excited-state properties. Limited configuration interaction calculations of the singlet type ultraviolet transitions are in respectable agreement with experimental data.

The C-8 position of purines is probably the one most easily substituted. For example, bromination of guanosine is simply done by adding Br_2 in water to the substrate.[1] The synthesis of numerous C-8 substituted derivatives has been reported, for example by Robins and coworkers[2] and by Ikehara and coworkers[3] among others. Because of the widespread interest in the chemical and physical properties of the purines, it would be of interest to have a handle on how such properties are affected by substituents of known electronic requirements. It is here suggested that C-8 substituted substrates serve this purpose well.

The theoretical approach employed was the CNDO/2 self-consistent field (SCF) molecular orbital method[4] in a version capable of treating second row elements. Application of this same method to the problem of DNA base protonation and the effects of such protonation on intermolecular interactions have already been reported from this laboratory.[5] The present contribution is concerned with the theoretical aspects of substituent effects in the guanine and adenine bases. This study was prompted, in part, by the appearance of experimental data on such substituent effects in adenine nucleosides[6] and results from this laboratory on C-8 substituted guanosines.[7]

The study serves multiple purposes. It attempts to correlate experimental and theoretical results on these substrates, point out where such correlations succeed or fail, and, from a purely theoretical viewpoint, provide insight into the perturbations caused in the electronic structure by the substituents studied.

Choice of Model Compounds and Geometries. Experimental data are available on 8-bromoadenosine and 8-methoxyadenosine[6] and on 8-bromoguanosine and 8-aminoguanosine[7] along with data on the unsubstituted substrates. Because of the capabilities of the theory employed,[4] calculations were performed on the 8-chloro and 8-amino derivatives of 9-methyladenine

and 9-methylguanine as models. The 9-methyl derivative was chosen instead of the nucleoside since X-ray data for both neutral and protonated unsubstituted substrates are available in the literature and because such planar structures will clearly demonstrate the electronic perturbations separately in the σ- and π-type molecular orbitals. To correlate with experimental data, it will be assumed that the trends for Cl and Br should be similar and that the uniform use of the 9-methyl group instead of the 9-ribose substituent will reflect proper trends.

The same substituent geometry parameters were used throughout. C–Cl and C–N bonds were constructed along the C–H bond vector with 1.70- and 1.43-Å bond lengths, respectively. The N–H bond lengths on the C-8 amino substituent were 1.00 Å uniformly and all bond angles around this N were assumed perfect trigonal (120°).[8] The geometries of the 9-methyl substrates were taken from their X-ray crystallographic structures: 9-methyladenine,[9] 9-methyladenine·H^+,[10] 9-methylguanine,[11] and 9-methylguanine·H^+[12] projected onto a plane.

Results and Discussion

Charges and Dipole Moments. The essential theoretical results are presented in Tables I–III. Some experimental results are quoted in Table IV.

Adenines. The chlorine substituent decreases the dipole moment significantly, while the amino group leads to a 50% increase. That the dipole moments for the unsubstituted molecules are qualitatively correct has been discussed by others.[5a]

The π and net atomic charges are worthwhile considering. So far as net charges are concerned, the amino group has an electron-donating effect compared to C-8 H, except to N-1 (very slightly) and to C-8, the atom of attachment. The chlorine, on the other hand, consistently withdraws (supposedly purely inductively) from every position. The effect of the chlorine is very much smaller than that of the amino group. The π-

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Table I. Net Atomic Charges and π Charges on C-8 Substituted 9-Methyladenine

