Theoretical Investigation of Adenine Radicals Generated in Irradiated DNA Components

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Density functional theory is used to investigate various hydrogenated, dehydrogenated, and hydroxylated radicals formed upon irradiation of adenine. The relative energies, geometries, and hyperfine coupling constants of possible radicals are discussed. The hyperfine couplings are compared to experimental data in order to confirm the assignment of particular couplings to specific radicals. The C8-hydrogenated and the N9-dehydrogenated radicals are the lowest energy radicals in their respective classes. Several degrees of geometrical distortion are exhibited in the adenine radicals. For example, the C2- and C8-hydrogenated radicals are only slightly distorted, whereas the C4- and the C5-hydrogenated radicals are severely puckered. Several N1-protonated radicals are also examined. In particular, the differences in the HFCCs for the various protonated forms of the C2- and the C8-hydrogenated radicals are well described by an appropriate level of density functional theory.

Introduction

The effects of radiation on DNA have attracted much interest in the last few decades. Ionizing radiation can cause drastic effects such as genetic mutations or the death of a living entity. The majority of radiation damage occurs via the formation of base and sugar radicals which subsequently undergo reactions that lead to DNA−protein cross links[1−3] or single-strand breaks.[4] These radicals can be characterized through the determination of their hyperfine coupling constants (HFCCs) by electron spin resonance (ESR). The examination of full DNA through ESR techniques, however, is very difficult, since the spectrum is composed of many overlapping components that are difficult to assign to particular base or sugar radicals. Thus, experimental studies have evolved around single-crystal models in order to determine which radicals are formed, the mechanism for formation, and how they subsequently react.[5] However, even with single crystals, more sophisticated experimental techniques, such as electron nuclear double resonance (ENDOR), must be implemented to aid in the assignment of the spectra to the appropriate radical products.
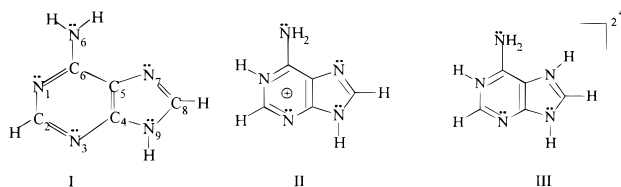
In addition to experimental studies on DNA radicals, a few theoretical investigations have appeared in the literature in which the HFCCs are examined. The first studies investigated the radicals formed in the sugar moiety of DNA at the Hartree−Fock (HF) level.[6,7] However, it is well-known that HFCCs calculated at this level of theory are overestimated by at least a factor of 2.[8] More recently, investigations of the radiation products in cytosine,[9] thymine/1-methylthymine/uracil,[10] and the sugar group present in full DNA[11] have been undertaken through the use of density functional theory (DFT). DFT is an appropriate method for this problem since it not only yields reliable HFCCs but it is also computationally efficient for models of the size considered when investigating biological molecules. The promising results obtained for the pyrimidines and the sugar group prompted the current investigation of adenine, as well as a study on guanine.[12]

Even though new experimental data is constantly appearing in the literature, the various adenine derivatives have been investigated experimentally to a lesser extent than the derivatives of any other base. This is due to the fact that early ESR investigations indicated that thymine and guanine are affected by radiation to a greater extent than the other bases, forming thymine anions and guanine cations. Later, it was also discovered that cytosine is in close competition with thymine for electrons generated during irradiation. In addition, due to solubility problems, few single-crystal studies on adenine derivatives have been performed since they are extremely difficult to prepare.[5] However, it is important to learn more about the radiation damage in adenine samples in order to better understand the effects of radiation on full DNA, and theory may be able to play an important role. Hence, the present study uses DFT to investigate the various radicals formed when adenine is subjected to radiation.

Theoretical Details

All geometries were optimized using Becke's three-parameter exchange functional (B3)[13] in combination with Lee, Yang, and Parr's correlation functional (LYP)[14] and Pople's 6-31G(d,p) basis set.[15] Frequency analyses were performed to ensure that stationary points were local minima and to correct the relative energies for the zero-point vibrational energy (ZPE). Subsequent single-point calculations were performed at the B3LYP level with Pople's 6-311G(2df,p) basis set[15] to obtain relative energies, spin densities, and dipole moments of the global minima. These calculations were accomplished using Gaussian 94.[16] The hyperfine coupling constants were obtained with Perdew and Wang's nonlocal exchange (PW),[17] Perdew's nonlocal correlation functional (P86),[18] and Pople's 6-311G-(2d,p) basis[15] through the deMon program.[19] The units of gauss

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Figure 1. The structures of adenine (**I**), N1 singly protonated adenine (**II**), and N1, N7 doubly protonated adenine (**III**).

(G), which can be converted to megahertz (MHz) by using a factor of 2.8025, are used throughout.

Many good reviews on the accurate calculation of HFCCs exist in the literature,[20,21] and thus, the details of theoretical requirements and methodology will not be reviewed here. However, to provide a fair and accurate interpretation of the results presented within, a few points should be addressed. Accurate isotropic HFCCs require both a good description of electron correlation and a well-defined basis set, whereas satisfactory anisotropic HFCCs can be obtained with almost any theoretical method and basis set, provided the structure is qualitatively correct. Thus, comparison of anisotropic hyperfine tensors can be used as an accurate guide to identify radical sites even when less satisfactory agreement is obtained for the isotropic component.

Results and Discussion

The chemical numbering of adenine used throughout this study is indicated in structure **I** of Figure 1. Experimentally, various crystalline samples have been used in order to investigate the effects of radiation on adenine. In some crystal structures, the parent adenine molecule is protonated at the N1 position (Figure 1, structure **II**) or doubly protonated at the N1 and N7 positions (Figure 1, structure **III**). It is important to study both radicals generated from the neutral molecule as well as those formed from various protonated parent molecules in order to better understand the dependence of radical formation on the environment. In particular, the importance of understanding proton transfer in DNA has been discussed. Nelson et al.[22] have suggested that experimental studies on different crystalline environments will aid in gaining a better understanding of the environmental effects on protonation and deprotonation behaviors in adenine molecules. This is of interest when transferring results obtained from single-crystal studies to full DNA samples. The present study mainly focuses on the radicals generated from the neutral adenine molecule. However, some of the radicals that have been proposed to be generated from protonated forms of adenine are also discussed when experimental data is available.

Adenine Anion and Cation. The adiabatic electron affinity (EA) and ionization potential (IP) of adenine are displayed in Table 1, along with the relative energies of other possible radicals. The spin density distributions in the anion and cation are displayed in Table 2. In a review of ab initio studies on DNA, Colson and Sevilla[23] report a negative value for the EA of adenine (−7.2 kcal/mol). This value is an estimate for the adiabatic EA, which was obtained by scaling the vertical EAs calculated at the ROHF/D95v//ROHF/6-31G* level.[24] Direct calculation of the adiabatic EA through the use of DFT and correcting for the zero-point vibrational energy, on the other hand, yields a value of −20.8 kcal/mol. The molecular geometry of the adenine anion furthermore indicates that considerable distortion occurs upon addition of an electron. The pyrimidine (six-membered) ring remains planar with the amino group located out of the molecular plane. The imidazole (five-

TABLE 1: Relative Energies (kcal/mol) of Adenine Radicals

radical	relative energy
IP	182.3
EA	−20.8
C8-hydrogenated	0
C2-hydrogenated	8.7
N7-hydrogenated	14.2
N3-hydrogenated	15.7
N1-hydrogenated	16.1
C5-hydrogenated	18.2
C4-hydrogenated	23.0
N9-dehydrogenated	0.0
N6-dehydrogenated	2.7
C2-dehydrogenated	9.2
C8-dehydrogenated	16.9
C8-hydroxylated	0
C2-hydroxylated	12.4
C4-hydroxylated	21.0
C5-hydroxylated	21.5

membered) ring, on the other hand, is puckered at the C8 position, and the hydrogen attached to N9 is also located out of the molecular plane. The puckering at the C8 position leads to a concentration of the spin density on this atom (0.43). In the pyrimidine ring, on the other hand, the spin density is distributed throughout the ring with the majority located on C6 (0.25), C2 (0.18), and N3 (0.09).

The adenine anion has not been proposed to exist as a radiation product in experiments to date. However, due to the complexity of experimental spectra, it is of interest to calculate the HFCCs for this radical, and the results are displayed in Table 3. From the results, it can be seen that a large isotropic coupling (10.0 G) would be expected for the C8 hydrogen in this radical, as well as a small coupling (1.2 G) for N9−H. Both of these couplings are due to the large amount of spin density at C8. In addition, considerable couplings would be predicted for C2−H (−5.3 G) and the two amino hydrogens (4.1 G).

