One-Electron Redox Potentials of Purines and Pyrimidines

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One-electron redox potentials of some purine and pyrimidine derivatives were determined by pulse radiolysis from electron transfer equilibria involving their and other free radicals. The redox potentials were determined at pH 13 by using *p*-methoxyphenol (E = 0.4 V), Trolox C (E = 0.19 V), and tryptophan (E = 0.56 V) as references. The lowest oxidation potential measured for DNA bases was for guanosine (E = 0.72 V vs. NHE), and the highest was for 1-methylpyrimidines ($E \sim 1.6$ V). Uric acid (E = 0.26 V) and isobarbituric acid (E = 0.13 V) were found to have the lowest potentials.

Introduction

One-electron transfer processes in biological systems induced by various exogenous agents, such as light and ionizing radiation, and endogenous processes, such as autoxidation and metabolic reactions, are not fully understood in spite of numerous studies.[1-3] For example, it is still an open question which DNA bases are damaged by direct action of ionizing radiation. Which base may act as a sink for the positive hole? Which particular compounds may act as repairing agents of the positive holes and their daughter radicals? Two features are of particular importance. One-electron redox potentials provide predictability for the possible direction of flow of the electrons. Secondly, rate constants allow definitive determination of preferred processes. These two parameters, while being exact in simpler systems, are still of considerable value even for large biological systems consisting of numerous subcomponents.

In this work one-electron redox potentials of various purines and pyrimidines were determined by pulse radiolysis measurements in aqueous solutions at pH 13.

Experimental Section

Chemicals were of the highest purity available and were used without further purification. Guanosine, adenine, adenosine, xanthine, uric acid, barbituric acid, isobarbituric acid, uracil, cytosine, thymine, and *p*-methoxyphenol were obtained from Sigma,[20] guanine, hypoxanthine, and tryptophan were from Aldrich, 1-methylcytosine was from Heterocyclic Chemical Corp., 1- and 3-methyluracil were from Chemical Dynamics Corp., and Trolox C was from Hoffman La Roche. Water was purified by a Millipore Milli-Q system, and solutions were freshly prepared before each experiment. The pH was adjusted by adding sodium hydroxide to N_2O-saturated water solutions in order to minimize chemical changes of solutes before irradiation.

The pulse radiolysis experiments were conducted on the Febetron 705 pulse radiolysis setup,[4] which allowed single-pulse transient spectra measurements and simultaneous absorbance vs. time readings at a fixed wavelength with the lowest time resolution of 1 μs. A Suprasil quartz cell with 2-cm path length was used in all experiments. Doses were in the range of 5-50 Gy, as determined by thiocyanate dosimetry.[5]

The procedure used in the study of electron transfer equilibria of investigated redox couples is best illustrated by the data presented in Figure 1 and Table I, obtained for the reaction of the uric acid radical with Trolox C. The experimentally obtained traces A-C in Figure 1 clearly indicate the establishment and measurement of equilibria in the reactions studied. The absorbances (corrected if both radicals absorb at the same wavelength) of the uric acid radical and chromanoxyl radicals measured separately allow the determination of the ratio of radical concentrations at the equilibrium. The equilibrium constant, K_{abs}, is then calculated from this ratio and concentrations of parent compounds in the solution.

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TABLE I: Determination of Equilibrium Constant for Electron Transfer Reaction, at pH 13.0 and 20 °C

$$(\text{uric acid})^{2-}\cdot + \text{Chr-O}^- \rightleftharpoons (\text{uric acid})^{3-} + \text{Chr-O}\cdot$$

[(uric acid)$^{3-}$], mmol dm^{-3}	[Trolox C as Chr-O$^-$], mmol dm^{-3}	A_{430}[a]	k_{app},[a] s^{-1}	K_{abs}[b]
7.65	0	0.007		
0	6.2	0.027		
7.06	0.426	0.017	7 300	28
6.20	1.05	0.022	11 000	26
4.29	2.44	0.0255	21 500	30
3.11	3.87	0.027	31 700	
2.08	4.43	0.027	35 300	

[a] The data on absorbances at 430 nm and measured rates represent the average of three experimentally determined values. [b] Calculated according to $K_{abs} = \{[(\text{uric acid})^{3-}][\text{Chr-O}\cdot]\}/\{[\text{Chr-O}^-][(\text{uric acid})^{3-}]\}$.