Atom	C-8 H π	C-8 H Total	N-1 H+, C-8 H π	N-1 H+, C-8 H Total	C-8 NH$_2$ π	C-8 NH$_2$ Total	N-1 H+, C-8 NH$_2$ π	N-1 H+, C-8 NH$_2$ Total	C-8 Cl π	C-8 Cl Total	N-1, H+, C-8 Cl π	N-1, H+, C-8 Cl Total
N-1	-0.272	-0.289	-0.615	-0.130	-0.264	-0.286	-0.609	-0.129	-0.272	-0.285	-0.615	-0.131
C-2	0.115	+0.216	0.092	0.237	0.102	0.208	0.084	0.231	0.122	0.220	0.060	0.238
N-3	-0.216	-0.242	-0.140	-0.161	-0.209	-0.238	-0.137	-0.158	-0.219	-0.238	-0.141	-0.156
C-4	0.045	0.198	0.052	0.207	0.030	0.191	0.055	0.211	0.051	0.204	0.057	0.215
C-5	-0.153	-0.056	-0.146	-0.031	-0.134	-0.045	-0.125	-0.023	-0.158	-0.055	-0.148	-0.032
C-6	0.164	0.270	0.221	0.341	0.149	0.262	0.198	0.327	0.168	0.277	0.220	0.342
N-6	0.157	-0.247	0.243	-0.210	0.152	-0.248	0.234	-0.214	0.161	-0.245	0.247	-0.208
N-7	-0.183	-0.209	-0.206	-0.172	-0.319	-0.281	-0.361	-0.261	-0.169	-0.187	-0.213	-0.170
C-8	-0.006	0.147	0.026	0.173	0.083	+0.301	0.126	0.330	-0.010	0.217	0.034	0.228
N-9	0.372	-0.095	0.479	-0.045	0.338	-0.125	0.425	-0.078	0.371	-0.087	0.477	-0.030
C-9		0.076		0.059		0.082		0.063		0.079		0.060
C-8 X[a]		0.006		0.044	0.095	-0.275	0.117	-0.265	0.031	-0.152	0.039	-0.064
N-1 H				0.168				0.167				0.171
C-2 H		-0.029		0.044		-0.030		0.042		-0.024		0.046
N-6 H-1		0.115		0.162		0.113		0.159		0.119		0.164
N-6 H-2		0.127		0.193		0.125		0.191		0.130		0.195
C-9 H-1		-0.002		0.043		-0.005		0.035		0.006		0.049
C-9 H-2		+0.012		0.039		0.013		0.034		0.014		0.041
C-9 H-3		-0.002		0.040		-0.005		0.039		0.006		0.041
N-8 H-1						0.127		0.152				
N-8 H-2						0.117		0.149				

[a] The atom attached to C-8.

Table II. Net Atomic Charges and π Charges on C-8 Substituted 9-Methylguanine

Atom	C-8 H π	C-8 H Total	N-7, H+, C-8 H π	N-7, H+, C-8 H Total	C-8 NH$_2$ π	C-8 NH$_2$ Total	N-7, H+, C-8 NH$_2$ π	N-7, H+, C-8 NH$_2$ Total	C-8 Cl π	C-8 Cl Total	N-7 H+, C-8 Cl π	N-7 H+, C-8 Cl Total
N-1	0.287	-0.220	0.261	-0.250	-0.291	-0.220	0.263	-0.250	0.283	-0.219	0.262	-0.248
C-2	0.200	0.383	0.239	0.421	0.193	0.377	0.236	0.419	0.207	0.388	0.241	0.423
N-2	0.172	-0.250	0.203	-0.243	0.169	-0.252	0.200	-0.244	0.176	-0.249	0.200	-0.242
N-3	-0.390	-0.330	-0.431	-0.311	-0.383	-0.325	-0.428	-0.316	-0.394	-0.328	-0.434	-0.314
C-4	0.076	0.208	0.087	0.249	0.062	0.202	0.089	0.248	0.084	0.215	0.086	0.247
C-5	-0.235	-0.109	-0.247	-0.091	-0.221	-0.106	-0.262	-0.098	-0.237	-0.106	-0.237	-0.082
C-6	0.191	0.357	0.156	0.381	0.188	0.353	0.161	0.383	0.189	0.359	0.156	0.382
O-6	-0.463	-0.385	-0.363	-0.302	-0.468	-0.388	-0.377	-0.314	-0.454	-0.376	-0.360	-0.300
N-7	-0.129	-0.158	-0.475	-0.011	-0.261	-0.238	-0.593	-0.075	-0.109	-0.135	-0.485	-0.008
C-8	-0.054	0.130	0.096	0.214	0.035	0.281	0.183	0.365	-0.062	0.193	0.093	0.245
N-9	0.378	-0.100	0.478	-0.038	0.347	-0.122	0.388	-0.097	0.377	-0.093	0.470	-0.040
C-9		0.060		0.069		0.062		0.075		0.063		0.069
C-8 X[a]		-0.011		0.073	0.085	-0.280	0.151	-0.248	0.027	-0.167	0.052	0.017
N-1 H		0.116		0.180		0.115		0.178		0.120		0.181
N-2 H-1		0.126		0.173		0.125		0.170		0.130		0.173
N-2 H-2		0.147		0.166		0.146		0.164		0.150		0.166
N-7 H				0.188				0.179				
C-9 H-1		0.008		0.044		0.006		0.035		0.013		0.046
C-9 H-2		0.012		0.038		0.008		0.025		0.023		0.043
C-9 H-3		0.017		0.051		0.018		0.048		0.019		0.051
N-8 H-1						0.125		0.165				
N-8 H-2						0.112		0.173				

[a] The atom attached to C-8.

electron distribution, on the other hand, is not affected in a regular manner; rather, the effects alternate going around the ring compared to the π charges on the unsubstituted ring. For example, the amino group decreases the π-electron density at C-8, increases it at N-7, decreases at C-6, etc. The effects of the amino and chlorine substituent on the π-electron densities are in opposite directions compared to the unsubstituted ring.