The puckered geometry obtained for the anion in the present study may not be possible in the crystal due to the hydrogen-bonding structure. For this reason, the C_s geometry of the anion was obtained through a constrained optimization. From B3LYP/6-311G(2df,p) single-point calculations on the B3LYP/6-31G-(d,p) optimized geometries, it was determined that the planar anion was predicted to be 4.7 kcal/mol higher in energy than the nonplanar radical anion. The spin density and HFCCs for the planar anion are displayed in Tables 2 and 3 under the heading C_s anion. The magnitude of the spin density on C2, N3, and C6 in the planar radical is larger than that in the nonplanar form, whereas the spin density on C8 is slightly smaller in magnitude for the planar radical. The major difference between the couplings in the two forms of the adenine anion is the sign of the C8−H isotropic component. Comparison of the calculated HFCCs of the planar and nonplanar radical anion with future experimental spectra will be useful in order to eliminate the possibility of the anion being formed but its spectra not being detected.

The adiabatic IP, corrected for the zero-point vibrational energy, was calculated to be 182.3 kcal/mol in the present study. This is in good agreement with the experimental value of 190.4 kcal/mol[25] and slightly smaller than the value obtained at the MP2/6-31+G(d)//MP2/6-31G(d) level (199.6 kcal/mol).[23] Unlike the anion, the adenine cation remains planar, including the amino group, upon formation. The spin density in the cation resides primarily on N3 (0.19), C5 (0.20), N6 (0.27), and C8 (0.18).

The adenine cation has been proposed to be formed upon irradiation of adenine hydrochloride hemihydrate (Ad·HCl·

TABLE 2: Spin Density Distributions in Adenine Radicals

radical	N1	C2	N3	C4	C5	C6	N6	N7	C8	N9
anion		0.18	0.09			0.25			0.43	
C_s anion		0.28	0.13			0.30			0.39	
cation			0.19		0.20		0.27		0.18	
N1-hydrogenated			0.12	0.15		0.60			0.10	
C2-hydrogenated	0.20		0.43	−0.10	0.33				0.23	
N3-hydrogenated		0.49	0.11			0.32				
C_s N3-hydrogenated		0.53	0.12			0.26	0.10		0.13	
C4-hydrogenated	0.14		0.10		0.44				0.20	0.12
C5-hydrogenated		0.44	−0.10	0.31		0.23	0.10			
N7-hydrogenated								0.11	0.64	0.10
C8-hydrogenated		0.18		0.10		0.16	0.10	0.49		0.09
C2-dehydrogenated		0.84								
N6-dehydrogenated	0.17	−0.10	0.23		0.21	−0.15	0.59		0.14	
C8-dehydrogenated									0.83	
N9-dehydrogenated		0.18		0.15		0.10	0.16	0.27	0.10	
C2-hydroxylated	0.17		0.43	−0.10	0.34				0.23	
C4-hydroxylated					0.46				0.24	
C5-hydroxylated		0.38		0.32		0.23	0.12			
C8-hydroxylated		0.18		0.12		0.15	0.11	0.46		

TABLE 3: Calculated HFCCs (G) in the Adenine Anion and Cation Radicals

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
anion	C2−H	−5.3	−3.0	−0.2	3.2
	N6−H	4.1	−0.8	−1.0	0.9
	N6−H	4.1	−0.8	−0.5	1.3
	C8−H	10.0	−5.6	−0.4	5.9
	N9−H	1.2	−1.5	−1.2	2.7
C_s anion	C2−H	−7.7	−4.4	0.0	4.4
	N6−H	−1.6	−1.1	−0.6	1.6
	N6−H	−1.3	−1.4	−0.6	2.0
	C8−H	−10.5	−6.2	0.9	5.4
	N9−H	−1.8	−1.3	−0.9	2.3
cation	N6−H	−6.3	−4.7	−1.5	6.2
	N6−H	−6.6	−5.2	−1.4	6.6
	C8−H	−5.5	−3.0	−0.7	3.7

$^1/_2$H$_2$O).[26] In this crystal structure, the parent molecule is protonated at the N1 position, and it was proposed that an observed radical was formed via removal of a hydrogen atom from the N1 position. This is structurally equivalent to the cation of the neutral adenine molecule. The spin density distribution in this radical was determined to be located primarily on N6 (0.25) and C8 (0.17/0.21), which is in excellent agreement with the calculated values. The experimental HFCCs obtained for the proposed cation radical (Table 4) consist of two N6−H couplings (−7.0/−7.3 G), which have considerable anisotropy ($T_{XX} = -4.5/-5.2$, $T_{YY} = -1.3/-1.1$, $T_{ZZ} = 5.8/6.3$ G), and one C8−H coupling (−4.9 G). These values are also in excellent agreement with those obtained in the present study (Table 3). In particular, the calculated and experimental anisotropic HFCCs for all three hydrogens are in extraordinary agreement, and the isotropic HFCCs differ by less than 1 G. Thus, our results strongly support the assignment of the experimental spectrum of Ad·HCl·$^1/_2$H$_2$O to the adenine radical cation.

Adenine Hydrogenated Radicals. The ZPE corrected relative energies of various adenine radicals obtained by net hydrogen addition are listed in Table 1. The results indicate that the radical formed by addition of a hydrogen to the C8 position is the lowest energy radical in this class. The C2-hydrogenated radical lies 8.7 kcal/mol higher in energy than the corresponding C8 radical. Radicals formed through addition of a hydrogen to any of the nitrogens are much higher in energy, on average 15.3 kcal/mol above the C8-hydrogenated radical. The C4 and C5 hydrogen addition radicals are the highest in energy by on average 19.3 kcal/mol. The spin densities and

the HFCCs for the various hydrogenated radicals included in the present study are listed in Tables 2 and 5, respectively. The corresponding experimental HFCCs are presented in Table 4.

Nitrogen Hydrogenated Radicals. The N1-hydrogenated radical undergoes minor geometric alterations at the C6 position upon radical formation such that C6 is displaced slightly to one side of the molecule and the amino group rotates to bring the nitrogen and one hydrogen to one side of the plane and the second hydrogen to the opposite side. This distortion forces a large amount of the spin density to be localized on C6 (0.60), with small amounts distributed around the planar parts of the molecule at N3 (0.12), C4 (0.15), and C8 (0.10). As a result of the nonplanar arrangement at the amino group, the calculated HFCCs in this radical consist of two large N6−H isotropic couplings (29.0 and 16.4 G). In addition, two relatively small couplings were obtained for the C8 and N1 hydrogens. This radical has not been assigned in any experimental studies to our knowledge.

The radical formed by hydrogen addition to the N3 position also undergoes significant geometric alterations upon radical formation. The hydrogen that is added to the N3 position is located out of the molecular plane, and the amino group is puckered with both hydrogens displaced out of the plane. Roughly half of the radical's spin density is located on C2 (0.49), with the rest of the spin distributed about the pyrimidine ring (N3 (0.11) and C6 (0.32)). The calculated HFCCs indicate that the large spin density at C2 leads to a significant isotropic C2−H coupling (−12.9 G) that has considerable anisotropy (−7.3, 0.4, 6.9 G). Substantial couplings are also observed for N3−H (15.2 G) due to its location out of the molecular plane, as well as for both of the N6 hydrogens (1.3/−1.5 G) and C8−H (−3.0 G). All the latter couplings have relatively small anisotropies.

The N3-hydrogenated radical has been observed experimentally in various adenine crystals, such as anhydrous deoxyadenosine,[22] adenosine,[27] deoxyadenosine monohydrate,[28] the cocrystals of adenosine and 5-bromouracil,[29] and 9-methyladenosine.[30] The N3 protonated radical observed in adenosine was speculated to have the spin density located primarily on C2 (0.41) with notable amounts located on N3 (0.12) and C8 (0.14). This is in agreement with the calculated results for C2 and N3, which were discussed previously, although the calculations indicate that significant spin density is located on C6 (0.32) rather than on C8. The calculated anisotropic C2−H and C8−H couplings are in good agreement with experiment. The absolute magnitudes of the isotropic HFCCs determined experimentally