The measured pseudo-first-order rates, k_{app}, of buildup of chromanoxyl radicals at different ratios of concentrations of uric acid and Trolox C may be used for determination of forward, k_f, and reverse, k_r, rate constants of the electron transfer equilibrium. The slope of the plot of k_{obsd}/[uric acid] vs. [Trolox C]/[uric acid] equals k_f,[1] whereas the intercept equals k_r. It can be seen from Table I and the values shown in Figure 1 that the equilibrium constant, K_{kin}, calculated from the measured forward and reverse rates agrees reasonably well with the average equilibrium constant derived from absorbances of radicals at equilibrium.

Results and Discussion

One-electron redox reactions of purine and pyrimidine derivatives were determined in their aqueous solutions at pH 13.0. Hence, all compounds studied were in deprotonated ionic forms. This simplified the redox reactions by making them a simple electron exchange. Initial one-electron oxidations of the purines and pyrimidines were carried out using bromide radical anion ($Br_2^-\cdot$), a strong oxidizing agent with E = 1.7 V.[6] The bromide radical anion was generated in N_2O-saturated solutions containing 0.1 mol dm^{-3} KBr:

$$\cdot\text{OH} + \text{Br}^- \rightarrow (\text{BrOH})^-\cdot \quad (1)$$

$$(\text{BrOH})^-\cdot \rightleftharpoons \text{Br}\cdot + \text{OH}^- \quad (2a)$$

$$\text{Br}\cdot + \text{Br}^- \rightleftharpoons \text{Br}_2^-\cdot \quad (2b)$$

with $k_1 = 1.1 \times 10^{10}$ dm^3 mol^{-1} s^{-1},[7] $K_{2a} = 320$ mol dm^{-3}, and $K_{2b} = 1.1 \times 10^5$ mol^{-1} dm^3.[8] Under these conditions $Br_2^-\cdot$ radicals

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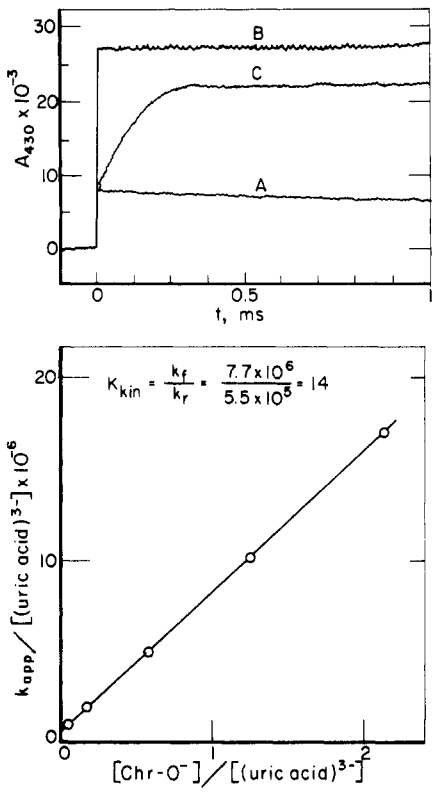


Figure 1. Redox equilibrium for uric acid and Trolox C radicals generated by $Br_2^{-}\cdot$ in aqueous solutions at pH 13. Conditions: N_2O-saturated solutions, 0.1 mol dm^{-3} KBr, dose/pulse = 5 Gy, 20 °C, $\lambda_{monitoring}$ = 430 nm. (top) Trace A: absorption of uric acid radical alone; 7.65 mmol dm^{-3} uric acid. Trace B: absorption of chromanoxyl radical; 6.2 mmol dm^{-3} Trolox C. Trace C: equilibrium absorption of chromanoxyl and uric acid radicals, resulting from electron transfer from Trolox C to uric acid radical; 6.2 mmol dm^{-3} uric acid; 1.05 mmol dm^{-3} Trolox C. (bottom) A plot of the dependence of measured rate of buildup of chromanoxyl radical on the ratio of concentrations of solutes.

