culated dipole moments of hydrocarbons can be predicted in from fair to good agreement with observations. From the work presented here, we see that variations in the C-H bond strength of hydrocarbons can also be explained in terms of pure electrostatic effects. It has also been possible to demonstrate[2a] that the barrier to rotation in ethane barrier and the instability of the gauche conformation relative to trans conformation in *n*-butane could not be explained by electrostatic interactions. These phenomena require other explanations, and the simplest is a nonbonded H · · · H repulsion of the form originally proposed by Huggins.[7] According to Huggins, a pair of H atoms attached to two different C atoms will repel each other if the distance between them is smaller than 2.7 Å. The energy associated with this repulsion can be as high as 1.0 kcal mol^{-1} for every interaction at a distance of ~2.3 Å. Once this repulsive potential is added to the electrostatic potential, an excellent agreement is obtained between the model and the experimental value for the barrier to rotation along C-C axis and for the relative instability of the gauche conformation. In some hydrocarbon molecules, the electrostatic model had predicted a heat of formation more negative than the experimental value. In all these cases, we found that at least one pair of nonbonded H atoms is separated by less than 2.5 Å. Adding the repulsion energy associated with this interaction, a better agreement with the experimental observation is obtained.

The various formal charges that we have selected in this series to explain the electrostatic stability of hydrocarbons are only an approximation, but they were consistent in all cases within the experimental uncertainty. The actual formal charges can be slightly different. This could be determined only when one will make the exact geometrical model and will take into account the exact energetic values for the H · · · H nonbonded interaction, the electrostatic interactions, and the potential function for the structure.

A preliminary calculation of $\Delta H_f°$ for compounds containing heteroatoms has already shown that this simple model by itself will not give as good an agreement with the observed results. Once an atom with a lone pair of electrons is introduced into the molecule, it appears that it is necessary to take into account the interaction between the dipole moment associated with the lone pair and the other formal charges in the molecule. Polarization effects also became significant energetically and, at the moment, we have not sorted them out.

Appendix I

The heats of formation of various free radicals, in kcal mol^{-1}, as a function of the formal charges y_r and δ_r ($|y| = 0.28 \times 10^{-10}$ esu) are given in Table VI.

References and Notes

(1) Postdoctoral Research Associate.
(2) (a) Part I of this series: S. W. Benson and M. Luria, *J. Am. Chem. Soc.*, **97**, 704 (1975); (b) part II: S. W. Benson and M. Luria, preceding paper in this issue.
(3) H. E. O'Neal and S. W. Benson in Free Radicals, Vol. II; J. Kochi, Ed., Wiley-Interscience, New York, N.Y., 1973, Chapter 17.
(4) S. W. Benson, "Thermochemical Kinetics", Wiley, New York, N.Y., 1968.
(5) D. M. Golden and S. W. Benson, *Chem. Rev.*, **69**, 125 (1969).
(6) C. W. Larson, E. A. Hardwidge, and B. S. Rabinovitch, *J. Chem. Phys.*, **50**, 2769 (1969).
(7) M. L. Huggins in "Structural Chemistry and Molecular Biology", A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968, p 761.
(8) Calculated heats of formation of the free radicals based on the different selections of formal charges are summarized in Appendix I.
(9) Assuming polarizabilities of 1 and 2 Å^3 for C and Ċ, respectively.

Free-Radical Intermediates Produced from the One-Electron Reduction of Purine, Adenine, and Guanine Derivatives in Water

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Abstract: The one-electron reduction of purine (PH), 9-methylpurine (MP), adenosine (A) and 1-methylguanosine (MG) in water was studied using the fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry. The hydrated electron, e_{aq}^-, and the acetone ketyl radical, $(CH_3)_2\dot{C}OH$, were used as the reducing agents. The reaction rate constants of the purine derivatives with e_{aq}^- and the $(CH_3)_2\dot{C}OH$ radical were determined at different pH values, consistent with the pK_a values of these compounds. The rate constants with e_{aq}^- were close to the diffusion-controlled limit, $k \leq 2.0 \times 10^{10}$ M^{-1} sec^{-1}. The electron transfer reaction from $(CH_3)_2\dot{C}OH$ was found to be strongly dependent on the acid–base properties of the purines and on the nature of the substituents. A correlation between the reaction rate constants with $(CH_3)_2\dot{C}OH$ and the redox potential of the purines is suggested. The transient optical absorption spectra of the free-radical intermediates produced from the reduction of the purine derivatives were determined over the pH range 0–14. The extinction coefficients and decay kinetics are also presented. These radicals undergo acid–base dissociation reactions. The pK_a values for the purine radicals $PH_4^{\cdot 2+}$, $PH_3^{\cdot +}$, $PH_2^{\cdot}$, and $PH^{\cdot -}$ are 3.2 ± 0.1, 8.5 ± 0.2, 9.9 ± 0.2, and 12.5 ± 0.2, respectively. For 9-methylpurine, the $MPH_3^{\cdot 2+}$, $MPH_2^{\cdot +}$, and $MPH^{\cdot}$ radicals have pK_a values of 2.9 ± 0.2, 6.3 ± 0.1, and 13.1 ± 0.2, respectively. For adenosine, the $AH_3^{\cdot 2+}$ and $AH_2^{\cdot +}$ radicals have pK_a values of 4.6 ± 0.1 and 10.5 ± 0.1, respectively, while the ionization of $AH^{\cdot}$ is not observed up to pH 13.6. For 1-methylguanosine, two pK_a (radical) values of ~7.0 and ≥13.0 are observed. These and other results are discussed, and tentative assignments are suggested for the various radical intermediates.