Next, one can consider the relative rates of hydrolysis of the glycosidic C–N bond of the corresponding nucleosides. The experimental evidence strongly suggests an A-1 mechanism (Table IV, ref 6). The purine ring is protonated in a prior equilibrium step (fast) followed by rate-limiting unimolecular rupture of the C–N bond. Such a mechanism would be favored by decreased electron density at N-9, the atom which would pick up the electron pair upon heterolytic bond cleavage. The smaller the electron density at N-9 the more readily this atom would accept the electron pair. Furthermore, the pH dependence of the rate strongly implies the possibility that the protonated species reacts much faster than the neutral one and most likely the diprotonated one reacts faster yet. The relative rates are: 8-bromoadenosine faster than adenosine which in turn is faster than 8-methoxyadenosine. The calculated values confirm both experimental results qualitatively. In all three models the electron density at N-9 diminishes greatly upon protonation at N-1.

Table III. Miscellaneous Molecular Orbital Results[a]

Molecule	E_{total}, au[b]	Binding energy, au	Proton affinity,[c] kcal/mol	IP, eV[d]	Dipole moment, D[e]
		9-Methyladenine			
C-8 H	−106.0378	−9.1924	−119.04	10.34	2.45
C-8 H, N-1 H⁺	−106.2276	−8.7434		16.00	
C-8 NH₂	−118.5624	−10.0013	−168.34	9.75	3.72
C-8 NH₂, N-1 H⁺	−118.8308	−9.6310		14.88	
C-8 Cl	−121.5268	−9.2734	−160.94	10.59	0.40
C-8 Cl, N-1 H⁺	−121.7834	−8.8912		15.83	
		9-Methylguanine			
C-8 H	−124.5791	−9.6517	−175.05	9.50	7.36
C-8 H, N-7 H⁺	−124.8582	−9.2920		15.49	
C-8 NH₂	−137.0369	−10.3939	−197.44	8.83	7.28
C-8 NH₂, N-7 H⁺	−137.3517	−10.0700		14.89	
C-8 Cl	−140.0082	−9.6728	−177.87	9.75	8.61
C-8 Cl, N-7 H⁺	−140.2918	−9.3177		15.48	

[a] 1 au = 627 kcal/mol = 27.2 eV. [b] Including nuclear repulsions. [c] $E_{total\ (protonated)}$ − $E_{total\ (neutral)}$. [d] Negative of the energy of the highest occupied molecular orbital, ionization potential according to Koopmans' theorem. Point dipole + hybrid contribution as in ref 4.

Table IV. Experimental Results[a]

Molecule	pK_a[b]	λ_{max}, nm, pH 1	λ_{max}, nm, neutral	Hydrolysis rates[c]
Adenosine	3.62	257	259.5	Standard
8-Bromoadenosine	4.02	262	264	Fast
8-Methoxyadenosine	3.85	261	259	Slow
Guanosine	2.30	257.5	252.5	Standard
8-Bromoguanosine	0.20	261.6	270	Fast
8-Aminoguanosine	4.50	252	258.5	Slow
		289	293	

[a] Adenosine results from ref 6; guanosine results from this laboratory. [b] For deprotonation of protonated base. [c] Relative rates compared to the unsubstituted nucleoside hydrolysis rate at the same temperature and pH values.

Also, the 8-amino model increases the electron density; the 8-chloro decreases it at N-9 compared to the unsubstituted molecule both in the neutral and protonated structures. Obviously, no quantitative correlations are feasible with simple theory, but the qualitative comparisons are quite satisfactory for these adenines.

Guanines. The variation in dipole moments upon C-8 substitution is predicted to be very much smaller in the guanines than in the adenines. The 8-amino group causes a slight decrease (to 7.28 D), the 8-chloro an increase (to 8.61 D), compared to the moment of 9-methylguanine (7.36 D). Again, the amino group donates electrons to most of the ring; the chlorine, on the other hand, withdraws from the ring. π charges again show alternating electron density enhancement or decrease going around the ring compared to the unsubstituted substrate. The chlorine and amino substituents show opposite trends to each other with respect to this effect.

Now the glycosidic hydrolysis of guanosine nucleosides will be considered. Assuming N-7 protonation of the base, an increased electron density would imply easier protonation at this atom. The C-8 amino group places the largest electron density at N-7, the C-chloro the smallest. The preliminary experimental data confirm this trend well as shown in Table IV.[7] There seem to be vast differences among the pK_a's of the molecules studied. Another prediction from theory is that the second protonation pK_a's should be closer to each other. This is currently being investigated.