TABLE II: Reactivities of Various Purine and Pyrimidine Derivatives with Bromide Radical Anion ($Br_2^{-}\cdot$), at pH 13.0 and 20 °C

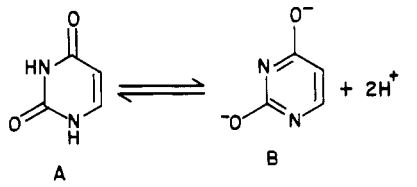
solute (S)	pK_a[a]	$k(Br_2^{-}\cdot + S)$,[b] $dm^3\ mol^{-1}\ s^{-1}$
1-methyluracil (**1**)	9.7	2.2×10^6
3-methyluracil (**3**)	9.85	2.8×10^8
uracil (**2**)	9.5	2×10^8[c]
thymine (**4**)	9.94	2×10^8[c]
barbituric acid (**8**)	4.0, 12.5	1.1×10^9
isobarbituric acid (**7**)	8.11, 11.5	1.3×10^9
1-methylcytosine (**5**)		2×10^6
cytosine (**6**)	4.6, 12.16	2×10^8[c]
hypoxanthine (**10**)	1.91, 8.96, 12.18	2.6×10^8
adenine (**11**)	4.15, 9.8	4.6×10^7
adenosine	3.3, 12.5	4.5×10^6
guanine (**14**)	3.3, 9.2, 12.3	2.5×10^8
guanosine (**12**)	1.9, 9.25, 12.33	2.5×10^8
xanthine (**15**)	7.53, 11.63	8.8×10^8
uric acid (**16**)	5.4, 5.54, 12.73	1.5×10^9

[a] From ref 15–17. [b] Estimated to be accurate to ±10%. [c] The same as in ref 9.

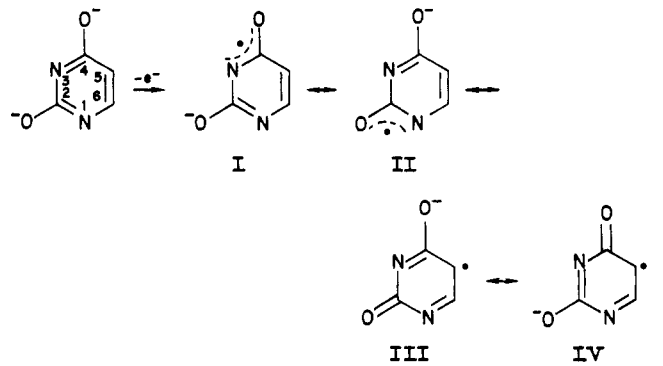
were formed in less than 0.1 μs, and in the absence of any other solutes they decayed on a millisecond time scale (as monitored at λ = 360 nm and $\epsilon = 1.2 \times 10^4$ $dm^3\ mol^{-1}\ cm^{-1}$).[7] In the presence of purine or pyrimidine derivatives in concentrations of 0.05–5 mmol dm^{-3}, the decay was on a microsecond time scale. This increase in decay rate is a consequence of one-electron oxidations of purines and pyrimidines, as follows

$$Br_2^{-}\cdot + S^{n-} \rightarrow S^{(n-1)-}\cdot + 2Br^- \qquad (3)$$

SCHEME I



SCHEME II



where S denotes purine or pyrimidine derivative and n the number of negative charges in the molecule. The pseudo-first-order rates derived from the decay of $Br_2^{-}\cdot$ radicals allow the determination of the second-order rates, which are summarized in Table II.