A great number of biochemical processes occur through a mechanism involving an electron transfer from a donor molecule to an acceptor molecule. The study of the one-electron reduction of the constituent bases of nucleic acids has received relatively little attention. The electrochemical reduction of pyrimidine and purine bases in aqueous and nonaqueous solutions has received the most attention, particularly through the investigations of Elving and coworkers.[2-6]

Table I. Reaction Rate Constants of Purine, Adenine, and Guanine Derivatives with One-Electron Reducing Agents in Aqueous Solution

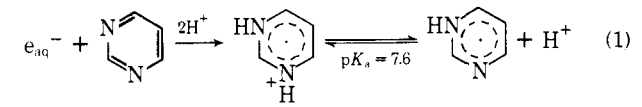
System	pK_a	Reducing agent	pH	k, M^{-1} sec^{-1} [a]
Purine	2.4, 9.0	e_{aq}^-	6.0	2.1×10^{10} (1.6×10^{10})
		e_{aq}^-	13.0	6.5×10^9 (8.2×10^9)
		$(CH_3)_2\dot{C}OH$, CO_2^-	6.0	$<10^7$
		$(CH_3)_2\dot{C}OH$	~0	2.7×10^9
		$(CH_3)_2\dot{C}O^-$	13.3	$<10^7$
9-Methylpurine	2.4	e_{aq}^-	8.5	1.9×10^{10}
		$(CH_3)_2\dot{C}OH$	8.6	1.7×10^8
		$(CH_3)_2\dot{C}OH$	~0	1.9×10^9
		$(CH_3)_2\dot{C}O^-$	13.6	8.7×10^8
		$CH_3\dot{C}HOH$	8.2	$<2.0 \times 10^7$
		$CH_3\dot{C}HO^-$	13.6	5.1×10^8
Adenosine	3.6, 12.4[b]	e_{aq}^-	7.0	1.2×10^{10} (9.2×10^9)
		$(CH_3)_2\dot{C}OH$	7.0	$<10^6$
		$(CH_3)_2\dot{C}OH$	2.2	4.7×10^7
		$(CH_3)_2\dot{C}O^-$	13.6	$<10^6$
Guanosine	1.6, 9.2, 12.4[b]	e_{aq}^-	6.7	6.0×10^9
1-Methylguanosine	~2.4	e_{aq}^-	9.2	7.7×10^9
		$(CH_3)_2\dot{C}OH$	0.5	8.0×10^7

[a] Values in parentheses are from ref 12. [b] Ionization of N_9 side chain.

They found that in nonaqueous media purine and 6-substituted purines undergo a one-electron reduction to form the corresponding free-radical anions. In aqueous media, free-radical formation was not observed (due to the short lifetimes of these intermediates) and initial multiple electron (2e or 4e) reduction occurred.

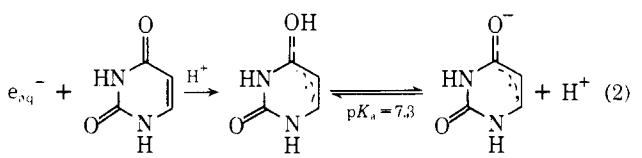
This work deals with the study of the intermediates produced from the one-electron reduction of purine and purine derivatives in water using hydrated electrons, e_{aq}^-, and acetone ketyl radicals, $(CH_3)_2\dot{C}OH$, as the reducing agents. The fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry afforded means to monitor the spectral and acid–base properties of the free-radical intermediates formed.

Previous pulse radiolysis studies have been carried out with pyrimidine,[7] pyrimidine derivatives,[8] and imidazole.[9] It was found that the intermediate produced from the reduction of pyrimidine[7] by e_{aq}^- is rapidly protonated in neutral aqueous solutions to form the dihydro radical cation. It ionizes with a $pK_a = 7.6$:



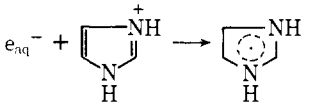
(1)

Addition of e_{aq}^- to uracil[8] produces a ketyl radical, which ionizes with a $pK_a \sim 7.3$:



(2)

Similarly, the reaction of e_{aq}^- with imidazole[9] has been suggested to occur via reaction 3:


(3)

Experimental Section

The pulse radiolysis experimental set-up and conditions used have been described.[10,11] Single pulses of electrons of 2.3 MeV energy and ~30 nsec duration were absorbed by water. The radiation chemistry of water produces e_{aq}^-, H, and OH radicals

$$H_2O \longrightarrow e_{aq}^- (2.8),\ OH\ (2.8),\ H\ (0.6)$$

where the numbers in parentheses are the G values (number of radicals produced per 100 eV of energy absorbed).

One-electron reduction experiments were carried out under two conditions: (1) reduction by e_{eq}^-, and (2) reduction by acetone ketyl radicals. Using condition 1 solutions contained ~1.0 M *tert*-butyl alcohol to scavenge the OH radicals. The β-alcohol radical produced[10] absorbs weakly at $\lambda > 280$ nm and was found not to interfere with the observations to be reported below. Using condition 2 solutions contained 1–2 M isopropyl alcohol and were saturated with N_2O (2.2×10^{-2} M). Under these conditions, e_{aq}^- were converted by N_2O to OH radicals and $(CH_3)_2\dot{C}OH$ was formed via reaction 4.

$$OH\ (\text{or H}) + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O\ (\text{or } H_2) \quad (4)$$

The chemicals used were the highest purity commercially available and were obtained from Calbiochem, Sigma Chemical, and Cyclochemical. Reagents employed were obtained from Aldrich, Mallinckrodt, Eastman and Baker, and Adamson.