TABLE 4: Experimental HFCCs (G) in Adenine Radicals

radical	molecule	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
Cation	adenine·HCl·$^1/_2$H$_2$O[26]	N6−H	−7.0	−4.5	−1.3	5.8
		N6−H	−7.3	−5.2	−1.1	6.3
		C8−H	−4.9	−2.5	−0.4	2.9
C2-hydrogenated	deoxyadenosine monohydrate[28]	C2−H	32.8	−1.5	−0.5	2.0
		C2−H	54.3	−1.4	0.0	1.4
		C8−H	−6.4	−3.5	−0.1	3.6
	deoxyadenosine monohydrate[32]	C2−H (2)	43.7			
	deoxyadenosine monohydrate[33]	C2−H (2)	42.0			
		C8−H	9.5			
	adenosine[31]	C2−H (2)	44.0			
		C8−H	10.0			
	adenosine·5-bromouracil[29]	C2−H (2)	40			
	anhydrous deoxyadenosine[22]	C2−H	38.9			
		C2−H	47.5			
		C8−H	−6.4	−3.4	0.0	3.4
N3-hydrogenated	adenosine[27]	C2−H	10.6	−5.0	−0.7	5.8
		N3−H	3.5	−4.0	0.6	3.4
		C8−H	4.1	−2.1	−0.4	2.5
	deoxyadenosine monohydrate[28]	C2−H	−10.5	−7.4	0.6	6.8
		C8−H	−3.4	−2.1	0.3	1.8
	adenosine·5-bromouracil[29]	C2−H	−10.6	−5.9	0.2	5.7
		N3−H	−3.9	−3.1	−0.9	4.0
		C8−H	−4.4	−2.4	0.3	2.1
	anhydrous deoxyadenosine[22]	C2−H	−10.9	−6.8	0.9	5.9
		C8−H	−4.0	−2.5	0.3	2.2
	9-methyladenine[30]	C2−H	−11.1	−6.6	0.8	5.9
		C8−H	−4.0	−2.4	0.4	2.0
C8-hydrogenated	deoxyadenosine monohydrate[28]	C2−H	−4.8	−2.6	0.0	2.6
		C8−H	36.3	−1.5	−0.4	1.9
		C8−H	41.6	−1.1	−0.7	1.8
	deoxyadenosine monohydrate[33]	C8−H (2)	38.0			
	adenosine[31]	C8−H (2)	39.0			
	anhydrous deoxyadenosine[22]	C2−H	−4.8	−2.7	0.1	2.6
		C8−H	36.7	−1.9	−0.3	2.2
		C8−H	40.9	−1.6	−0.6	2.2
	9-methyladenine[30]	C2−H	−4.6	−2.5	0.1	2.5
		C8−H	38.4	−1.2	−0.4	1.6
		C8−H	41.0	−1.2	−0.4	1.6
N6-dehydrogenated	adenosine[27]	N6−H	12.1	−9.1	1.2	7.9
		C8−H	4.4	−2.1	−0.1	2.3
	adenosine·5-bromouracil[29]	N6−H	−10.1	−11.7	4.7	6.9
	anhydrous deoxyadenosine[22]	N6−H	−11.5	−8.3	−1.2	9.4
		C8−H	−4.0	−2.4	−0.2	2.6

are in qualitative agreement with the calculated values for the C2−H (10.6 G) and C8−H (4.1 G) couplings (calculated results are −12.9 and 3.0 G, respectively). Comparison of the calculated and experimental isotropic C2 and C8 hydrogen couplings assigned in adenosine, as well as comparison with the values obtained in other experiments (Table 4), indicates that the sign of these experimental isotropic couplings should be negative. The major difference between theory and experiment is the value of the N3−H isotropic coupling. Experimentally, a small coupling (3.5 G) was observed for this radical. Theoretically, on the other hand, a large HFCC (15.2 G) was obtained due to the distortion at the N3 position. It is possible that hydrogen bonding in the crystal structure forces the N3 hydrogen to remain in the molecular plane, thus leading to a small isotropic HFCC. The experimental and theoretical anisotropic results are in fair agreement with each other. In addition, no N6−H couplings were detected experimentally. These couplings were calculated to be quite small and should therefore be difficult to detect. Overall, however, fair agreement between theory and experiment was obtained.

The N3-hydrogenated radical was also observed in deoxyadenosine monohydrate.[28] In this crystal, the spin density at C2 was estimated to be 0.37, which is slightly smaller than the calculated value (0.49). In addition, significant spin density

was observed experimentally at C8 (0.13). HFCCs were reported for both the C2 and the C8 hydrogens. The experimental C2−H isotropic value (−10.5 G) is slightly smaller than the calculated value (−12.9 G). However, excellent agreement is obtained for the anisotropic couplings in this crystal as opposed to the values previously discussed for adenosine [−7.4, 0.6, 6.8 G (experimental) and −7.3, 0.4, 6.9 G (calculated)]. The good agreement between the calculated and experimental C8−H couplings also supports the experimental assignment of the N3-hydrogenated radical. Nearly identical C2−H and C8−H couplings were obtained by the same group in crystals of anhydrous deoxyadenosine[22] and 9-methyladenine.[30] In those studies, it was noted by the authors that the N3−H HFCCs are not observed in all of the experimental studies of adenine crystals since very strong signals are required for the detection of this coupling. This further supports our hypothesis that in the crystalline samples the N3 hydrogen is kept in the molecular plane, leading to a small isotropic HFCC.

The final environment in which this radical was isolated involves the cocrystal of adenosine and 5-bromouracil.[29] The spin densities acquired from this sample indicate that a large part of the spin is located on C2 (0.35), as seen in the calculated results, and the rest of the spin density is located over C8 (0.15) and N3 (0.14). From the HFCCs in Table 4, it is evident that

TABLE 5: Calculated HFCCs (G) in Adenine Hydrogenated Radicals

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
N1-hydrogenated	N1−H	−2.1	−2.2	−1.4	3.6
	N6−H	16.4	−1.7	−1.3	3.0
	N6−H	29.0	−1.4	−1.0	2.4
	C8−H	−4.4	−2.5	0.0	2.5
N3-hydrogenated	C2−H	−12.9	−7.3	0.4	6.9
	N3−H	15.2	−2.9	−1.5	4.4
	N6−H	1.3	−2.0	−0.6	2.6
	N6−H	−1.5	−1.8	−0.9	2.8
	C8−H	−3.0	−1.9	−0.2	2.1
C_s N3-hydrogenated	C2−H	−14.0	−7.9	0.2	7.7
	N3−H	−3.6	−3.3	−1.1	4.5
	N6−H	−2.8	−2.3	−0.7	2.9
	N6−H	−2.4	−2.0	−0.9	2.9
	C8−H	−5.1	−2.9	0.2	2.7
N7-hydrogenated	N6−H	1.8	−0.6	0.1	0.5
	N6−H	−0.2	−0.6	−0.4	1.0
	C8−H	22.5	−8.2	−1.3	9.5
	N7−H	13.4	−3.1	−2.2	5.3
	N9−H	1.2	−2.3	−1.6	3.9
C2-hydrogenated	C2−H	45.5	−1.4	−0.5	1.9
	C2−H	43.3	−1.4	−0.5	1.9
	C8−H	−6.7	−3.9	−0.2	4.1
	N9−H	−1.2	−1.0	−0.6	1.6
C4-hydrogenated	C4−H	62.9	−0.9	−0.5	1.4
	C8−H	−6.0	−3.7	−0.6	4.3
	N9−H	−3.3	−3.2	−1.2	4.4
C5-hydrogenated	C2−H	−11.6	−6.5	−0.3	6.8
	C5−H	51.2	−0.7	−0.1	0.8
C8-hydrogenated	C2−H	−5.6	−3.0	−0.1	3.1
	N6−H	−2.6	−1.8	−1.1	2.8
	N6−H	−2.7	−2.9	−0.8	3.7
	C8−H	38.9	−1.0	−0.5	1.5
	C8−H	39.1	−1.0	−0.5	1.5
	N9−H	−3.7	−3.0	−1.0	4.0

the results obtained in this study are highly similar to the results previously discussed for adenosine. Hence, the conclusion that the calculations support the experimental assignment of this radical in adenosine can also be applied to adenosine·5-bromouracil.

To test the hypothesis that the discrepancies between theory and experiment arise due to the fact that the N3-hydrogenated radical remains planar upon formation, the geometry of this radical was obtained in a planar form. The fully optimized C_s structure is only 1.7 kcal/mol above the nonplanar form at the B3LYP/6-31G(d,p)//B3LYP/6-311G(2df,p) level. A frequency analysis on this structure indicates that it has two imaginary frequencies. The spin density and HFCCs for the C_s N3-hydrogenated radical are displayed in Tables 2 and 5. The spin density distribution for this radical is in better agreement with experiment than that for the nonplanar radical due to the calculated C8 spin density of 0.13 in the planar form. The calculated C2 and C8 hydrogen HFCCs are very similar for both radical forms with an average deviation of 1.6 and 0.8 G in the isotropic and anisotropic components, respectively. The main difference in the two sets of computed couplings is in the magnitude of the N3−H isotropic HFCC. In the C_s N3-hydrogenated radical, the N3−H isotropic component was calculated to be −3.6 G, compared to 15.2 G in the puckered form. Experimentally, this coupling was determined to be on average −3.7 G. Hence, it can be concluded that in the crystal the N3-hydrogenated radical is likely to remain in its planar form.

The radical formed through addition of hydrogen to N7 has not been claimed to be observed in the spectrum of any unprotonated adenine crystal.[5] Upon radical formation, the parent adenine molecule is significantly distorted at C8 and N6.

The spin density distribution (Table 2) indicates that the majority of the spin density resides on C8 (0.64) and the remaining spin density is distributed between N7 (0.11) and N9 (0.10). The HFCCs (Table 5) reflect this spin density distribution in that there exists a large isotropic C8−H coupling (22.5 G) that has considerable anisotropy (largest component of the tensor, 9.5 G). A smaller, yet significant, coupling was also obtained for N7 (13.4 G). Comparison with experimental results is not possible at this time due to the lack of experimental data for this species.

