Antioxidation Mechanisms of Uric Acid†

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Abstract: One-electron oxidation of uric acid generates the urate radical, which was studied in aqueous solution by pulse radiolysis and oxygen-uptake measurements. Acid–base properties of the uric acid radical were determined, i.e., $pK_{a1} = 3.1 \pm 0.1$ and $pK_{a2} = 9.5 \pm 0.1$. The reaction of the radical with oxygen was too slow to be measured, $k < 10^{-2}$ dm^3 mol^{-1} s^{-1}. The one-electron-redox potential vs NHE, $E_7 = 0.59$ V, was derived from the pH dependence of the redox potential, which was fitted through the values measured at pH 7 and 8.9 and those previously determined at pH 13. Rapid reactions of uric acid with oxidizing species and peroxy radicals were indicative of uric acid as a possible water-soluble physiological antioxidant. Rapid reaction of uric acid with the guanyl radical indicates that uric acid may also act as a repair agent of oxidative damage to DNA bases.

Free-radical processes are increasingly invoked in many deleterious biological effects,[1] such as replicative inactivation of DNA,[2] mutation,[3] carcinogenesis,[4] atherosclerosis,[5] arthritis,[6] and aging.[7] The generation of free radicals in biosystems exposed to ionizing radiations[2] or in systems undergoing autoxidation[5] has been firmly established by numerous direct observations of free radicals and their reactions in appropriate model systems.[8] The involvement of free radicals in normal physiology,[9] pathophysiology,[10] and aging,[7] although lacking direct proof, is backed by strong indirect evidence. Direct monitoring of free radicals in vivo is much more complex than detecting their presence in model biosystems,[11] which is in any case considerably limited. Consequently, in addition to indirect-measurement approaches in vivo, comparison to model systems is crucial in assessing free-radical processes in biological media. In this work, the energetics, kinetics, and mechanisms of the antioxidant properties of uric acid were investigated by pulse and steady-state radiolysis in model aqueous solutions.

Various sulfhydryls and antioxidants may act as efficient free-radical scavengers and repair agents of specific-free-radical damage to biomolecules.[4] On the basis of these properties and the effects they exhibit, therefore, these two classes of compounds are considered to play an important role in anticarcinogenesis and chemical protection from ionizing radiations.[4] Their role in the overall defense system of humans, and specifically as dietary anticarcinogens, has been discussed by Ames.[10] He proposed optimizing the physiological defense system through planned dietary uptake as an important consideration in carcinogenesis. He also suggested that uric acid, which is present in plasma in fairly high concentrations (0.3 mmol dm^{-3}), may be an important anticarcinogen. This suggestion was based on the previously published hypothesis[12] that uric acid may act in the human defense system as an antioxidant thereby protecting living organisms against oxidative and free-radical damage. Specifically, it was shown that urate acts as a singlet oxygen, $^1O_2$, $^{\bullet}OH$ radical, and oxo–heme scavenger. It was also demonstrated that urate protects erythrocyte membrane ghosts from peroxidation and intact erythrocytes from lysis by peroxides.[12] Furthermore, this protective role of uric acid was cited as an important factor in the prevention of aging and cancer.[12]

More recently, the role of urate as an agent in the human defense system has been questioned because urate appears to enhance the free-radical inactivation of alcohol dehydrogenase.[13] To resolve some of these apparent inconsistencies and to provide a better mechanistic understanding of what uric acid can do against oxidative damage, we have investigated the repairing and protective efficiency of urate. The interaction of the urate free radical, resulting from such reactions, with some other endogenous antioxidants was also investigated.

Materials and Methods

All chemicals were analytical grade and were used without further purification. Uric acid potassium salt, guanosine, and DNA (type XIV) were obtained from Sigma;[43] KBr, phosphate, and borate were from Fisher; carbon tetrachloride, 2-propanol, $HClO_4$, and NaOH were from Baker; and Tl_2SO_4, 2,6-dimethoxyphenol, 3,4-dimethoxyphenol, 3,5-dimethoxyphenol and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine hydrochloride were from Aldrich. Water was purified by the Millipore Milli Q system. Solutions were freshly prepared before each experiment. The pH was adjusted by $HClO_4$ or NaOH or maintained by phosphate and borate buffers. High-purity nitrous oxide (Matheson or Lek, Ljubljana) was used to saturate aqueous solutions.