Solutions were buffered with perchloric acid, potassium hydroxide, phosphates, and tetraborate salts. All the transient absorption spectra presented below were measured at ~0.2 μsec after the pulse (unless stated otherwise) and were corrected for depletion of the purine derivatives used, at the appropriate pH values and wavelengths. The extinction coefficients were derived based on the KCNS dosimetry (see ref given in ref 10) and the G values of the radical given above.

The H atoms present in solution presumably add to the substrates. The intermediates produced appear to have relatively low extinction coefficients compared to those produced from reaction with e_{aq}^- and $(CH_3)_2\dot{C}OH$ radicals.

Results and Discussion

Reactivity Toward e_{aq}^-. The reaction rate constants of e_{aq}^- with purine and derivatives were determined by monitoring the decay kinetics of e_{aq}^- at 700 nm. From the pseudo-first-order decays, the second-order rate constants were calculated and are given in Table I. These are compared with literature values.[12]

The $k(e_{aq}^- + \text{purine}) = 2.1 \times 10^{10}$ M^{-1} sec^{-1} at pH 6.0. On ionization of N_9H in purine ($pK_a = 9.0$), the rate constant decreases to 6.5×10^9 M^{-1} sec^{-1}. The rate constant of e_{aq}^- with 9-methylpurine is 1.9×10^{10} M^{-1} sec^{-1}, and independent of pH in the range 6–13 as this molecule does not ionize in this region. The reactivity of e_{aq}^- with adenosine is 1.2×10^{10} M^{-1} sec^{-1} at pH 7; while with 1-methylguanosine $k = 7.7 \times 10^9$ M^{-1} sec^{-1} at pH 9.2. The latter lower rate constant may be due to the presence of the electron-donating substituents NH_2, OH, and CH_3 in the pyrimidine ring. The reactivities of guanosine and 1-methyl-

Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and Ionization Constants of Free-Radical Intermediates Produced from the One-Electron Reduction of Purines in Water

System[a]	pH	λ_{max}, nm	ϵ, mM^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1}	pK_a (radical)	Suggested radical
Purine, PH	2 *M* $HClO_4$[b]	317, 470	3.3, 1.0	3.9×10^8[b]		
	1.0[b]	318, 355, 465	4.0, 3.7, 1.2	*d*	3.2 ± 0.1	$PH_4^{\cdot 2+}$
	5.2	315, ~430	3.6, 0.7	*c*	8.5 ± 0.2	$PH_3^{\cdot +}$
	9.5	~310, ~470	3.4, 1.0	*c*	9.9 ± 0.2	$PH_2^{\cdot}$
	11.0	~312, ~470	3.6, 1.1	*c*	12.5 ± 0.2	$PH^{\cdot -}$
	13.3	332, ~440	3.8, 1.0	*c*		$P^{\cdot 2-}$
9-Methylpurine, MP	2 *M* $HClO_4$[b]	317, 475	2.8, 1.1	3.7×10^8[b]		
	1.0[b]	363, 445, ~520	6.2, 2.1, 1.5	*e*	2.9 ± 0.2	$MPH_3^{\cdot 2+}$
	5.0	308, 318, 550	2.2, 2.2, 1.2	2.4×10^8[b]	6.3 ± 0.1	$MPH_2^{\cdot +}$
	10.2	325, 475	2.6, 2.2, 1.0	1.2×10^9[b]	13.1 ± 0.2	$MPH^{\cdot}$
	13.6	320, 330, 535	4.7, 4.6, 1.2	5.5×10^8[b]		$MP^{\cdot -}$
Adenosine, A	2 *M* $HClO_4$[b]	347, ~430	5.0, ~2	5.8×10^7[b]		
	1.9	320, 360, ~430	3.7, 4.4, ~2	*f*		$AH_3^{\cdot +}$
	5.8	320, 360, ~560	4.9, 2.5, ~0.7	1.7×10^8	4.6 ± 0.1	$AH_2^{\cdot +}$
	13.3	360, ~450	11.6, ~1.2	2.5×10^8	10.5 ± 0.1	$AH^{\cdot}$
1-Methylguanosine, MG	0.5	330, ~380, ~470	10.5, 3.7, 2.8	*g*	*g*	$MGH_2^{\cdot +}$, $MGH^+(OH)^{\cdot}$
	5.2	≤315, ~370, 465	2.3, 0.8, 0.6	*g*	*g*	$MGH^{\cdot}$, $MG(OH)^{\cdot}$
	9.0	~330, 470	1.8, 0.9	*g*	7.0 ± 0.2[h]	$MGH^{\cdot}$, $MG(O^-)^{\cdot}$
	13.2	~330, 470	2.1, 0.9	*g*	≥13.0[i]	$MG^{\cdot -}$, $MG(O^-)^{\cdot}$

[a] Experiments carried out in oxygen-free 1.0 *M* *t*-BuOH solutions. [b] Isopropyl alcohol was used instead of *t*-BuOH. [c] Intermediates decay with mixed kinetics. [d] Decays pseudo-first order with $[H^+]$, $k = 3.0 \times 10^6 M^{-1}$ sec^{-1}, to give same species observed in 2.0 *N* $HClO_4$. [e] Decays pseudo-first order with $[H^+]$, $k = 7.5 \times 10^6 M^{-1}$ sec^{-1}, to give the species observed in 2 *N* $HClO_4$. [f] Probably decays by reaction with H^+, as was found for purine and 9-methylpurine. [g] Not determined. [h] pK_a of $MG(OH)^{\cdot}$ radical. [i] pK_a of $MGH^{\cdot}$ radical.

guanosine are close to those of cytosine (8.0×10^9 M^{-1} sec^{-1}) and uracil (8.9×10^9 M^{-1} sec^{-1}), while that of adenosine is comparable to 4-aminopyrimidine (1.1×10^{10} M^{-1} sec^{-1}). The imidazole ring has no great affinity for electrons ($k \sim 4 \times 10^7$ M^{-1} sec^{-1}).