Carbon-Hydrogenated Radicals. The radical formed through the addition of hydrogen to the C2 position has been observed on numerous occasions experimentally. It has been proposed that the couplings in this radical depend on the protonation state of the parent molecule.[31] In deoxyadenosine monohydrate, Lichter and co-workers determined that the C2-hydrogenated radical was present rather than the corresponding C8 radical.[32] The spin density was determined to reside mainly on N1 (0.17) and N3 (0.37). This is in agreement with the calculated results (N1 (0.20) and N3 (0.43)), although significant spin density is also found on C5 (0.33), C8 (0.23), and C4 (−0.10). Two equivalent C2−H couplings (43.7 G) were observed in the experimental spectrum. In another study of deoxyadenosine monohydrate crystals, Zehner et al.[33] also observed two identical C2−H couplings (42.0 G), as well as a significant C8−H coupling (9.5 G). In a later study, Zehner and co-workers[31] obtained very similar results for adenosine. The observed spectra consisted of two equivalent C2−H couplings (44.0 G) and one C8−H coupling (10.0 G). Kar and Bernhard also observed two equivalent C2−H couplings (40 G) in cocrystals of adenosine and 5-bromouracil that were assigned to the C2-hydrogenated radical.[29]

In a recent ESR/ENDOR study of deoxyadenosine monohydrate by Close et al.,[28] a very accurate set of full couplings was determined and assigned to the C2-hydrogenated radical. The experimental isotropic (−6.4 G) and anisotropic (−3.5,−0.1,3.6 G) C8−H couplings are in excellent agreement with those calculated in the present study (A_{iso} = −6.7 G; −3.9, −0.2, 4.1 G), as well as with the values obtained by the same group in a more recent study of anhydrous deoxyadenosine[22] (A_{iso} = −6.4 G; −3.4, 0, 3.4 G). The agreement between the calculated and experimental results for these studies is far better than that obtained for the experimental work discussed previously. In addition, the calculations indicate that the C8−H coupling reported in earlier work should be negative. However, the experimental work of Close and co-workers[22,28] and the theoretical results obtained in the present study differ in the magnitude of the two C2−H isotropic HFCCs. In the calculations, the molecule remains nearly planar upon radical formation, with the exception of the amino group, and the two hydrogens at C2 are distributed equally on either side of the molecular plane. This arrangement of the two C2 hydrogens leads to two nearly equivalent (43.3 and 45.5 G) isotropic couplings. In the careful ESR/ENDOR work of Close et al.,[22,28] on the other hand, the difference in the two HFCCs for the C2 hydrogens is greater (32.8/38.9 and 54.3/47.5 G in deoxyadenosine monohydrate/anhydrous deoxyadenosine). The experimental results indicate that the distribution of the hydrogens at the C2 center is more unsymmetric than that modeled by the theoretical methods implemented in this study.

The problem of theoretical methods inadequately describing ring puckering resulting from the addition of a hydrogen has been observed previously for thymine, 1-methylthymine, and uracil.[11] It is possible that insufficient ring puckering is also

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TABLE 6: Dihedral Angles (deg) Which Define the Distortion in the C4 and C5 Hydrogen and Hydroxyl Radical Addition Products

	C4-hydrogenated	C5-hydrogenated	C4-hydroxylated	C5-hydroxylated
N3C5C4N7	136.0	−156.3	134.9	−155.8
N3C5C4C6	−30.6	−30.7	−30.0	−31.8
N9C5C4N7	10.0	14.8	12.2	14.8
N9C5C4C6	−156.7	140.4	−152.7	138.8

responsible for the disagreement between theory and experiment in the adenine C2-hydrogenated radical. The disagreement between the most recent experimental studies and those which appeared in the earlier literature, which indicate equal couplings for both hydrogens, can be understood through the use of a more detailed experimental technique in the later studies. Another possible explanation for this could be a reduced vibrational averaging in the later experiments. Note that the average value of the two unequal C2−H couplings generated from the most recent experiments (~43.6 G) is equal to the value obtained in earlier experiments and highly similar to their computed average (44.4 G).

As noted earlier, the anisotropic couplings can be calculated with a great degree of accuracy and, hence, comparison of the anisotropic HFCCs can aid in the identification of the radical in question. Comparison of the experimental (Table 4) and theoretical (Table 5) anisotropic C2−H couplings indicates that they are in excellent agreement, and the assignment of the observed couplings to the C2-hydrogenated radical is supported by the theoretical results.

The C4-hydrogenated radical has not been assigned in the spectrum of the nonprotonated adenine crystals (Figure 1, **I**), although it has been detected in the protonated crystals (Figure 1, **II**) discussed below. The addition of hydrogen to the C4 position leads to considerable geometrical distortion at this position, as previously observed by Colson and Sevilla.[34] They described the geometry in these types of radicals as a "butterfly" conformation, where the pyrimidine and imidazole rings remain planar but are tilted about the C4C5 bond toward each other. The dihedral angles obtained in the present study, which define this distortion about the C4C5 bond and the resulting "butterfly" conformation, are displayed in Table 6. Even though the molecule is distorted upon radical formation, the spin density is distributed throughout both of the rings with the majority of the spin density located at C5 (0.44) and significant amounts residing on N1 (0.14), N3 (0.10), C8 (0.20), and N9 (0.12). The HFCCs consist of a very large isotropic C4−H coupling of 62.9 G. In addition, significant couplings were also calculated for the C8 (−6.0 G) and N9 (−3.3 G) hydrogens. Comparison with experimental data is not possible at this time. Colson and Sevilla also discussed a higher energy conformer, which was not considered in the present work, in which the rings are tilted to opposite sides of the C4C5 bond.

Similar to the C4-hydrogenated radical, the C5-hydrogenated radical also undergoes considerable distortion upon radical formation leading to the previously mentioned "butterfly" conformation. The dihedral angles defining this conformation are displayed in Table 6. The degree of distortion in both the C4- and C5-hydrogenated radicals is highly similar. The largest part of the spin density in the C5-centered radical is shared between C2 (0.44) and C4 (0.31), with considerable spin density also located on C6 (0.23), N3 (−0.10), and N6 (0.10). The coupling constants calculated for this radical include a large C5−H coupling (51.2 G) and a smaller C2−H coupling (−11.6 G). To date, no experimental couplings have been isolated for this radical.

The final carbon-hydrogenated radical to be discussed is the radical formed through addition of a hydrogen to the C8 position. This radical has been observed in numerous studies in the literature, and the couplings, similar to the C2-hydrogenated radical, have been shown to depend on the protonation state of the parent molecule. The effects of protonation on the HFCCs will be discussed further in a later section. The HFCCs in the C8- and C2-hydrogenated radicals have been determined in the past to be almost identical, and thus, discussions have appeared in the literature disputing to which position the hydrogen primarily attaches.

The C8-hydrogenated radical was determined to be present in deoxyadenosine monohydrate by Zehner et al.,[33] where two equivalent C8−H couplings were isolated (38.0 G). In a later paper, Zehner and co-workers[31] extracted two equivalent C8 hydrogen couplings in the adenosine crystals (39.0 G). The HFCCs in both of these studies are in excellent agreement with the calculated couplings (38.9/39.1 G). In addition, the calculated values in Table 5 indicate that there also exists other significant couplings in this radical, the largest of which occurs for the C2 hydrogen (−5.6 G).

Close and co-workers also examined the C8-hydrogenated radical in deoxyadenosine monohydrate[28] and anhydrous deoxyadenosine.[22] In their studies, the hyperfine tensor for C2−H was extracted and shown to carry a small isotropic coupling (−4.8 G) and significant anisotropy (−2.6, 0.0, 2.6 G). This coupling is in agreement with that calculated for C2−H (A_{iso} = −5.6 G; −3.0, −0.1, 3.1 G). The main difference between the theoretical results or the results obtained from previous experimental studies and the experimental work of Close et al. is the magnitude of the isotropic C8−H couplings. Experimentally, two unique couplings of 36.3 (36.7) G and 41.6 (40.9) G were obtained in deoxyadenosine monohydrate (anhydrous deoxyadenosine), compared to the two nearly equal C8−H couplings that were obtained from the calculations (38.9 and 39.1 G). Theoretically, radical formation leads to little alteration in the molecular geometry, and the two hydrogens at the C8 position are distributed symmetrically on either side of the molecular plane. The amino group in this radical is nonplanar. From the experimental results, it is apparent that the two C8 hydrogens must be unsymmetrically oriented, which is probably caused by alterations in the ring geometry at the C8 position. This is an identical situation to that which was observed for the adenine C2-hydrogenated radical. Once again, the experimental (Table 4) and theoretical (Table 5) anisotropic couplings are in good agreement, and thus, it can be concluded that the theoretical results support the experimental assignment of the C8-hydrogenated radical. It is interesting to note that the two experimental couplings in the C8-hydrogenated radical are closer in magnitude than those obtained in the C2-hydrogenated radical, indicating smaller geometrical alterations upon formation of the C8 hydrogen addition radical. Very similar experimental couplings were also obtained for 9-methyladenine (Table 4).[30]