N-9-Methyladenine

N-9-Methylguanine
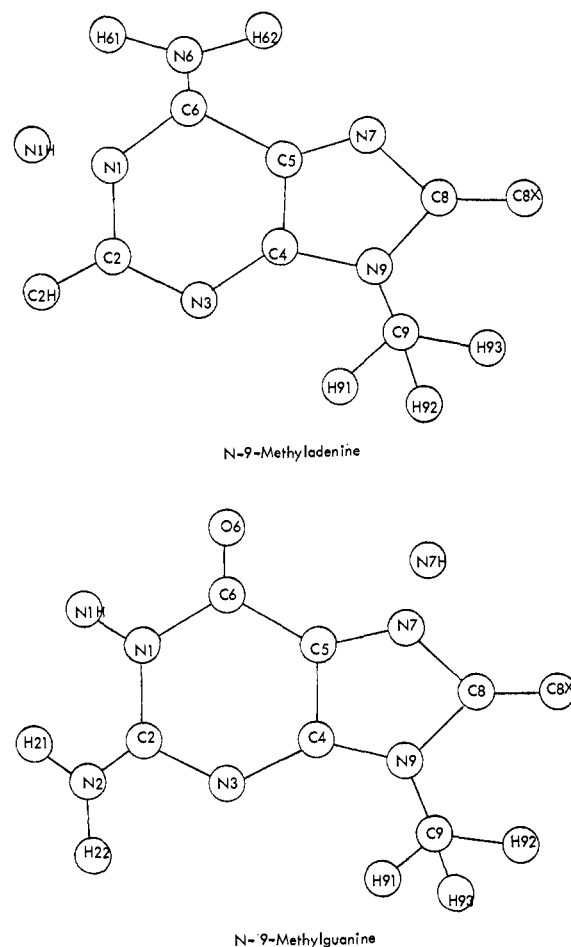
Figure 1.

The rate of hydrolysis of guanosine nucleosides follows the relative order: 8-bromoguanosine faster than guanosine which in turn is faster than 8-aminoguanosine. In the neutral substrates indeed the C-8 amino places the largest negative charge at N-9 (hence, supposedly reducing the rate), the C-8 chloro the smallest. Among protonated substrates the C-8 amino group still places the largest negative charge onto N-9, with 8-H and 8-Cl substituents being roughly equal. That N-7 protonated guanosine undergoes faster hydrolysis

than does the neutral molecule has been substantiated by Zoltewicz and coworkers.[13]

On the Sites of Base Protonation. Considerable interest has been evident in the definition of the sites of proton and metal ion attachment in adenine and guanine. This interest has engulfed experimentalists as well as theoreticians. Experimental tabulations of the values and probable sites of attachment are available[14] and nmr assignments of the sites in strong acid were recently given.[15]

Theoretically, one can consider the problem with varying degrees of sophistication. As a first approximation, the net charges at the heteroatoms may be related to their basicities. The first qualification which must be made is that solvation is unfortunately not accounted for by most current calculations. Thus the results can only be applied to gas-phase properties. Nonetheless, some theories have correlated with solution results quite well and thus are of interest. A second theory employed a perturbation theory to the problem.[16] The most promising theory to date involves the calculation of the potential of a proton approaching the base at various positions and associating the most favorable approach with the most favorable potential.[17] While the last method is quite rigorous, it does not account for nonbonding interactions and supposedly the ultimate answer lies in a total quantum chemical (*ab initio*) calculation of the entire surface.

In the present study the simplest method will be examined first; then yet another qualitative correlation will be proposed which while requiring no further calculations (past that of the density matrix) still gives respectable agreement with experimental data.

Based on net charges only N-1 of adenine is the most likely site for protonation in all three models studied; in fact, the pK_a's for the deprotonation of the adeninium cation are similar for the unsubstituted, 8-methoxy-, and 8-bromoadenosines (see Table IV). The calculations assign essentially identical N-1 charges for the three molecules studied. In 8-aminoadenine N-7 appears to be a close competitor for the proton. While the N-1 site has been suggested as the site of first protonation for the unsubstituted compound,[14,15] in the substituted molecules this site is as yet undefined. The site of the second proton has been suggested to be the N-7 atom.[15] The calculations indicate that upon protonation at N-1, the N-6 amino nitrogen is the primary target based on net charges. On the purine ring itself, however, N-7 indeed is the preferred site of second protonation (*i.e.*, among N-9, N-1, N-3, and N-7).

