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Pyrimidine Ring Opening in the Unimolecular Dediazoniation of Guanine Diazonium Ion. An Ab Initio Theoretical Study of the Mechanism of Nitrosative Guanosine Deamination

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DNA base deamination and interstrand cross-linking due to reaction with HNO_2 or NO has been linked to a variety of disorders in people.[1] Nitric oxide deaminates nucleosides, nucleotides, and DNA in vitro, and similar DNA damage also occurs in vivo.[2] The amino group of adenine can be eliminated via diazotization reactions,[3] and deamination of cytosine to uracil is a well-known mutagenic event.[4,5] The deamination of guanine with HNO_2 leads to xanthine formation (Scheme 1) or cross-linking[6,7] to a proximate guanine or adenine.[8]

The deaminations of the DNA bases are thought to involve diazonium ions as the crucial reactive intermediate, and the mechanistic hypotheses are based on product analyses and their rationalization in analogy to the chemistry of aromatic primary amines.[9] In contrast to aniline, however, the diazonium ions of the DNA bases have never been observed directly, and their properties, stabilities, and reactivities are not known. The mechanistic hypotheses for the reaction of water with the guanine diazonium ion GN_2^+ (**1**) are outlined in Scheme 1. The three principle mechanisms previously discussed *all* result in the replacement of the N_2^+ function by the OH group, followed by tautomerization to xanthine, and they differ in the timing of N_2 elimination and hydroxyl group addition ($+H_2O/-H^+$). If H_2O attacks the C-atom to which the diazonio function is attached, nucleophilic aromatic substitution occurs with N_2 loss and formation of xanthine in a uni- (S_NAr1) or bimolecular (S_NAr2) fashion. Nitroguanine is a known side product, and its formation is indicative of an S_N1 type process. Alternatively, a nucleophile may add to N_β and the diazene may undergo N_2 expulsion. In cross-link formations, it is thought that the amino group of another DNA base serves as the nucleophile, and the Shapiro mechanism is consistent with the Verly kinetic data.[10] The cross-linking was studied with oligodeoxynucleotide duplexes,[11a] and the observed sequence preferences were rationalized by proximity effects involving the diazonium ion[11a]

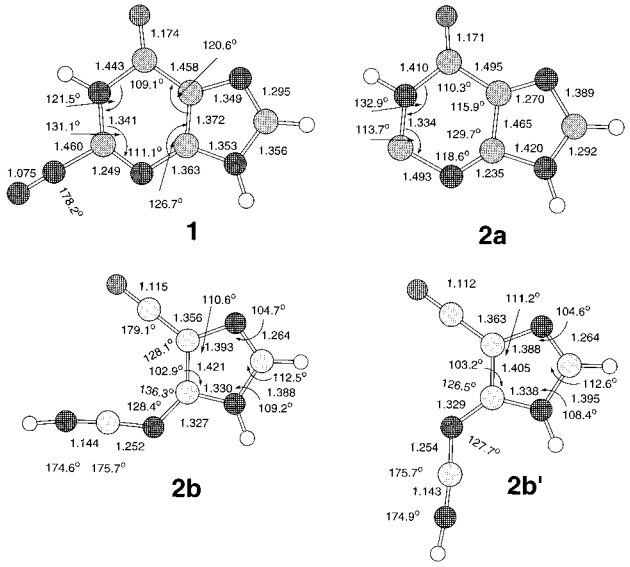
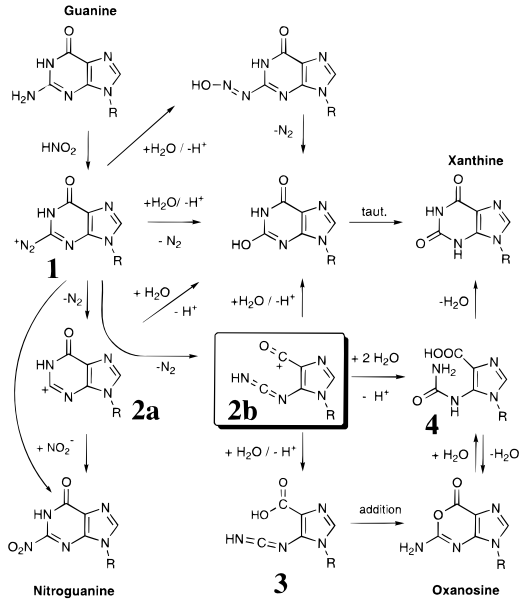


Figure 1. Molecular models of the optimized structures of the guanine diazonium ion **1** and of the dediazoniated cations **2a** and **2b**.