Adenine Dehydrogenated Radicals. The relative energies for the various adenine radicals formed through net hydrogen removal are displayed in Table 1. The results suggest that the

TABLE 7: Calculated HFCCs (G) in Dehydrogenated Radicals

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
C2-dehydrogenated	N6−H	1.3	−0.4	−0.3	0.8
N6-dehydrogenated	N6−H	−11.8	−9.7	−2.0	11.8
	C8−H	−4.0	−2.3	−0.3	2.6
C8-dehydrogenated	N9−H	−1.8	−3.0	−1.9	4.9
N9-dehydrogenated	C2−H	−5.2	−3.0	−0.6	3.5
	N6−H	−4.1	−2.8	−1.1	3.9
	N6−H	−4.1	−3.5	−0.9	4.4
	C8−H	−3.7	−2.2	−0.8	3.0

N9-dehydrogenated radical is the lowest lying radical in this class. The radical formed via abstraction of a hydrogen from the amino group lies 2.7 kcal/mol higher in energy. The two radicals formed through abstraction of a hydrogen from one of the carbons (C2 or C8) are 9.2 and 16.9 kcal/mol higher in energy than the lowest energy radical in this class. In DNA, the hydrogen at N9 in adenine is replaced by a sugar group. This implies that in full DNA samples, the N9-dehydrogenated radical is not possible and the lowest energy dehydrogenated radical would be formed through removal of a hydrogen from the amino group. In cocrystals of thymine and adenine derivatives, both of the amino hydrogens take part in hydrogen bonding and no amino hydrogen abstraction radical is observed. However, in DNA, only one of the amino hydrogens is involved in hydrogen bonding, which allows one hydrogen to be removed to form the N6-dehydrogenated radical. The HFCCs of the various dehydrogenated radicals are displayed in Table 7.

The C2- or C8-dehydrogenated radicals have not been assigned in any of the experimental spectra in the literature to date. The energetics of the dehydrogenated series indicate that these two radicals are higher in energy than any other dehydrogenated radical yielding a possible explanation as to why these radicals have been undetected. The spin density distribution in both radicals indicates that the majority of the spin density is located on the radical center (C2 (0.84) and C8 (0.83) in the C2- and C8-dehydrogenated radicals, respectively). In addition, the HFCCs in these radicals indicate that the only hydrogen coupling that could possibly be detected in these radicals is due to N6−H (1.3 G) in the C2-dehydrogenated radical or N9−H (−1.8 G) in the C8-dehydrogenated radical, respectively. However, these couplings are very small and, hence, would probably be difficult to detect even if the radicals are generated.

The N9-dehydrogenated radical was determined to be the lowest lying radical in this class even though it has not been observed in the experimental studies to date. This is not surprising, however, since most studies have been performed on adenosine crystals where a sugar group replaces the hydrogen at the N9 position. Hence, the formation of this radical is not possible. The optimized geometry of this radical indicates that the molecule remains planar upon radical formation. The amino group also lies in the molecular plane. The spin density distribution in the N9-dehydrogenated radical (Table 2) indicates that the spin density is evenly distributed around both molecular rings. The HFCCs (Table 7) indicate that the nuclei with the largest isotropic couplings are C2−H (−5.2 G), C8−H (−3.7 G), and both of the N6-hydrogens (−4.1 G). In addition, all of these couplings have significant anisotropic character (the largest average component of the anisotropic tensor is approximately 3−4 G).

The N6-dehydrogenated radical has been detected in a few experimental studies. Kar and Bernhard identified this radical in their study of the cocrystals of adenosine and 5-bromouracil.[29] They determined that the dominating isotropic coupling of

−10.0 G was due to the remaining hydrogen at N6, which is hydrogen bonded to O2 in uracil. It was suggested that the anisotropy associated with this coupling (−11.7, 4.7, 6.9 G) arose due to the hydrogen-bonding interactions. In addition, Close indicated that the N6−H hyperfine tensor obtained in the above study is not what one would expect for these interactions.[5] The theoretical results indicate that the N6-dehydrogenated radical would indeed give rise to a large isotropic coupling (−11.8 G) with significant anisotropy (−9.7, −2.0, 11.8 G). Differences between the two sets of data might be due to the hydrogen bonding in the crystal structures, although it is clear from the present calculations that the magnitude of the N6−H coupling tensor is also significant without hydrogen-bonding interactions. The geometry of the N6-hydrogenated radical was calculated to be planar with the remaining amino hydrogen also located in the molecular plane.

The radical formed via net removal of a hydrogen from N6 was also observed in an ESR/ENDOR analysis of adenosine single crystals studied by Close and Nelson.[27] The estimated spin densities in the observed radical indicated that the spin density is primarily located on C8 (0.16) and N6 (0.42). The authors also indicated that the two possible resonance contributors for this radical most probably lead to spin density on the cyclic nitrogens (N1 and N3). The calculated results indicate that a large part of the spin density resides on N6 (0.59) and that the rest of the spin density is distributed throughout the molecule (Table 2), including significant amounts on N1 (0.17) and N3 (0.23). The experimental data include a large N6−H coupling (12.1 G), which has a large degree of anisotropy (−9.1, 1.2, 7.9 G), and a smaller C8−H coupling (4.4 G), with an anisotropic tensor of (−2.1, −0.1, 2.3 G). The C8−H coupling is in good agreement with the absolute magnitude of the calculated isotropic (−4.0 G) and anisotropic (−2.3, −0.3, 2.6 G) HFCCs. The N6−H calculated isotropic coupling (−11.8 G) is also in good agreement with experiment, whereas the calculated anisotropic N6−H tensor (−9.7, −2.0, 11.8 G) is slightly larger than that obtained experimentally. Comparison of the experimental and the calculated isotropic couplings indicates that the sign of the experimental couplings should be negative. Overall, however, it can be concluded that the calculated results support the experimental assignment of this radical.

Nelson and co-workers[22] indicated that the N6-dehydrogenated radical has not been observed in many of the adenine samples that have been investigated in the literature. In their study, they examined anhydrous deoxyadenosine and concluded that the N6-dehydrogenated radical is produced in this crystal and that couplings very similar to those previously discussed for adenosine (Table 4) were obtained. Upon careful consideration of the crystal structures they furthermore concluded that the N6-dehydrogenated radical is formed via a concerted proton transfer where the hydrogen is "shuffled" away from the charged site. In addition, it was concluded that hydrogen bonding could control the deprotonation site.

Adenine Hydroxylated Radicals. Little experimental evidence exists for the formation of hydroxylated radicals in adenine samples. However, the idea of the formation of this type of radical is not new. Various studies on DNA bases in the liquid phase have appeared in the literature that indicate the possibility of OH radical addition to the parent base molecule. In addition, crystal studies on guanine have indicated that various radicals have been detected in which the net result is OH addition to the parent base molecule.[35,36] Thus, the present study investigates the geometry and the HFCCs in hydroxylated

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TABLE 8: Calculated HFCCs (G) in Hydroxylated Radicals

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
C2-hydroxylated	C2−H	40.0	−1.1	−0.4	1.6
	C8−H	−6.7	−4.0	−0.3	4.3
	N9−H	−1.2	−1.0	−0.6	1.6
C4-hydroxylated	N6−H	−1.1	−1.1	−0.4	1.4
	C8−H	−7.3	−5.0	2.2	2.8
	N9−H	−2.4	−2.4	−1.0	3.4
C5-hydroxylated	C2−H	−9.9	−5.6	−0.4	6.0
	C8−H	−2.2	−1.3	−0.5	1.8
C8-hydroxylated	C2−H	−5.6	−3.0	−0.1	3.2
	N6−H	−4.0	−1.4	−1.2	2.6
	N6−H	−3.7	−2.8	−0.1	2.9
	C8−H	28.8	−0.8	−0.4	1.2
	N9−H	−3.0	−2.6	−0.9	3.5

TABLE 9: Relative Energies of Protonated Adenine Radicals (kcal/mol)

radical	N1-protonated	N1,N7-diprotonated
C2-hydrogenated	0	5.8
C8-hydrogenated	0.3	0
N7-hydrogenated	6.1	
N3-hydrogenated	6.8	
C4-hydrogenated	14.6	
N6-dehydrogenated	0	

radicals in anticipation that they may be used in future studies on hydrated adenine crystals in order to determine whether it is possible to form OH addition radicals in adenine samples.

The most likely site of addition for the hydroxyl group would be at one of the carbons involved in a double bond, namely C2, C4, C5, or C8. These centers have already been investigated as possible sites for hydrogen addition, and the energetics have been discussed. It is of interest to investigate differences resulting from OH addition compared with the corresponding hydrogen addition at these sites. The relative energies of the hydroxylated radicals are displayed in Table 1. From the results, it can be seen that the relative stability of the hydroxylated and hydrogenated radicals is very similar. For example, the C8-hydroxylated radical is the lowest energy species in this class. The next lowest energy radical is the C2-hydroxylated radical, which lies 12.4 kcal/mol higher in energy. The C4- and C5-hydroxylated radicals are the highest in energy, lying 21.0 and 21.5 kcal/mol above the corresponding C8-hydroxylated radical, respectively. In the hydrogenated radicals, the order of the relative energies of the C4 and C5 radicals is reversed.