In guanines oxygen has the largest negative charge (−0.385), followed by N-3 (−0.330) and the N-2 amino nitrogen (−0.250). Experimentally, N-7 appears to be the site of attachment of the first proton, followed by N-3 and then O-6. Parenthetically, the perturbation approach also failed on guanine.[16] Of the ring nitro-

gens N-3 appears to be the most negative atom, as was also found by Glassman, *et al.*[16] While Ca(II) and Mg-(II) probably bind to the pyrimidine ring portion at or very near N-3[18] (as observed by the nearly equal diamagnetic shifts induced in the N-1 and N-2 protons upon binding of these metals), transition metals bind at the N-7 atom of guanine derivatives.[19] A serious question arises. Do *all* theories fail on this count? Is there perhaps some special and specific solvation of the N-3 site in aqueous medium which solvation hinders protonation? It is disheartening and not totally convincing to accept that the theories fail. In another study, for example, it was pointed out that upon placing a proton on N-3 or N-7, the electronic structure of the N-7 protonated guanine was supported by experimental evidence, while N-3 protonation led to some fundamental differences in electron density trends.[20] Thus, the theories appear to have some internal consistencies. Perhaps a reconsideration of the problem of the approach of the proton is in order. It is well accepted that the nucleic acid bases are aromatic rings. Because of this aromaticity, protonation is most likely to occur in the σ plane (as evidenced by the solid-state data employed in this study) so as not to disturb the aromaticity significantly. Since the present model molecules are all essentially planar, a σ–π separation is simple. A search was made for characteristics in the σ properties which could be correlated with the experimentally suggested sites of protonation. One can then arbitrarily decide that approach in the σ plane should be correlated with such a property, whereas approach in the π cloud (above the σ plane) correlates with π characteristics or net charges. It should also be pointed out that there is considerable evidence indicating that the substituents, such as the C-2 amino group and the C-6 oxygen, are part of the aromatic or conjugated system; therefore, protonation on these should also occur in the σ plane. Based on this analysis of the problem two quantities are proposed as qualitative indices of the preferred sites of protonation, both associated with the σ portion of the diagonal elements of the density matrix (*i.e.*, orbital electron densities). One is the total σ-electron density on the atom considered (it should be recalled that in the CNDO theory C, N, and O are assigned basis sets consisting of one 2s and three 2p orbitals of which in a planar system one 2p orbital participates in the aromatic or conjugated π-type molecular orbital); the other quantity is the per cent 2s character in the total σ-electron density. Qualitatively, the larger the total σ-electron density or the larger the per cent 2s character in the σ density, the more likely is the atom to be the preferred site of protonation. Both quantities are well applicable to the same type of atom but the per cent 2s criterion is, in principle, applicable to a comparison of any two atoms. Both quantities lead to more consistent predictions than do the net atomic charges. Table V provides a comparison of these various quantities at sites of interest in both adenines and guanines here reported on. On guanines the results indicate that with the σ criteria here proposed N-7 emerges as a close contender for the proton with N-3 based on the

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per cent 2s results and wins clearly based on total σ-density values (taking into account that the nuclear charge on O is $+8$ and on N is $+7$, of which the core electrons neutralize two units). Upon first base protonation at N-7 the per cent 2s results imply preference for N-3 for second protonation. Introducing the C-8 substituents always leads to preference for N-7 for first protonation based on the per cent 2s values. Also striking is the result assigning third preference for protonation to O-6, again as suggested by experiment.[15]

Table V. Quantities Correlated to Sites of Protonation

Molecule	Atom	Net charge[a]	σ-Electron density[b]	%2s in σ[c]
9-Methylguanine	N-1	-0.220	3.508	35.1
	N-3 (B)[d]	-0.330	3.940	36.9
	N-7 (A)[d]	-0.158	4.028	36.7
	N-9	-0.100	3.479	34.0
	N-2	-0.250	3.422	35.0
	O-6 (C)[d]	-0.385	4.921	35.3
9-Methylguanine·N-7 H$^+$	N-1	-0.250	3.511	33.8
	N-3	-0.311	3.880	37.8
	N-9	-0.038	3.516	33.6
	N-2	-0.243	3.446	33.7
	O-6	-0.302	4.939	34.7
8-Chloro-9-methylguanine	N-1	-0.219	3.508	35.1
	N-3	-0.328	3.934	36.9
	N-7	-0.135	4.026	36.7
	N-9	-0.093	3.471	34.1
	N-2	-0.249	3.424	35.0
	O-6	-0.376	4.922	35.3
8-Amino-9-methylguanine	N-1	-0.220	3.510	35.2
	N-3	-0.325	3.942	36.8
	N-7	-0.238	3.977	37.1
	N-9	-0.122	3.469	34.1
	N-2	-0.252	3.421	35.0
	N-8	-0.280	3.365	36.2
	O-6	-0.388	4.920	35.3
9-Methyladenine	N-1 (A)[d]	-0.289	4.016	36.6
	N-3 (C)[d]	-0.242	4.026	36.1
	N-7 (B)[d]	-0.209	4.027	36.9
	N-9	-0.095	3.467	33.8
	N-6	-0.247	3.403	34.1
9-Methyladenine·N-1 H$^+$	N-3	-0.161	4.022	36.0
	N-7	-0.172	3.966	37.7
	N-9	-0.045	3.524	33.1
	N-6	-0.210	3.454	32.9
8-Chloro-9-methyladenine	N-1	-0.285	4.013	36.6
	N-3	-0.238	4.019	36.1
	N-7	-0.187	4.017	36.9
	N-9	-0.087	3.458	33.9
	N-6	-0.245	3.406	34.1
8-Amino-9-methyladenine	N-1	-0.286	4.022	36.5
	N-3	-0.238	4.029	36.0
	N-7	-0.281	3.962	37.3
	N-9	-0.125	3.463	33.9
	N-6	-0.248	3.400	34.1
	N-8	-0.275	3.370	36.1