Pulse-radiolysis experiments were performed on the Febetron 705 pulse-radiolysis setup at NIST described in ref 14a and on the Boris Kidric Institute Febetron 707 setup described in ref 14b. A Suprasil quartz cell was used as an irradiation vessel. Dose rates ranged from 5 to 50 Gy as determined by thiocyanate dosimetry, using $G(SCN)_2^{\bullet-} = 6.0$ in N_2O-saturated aqueous solutions and $A_{480} = 7600$ dm^3 mol^{-1} cm^{-1}.[16]

One-electron oxidation of uric acid, which resulted in the generation of the uric acid radical, was accomplished by bromide, $Br_2^{\bullet-}$, and thiocyanate, $(SCN)_2^{\bullet-}$, radical anions as well as Tl(II) intermediates. These

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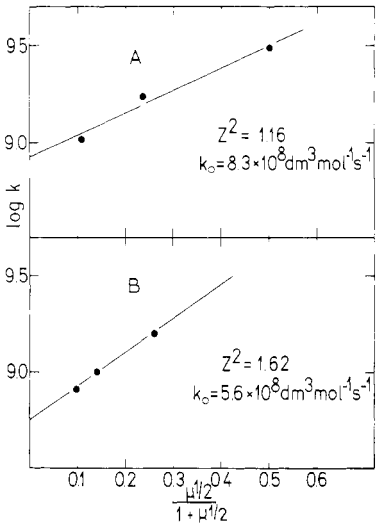


Figure 1. Ionic-strength dependence of the rate of oxidation of uric acid studied by pulse radiolysis of aqueous solutions at 5 Gy, 20 °C. (ionic strength was varied with KBr and KSCN): (A) oxidation of urate by $Br_2^{\bullet-}$ at pH 7, and (B) oxidation of urate by $(SCN)_2^{\bullet-}$ at pH 11.16.

transient oxidants were generated by pulse radiolysis in aqueous solutions of KBr, KSCN, and Tl_2SO_4 from the reactions of •OH radicals with Br^-,[15] SCN^-,[16] and Tl^+,[17] respectively.

Results and Discussion

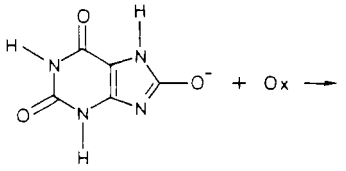
Acid–Base Properties. The potential of uric acid to act as an antioxidant is associated with its ability to inactivate an oxidant (e.g., peroxy radical) via an electron transfer—before the oxidant damages the target biomolecule. This ability to donate an electron is usually affected by the protonation state of the parent molecule and the free-radical intermediate.

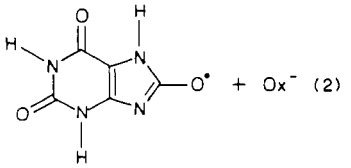
There is a misleading controversy in the literature regarding the dissociation constants of uric acid. One source[18a] lists $pK_{a1} = 5.4$ and $pK_{a2} = 5.54$, whereas the other[18b] cites $pK_{a1} = 5.4$ and $pK_{a2} = 10.3$.

The potentiometric titration of uric acid with aqueous NaOH at 20 °C gave $pK_{a1} = 5.40 \pm 0.02$ and $pK_{a2} = 9.80 \pm 0.02$. The ionization state of the uric acid molecule was derived from the ionic-strength dependence of the rate constants for the oxidation with $Br_2^{\bullet-}$ at pH 7.0 and $(SCN)_2^{\bullet-}$ at pH 11.16, which are presented in Figure 1. On the basis of these results, the uric acid molecule, UH_3, has the following acid–base equilibria:

$$UH_3 \underset{pK_{a1} = 5.4}{\rightleftharpoons} UH_2^- \underset{pK_{a2} = 9.8}{\rightleftharpoons} UH^{2-} \qquad (1)$$

Hence, in neutral media uric acid has a single negative charge and its oxidation may be envisaged as follows:





The loss of an electron from O^8 is assumed because this hydroxy group is the strongest acid.[18]