The reactivity of $Br_2^{-}\cdot$ radicals with various purine and pyrimidine derivatives may vary by as much as 3 orders of magnitude, i.e. from 1×10^6 to 1×10^9 $dm^3\ mol^{-1}\ s^{-1}$. The 1-methyl-substituted pyrimidines, which may serve as the most appropriate models for pyrimidine bases in DNA, enter equilibrium with $Br_2^{-}\cdot$ radicals

$$Br_2^{-}\cdot + \text{1-MePyr}^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \text{1-MePyr}\cdot + 2Br^- \qquad (4)$$

with $k_f = 2 \times 10^6$ $dm^3\ mol^{-1}\ s^{-1}$. In alkaline solutions, due to the fast second-order decay of $Br_2^{-}\cdot$ radicals ($2k \sim 4 \times 10^9$ $dm^3\ mol^{-1}\ s^{-1}$), only an approximate equilibrium constant and redox potential difference can be obtained, i.e., $K \sim 20$–50; $\Delta E \sim 0.05$–0.1 V.

Kinetic and redox properties of uracil and *N*-methyl-substituted uracils were compared. Uracil in its enol form **B** (Scheme I) can be readily oxidized by the bromide radical. The resulting mesomeric forms of the uracial radical are shown in Scheme II. 3-Methyluracil behaves similarly to uracil and has similar mesomeric forms. 1-Methyluracil radical is devoid of forms III and IV which reflects in its lower reactivity with $Br_2^{-}\cdot$.

The bromide radical was found to oxidize cytosine, thymine, and uracil at similar rates ($k = 2 \times 10^8$ $dm^3\ mol^{-1}\ s^{-1}$, same as in ref 9). The hydroxy derivatives of uracil, isobarbituric and barbituric acid, react with $Br_2^{-}\cdot$ an order of magnitude faster, at nearly diffusion-controlled rates $k \sim 1 \times 10^9$ $dm^3\ mol^{-1}\ s^{-1}$. The higher reactivity of isobarbituric and barbituric acid is due to an increased electron-donating ability of the deprotonated hydroxy group at positions C5 and C6, respectively.

The rates of oxidations of the investigated purine derivatives were found to be in the range of $k = 4 \times 10^7$ to 1.5×10^9 $dm^3\ mol^{-1}\ s^{-1}$. The variation in oxidizability of purine derivatives may also be attributed to differences in the substitution of electron-donating groups as well as to different electron-donating abilities of amino and hydroxy groups. For example, monohydroxy (hypoxanthine)- or amino-substituted (adenine) forms of purines are less readily oxidized than dihydroxy (xanthine) and aminohydroxy (guanine) derivatives, whereas uric acid, a 2,4,8-trihydroxypurine, reacts with $Br_2^{-}\cdot$ at nearly diffusion-controlled rate.

Optical spectra of transients produced in $Br_2^{-}\cdot$ mediated oxidation of purine or pyrimidine derivatives at pH 13.0 and 20 °C are presented in Figure 2. The spectra were utilized for kinetic

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TABLE III: One-Electron Redox Reactions of Various Purine and Pyrimidine Derivatives in Aqueous Solutions at pH 13.0 and 20 °C[f]

$$A\cdot + d^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} A^- + D\cdot$$

electron acceptor,[a] radical A· from	electron donor, solute D⁻	k_f,[b] dm³ mol⁻¹	k_r,[b] s⁻¹	K_{abs}[c]	K_{kin}[c]	ΔE,[d] V
p-methoxyphenol	uric acid	2.1×10^8	5.5×10^5	n.m.	382	0.15
uric acid	Trolox C	7.7×10^6	5.5×10^5	28	14	0.08
xanthine	*p*-methoxyphenol	2.7×10^8	1.6×10^5	n.m.	1700	0.19
hypoxanthine	tryptophan	9.1×10^8	1.5×10^6	950	610	0.17
	guanine	8.1×10^8	2.2×10^6	n.m.	370	0.15
guanine	xanthine	1.4×10^6	5×10^5	n.m.	3	0.03
	tryptophan	2.2×10^6	7.2×10^5	20	30	0.08
guanosine	tryptophan	2.4×10^7	2×10^5	500	120	0.15
adenine	guanine	3.2×10^7	3×10^5	150	110	0.12
barbituric acid	guanine	4.9×10^7	1.5×10^5	600	320	0.16
	hypoxanthine	8.6×10^5	1.4×10^5	4	6	0.04
adenosine	uracil	7×10^5	9.9×10^6	0.06	0.07	−0.07
thymine	hypoxanthine	2.5×10^6	6×10^5	8	4	0.04
	guanine	7.5×10^8	8.8×10^5	600	852	0.17
cytosine	hypoxanthine	1×10^7	2.8×10^5	42	36	0.09
	adenine	2.2×10^6	1.3×10^6	6	2	0.04
uracil	hypoxanthine	5.9×10^8	3.4×10^6	n.m.	174	0.13
	adenine	8.4×10^7	2.7×10^6	70	31	0.10
	barbituric acid	8.7×10^8	6×10^6	280	145	0.14
Br^-[e]	1-methyluracil	2.2×10^6	n.m.	~50		~0.1
	1-methylcytosine	2×10^6	n.m.	~20		~0.08