Reactivity Toward $(CH_3)_2\dot{C}OH$ Radicals. The reaction rate constants of $(CH_3)_2\dot{C}OH$ radical with purine derivatives were determined by monitoring the formation kinetics of the free-radical intermediates produced from this electron-transfer reaction, at the appropriate wavelengths. From the pseudo-first-order rate, the second-order rate constants were calculated, see Table I.

No reaction could be observed between $(CH_3)_2\dot{C}OH$ and purine ($pK_a^1 = 2.4$, $pK_a^2 = 9.0$) at pH 6.0. This is not surprising since the redox potential of purine (PH) at pH 7.0 is $E^{01} = -1.03$ V (ref 3-5), while the kinetic potential[13] of the $(CH_3)_2\dot{C}OH$ radical is $E_k^{01} = -0.82$ V. In ~1.0 *N* $HClO_4$, reaction between $(CH_3)_2\dot{C}OH$ and PH_2^+ was observed, $k = 2.7 \times 10^9$ M^{-1} sec^{-1} (see Table I). At this pH, the redox potential of PH_2^+ is expected[14] to be significantly more positive than that of PH. In alkaline solutions (at pH ≥10), purine is present as the anion P^- whose redox potential is much more negative. In fact, purine is not reduced at the dropping mercury electrode in alkaline solutions. Since the $(CH_3)_2\dot{C}O^-$ radical, with $E_k^{01} \sim -1.6$ V[13], reduces P^- only very slowly one must conclude that the redox potential of P^- is more negative than −1.6 V.

The redox potential of 9-methylpurine (MP) is not known. The acetone ketyl radical reacts with MP and MPH^+ with k values of 1.7×10^8 M^{-1} sec^{-1} (at pH 8.6) and 1.9×10^9 M^{-1} sec^{-1} (in 1.0 *N* $HClO_4$), respectively. From these values, one can infer that the redox potential of MP is more positive than that of PH.

No reaction occurs with adenosine (A) at pH 7.0. This agrees with the low redox potential (−1.18 V) for this molecule. On protonation of A a relatively slow reaction occurs with $k = 4.7 \times 10^{7\ -1}$ sec^{-1} at pH 2.2 (Table I). At pH 0.5, the 1-methylguanosine cation can be reduced by $(CH_3)_2\dot{C}OH$ radicals with $k = 8.0 \times 10^7$ M^{-1} sec^{-1}. As in the case of the reaction with e_{aq}^-, these lower rates can be due to the effect of the electron-donating substituents in the molecule.

Purine. Purine (PH) has two ionization constants: $pK_a^1 = 2.39$ due to deprotonation of the cation mainly at the N_1 position, and $pK_a^2 = 8.96$ due to deprotonation of the neutral molecule at the N_9 position.

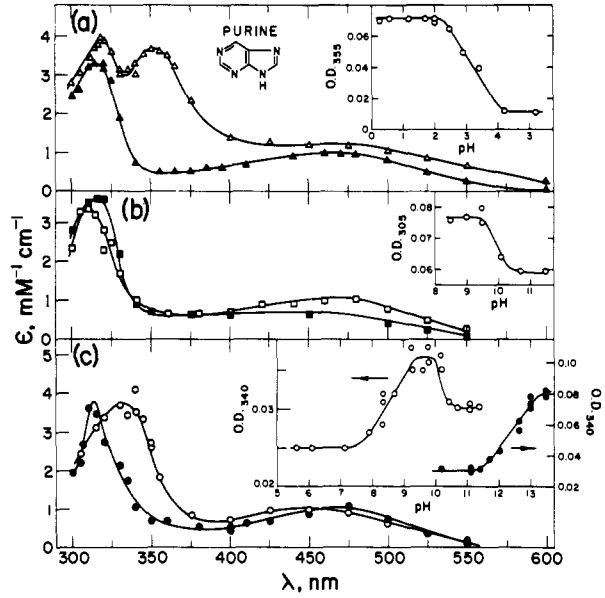


Figure 1. Absorption spectra of intermediates produced from the one-electron reduction of purine (5×10^{-4} *M*) by (a) $(CH_3)_2\dot{C}OH$ radicals at pH 1.0 (△) and 2.0 *M* $HClO_4$ (▲) (solutions contained 1.0 *M* *i*-PrOH, 1 atm argon). Insert: change in absorbance at 355 nm with pH (3×10^{-3} *M* purine, 2.0 *M* *i*-PrOH, 1 atm argon; at pH >4.0 solutions contained *t*-BuOH instead). (b) e_{aq}^- at pH 5.2 (■) and pH 9.5 (□) (solutions contained 0.5 *M* *t*-BuOH, 1 atm argon). Insert: change in absorbance at 305 nm with pH. (c) e_{aq}^- at pH 11.0 (●) and pH 13.3 (○) (solutions contained 0.5 *M* *t*-BuOH, 1 atm argon). Insert: change in absorbance at 340 nm with pH. Total dose ~1.5-5.0 krads/pulse.