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Table I. Reactivities of Transient Oxidants with Uric Acid at Different pH

parent solute	one-electron oxidant[a]	k,[b] $dm^3\ mol^{-1}\ s^{-1}$		
		pH 3	pH 7	pH 12
Tl(I)	Tl(II)	1.0×10^9	1.4×10^9[c]	
Br^-	$Br_2^{\bullet-}$	2.6×10^8[d]	8.3×10^8[d]	
SCN^-	$(SCN)_2^{\bullet-}$		3.0×10^8[d]	5.6×10^8[d]

[a] Generated by the •OH radical reaction with the parent solute. [b] Estimated to be accurate to ±10%. [c] The ionic strength of the solution was $\mu = 0.01$ mol dm^{-3}. [d] Extrapolated to zero ionic strength.

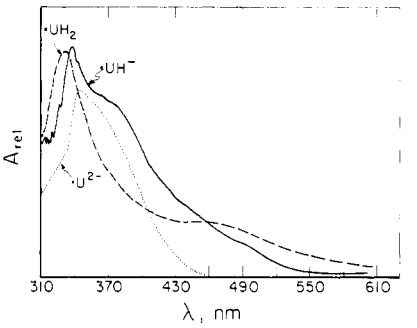

Figure 2. The absorption spectra of different forms of the urate radical obtained in N_2 saturated aqueous solutions of 0.1 mol dm^{-3} KBr and 1 mmol dm^{-3} uric acid, at 20 °C, dose/pulse = 50 Gy: broken line, the neutral urate radical, $\cdot UH_2$, at pH 2; full line, urate radical anion, $\cdot UH^-$, at pH 7; and dotted line, double-deprotonated urate radical, $\cdot U^{2-}$, at pH 13.

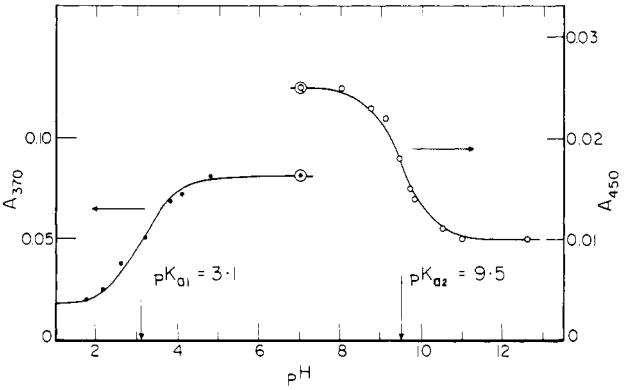

Figure 3. The pH profiles of absorbance readings taken in N_2O-saturated aqueous solution of 0.1 mol dm^{-3} KBr and 1 mmol dm^{-3} uric acid at 20 °C, dose/pulse = 34 Gy: (●) at 370 nm and (○) at 450 nm. The full lines represent the computer fits of experimental data according to indicated pK_a values.

Strong oxidants such as bromide radical anion, $Br_2^{\bullet-}$, with the redox potential $E_7 = 1.69$ V[19] and Tl(II) with $E_7 = 1.98$ V[20] react with urate at diffusion-controlled rates at pH 7 (Table I). [The term "redox potential" is used in this paper to indicate one-electron-redox potential of a given redox couple vs the redox potential of normal hydrogen electrode (abbreviated by NHE). The pH pertinent to the redox potential is given as a subscript.] The thiocyanate radical anion, $(SCN)_2^{\bullet-}$, which is a weaker oxidant ($E_7 = 1.29$ V),[21] is somewhat slower.

The uric acid radicals generated in reaction 2 have broad and structured transient absorption spectra with a high molar absorptivity. The spectral properties of the urate radical are similar to other purine radical spectra.[20] Absorption spectra of specific urate radicals at different pH are shown in Figure 2. From the absorption vs pH measurements (Figure 3), pK_a values for these radicals were derived (using computerized data analysis). The charges of different forms of the urate radical were determined