Scheme 1



and corroborated by theoretical study.[11b] On this background, Makino et al.[12] have discovered that in excess of 20% of 2′-deoxyoxanosine was formed in the nitrosations of 2′-deoxyguanosine, oligodeoxynucleotide, and calf thymus. No currently accepted mechanism for guanine deamination accounts for the oxanosine product, and no postulates have been advanced. Here, we report the results of an ab initio study of the unimolecular dediazoniation of **1** (Figure 1) that explains the experimental findings by Makino et al.[12] and provides a mechanistic hypothesis for future experimental investigations.

Structure optimizations and vibrational analyses were carried out at the RHF/6-31G* level, and electron correlation effects were approximated with third-order Møller−Plesset perturbation theory in the frozen core approximation and with the RHF/6-31G* structures (Table 1). Systematic studies of theoretical model dependencies of RN_2^+ (R = H,[13b] Me,[13a,c] Et,[13a] Ph[13d]) show excellent agreement between experiment and theory at this theoretical level [MP3(fc)/6-31G*//RHF/6-31G*+0.9ΔVZPE-

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Table 1. Binding, Relative, and Vibrational Zero-Point Energies[a]

	VZPE	$E_{rel(RHF)}$	$E_{rel(MP2)}$	$E_{rel(MP3)}$	ΔVZPE
1	66.27	18.11[b]	52.80[b]	37.66[b]	−6.07
2a	55.59				
2b	56.79	−60.23[c]	−75.76[c]	−63.06[c]	1.18
2b′	56.77	−1.18[d]	−0.08[d]	−0.43[d]	−0.02
N_2	3.94				

[a] All data based on RHF/6-31G* structures. Vibrational zero-point energies (VZPE, not scaled), VZPE corrections (ΔVZPE, scaled, factor 0.9), and relative energies are in kilocalories per mole. $E_{rel}^{corr} = E_{rel} + \Delta VZPE$. [b] Relative to **2a** + N_2. [c] Relative to **2a**. [d] Relative to **2b**.

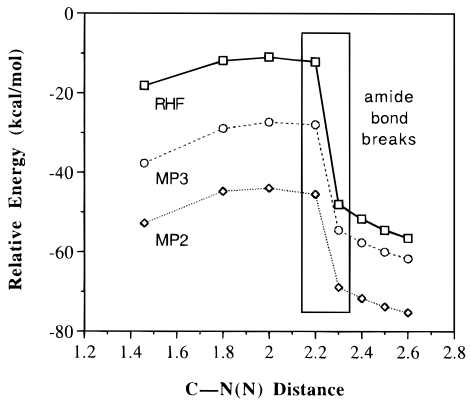
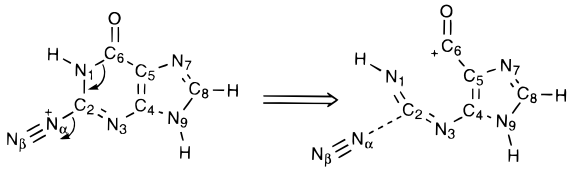


Figure 2. Potential energy diagrams for the unimolecular dediazoniation of **1** as a function of the CN_2 bond length.