The molecular geometry of adenine undergoes significant alterations upon formation of the C4- and C5-hydroxylated radicals, forming the same types of "butterfly" conformers previously discussed for the hydrogenated species. The dihedral angles defining this conformation are displayed in Table 6 along with the dihedral angles observed for the corresponding hydrogen addition adducts. The degree of distortion observed for both the C4 and C5 hydrogen and hydroxyl radical addition products is highly similar. The largest difference in the distortion between the various products occurs for the N9C5C4C6 dihedral angle, which differs by 4° in the C4-hydroxylated product relative to the C4-hydrogenated product. All other dihedral angles differ by no more than 2° in the hydroxylated products relative to the hydrogen addition adducts. The observed distortion in the hydroxylated radicals causes a significant amount of the spin density to be localized at C5 (C2/C4) for the C4(C5)-hydroxylated radical. The C2- and C8-hydroxylated radicals, on the other hand, undergo only slight geometrical alterations upon addition of OH, whereby the addition center is displaced slightly out of the molecular plane. The amino groups in all of the hydroxylated radicals are nonplanar at the nitrogen.

The HFCCs for these radicals are displayed in Table 8. Perhaps the most interesting feature is the magnitude of the C2−H (40.0 G) and C8−H (28.8 G) couplings in the C2- and C8-hydroxylated radicals, respectively. In particular, the C2−H coupling is very similar to those calculated for the C2-hydrogenated radical, whereas the C8−H coupling is much smaller in magnitude than those determined for the corresponding C8-hydrogenated radical.

Gregoli et al.[37] observed a radical in frozen aqueous solutions of deoxyadenosine 5′-monophosphate that was thought to be formed through addition of a hydroxyl radical to the C8 position. The mechanism proposed for the formation of this radical was obtained through computer analysis of the transformations of the ESR spectra upon annealing. One isotropic HFCC of 29 G was obtained for this radical. Comparison with the calculated results for the C8-hydroxylated radical (Table 8) indicates that this coupling is indeed due to the C8 hydrogen whose calculated HFCC is 28.8 G. The results in Table 8 also indicate that a better resolved spectra would yield experimental couplings for C2−H and N9−H, as well as for both of the amino hydrogens.

N1-Protonated Adenine Radicals. As previously discussed, it is important to gain a better understanding of factors that affect the protonation of the DNA bases. The present study investigates some of the radicals, similar to those previously discussed, which are protonated at the N1 position and for which accurate experimental data exists. The majority of the experimental studies that have been performed on protonated systems involve adenine hydrochloride crystals, where a chlorine ion participates in a hydrogen bond with the protonated N1 position in adenine.

The relative energies of a selection of N1-protonated radicals are displayed in Table 9, and the corresponding spin densities are shown in Table 10. It is interesting to note that for the nonprotonated radicals, the C2-hydrogenated radical was 8.7 kcal/mol higher in energy than the C8-hydrogenated radical. However, the relative energies for the various N1-protonated radicals indicate that the C2-hydrogenated radical is slightly lower in energy (0.3 kcal/mol) than the C8-hydrogenated species. The influence of varying the degree of protonation of the parent adenine base on the HFCCs of the C2- and C8-hydrogenated radicals will be discussed in a later section.

The N1-protonated N3-hydrogenated radical has been observed in crystals of adenine hydrochloride hemihydrate.[26] The spin density on C2 was determined to vary between 0.55 and 0.65, depending on whether it was calculated from the isotropic or the anisotropic couplings, respectively. Theoretically, it was determined that the spin density on C2 is much higher (0.71). The discrepancy between the experimental spin density obtained via the two different methods was suggested to arise due to one of two reasons. First, it was proposed that there may exist significant spin density at N3, which would be accounted for in the dipolar couplings, and hence, a larger value is obtained from the anisotropic couplings. Second, it was speculated that since net hydrogen addition removes the double bond between N3 and C2, the C2 center may become more pyramidal leading to a smaller isotropic value. The geometry optimization in this study indicates that the radical is distorted at C2, where this position is located out of the molecular plane. The amino hydrogens, on the other hand, remain in the molecular plane. In addition, the calculated spin density distribution in this radical (Table 10) indicates that there does exist significant spin density at N3 (0.13). Thus, the spin density at N3 and the distortion at C2 are probably jointly responsible for the discrepancies in the experimental spin densities.

TABLE 10: Spin Density Distributions in N1-Protonated Adenine Radicals

radical	N1	C2	N3	C4	C5	C6	N6	N7	C8	N9
C2-hydrogenated			0.45	−0.11	0.29				0.17	
N3-hydrogenated		0.70	0.13		0.09					
C_s N3-hydrogenated		0.72	0.14		0.09					
C4-hydrogenated					0.30	0.11	0.13	0.11	0.11	0.14
N7-hydrogenated	0.12		0.11	0.11		0.45			0.32	
C_s N7-hydrogenated						0.28	0.10		0.44	0.11
C8-hydrogenated		0.14				0.17	0.11	0.49		0.10
N6-dehydrogenated			0.10	0.10	0.24	−0.14	0.47			

A large isotropic C2−H coupling (−14.2 G) was extracted from the experimental spectrum, as well as a large anisotropic tensor (−10.0, 1.0, 9.0 G). The anisotropic couplings are in excellent agreement with the calculated values (−9.4, −0.9, 10.3 G), while only a small isotropic HFCC was calculated (2.2 G). The calculated isotropic coupling for the nonprotonated N3-hydrogenated radical (−12.9 G) is in better agreement with the experimental isotropic coupling; however, the anisotropic couplings calculated for the nonprotonated radical are much too small (−7.3. 0.4, 6.9 G). Thus, since the anisotropic couplings can be predicted with a greater degree of accuracy, it can be concluded that the observed radical is the N3-hydrogenated radical that is protonated at the N1 position. The main difference between the protonated and nonprotonated radicals is the geometrical distortion at the C2 position. The protonated radical is distorted at this position, as previously mentioned, which leads to a small coupling, while the nonprotonated form is not distorted and possesses a larger isotropic C2−H coupling. It is possible that crystal interactions lead to a more planar radical experimentally, which would in turn lead to a larger isotropic C2−H coupling.

If crystal interactions are considered, a planar N1-protonated N3-hydrogenated radical may be possible. A full optimization and frequency analysis on a C_s N1-protonated N3-hydrogenated radical indicates that this radical is only 1.1 kcal/mol higher in energy than the equivalent nonplanar radical, but it possesses multiple imaginary frequencies. The spin density distribution (Table 10) varies only slightly between the planar and nonplanar radical forms. The HFCCs (Table 11) are also similar with the exception of the isotropic C2−H coupling. In the nonplanar radical, a C2−H coupling of 2.2 G was obtained, whereas the corresponding coupling in the planar radical is equal to −18.5 G. This is in much better agreement with the experimental coupling (−14.2 G) (Table 12), which indicates that this radical most probably possesses a planar form in the crystalline environment.

The doubly protonated adenine molecule (hydrogens at the N1 and N7) was investigated by Box and Budzinski[38] in the crystals of adenine dihydrochloride. It was determined that an anion of the doubly protonated base was responsible for the couplings observed in their study. This radical is equivalent to the N1-protonated N7-hydrogenated adenine radical. The spin density at C8 was determined to be 0.292/0.311, which is similar to the value calculated in the present study (0.32). The experimental C8−H hyperfine tensor was composed of an isotropic component of −8.7 G with an anisotropic part of −5.8, 0.8, 4.9 G. The present calculations yield both large isotropic (−10.4 G) and anisotropic components (−5.7, 0.2, 5.5 G) for the C8 hydrogen, in excellent agreement with experiment. In addition, large couplings were calculated for both hydrogens at N6 (18.8/18.9 G) and significant couplings were also calculated for N1−H (−3.2 G) and N7−H (−1.8 G). The large couplings calculated for the N6 hydrogens arise because the optimized geometry yields a planar molecular backbone with the amino group twisted such that one hydrogen is above the molecular