The transient optical absorption spectrum of the intermediate produced from the reaction of e_{aq}^- with purine at pH 5.2 is shown in Figure 1b. Absorption maxima at 315 and ~430 nm with extinction coefficients of 3.6×10^3 and 7×10^2 M^{-1} cm^{-1}, respectively, were found (see Table II).

The one-electron reduction of PH_2^+ at pH 1.0 was brought about by reaction with the $(CH_3)_2\dot{C}OH$ radical. The spectrum of the transient species produced is shown in

Figure 1a. This species decays by pseudo-first-order kinetics, dependent upon $[H^+]$, with $k_{H^+} = 3.0 \times 10^6\ M^{-1}$ sec^{-1}, to give a different transient spectrum (see Figure 1a). This latter spectrum (an identical spectrum is formed at pH ≤ 0 from the reduction of PH^+ with acetone ketyl radicals) decays by second-order kinetics with $2k = 3.9 \times 10^8\ M^{-1}$ sec^{-1} (Table II).

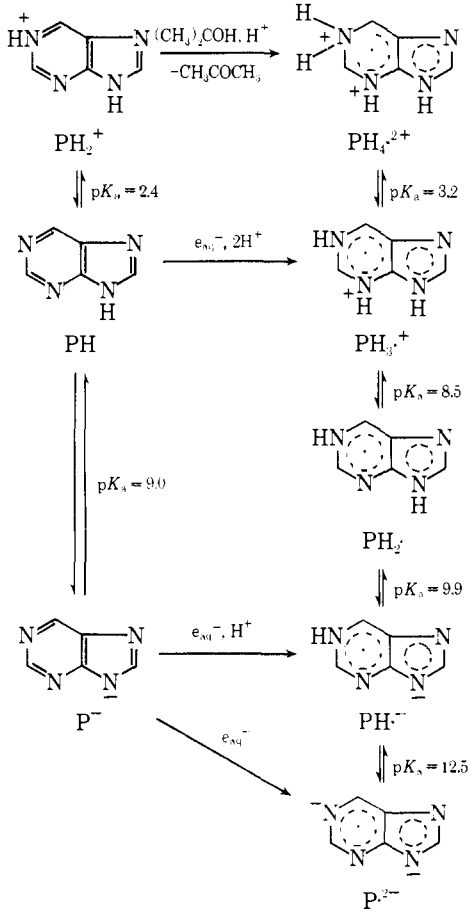
The reaction of e_{aq}^- with purine at pH 9.5, 11.0, and 13.3 gives rise to different intermediates, each having a characteristic absorption spectrum: Figures 1b and 1c and Table II.

The change in absorption with pH was monitored at different wavelengths. Typical titration curves were observed, and from the midpoints the following pK_a (radical) values were derived: 3.2 ± 0.1, 8.5 ± 0.2, 9.9 ± 0.2, and 12.5 ± 0.2; see inserts to Figure 1 and Table II.

A tentative set of reactions is suggested to explain the experimental observations presented above. The reaction mechanism was chosen on the basis of the known[15] basicities of purine (and purine derivatives) and the strong proton affinity of the radicals produced from aromatic nitrogen heterocyclic compounds.[7,9,16-18]

In Scheme I (as in the other schemes presented below) different isomeric forms of the free radicals are not presented, nor is any statement implied on the spin densities for any position in the purine structure.

Scheme I. Purine (PH)



It is interesting to point out that, under Scheme I, the $pK_a = 8.5$ for the $PH_3{\cdot}^+$ radical can be compared to $pK_a = 7.6$ for the dihydrocation radical of pyrimidine.[7] Similarly, while the neutral monohydro radical of pyrimidine does not ionize up to pH ≥13.0, the $PH{\cdot}^-$ radical has a $pK_a = 12.5$. The pK_a values of 3.2 and 9.9 are assumed to be due to deprotonation at N_1 and N_9 positions, as in the parent molecule.

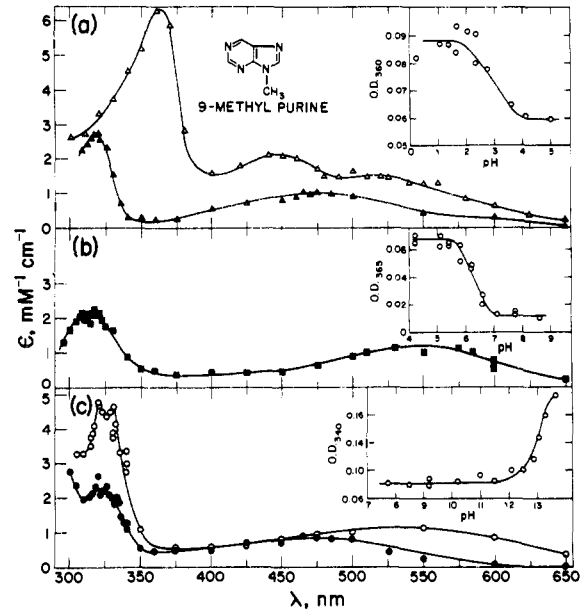


Figure 2. Absorption spectra of intermediates produced from the one-electron reduction of 9-methylpurine (5×10^{-4} *M*) by (a) $(CH_3)_2\dot{C}OH$ radicals at pH 1.0 (△) and 2.0 *M* $HClO_4$ (▲) (solutions contained 1.0 *M* *i*-PrOH, 1 atm argon). Insert: change in absorbance at 360 nm with pH. (b) e_{aq}^- at pH 5.0 (■) (solutions contained 0.5 *M* *t*-BuOH, 1 atm argon). Insert: change in absorbance at 565 nm with pH. (c) e_{aq}^- at pH 10.2, (●) and pH 13.6 (○) (solution contained 0.5 *M* *t*-BuOH, 1 atm argon). Insert: change in absorbance at 340 nm with pH. Total dose ~4–10 krads/pulse.