(RHF/6-31G*)]. The linear unimolecular dediazoniation path was examined, and several structures of **1** with fixed CN bond lengths were optimized. Molecular models of the stationary structures are shown in Figure 1, and cross sections of the potential energy surface (PES) as a function of r_{CN} are depicted in Figure 2. The PES cross section of **1** provided a surprise: For structures with CN bond lengths greater than 2.2 Å, cleavage of the C6−N1 bond occurred (Figure 2). The cation **2a**—the expected product of heterolytic CN bond cleavage—does exist as a stable structure on the potential energy surface. However,



2a is not formed in the unimolecular dediazoniation! Instead, the N_2 elimination is correlated with amide bond cleavage and leads to the pyrimidine-ring-opened cation **2b**. All attempts to find a reaction channel connecting **1** directly to **2a** failed. Structure **2b** (Figure 1) was optimized and is preferred over **2a** by 63.0 kcal/mol. The rotational isomer **2b′** is nearly isoenergetic. One might expect an allene type structure for the carbodiimide moiety of **2b** as for HN═C═NH itself,[14] but the planar structures **2b** and **2b′** are indeed minima. The *N*-inversion barrier of carbodiimide is known to be rather low.[15]

The binding energy of the benzenediazonium ion PhN_2^+ is the pertinent reference, and it is the reaction energy for dissociation to form singlet phenyl cation and N_2 which is the lowest energy path for dissociation. We determined $E_b(PhN_2^+) = 26.6$ kcal/mol[13f] at the well-correlated level QCISD(T,fc)/6-31G*//MP2(full)/6-31G*, and this value is in excellent agreement with experimental dissociation energies (25.8−28.3 kcal/mol).[9b] The reaction enthalpy of 31.6 kcal/mol for the process **1** → **2a** + N_2 would suggest that **1** is more stable toward N_2 loss than PhN_2^+. However, since the unimolecular dissociation leads to **2b** instead of **2a**, ion **1** is *thermodynamically unstable* by −30.4 kcal/mol with regard to the reaction channel **1** → **2b** + N_2 and this dediazoniation is *kinetically hindered* by only about 10 kcal/mol. The stability of **1** toward dediazoniation is not measured by the binding energy but rather by this kinetic barrier. The stabilities of the DNA base diazonium ions toward unimolecular dediazoniation follow the order CyN_2^+ (3.7 kcal/mol) < AdN_2^+ (9.0) ≈ GuN_2^+ (<10) ≪ PhN_2^+ (26.6). According to comparative kinetic analysis of nitrous acid deaminations,[16] the reactivities followed the order guanosine > adenosine > cytidine and parallel (with few exceptions) the reactivities observed in intact nucleic acids or whole viruses. In light of the computed stabilities of the diazonium ions, it appears that these reactivities are not determined by diazonium ion stabilities but more likely reflect the rates of their formations.

Our calculations suggest that only about 10 kcal/mol are required to elongate the CN_2 linkage to such a degree that C6−N1 amide bond cleavage occurs. Isotropic and anisotropic effects of the environment will have to be included in future refinements of the model, but it is very likely that the qualitative essentials will persist. This finding provides a consistent explanation for the formations of all observed products. Hydrolysis of **2b** to form the carboxylic acid **3** should be facile and strongly suggests intramolecular addition of the acid onto the carbodiimide as the most likely route to oxanosine. Twofold hydrolysis of **2b** to **4** followed by amide formation constitutes a reaction channel for *xanthine formation via* **2b** and a second path for oxanosine.[17] The formations of xanthine via S_N1 or S_N2 type reactions at C_{ipso} should by no means be considered exclusively, and xanthine might well arise from **2b**. Such condensation reactions are precedented and may occur under the reaction conditions used in the diazotizations. The direct S_N2 type replacement of the N_2 group by weak nucleophiles (H_2O, NO_2^-) as well as the intermediacy of **2a** both seem unlikely in light of the results presented. Instead, our results suggest that experimental and theoretical studies of guanine deamination should focus on investigations of the reactivity of **2b**. Preliminary results of electronic structure analyses indicate that **2b** is well described as a ketene−carbodiimide-substituted tertiary (C4-centered) carbenium ion and that this description is superior to the acylium ion resonance form. We are currently addressing regiochemical issues of the reactivity of **2b**.

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Supporting Information Available: Details of computations and results of electron density analysis of **2b** (6 pages). See any current masthead page for ordering and Internet access instructions.

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