[a] Generated in one-electron oxidation of substrates (reaction 4). [b] Estimated to be accurate to ±10% for the reactions proceeding in favorable direction, ±20% for the others. [c] Apparent equilibrium constants obtained from kinetic data and absorbances of radicals at equilibrium. [d] Difference in one-electron redox potentials, calculated from formula[7]. [e] Generated in reactions 1 and 2. [f] Ionic strength μ = 0.2 mol dm⁻³.

measurements and specification of the generated radicals.

Absorbances of purine radicals were generally higher than those of pyrimidine radicals, except for isobarbiturate and barbiturate radicals. This could be attributed to the higher aromaticity of purine radicals, which is also invoked by the similarity of the spectra in Figure 2 with the spectra of the well-known indolyl[10] and phenoxy[1] type radicals. The spectra of monosubstituted purine radicals exhibited two maxima in the 300–600-nm region. A higher absorption band at 340 nm and a lower band at higher wavelengths showed great similarity to the spectra of indolyl radicals. An additional absorption band at ~400 nm of radicals from disubstituted derivatives could be assigned to phenoxyl type radicals contribution, as observed for aminophenoxyl[11] and hydroxyindolyl[2] radicals. The substitution of the purine ring at position N9, as in guanosine, should inhibit the resonance stabilization of purine radicals through the imidazole part of the purine ring. Consequently, the spectrum of radicals derived from guanosine resembles more the spectra of pyrimidine radicals, e.g. cytosine (see Figure 2).

Optical absorption spectra of the radicals derived from thymine, cytosine, and uracil exhibit two maxima, one at about 390 nm and another at a higher wavelength (not shown in Figure 2). These spectra were similar to the spectra of transients generated by the $SO_4^{-}\cdot$ radical.[12,13] It was suggested[12] that these spectra could be ascribed to pyrimidine radical anions (Scheme II). The spectra of radicals derived from 1- and 3-methyl-substituted pyrimidines resemble the spectra of neutral uracil radicals obtained by $SO_4^{-}\cdot$ reaction with uracil at pH 8 and 12 as expected (see previous discussion on uracil radicals). Isobarbiturate radicals exhibit only one maximum at λ = 360 nm and $\epsilon = 3600 \pm 400$ dm³ mol⁻¹ cm⁻¹, similar to the spectrum previously reported.[1] The spectrum of barbiturate radicals is red-shifted, with the maximum at 590 nm.

One-Electron Redox Reactions of Purines and Pyrimidines. One-electron transfer reactions of purines and pyrimidines can be studied conveniently by using the procedure outlined in ref 1. Providing that the redox equilibrium

$$A\cdot + D^- \rightleftharpoons A^- + D\cdot \quad (5)$$

is achieved in the rate-determining step, the one-electron redox potential of either half-cell reactions

$$A\cdot + e^- \rightarrow A^- \quad (6a)$$

$$D\cdot + e^- \rightarrow D^- \quad (6b)$$

can be determined from the formula[1]

$$\Delta E^1_{13} = 0.059 \log K_5 \quad (7)$$

where E^1_{13} denotes one-electron redox potential difference between half-cells (6a) and (6b) at pH 13.0 and K_5 is the apparent equilibrium constant of reaction 5. If the redox potential of one of the half-cell reactions is known, the determination of the redox potential of the other half-cell is straightforward. We used *p*-methoxyphenol (E^1_{13} = 0.4 V),[1] Trolox C (E^1_{13} = 0.19 V),[1] and tryptophan (E^1_{13} = 0.57 V)[2] as primary references. The approach to equilibrium in redox reactions of purines and pyrimidines was evaluated by using both kinetic treatment and absorbance readings, similar to the one described in ref 1, as detailed in the Experimental Section. The results are summarized in Tables III and IV.