9-Methylpurine. Methylation of the N_9 position simplifies the nature and the number of dissociation constants. The free-radical intermediates formed at pH 1.0, 5.0, 10.2, and 13.6 are shown in Figure 2, and the results are given in Table II.

The intermediate produced at pH 1.0 decays by reaction with H^+ (as was found above for purine) with $k_{H^+} = 7.5 \times 10^6\ M^{-1}\ sec^{-1}$, to give a spectrum identical to the transient spectrum observed at pH ≤0. The second transient species formed decays with $2k = 3.7 \times 10^8\ M^{-1}\ sec^{-1}$ in 2 *M* $HClO_4$.

Four different intermediates formed from the one-electron reduction of 9-methylpurine were observed, with pK_a values of 2.9 ± 0.2, 6.3 ± 0.1, and 13.1 ± 0.2. These free radicals decay by second-order kinetics, Table II.

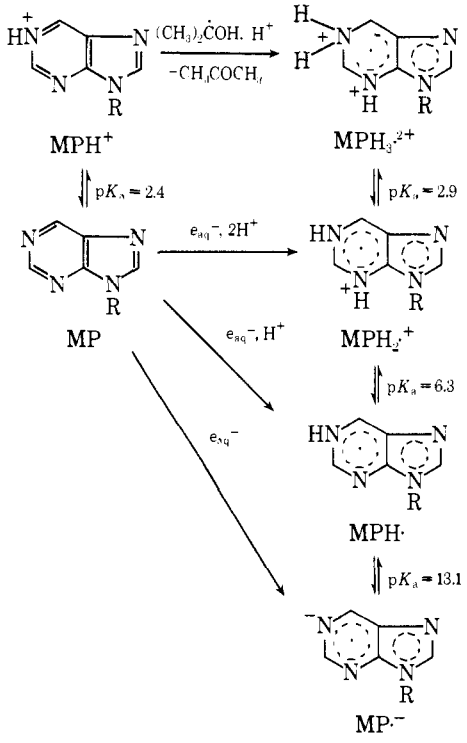
The suggested mechanism and nature of these free-radical intermediates is shown in Scheme II. The reactions have been presented in a manner consistent with those suggested above for purine. It may be noted that the pK_a of $MPH_2{\cdot}^+$ (6.3) is lower than that of $PH_3{\cdot}^+$ (8.5).

Adenosine. The site of protonation in adenine and adenosine ($pK_a = 3.6$) is mainly at the N_1 position,[19] as in purine. Electrochemical reduction has also been suggested to take place at this position.

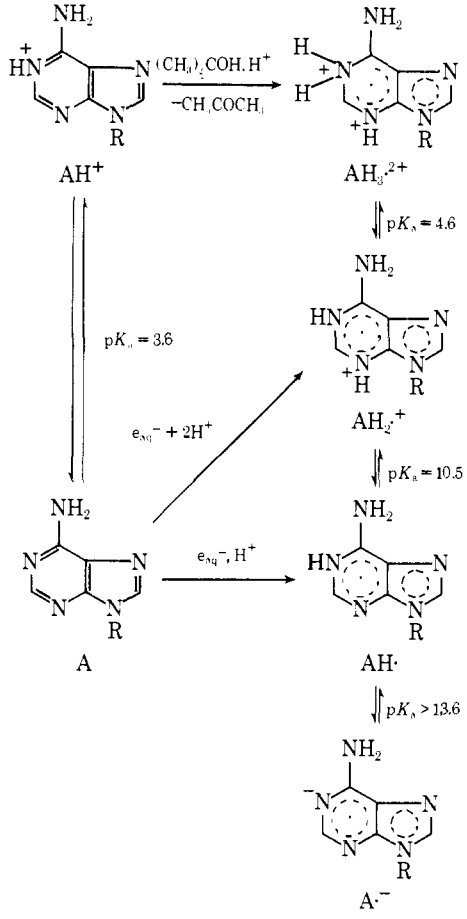
Figure 3 shows the transient absorption spectra of the free-radical intermediates produced from adenosine at pH 1.9, 5.8, and 13.3 and in 2.0 *N* $HClO_4$. Ionization constants of the free radicals were observed at 4.6 ± 0.1 and 10.5 ± 0.1; see inserts in Figure 3 and Table II. The suggested reactions for the one-electron reduction of adenosine are shown in Scheme III.

These assignments are consistent with those suggested above for purine and 9-methylpurine. The $pK_a = 10.5$ for $AH_2{\cdot}^+$ is higher than that for $MPH_2{\cdot}^+$ (6.3). The ionization of the $AH{\cdot}$ radical, as in the case for 4-NH_2 pyrimidine[18]

Scheme II. 9-Methylpurine (MP)



Scheme III. Adenosine (A)



and pyrimidine,[7] is not observed up to pH 13.6.

The purine, 9-methylpurine, and adenosine dication radicals react with H^+ with $k \sim 5 \pm 2 \times 10^6\ M^{-1}\ sec^{-1}$. This rate constant appears to be too slow for a true protonation reaction, but is not unreasonable for an acid-catalyzed rearrangement. Furthermore, as the protonation of the parent molecules is not exclusively at the N_1 position, the initial transient species produced at pH $\leq$ pK_a may be a mixture of radicals. Rearrangement by H^+ may follow.