[a] Total atomic electron density minus nuclear charge. [b] Sum of the diagonal density matrix elements on the atom except for the one partaking in the π system. [c] Per cent 2s contribution in quantity defined in footnote b. [d] Experimentally suggested order of preferences for protonation: A, most preferred (first site); B, second site; C, third site of protonation data in strong acid as in ref 15.

It was of course of interest to test these σ criteria on the remaining bases and detailed results are given in Table V for adenine. In 9-methyladenine according to the σ criteria N-7 wins over N-1, the latter being a close contender. There is significant evidence that many metal ions bind to N-7, including Mn(II) and others.[21] Assuming N-1 protonation, N-7 becomes the most likely candidate for the site of second protonation, followed by N-3 based on per cent 2s values again, in pleasing accord with experiment. Why does N-1 pick up the proton whereas metals prefer N-7? This question is indeed difficult to answer but attempts will be made in the future to do so by performing total energy chemical quality calculations.

Since CNDO/2 results are available for cytosine and thymine as well,[5b] the σ criteria were also applied to these systems. On cytosine the oxygen atom carries the largest negative charge but both the total σ and per cent 2s values criteria imply N-3 to be the site of first protonation as accepted experimentally. In thymine among the N-1, N-3, O-2, and O-4 atoms, the net negative charge is largest at O-4 as are the total σ-electron density and the per cent 2s character in the total σ values. This atom is the accepted site of first protonation in thymine. The criteria here suggested are generally much more successful than a consideration of net atomic charges alone. Particularly gratifying is the success of the method on molecules containing different types of heteroatoms. In these, the oxygen invariably carries the largest negative charge but is, more often than not, not the site of first protonation. Incidentally, on cytosine, for example, one finds that the criteria here proposed also perform with MINDO wave functions, which usually lead to incorrect predictions based on net charges only.

These correlations are interesting and enlightening and promise to be applicable to any heteroaromatic system posing such an uncertainty in protonation sites. The reason for this apparent success is not obvious to the author. Perhaps the implication is that the larger the per cent 2s character available the stronger will be the incipient hybrid orbital once the proton attaches itself. The correlation of stronger bonds resulting from an increased per cent 2s character in the contributing hybrid is of course a well-accepted phenomenon in organic chemistry.

Calculations of Some Other Ground-State Properties. Energy Properties. The first ionization potential of a closed shell system can be associated with the negative of the highest occupied molecular orbital energy level according to Koopmans' theorem. All ionization potentials determined this way are found to be associated with π-type molecular orbitals for both neutral and protonated substrates in this study. The chlorine atom raises and the amino group lowers the ionization potential of both 9-methyladenine and 9-methylguanine. As suggested in the previous contribution, the ionization potential increases some 50% upon protonation[5] (see Table IV).

Since the calculations apply, strictly speaking, to the gas phase, the energy differences between neutral and protonated substrates could be looked at as proton affinities. By definition, adding a proton to a substrate will lead to energy lowering. The greater such energy lowering is, the larger the proton affinity of the substrate and the larger the gas-phase basicity of a particular site. The present method undoubtedly is incapable

(21) M. Cohn and T. R. Hughes, *J. Biol. Chem.*, **237**, 176 (1962); H. Sternlicht, R. G. Schulman, and E. W. Anderson, *J. Chem. Phys.*, **41**, 3133 (1965).

of predicting such effects quantitatively (at least with the parametrization employed). The suggestions are, however, interesting and suggest some experiments. Guanine apparently becomes the stronger base in the gas phase, whereas in water adenine is protonated at some pH 1.5 unit higher than guanine. Among the adenine derivatives both C-8 amino and chlorine substituents lead to enhanced proton affinity compared to C-8 H. Among the guanines the amino group enhances the proton affinity; the chlorine changes very little if compared to the unsubstituted compound. Further theoretical work on this subject using *ab initio* methods is desirable. It should be noted that there are no gas-phase proton affinity data available on these substrates in the literature. As a rough reference, one could point to the -151 kcal/mol value for H_2O (going to H_3O^+) and -203 kcal/mol for NH_3 (going to NH_4^+).[22]