TABLE IV: One-Electron Redox Potentials of Various Purine and Pyrimidine Derivatives at pH 13.0 vs. NHE

derivative	E^1_{13},[a] V	derivative	E^1_{13},[a] V
1-methylcytosine	~1.63	adenine	0.75
1-methyluracil	~1.6	hypoxanthine	0.74
uracil	0.88	guanosine	0.71
adenosine	0.81	guanine	0.63
cytosine	0.81	xanthine	0.59
thymine	0.79	uric acid	0.26
barbituric acid	0.78	isobarbituric acid	0.13[b]

[a] Estimated to be accurate to ±0.02 V, except for 1-methylpyrimidines (see Results and Discussion). [b] From ref 1.

The effect of substitution at C5 of uracil on the redox potential is comparable to that of parasubstitution of phenols.[1] 5-Methyluracil (thymine) has a 0.1 V lower oxidation potential than uracil, whereas in the case of 5-hydroxyuracil (isobarbituric acid) the difference is as much as 0.75 V. This decrease in redox potential is in agreement with the resonance stabilization of uracil radical anions, since the substitution of electron-donating groups at positions of high unpaired electron density would, generally, decrease the redox potential of the compound. Hence, in the case

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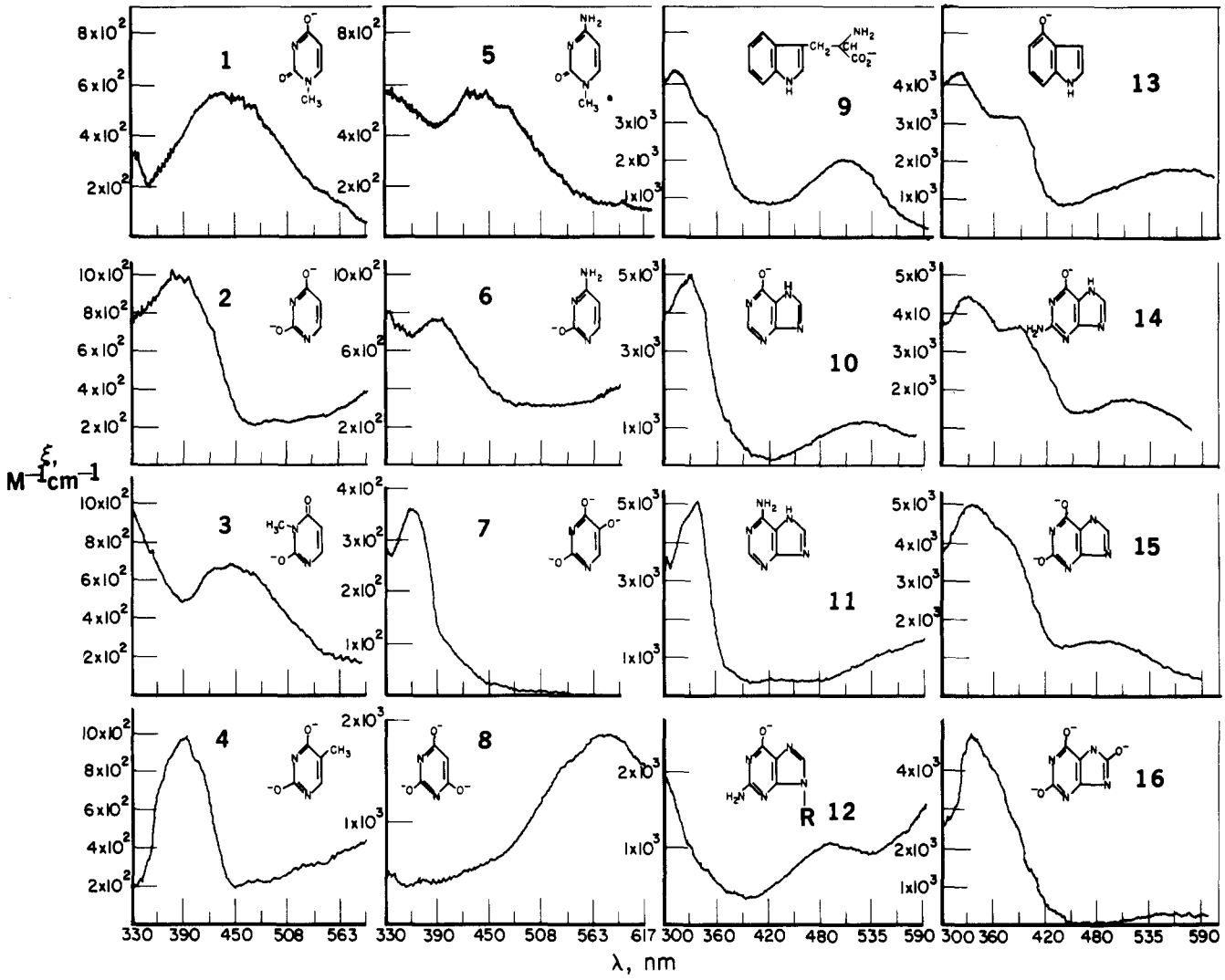