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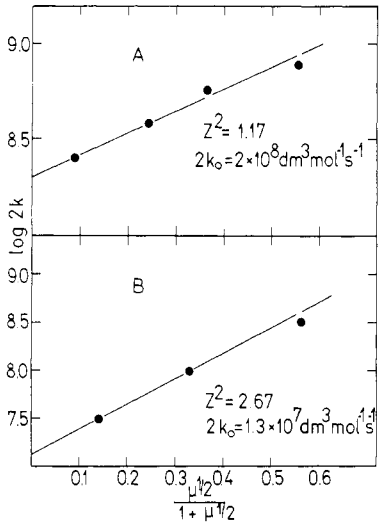


Figure 4. The ionic-strength dependence of decay rates of different forms of the urate radical generated upon oxidation of uric acid induced by $Br_2^{\bullet-}$ monitored at 330 nm at 10 and 23 Gy/pulse and 20 °C (ionic strength was varied with KBr): (A) pH 7.0, A_{330} = 8000 ± 800 dm^3 mol^{-1} cm^{-1} and (B) pH 11.2, A_{330} = 9500 ± 900 dm^3 mol^{-1} s^{-1}.

from the ionic-strength dependence of their decay rates (Figure 4). The urate radical has a single negative charge at pH 7 and a double negative charge at pH 11.16. Consequently, the following acid–base equilibria describe the protonation state of the urate radical:

$$\underset{}{^{\bullet}UH_2} \underset{pK_{a1} = 3.1}{\rightleftarrows} \underset{}{^{\bullet}UH^-} \underset{pK_{a2} = 9.5}{\rightleftarrows} \underset{}{^{\bullet}U^{2-}} \qquad (3)$$

The observed pK_a values of the radical are about 2.3 and 0.3 pH units lower than corresponding pK_a values of the parent molecule, which is the case for many other free radicals with an unpaired electron in the α position to the acidic group.[22]

Redox Potential of Uric Acid. The redox reactions of the urate radical were studied in the pH range from 7 to 14. The redox potential of the urate redox couple vs NHE was derived from the redox equilibria with the following secondary redox standards: 2,6-dimethoxyphenol, with E_7 = 0.49 V,[23] 3,4-dimethoxyphenol, with E_7 = 0.50 V,[23] 3,5-dimethoxyphenol, with E_7 = 0.65 V,[23] and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine hydrochloride (TMPD), with $E_{8.9}$ = 0.27 V.[24] The results are summarized in Table II.

Redox potentials of the urate redox couple at different pH values, E_7 = 0.51 ± 0.05 V at pH 7, $E_{8.9}$ = 0.47 ± 0.05 V at pH 8.9, and (previously measured[20]) E_{13} = 0.26 ± 0.02 V at pH 13, may be used to evaluate the pH profile of the redox potential. The basis for the evaluation of the pH dependence is the "electrode" reaction of the urate redox couple, which is given below:

$$^{\bullet}U^{2-} + e^- + H^+ \rightarrow UH^{2-} \qquad (4)$$

If the well-known general formula[25] is modified according to the above electrode reaction, the following expression will be obtained:

$$E_{pH=i} = E_{pH=0} + \log \{[(10^{-i})M]/R\} \qquad (5)$$

where $M = K_{a1}K_{a2} + K_{a1}10^{-i} + 10^{-2i}$ and $R = K_{r1}K_{r2} + K_{r1}10^{-i} + 10^{-2i}$. With use of eq 5 and the measured values, the pH profile of E can be calculated, and it is shown in Figure 5 (solid line). Actual experimental measurements of E at three different pHs are in good agreement with the calculated values.

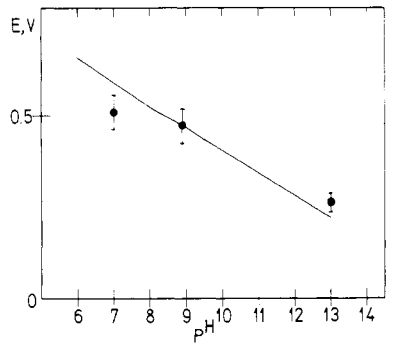


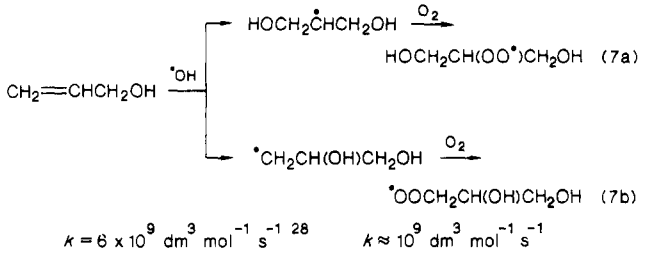
Figure 5. The pH profile of the redox potential of the urate redox couple calculated with formula 5 in the text. The points are experimental values.