Also of interest in these molecules is the interaction of the C-8 substituent with the purine ring. From the previous discussion it is obvious that the charges (diagonal density matrix elements) are significantly influenced. The off-diagonal density matrix elements also change upon C-8 substitution. Because of the planarity of the systems the π-bond orders can be discerned most easily. These are matrix elements (between directly bonded atoms) perpendicular to the molecular plane. Both the 8-chloro and 8-amino substituents affect π-bond orders only in the imidazole portion of the adenine ring. The π-bond orders in the pyrimidine ring appear larger than those in the imidazole excepting the C-8-N-7 π-bond order. π-Bond orders to N-9 are always fairly small. In the adenine derivatives the N-6 atom always has a significant π-bond order to C-6, always larger than the smallest in-ring π-bond order. The C-8 substituent also has π-bond order to C-8, the amino one being much larger than the chloro. The chlorine incidentally has 3p-type π-bond order as well as a small 3d-type π bond with the C-8 atom. The chlorine 3d orbitals are active participants in bonding to the adenine ring. Protonation of adenine at N-1 brings on quantitative π-bond order changes throughout the ring system.

Among guanine derivatives, C-8 substitution again alters the imidazole ring predominantly. The N-7–C-8 π-bond order is reduced by both the chloro and amino substituents. Again all substituents (O-6, N-6, Cl-8, or N-8) participate in the conjugated system as evidenced by a sizable π-bond order to N-2 and C-8 substituents and less than unit π-bond order to O-6. There is evidence again for strong 3d orbital participation in the C-8–Cl π bond. N-7 protonation of the guanine base again affects π-bond orders throughout the ring.

Lastly, one can remark that the methyl group invariably withdraws electrons from the π system of the purine ring (the π-electron density on the purine ring lacks perhaps 0.02–0.08 electron on the average), but, in terms of total electron density, the methyl group donates to the ring (*i.e.*, the methyl is a σ donor) perhaps 0.08–0.1 electron.

Calculation of Excited-State Properties. It has been suggested by many theoreticians that any calculations

Table VI. Calculations of Guanine and Adenine Singlet States[a]

Molecule	λ_{calcd}, nm	f_{calcd}[b]	λ_{obsd}, nm	ϵ_{obsd}, $\times$ 10^3	Polarization[c] x	y
Guanine	305.2	0.21	275	8.1	0.94	0.34
	244.7	0.07	246	10.7	0.76	−0.65
	235.0	0.05	224	3.6	0.24	−0.97
	198.1	0.15	196	22.1	0.70	−0.71
	188.4	0.07			−0.32	0.95
	179.1	0.14			0.99	−0.14
8-Aminoguanine	318.8	0.22	295	12.4	0.94	0.34
	252.4	0.10	255	7.4	0.78	−0.62
	244.9	0.06			0.25	−0.97
	201.9	0.05			0.67	−0.74
	194.2	0.10			−0.60	0.80
	180.5	0.15			0.96	−0.26
Adenine	278.2	0.01			−0.31	−0.95
	258.2	0.20	260.5	13.4	0.91	−0.41
	220.3	0.090	225.0	2.6	−0.59	−0.81
	205.5	0.32	207.0	23.2	−0.59	−0.81
	197.2	0.32	190.0	12.9	0.85	−0.53
8-Aminoadenine	284.3	0.03			0.08	−0.99
	268.9	0.24			0.97	−0.25
	226.9	0.04			−0.65	−0.76
	207.0	0.34			−0.48	−0.88
	198.0	0.31			0.90	−0.45

[a] Including the 30 lowest singly excited configurations; all transitions are π-π^*. [b] Oscillator strengths. [c] The molecules are constructed in the x-y plane with the C-4-C-5 bond along the y axis, C-5 having the more positive y value.

concerning excited-state properties (such as that of the ultraviolet spectrum) must include configuration interaction (CI). Recently Hug and Tinoco presented extensive calculations on the uv spectra of DNA bases.[23] These authors employed a modified CNDO parametrization and included 120 singly excited configurations in their CI calculations.

In the present work the effect of the C-8 amino substituent on the ultraviolet (uv) spectrum of adenine and guanine will be considered. The present calculations employed a much more limited number of singly excited configurations (30) in the CI treatment (mainly for purposes of economy) with the parametrization due to Del Bene and Jaffe.[24] This latter method was quite successful in predicting the uv spectral properties of pyridine and diazines and therefore was thought to be applicable to the problem at hand.

There are experimental data available on the uv spectra of guanine and 8-methylaminoguanine from this laboratory and on adenine[25] and 8-methoxyadenine[6] from other laboratories.

Adenines. It should be mentioned that the Del Bene–Jaffe (D–J) parametrization gives similar net atomic charge densities but lower ionization potentials than the original CNDO/2 parametrization[4] (Koopmans' theorem ionization potentials are 8.62 eV for adenine and 8.31 eV for C-8 aminoadenine, with the D–J method).