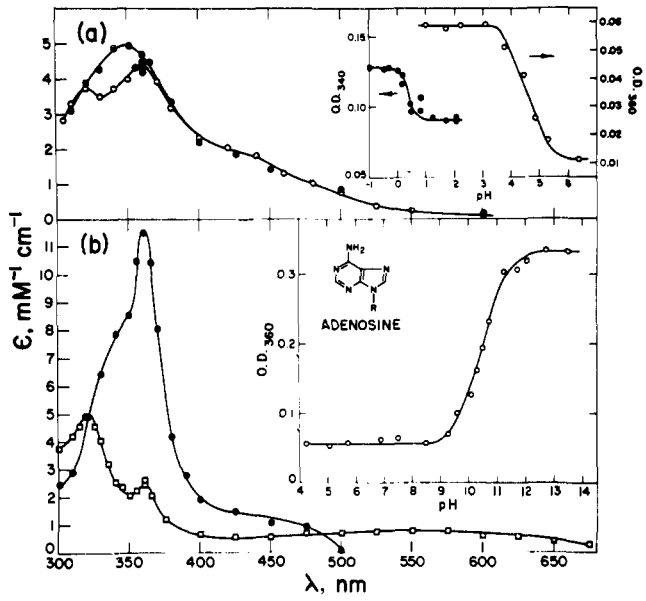


Figure 3. Absorption spectra of intermediates produced from the one-electron reduction of adenosine by (a) $(CH_3)_2\dot{C}OH$ radicals at pH 1.9 (○) and 2.0 M $HClO_4$ (●) (solutions contained 5×10^{-3} M adenosine, 1.0 M i-PrOH, 1 atm argon). Insert: change in absorbance at 340 and 360 nm with pH. (b) e_{aq}^- at pH 5.8 (□) and pH 13.3 (●) (solutions contained 10^{-3} M adenosine, 1.0 M t-BuOH, 1 atm argon). Insert: change in absorbance at 360 nm with pH. Total dose ~3–5 krads/pulse.

Deprotonation reactions of the free radical by OH^- ions could be observed in this case in the pH range ~10–12. The reaction rate constant was determined from the pseudo-first-order decay of the transient species at 360 nm, and $k_{OH^-} = 1.1 \times 10^8\ M^{-1}\ sec^{-1}$ was obtained.

1-Methylguanosine. 1-Methylguanosine (MG) has a pK_a ~ 2.4 and protonation has been assigned primarily to the N_7 position (i.e., in the imidazole ring). This molecule has a carbonyl group in the C_6 position which cannot be enolized due to the N_1CH_3 group. Consequently, there is less resonance conjugation in MG compared to the other purines studied.

The transient spectra observed from the one-electron reduction of MG, and the ionization constants of the free-radical intermediates, are shown in Figure 4 and Table II.

One-electron reduction of MG (the same would be true for guanosine and guanine) is suggested to occur at two different sites in the molecule: the pyrimidine and imidazole rings. The electron addition in the pyrimidine ring is suggested to form primarily a ketyl radical, see Scheme IV, as was proposed[8] for various substituted pyrimidines containing a carbonyl group. The $-\dot{C}(O^-H)-$ radical ionizes with a pK_a = 7.0. This value is to be compared to the pK_a ~ 7.3 observed[8] for the corresponding radical in uracil and thymine.

Electron reduction of the imidazole ring of MG is also suggested to occur. This is supported by the strong basicity[15] of the N_7 position in guanine. The neutral radical intermediate formed has a p$K_a \geq 13.0$ and ionizes to give the $MG\cdot^-$ radical anion, see Scheme IV.

Conclusions

The rate constants for the one-electron reduction of various purine derivatives by hydrated electrons and acetone

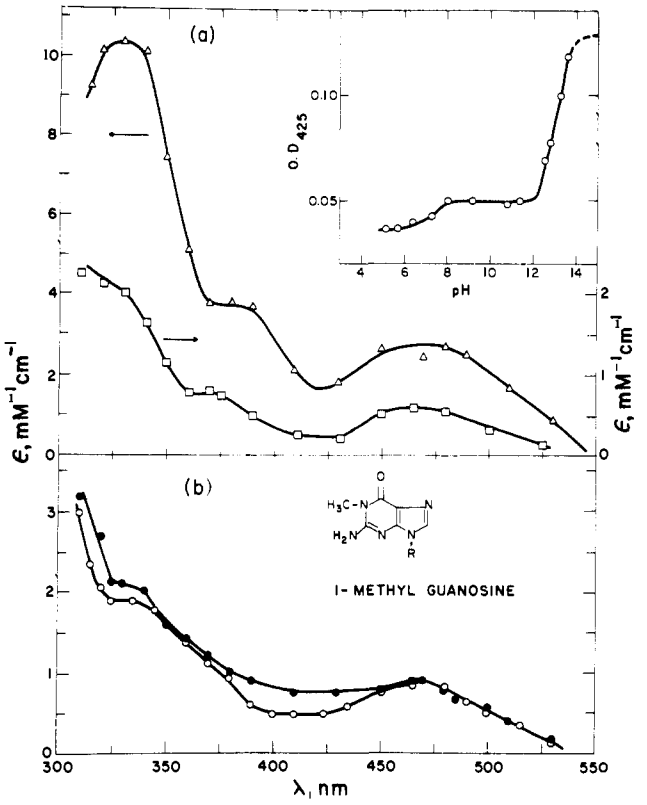


Figure 4. Absorption spectra of intermediates produced from the one-electron reduction of 1-methylguanosine (1×10^{-3} *M*) by (a) $(CH_3)_2\dot{C}OH$ radicals at pH 0.5 (△) (in 1.5 *M* *i*-PrOH) and (b) by e_{aq}^- at pH 5.2 (□), pH 9.0 (○), and pH 13.2 (●) (in 1.0 *M* *t*-BuOH, 1 atm argon). Insert: change in absorbance at 425 nm with pH. Total dose 2–4 krads/pulse.