TABLE 11: Calculated HFCCs (G) in Adenine N1-Protonated Radical Cations

radical	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
N3-hydrogenated	N1−H	−1.8	−2.1	−1.4	3.5
	C2−H	2.2	−9.4	−0.9	10.3
	N3−H	−3.2	−3.9	−1.8	5.8
	C8−H	−2.7	−1.6	−0.2	1.7
C_s N3-hydrogenated	N1−H	−1.2	−1.6	−1.1	2.7
	C2−H	−18.5	−10.7	0.4	10.4
	N3−H	−4.7	−4.1	−1.6	5.7
	C8−H	−3.4	−1.9	−0.1	2.0
N6-dehydrogenated	N1−H	−1.8	−1.1	−0.9	2.0
	C2−H	−2.2	−1.3	−0.7	2.0
	N6−H	−9.6	−8.0	−1.7	9.7
	C8−H	−6.5	−3.5	−0.5	4.0
N7-hydrogenated	N1−H	−3.2	−2.8	−1.1	3.9
	N6−H	18.8	−1.1	−0.9	2.0
	N6−H	18.9	−1.1	−0.9	2.0
	N7−H	−1.8	−1.2	−1.0	2.2
	C8−H	−10.4	−5.7	0.2	5.5
C_s N7-hydrogenated	N1−H	−2.4	−2.0	−0.8	2.8
	N6−H	−2.8	−2.5	−0.7	3.2
	N6−H	−2.6	−1.9	−0.9	2.9
	N7−H	−3.7	−2.8	−1.4	4.2
	C8−H	−12.8	−7.2	0.4	6.8
C2-hydrogenated	N1−H	−2.6	−2.0	−0.8	2.8
	C2−H	36.7	−1.4	−0.7	2.1
	C2−H	36.2	−1.4	−0.7	2.1
	C8−H	−5.8	−3.2	−0.5	3.7
	N9−H	−1.5	−1.0	−0.9	1.9
C4-hydrogenated	N1−H	−1.6	−1.5	−0.6	2.1
	C4−H	49.2	−0.9	−0.4	1.4
	N6−H	−2.9	−1.6	−1.3	2.9
	N6−H	−2.9	−2.9	−0.8	3.7
	C8−H	−3.9	−2.5	−0.9	3.4
	N9−H	−3.8	−3.4	−1.1	4.5
C8-hydrogenated	C2−H	−5.2	−2.8	−0.3	3.0
	N6−H	−2.9	−1.4	−1.1	2.6
	N6−H	−2.9	−2.7	−0.8	3.5
	C8−H	40.6	−1.3	−0.7	1.9
	C8−H	40.5	−1.3	−0.7	1.9
	N9−H	−3.1	−2.7	−0.9	3.6

plane and the other below. Hydrogen bonding in the crystal structure may force these hydrogens to remain in the molecular plane, and hence, these couplings are not observed experimentally.

The geometry of the planar N1-protonated N7-hydrogenated radical was obtained through a constrained optimization. The planar radical lies 2.8 kcal/mol above the nonplanar form. A greater spin density on C8 and a smaller spin density on C6 were calculated for the planar radical (Table 10) relative to the nonplanar form. As predicted, the couplings for both amino hydrogens are very small (approximately 3 G) if a planar geometry is considered. However, the isotropic and anisotropic C8−H couplings for the planar N1-protonated N7-hydrogenated radical are larger in magnitude than those obtained for the nonplanar form. The C8−H couplings in the planar radical are in poorer agreement with experiment, and thus, the geometry of this radical is difficult to determine. Future experimental

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TABLE 12: Experimental HFCCs (G) for N1-Protonated Adenine Radicals

radical	molecule	atom	A_{iso}	T_{XX}	T_{YY}	T_{ZZ}
N3-hydrogenated	adenine·HCl·^{1}/$_2$H$_2$O[26]	C2−H	−14.2	−10.0	1.0	9.0
N6-dehydrogenated	adenine·HCl·^{1}/$_2$H$_2$O[26]	N6−H	−9.4	−6.4	−1.4	7.9
		α-C−H	−6.2	−3.3	−0.1	3.4
	adenosine·HCl[26]	N6−H	−11.4	−7.9	−1.3	9.2
		C8−H	−5.8	−2.8	−0.1	2.9
N7-hydrogenated	adenine·2HCl[38]	C8−H	−8.7	−5.8	0.8	4.9
C2-hydrogenated	adenosine·HCl[26]	N1−H	−2.6	−2.0	−0.3	2.3
		C2−H	40.5	−1.4	−0.7	2.1
		C2−H	39.1	−1.6	−0.8	2.4
		C8−H	−5.5	−2.8	−0.2	3.0
C4-hydrogenated	adenosine·HCl[28]	C4−H	9.0	−4.3	0.1	4.3
C8-hydrogenated	adenosine·HCl[26]	C2−H	−4.3	−2.4	0.0	2.4
		C8−H	43.0	−2.1	0.7	1.5
		C8−H	40.9	−1.3	−0.6	2.0

studies that measure the N6−H couplings in this radical would be beneficial for the determination of its geometrical properties.

The next system to be discussed that has been detected in experimental spectra is the N1-protonated N6-dehydrogenated radical. The geometry of this radical was determined to be planar, including the remaining amino hydrogen. Nelson et al.[26] observed this N1-protonated N6-hydrogenated radical in crystals of both adenine hydrochloride hemihydrate and anhydrous adenosine hydrochloride. The couplings extracted from the spectra of irradiated adenine hydrochloride hemihydrate were of poor quality, and assignment of the observed couplings to the N6 hydrogen removal radical was stated to be tentative. The experimental results for this radical indicated that a spin density of 0.33 is located on a nitrogen atom (probably N6), but the calculated results indicate that a larger amount is located at this position (N6, 0.47) and that smaller amounts are located throughout the molecule on N3 (0.10), C4 (0.10), C5 (0.24), and C6 (−0.14). The experimental coupling extracted for the N6−H consisted of a substantial isotropic coupling (−9.4 G) with significant anisotropy (−6.4, −1.4, 7.9 G). The calculated isotropic coupling (−9.6 G) matches that obtained from the experiment. However, the calculated anisotropic couplings differ from experiment by on average 1.5 G. This difference could be due to the poorly resolved spectra, and hence, better agreement between theory and experiment is not expected. Further support for the assignment of the N6-dehydrogenated radical is obtained by examining another resolved hyperfine structure corresponding to a α-C−H nucleus. It consists of an isotropic component of −6.2 G and an anisotropic tensor of −3.3, −0.1, 3.4 G. These couplings are in excellent agreement with the calculated values for the C8−H coupling in this radical (A_{iso} = −6.5 G; −3.5, −0.5, 4.0 G).

Experimental spectra for the same species were obtained at a much greater resolution in anhydrous adenosine hydrochloride.[26] The results indicated that the spin density resides on C8 (0.21) and N6 (0.39). The calculated spin densities reveal that only a very small amount of the spin density is located on C8 and 47% of the spin density is located on N6. However, the spin densities in the nonprotonated N6-dehydrogenated radical indicate that this radical exhibits spin density on both C8 (0.14) and N6 (0.59). In addition, the C8−H couplings calculated for the deprotonated radical are in better agreement with the experimental couplings, whereas the N6−H couplings support the assumption that the radical is protonated. In particular, the largest component of the experimental N6−H anisotropic coupling (9.2 G) resembles that calculated for the protonated radical (9.7 G) more closely than it does that obtained for the nonprotonated form (11.8 G).

The protonated C4-hydrogenated radical was thought to be observed in adenosine hydrochloride.[26] The spin density at C8

was estimated to be 0.31, which is much larger than the value obtained in the calculations (0.11). A reasonably sized coupling was obtained experimentally for C4−H (9.0 G) with an anisotropic tensor of −4.3, 0.1, 4.3 G. However, the calculated results displayed in Table 11 for this radical indicate that the C4 hydrogen would have a much larger isotropic coupling (49.2 G) and a very small anisotropic coupling (−0.9, −0.4, 1.4 G). The authors are correct in their prediction that upon radical formation the C4 position becomes pyramidal, but the experimental and calculated C4−H couplings do not match. In addition, the experimental couplings obtained in the adenine radical would be expected to be close to those obtained for cytosine or thymine when a hydrogen is added perpendicular to the C5C6 double bond. It can hence be concluded that the C4-hydrogenated radical is unlikely to be responsible for the observed coupling. The assignment of the observed coupling to a particular nucleus or radical is difficult in this case since neither of the computed sets gives a perfect match for both isotopic and anisotropic data.

Various Prontonated C2- and C8-Hydrogenated Radicals. Surprising results were obtained in a study of the cocrystals of 1-methylthymine and 9-methyladenine.[39] In their investigation, Sagstuen et al. indicated that no products formed through oxidation of adenine were observed. The only two adenine radicals observed were the C2- and C8-hydrogenated radicals. An in-depth experimental study on various properties of the C2- and C8-hydrogenated radicals has been performed by Zehner and co-workers.[31] They examined the couplings of these two radicals in a variety of crystals that represent different protonation states of the parent adenine molecule and it was determined that the HFCCs depend strongly on the protonation state of the adenine base. It was also determined that the relative yield of the two radicals depends on the environment. More specifically, in crystals of 9-methyladenine, where the crystal interactions depend on van der Waals forces, only the C8 hydrogen addition radicals were observed, whereas in crystals that involve small polar molecules or extensive hydrogen bonding, both the C2 and the C8 hydrogen addition radicals were observed. On the other hand, in adenine dihydrochloride (doubly protonated), the concentration of the C8 radicals is much larger than the concentration of the C2 radicals. This agrees perfectly with the computed relative energies of the various species listed in Tables 1 and 9. For the free base, C8−H lies 8.7 kcal/mol below the C2−H radical. On the other hand, the energy difference in the N1-protonated systems is only 0.3 kcal/mol. In addition, in the doubly protonated system C8 is lower in energy than C2 by 5.8 kcal/mol.