Figure 2. Transient absorption spectra and molar absorbances of free radicals obtained on one-electron oxidation of various purine and pyrimidine derivatives by $Br_2^{-\cdot}$. Radicals were generated in aqueous solutions containing 1 mmol dm^{-3} of substrates and 0.1 mol dm^{-3} KBr, at pH 13.0 and 20 °C. The numbers correspond to those in Table II. Spectra of radicals obtained from tryptophan (**9**) and 4-hydroxyindole (**13**) are presented for comparison.

of 6-hydroxyuracil (barbituric acid), the decrease in redox potential is only 0.13 V, because the electron-donating $-O^-$ group is in the meta position to $-O^-$ groups of uracil. Furthermore, there is only a minor effect of the replacement of hydroxy group of uracil (2,4-dihydroxypyrimidine) for an amino group as in cytosine (2-hydroxy-4-aminopyrimidine) on the redox potential. In fact, the redox potential of uracil is slightly higher than that of cytosine, while the opposite is found for purines (see Table IV). It is suggested that this lack of effect of different electron-donating abilities of amino and hydroxy groups might be attributed to low unpaired electron density at positions C2 and C4, as compared to positions N1, N3, and C5 of uracil radicals, in agreement with ESR measurements.[14]

Oxidation potentials of purine derivatives were found to be more sensitive to substitution than those of pyrimidine derivatives. As discussed previously for the optical spectra of the purine radicals, the substitution effects could be rationalized on the basis of highly delocalized structures of purine radicals. The substitution at position N9 was found to increase the redox potential of a parent compound (from 0.62 V for guanine to 0.72 V vs. NHE for guanosine). This increase in redox potential is probably a consequence of the inhibition of resonance stabilization of the guanosine radicals through the π-electrons of the imidazole ring. Hence, both the spectra and the redox properties of guanosine radicals could be envisaged as those of substituted cytosine radicals, which is indeed observed (see Figure 2 and Table IV). On the other hand, a gradual decrease in the redox potential of purine derivatives was found when electron-donating groups were substituted at positions C2, C5, and C8. This is best illustrated by an approximately 0.25-V decrease in the redox potential following hydroxy substitution in the hypoxanthine–xanthine–uric acid series. Consequently, it may be suggested that there is considerable unpaired spin density at positions C2, C5, and C8 of a purine ring of the radicals. This is further supported by the difference in the redox potentials of amino- and hydroxypurines which follows the difference in their electron-donating abilities. However, the difference between the redox potentials of amino- and hydroxypurines is only ~0.05 V, much less than between amino- and hydroxyphenols (see ref 1). Hence, it might be suggested that purine radicals, similarly to pyrimidine analogues, would have higher spin density at positions N1, C3, N5, N7, and N9.