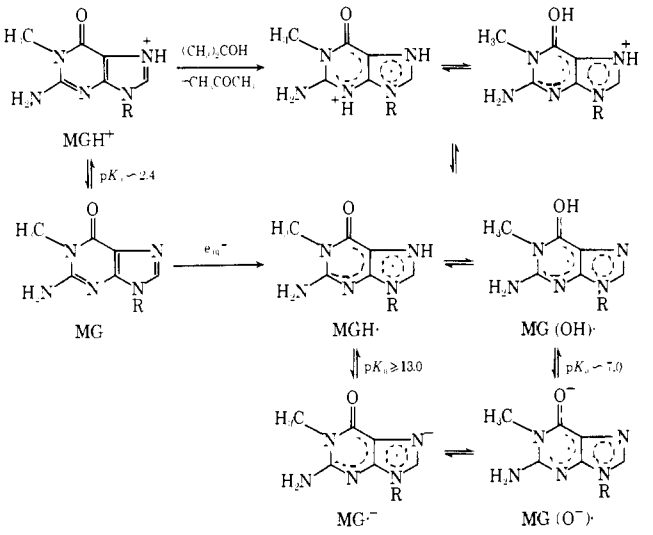
ketyl radicals have been interpreted on the basis of substituent effects.

Tentative reaction schemes have been proposed for the sites of one-electron reduction, followed by subsequent rapid protonation of purine, 9-methylpurine, adenosine, and 1-methylguanosine in water. Electron localization on nitrogen and/or carbon atoms is suggested. The positions for loss of a proton on ionization of the free-radical intermediates observed have also been suggested. Purine, 9-methylpurine, and adenosine appear to undergo reduction primarily in the pyrimidine ring. 1-Methylguanosine is suggested to be reduced in both the pyrimidine and imidazole rings.

Many of the free-radical intermediates decay by second-order kinetics. A disproportionation reaction probably occurs with the formation of the dihydro derivatives (e.g., in the case of purine, 1,6-dihydropurine may be formed).

It is of interest to point out that the intermediate produced at pH 7.0 from the reduction by e_{aq}^- of adenine is a

Scheme IV. 1-Methylguanosine (MG)



powerful reducing agent.[21] It has a kinetic potential $E_k^{01} \sim -1.5$ V. With guanosine, two species are observed[21] with E_k^{01} values of −0.26 and +0.04 V. This marked difference in the reducing properties of the radicals produced from purine derivatives may be of significance in biochemical electron-transfer reactions, as well as in radiosensitization processes.

References and Notes

(1) Visiting Scientist from the Bhabha Atomic Research Center, Chemistry Division, Trombay, Bombay 400285, India.
(2) B. Janik and P. J. Elving, *J. Electrochem. Soc.*, **116**, 1087 (1969); *J. Am. Chem. Soc.*, **92**, 235 (1970).
(3) J. W. Webb, B. Janik, and P. J. Elving, *J. Am. Chem. Soc.*, **95**, 8495 (1973).
(4) P. J. Elving and J. W. Webb, "The Purines-Theory and Experiment", Israel Academy of Sciences and Humanities, Jerusalem, 1972.
(5) P. J. Elving, S. J. Pace, and J. E. O'Reilley, *J. Am. Chem. Soc.*, **95**, 647 (1973).
(6) K. S. V. Santhanam and P. J. Elving, *J. Am. Chem. Soc.*, **96**, 1653 (1974).
(7) P. N. Moorthy and E. Hayon, *J. Phys. Chem.*, **78**, 2615 (1974).
(8) E. Hayon, *J. Chem. Phys.*, **51**, 4881 (1969).
(9) P. S. Rao, M. Simic, and E. Hayon, *J. Phys. Chem.*, in press.
(10) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969).
(11) J. P. Keene, E. D. Black, and E. Hayon, *Rev. Sci. Instrum.*, **40**, 1199 (1969).
(12) P. C. Schragge, H. B. Michaels, and J. W. Hunt, *Radiat. Res.*, **47**, 598 (1971).
(13) P. S. Rao and E. Hayon, *J. Am. Chem. Soc.*, **96**, 1287 (1974); *ibid.*, **97**, 2986 (1975).
(14) See ref 3, 5, and 6.
(15) B. Pullman and A. Pullman, "Quantum Biochemistry", Interscience, New York, N.Y., 1963, p 232.
(16) D. V. Bent, E. Hayon, and P. N. Moorthy, *J. Am. Chem. Soc.*, in press.
(17) P. N. Moorthy and E. Hayon, *J. Am. Chem. Soc.*, **97**, 2048 (1975).
(18) P. N. Moorthy and E. Hayon, *J. Phys. Chem.*, in press.
(19) R. M. Izatt and J. J. Christensen, *J. Phys. Chem.*, **66**, 359 (1962).
(20) P. N. Moorthy and E. Hayon, *J. Phys. Chem.*, submitted.
(21) P. S. Rao and E. Hayon, *J. Phys. Chem.*, submitted.