The above characteristics were rationalized by the hypothesis that the C2-hydrogenated radical requires a specific environment to be stabilized.[29] In particular, it was determined from INDO

TABLE 13: Calculated and Experimental Isotropic HFCCs (G) and Calculated Dipole Moments (D) in Protonated C2- and C8-hydrogenated Radicals

radical		C2−H	C2−H	C8−H	C8−H	dipole moment
		C2-hydrogenated radical				
free base	calculated	43.3	45.5	−6.7		2.8
	experimental[31]	44.0	44.0	10.0		
N1-protonated	calculated	36.7	36.2	−5.8		3.1
	expermental (HCl)[31]	39.0	39.0	6.5		
	experimental (HCl·$^{1}/_{2}$H$_2$O)[31]	40.0	40.0	6.5		
	experimental (HCl)[26]	39.1	40.5	−5.5		
N1,N7-protonated	calculated	50.0	50.4	−2.5		4.9
	experimental[31]	45.0	45.0			
		C8-hydrogenated radical				
free base	calculated		−5.6	38.9	39.1	2.3
	experimental[31]			39.0	39.0	
N1-protonated	calculated		−5.2	40.6	40.5	3.5
	experimental (HCl)[31]			40.0	40.0	
	experimental (HCl·$^{1}/_{2}$H$_2$O)[31]		6.0	42.0	42.0	
	experimental (HCl)[26]		−4.3	43.0	40.9	
N1,N7-protonated	calculated		−2.5	46.5	46.6	4.9
	experimental[31]			41.0	41.0	

calculations that the dipole moment of the C2-hydrogenated radical (2.7 D) is larger than that of the corresponding C8 radical (1.7 D) and, thus, this property could lead to the greater stability of the C2-hydrogenated radical in ionic environments. The dipole moments obtained in the present study for the various C2- and C8-hydrogenated radicals are displayed in Table 13. The results for the nonprotonated radicals indicate that the C2 radical's dipole moment (2.8 D) is larger than the C8 radical's dipole moment (2.3 D), but not to the extent indicated in the above-mentioned INDO calculations. Similarly, the dipole moments calculated for the N1-protonated radical indicate that the C8 radical possesses only a slightly larger dipole by 0.4 D and the N1,N7-protonated C2 and C8 radicals have identical dipole moments.

The HFCCs calculated for the C2- and C8-hydrogenated radicals with varying degrees of protonation are displayed in Table 13. From the results, it is apparent that the disagreement between theory and experiment increases as the number of protons added to the parent molecule increases. However, even though the absolute magnitude of the results may not agree with experiment, the trend in the couplings of these protonated radicals is clearly described by the calculations. For example, for the C2-hydrogenated radical, the magnitude of the C2−H couplings increases as one goes from the N1-protonated radical to the free base to the N1,N7-protonated radicals in both the experimental and theoretical results. Similarly, the magnitude of the C8−H couplings in the C8-hydrogenated radical increases from the free base to the N1-protonated and to the N1,N7-protonated radicals. In addition, the relative magnitudes of the C2−H and C8−H couplings in the C2 and C8 radicals are also well described by the calculations. For example, the C2−H couplings in the C2-hydrogenated radical are larger than the C8−H couplings in the C8-hydrogenated radical for the free base, but not for the protonated form. It is interesting to note how the couplings in these two radicals differ with changes in the protonation state of the parent molecule. Comparison of calculated and experimental couplings indicates that the isotropic HFCC for C2−H and C8−H in the C8- and C2-hydrogenated radicals, respectively, are most probably negative.

Perhaps the most complete set of HFCCs for the N1-protonated C2- and C8-hydrogenated radicals has been obtained in the more recent work by Close and co-workers[26] in adenosine hydrochloride, and the full results obtained in their study are displayed in Table 12. The values obtained for the N1-protonated C2-hydrogenated radical indicate that the two C2

hydrogens have slightly different couplings (40.5/39.1 G). These isotropic values are slightly larger than those calculated for this radical (36.7/36.2 G). However, the anisotropic C2−H couplings, as well as the full tensors obtained for C8−H, and the N1−H HFCCs are in remarkable agreement.

It is interesting to note that the calculated coupling tensors of the nonprotonated C2-hydrogenated radical (45.5/43.3 G) differ from the HFCCs in the N1-protonated form (36.7/36.2 G). In addition to the differences in the magnitude of the couplings, the two couplings obtained for the hydrogens at C2 in the nonprotonated radical are different from each other. Also, the average of the calculated isotropic HFCCs obtained for the protonated and the nonprotonated, 40.4 G, radicals is in astonishing agreement with the magnitude of the couplings obtained experimentally for the singly protonated system (40.5/39.1 G). Thus, it is possible that experimentally an averaging of the protonated and nonprotonated HFCCs is observed.

The values obtained by Close et al.[26] for the complete HFCC tensor for the C2−H, as well as the anisotropic couplings for the C8−H, in the C8-hydrogenated radical are in excellent agreement with the calculated values for the singly protonated systems (Table 11). The calculated isotropic C8−H HFCCs (40.6/40.5 G) are also in good to fair agreement with the experimental values (43.0/40.9 G), even though both couplings are calculated to be of equal magnitude, whereas experiment indicates that there is a slight difference between the two hydrogens. The calculations clearly indicate, unlike for the C2-hydrogenated radical, that the observed C8-hydrogenated radical is protonated at the N1 position.

Conclusion

The geometries, spin density distributions, and hyperfine coupling constants in radicals that could be formed upon irradiation of adenine have been studied through the use of density functional theory. Possible hydrogenated, dehydrogenated, and hydroxylated radicals were examined, and the coupling constants were compared to those obtained from detailed ESR/ENDOR studies on single crystals of numerous adenine derivatives. In addition to the neutral radicals, various protonated radicals were also examined. These radicals involved mainly protonation at the N1 position and, in the case of the C2 and C8 radicals, the N1 and N7 doubly protonated radicals.

The geometry of the adenine anion revealed that this radical deviates substantially from planarity, and the calculated cou-

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plings for this radical, as well as a planar form, were not observed in any experimental spectra. On the other hand, adenine does not undergo any significant geometrical alterations upon the formation of the cation, and this radical has been observed experimentally. The N9-dehydrogenated radical was shown to be the lowest energy radical in its class. In full DNA, this radical is not possible and the adduct formed by an abstraction of a hydrogen from the amino group would be favored. Radicals generated by abstraction of a hydrogen from the C2 or C8 position are much higher in energy.

The C2 and C8 hydrogen addition radicals were determined to have the lowest energy in their class. The geometrical effects due to the formation of these radicals (local puckering at the site of addition) are difficult to describe theoretically. Thus, the HFCCs of the C2 (C8) hydrogens were calculated to differ by only 2 G (0 G), while experimentally these couplings deviate by approximately 10 G (4 G) in the two hydrogen adducts. The nitrogen-hydrogenated radicals undergo significant geometrical alterations upon formation, with great distortion noted for the radical formed by addition of a hydrogen to N3. The distortion in the N3-hydrogenated radical results in an out of plane position for the N3 hydrogen and, hence, a large isotropic HFCC. Experimentally, this hydrogen yields only a small HFCC, and thus, it is speculated that interactions must be occurring in the crystals that lead to an in-plane position for the N3 hydrogen and a subsequently small HFCC. Calculations on a constrained, planar geometry for this radical confirmed this to be the case. The C4 and C5 hydrogen addition radicals are the highest in energy of the radicals formed via hydrogen addition. These two radicals adopt the "butterfly" conformation that has been previously discussed in the literature.[34]

The C2 and C8 hydroxyl addition radicals are lower in energy than those radicals formed via addition of hydroxyl to either the C4 or the C5 position. In addition, the C2 and the C8 addition radicals undergo only slight geometrical alterations, whereas the radicals formed by hydroxyl addition to C4 and C5 adopt conformations identical to those observed for the corresponding hydrogen adducts. Only the C8 hydroxyl addition radical has as yet been observed in the adenine crystals that have been examined experimentally. However, the comparison of the calculated HFCCs for the other possible hydroxylated radicals with the experimental spectra will make it easier to determine whether these radicals may actually be formed or not.

A few N1-protonated and N1,N7-diprotonated radicals were also examined in the present study, and the energetics of these radicals were similar to those of the nonprotonated forms. The only difference between the protonated and the nonprotonated radicals is the relative stabilities of the C2 and C8 hydrogen addition radicals, which is perfectly reproduced by the calculated energetics. Comparison of theoretical and experimental results leads to the conclusion that the protonated C4-hydrogenated radical has not been detected in the experimental studies to date. The differences in the HFCCs of various protonated forms of the C2- and C8-hydrogenated radicals observed experimentally were well reproduced with DFT.

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