Conclusion

One-electron oxidation potentials of various purines and pyrimidines were found to be in the range of 0.13 to ~1.63 V vs. NHE at pH 13.0. Substitution of pyrimidines at the N1 position

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results in a marked increase in the redox potential, e.g. from 0.81 V for cytosine to ~1.63 V vs. NHE for 1-methylcytosine. a similar, but smaller, effect is observed when purine derivatives were substituted at the N9 position. From the data presented in Table IV it may be concluded that purines are the more likely targets of oxidative damage to DNA. In an event that a pyrimidine base was somehow oxidized, it would be rapidly repaired by its purine counterpair by transfer of an electron. Since there is a redox potential difference between adenine and guanine of 0.1 V, it may be suggested that a "positive hole" sink in DNA would be guanine, in agreement with previous ESR measurements.[18,19]

Registry No. **1**, 615-77-0; **2**, 66-22-8; **3**, 608-34-4; **4**, 65-71-4; **5**, 1122-47-0; **6**, 71-30-7; **7**, 496-76-4; **8**, 67-52-7; **10**, 68-94-0; **11**, 73-24-5; **12**, 118-00-3; **14**, 73-40-5; **15**, 69-89-6; **16**, 69-93-2; *p*-$MeOC_6H_4OH$, 150-76-5; Br^-, 12595-70-9; adenosine, 58-61-7; trolox C, 56305-04-5; L-tryptophan, 73-22-3.

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Studies on Diamagnetic Susceptibility of Biologically Active Heterocycles. 1. Diamagnetic Susceptibility of Phenothiazines

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Diamagnetic susceptibilities for a number of phenothiazines are reported. A theoretical method to estimate the diamagnetic susceptibility of these biologically active heterocycles is presented. Phenothiazines have been considered to be composed of two units: one consisting of a benzene nucleus containing amino and thio groups at ortho positions to the substituents and the other consisting of a benzene ring with substituents. The diamagnetic contributions of these two units have been obtained from the diamagnetic susceptibilities of substituted *o*-aminobenzenethiols and benzenes and have been used in estimating the diamagnetic susceptibilities of phenothiazines. It has provided excellent theoretical results. Such an excellent agreement between measured and estimated values is due to the fact that the interactions between substituents which affect the diamagnetism have been duly accounted for in such calculations.

Recently we have reported some interesting findings on the diamagnetic susceptibilities of aliphatic compounds[1-3] and considered it worthwhile to investigate the diamagnetic susceptibilities of biologically active heterocycles. Phenothiazines form an interesting series of biologically active heterocycles and find several applications as medicinal agents (anticancer agents,[4-7] antipsychotics,[8-10] antihistaminic,[11] antiinflammatory[12]). Significant research work has been carried out by mass spectrometry[13-17] and photoelectron spectroscopy[18] to throw light on their biological activities and mode of action. But surprisingly, there is no report on the diamagnetism of such a medicinally important class of heterocycles. Therefore, in the present work we have made an attempt to present an account of the diamagnetic behavior of phenothiazines.

Diamagnetic susceptibilities of phenothiazines have been determined by the Gouy method[1] and summarized in Table I. The phenothiazines studied have been synthesized by Smiles rearrangement by condensing substituted *o*-aminobenzenethiols and substituted *o*-halonitrobenzenes.[19,20]

To analyze the measured diamagnetic susceptibilities in relation to the existing structural environments present in these molecules, one must make theoretical estimates of the diamagnetic susceptibility. Phenothiazines contain substituents which involve additional interactions and affect the diamagnetism.[21] The diamagnetic susceptibility of such molecules cannot be calculated by incremental systems on the basis of atomic and bond susceptibility concepts as the effect of the interactions between substituents cannot be accounted for. For such molecules wave-mechanical calculations by the Baudet method[22] cannot be made due to the nonavailability of the susceptibility data required. The semiempirical approach which has provided excellent results for aliphatics,[1-3,23] aromatics,[24,25] and nitrogen heterocycles[26] cannot

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