Table VI lists the predicted longest wavelength uv absorption positions along with their oscillator strengths and directions of polarization, together with some ex-

(22) (a) See A. C. Hopkinson, N. K. Holbrook, K. Yates, and I. G. Csizmadia, *J. Chem. Phys.*, **49**, 3596 (1968), for early calculations; (b) see E. M. Arnett, *Accounts Chem. Res.*, **6**, 404 (1973), for a recent review of gas-phase proton transfers.

(23) W. Hug and I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **95**, 2803 (1973); **96**, 665 (1974).
(24) J. Del Bene and H. H. Jaffe, *J. Chem. Phys.*, **48**, 1807 (1968). The program is available from the Quantum Chemistry Program Exchange, No. 174.
(25) D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, **1**, 193 (1963).

perimental data. In adenine the predicted wavelengths are always within 7 nm of the experimental peaks. Electron donation by the C-8 amino group shifts the longest wavelength peaks to longer wavelengths according to theory and in accord with the data on the related 8-methoxyadenine.[6] A detailed analysis of the configuration interaction matrix indicates that all those states quoted are essentially totally transitions.

Guanines. Table VI lists some results on guanine and 8-aminoguanine. The amino group again red shifts the longest wavelength absorption. The agreement between predicted and experimental results is not as good as in adenines, yet infinitely better than without CI. All the listed absorptions are predicted to be of the $\pi \rightarrow \pi^*$ type transitions again principally. In defense of the method one should emphasize that even subtle features such as the long wavelength shoulder in adenine (near 275 nm) are implied by the calculations.

This author feels that even the limited CI calculation reproduces the qualitative features of the uv spectra respectably and is thus of considerable value when one wants to interpret the spectra of molecules for which the transitions are not well defined.

Conclusions

Apparently ground-state trends are better predicted with the CNDO/2 method than excited-state properties. While use has already been made of the present series of substituents by others as well as the author's group, these series could be employed in a variety of other ways also. For example, in studying the theoretical origins of hydrogen bonding and stacking interactions one could compare the thermodynamics of self-association of adenosine and 8-bromoadenosine (or corresponding nucleotides). 8-Bromoadenine appears to have a very small dipole moment compared to adenine, whereas the C–Br bond, of course, is much more polarizable than the C–H bond. This would provide a handle on sorting out dipole–dipole interactions from induced dipole types (such as dispersion and dipole–induced dipole type), the latter mainly influenced by polarizability.

Considerable experimental work remains to be performed on the definition of proton and metal binding sites in the DNA bases. The theoretical definition is still lacking also, since there is not yet any unified theory correctly predicting protonation and metal binding sites on *all* bases simultaneously.

Finally, with the advent of volatile purine derivatives it would be fascinating to see whether or not the gas-phase proton affinities of these molecules follow the order of basicities found in aqueous medium.

Importance of Solvent Cohesion and Structure in Solvent Effects on Binding Site Probes

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Abstract: The solvent effect on the corrected emission frequency of the fluorescent probe, 2-toluidinonaphthalene-6-sulfonate (TNS), and on the free energy of tautomerization of the dye probe, 4-phenylazo-1-naphthol-2,4'-disulfonate (PND), has been studied using a wide range of nonpolar, dipolar aprotic, and hydrogen-bonding solvents. There is good correlation between $\bar{\nu}_{max}$ for TNS and ΔG° for PND in hydrogen-bonding solvents, showing that the transition energy and free energy of the respective probes are subject to similar solvation effects. $\bar{\nu}_{max}$ for TNS in solvents covering a range of dielectric constants from 2.2 to 182.4 is correlated poorly by ϵ and by several empirical polarity scales but is correlated well by the solubility parameter, δ. ΔG° for the tautomerization of PND is also correlated by δ and by the solvent surface tension in water and low-molecular-weight amides and alcohols. The results indicate that specific polar or hydrogen-bonding interactions between solvent and probe make a smaller contribution to the solvent effects than the work of cavity formation in the solvents. It is suggested that the spectral changes that accompany binding to a macromolecule from aqueous solution result as much from loss of aqueous solvation characteristic of structured bulk water as from changes in polarity of the microenvironment.

The purpose of this study was to assess the importance of various solvent parameters that contribute to the effect of solvents on binding site probes and to solvatochromic effects in general. Dyes and fluorescent probes are commonly used to investigate the binding of organic ions to macromolecules and to gain insight into the nature of binding sites. Their utility results from the fact that the absorption curves, emission frequencies, or fluorescent quantum yields are significantly different in the bound state from the properties in aqueous solution. Since the spectral changes that accompany binding can be duplicated by transfer to organic solvents or by addition of polar organic solvents to aqueous solutions of the probes, efforts have