The redox potential of uric acid at pH 7, E_7 = 0.59 V, is considerably higher than the redox potential of ascorbate,[24] E_7 = 0.28 V. Consequently, ascorbate is expected to repair the urate radical and thus prevent its deleterious effect on some enzymes. The electron transfer from ascorbate to the urate radical was studied in aqueous solutions at pH 7. The urate radical was generated on oxidation of uric acid induced by $Br_2^{\bullet-}$. In the absence of any redox solutes, the urate radical, $^{\bullet}UH^-$, decays by the second-order kinetics with $2k = (2.0 \pm 0.2) \times 10^8$ dm^3 mol^{-1} s^{-1} at zero ionic strength, as monitored at 330 nm. On addition of ascorbate, the electron transfer from ascorbate to the urate radical changed the second-order decay of the urate radical to pseudo-first-order. The reaction was monitored at 420 nm because of the spectral overlap of urate and ascorbate radicals in the 300–400-nm region. The electron transfer from ascorbate to the urate radical is shown below:

$$^{\bullet}UH^- + AH^- \rightarrow UH_2^- + {}^{\bullet}A^- \qquad (6)$$

$$k = (1.0 \pm 0.3) \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

Uric Acid as an Antioxidant. As already indicated by reaction 2, uric acid can inactivate strong transient oxidants at diffusion-controlled rates (Table I). Corresponding reaction rate constants of weaker oxidants, e.g., peroxy radical, are considerably lower. Peroxy radicals were generated from allyl alcohol:



In neutral solutions these peroxy radicals are inactivated by uric acid at moderate rates:

$$ROO^{\bullet} + UH_2^- \rightarrow ROO^- + {}^{\bullet}UH^- + H^+ \qquad (8)$$

$$k = (3.0 \pm 0.5) \times 10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

The ROO^- ion protonates rapidly (pK_a = 12.8)[29] to give hydroperoxides, ROOH.

Reaction 8 is a classical reaction of an antioxidant, provided the resulting antioxidant radical does not react with oxygen, to give another peroxy radical which would propagate the chain. The reactivity of the urate radical with oxygen was tested using an oxygen electrode. The radical was generated in a KBr (0.1 mol dm^{-3}), uric acid (1 mmol dm^{-3}), and N_2O:O_2 = 5:1 system at 0.73

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Table II. One-Electron-Transfer Reactions of the Urate Redox Couple, Measured by Pulse Radiolysis of Aqueous Solutions at 20 °C, μ = 0.1 mol dm^{-3}

$$^{\bullet}A + B^- \underset{k_r}{\overset{k_f}{\rightleftharpoons}} {}^{\bullet}B + A^-$$

radicals, $^{\bullet}$A, from	solute, B$^-$	pH	k_f[a]	k_r[a]	K_{kin}[b]	K_{abs}[b]	ΔE, V[c]
uric acid	TMPD	8.9	1.5×10^9	6×10^5	2500	2800	0.2
3,5-dimethoxyphenol	uric acid	7.0	1.1×10^8	1×10^6	610	800	0.17
p-methoxyphenol	uric acid	7.0	$<2 \times 10^6$	$<2 \times 10^6$			
uric acid	2,6-dimethoxyphenol	7.0	7.4×10^5	2.8×10^5	3	8	0.04
uric acid	3,4-dimethoxyphenol	7.0	1×10^6	5×10^5	2	4.5	0.03

[a] Rate constants in dm^3 mol^{-1} s^{-1}, estimated to be accurate to ±10% for the reactions in favorable direction and to ±20% for the others. [b] Equilibrium constants derived from the kinetics and absorbances of the radicals at equilibrium. See ref 24 for details. [c] The redox potential difference calculated from the mean of the equilibrium constants by the Nernst equation, $\Delta E = 0.059 \log K$.

Gy/min, 20 °C. In this system all OH radicals (G = 5.6) react with Br$^-$ to generate the bromide radicals which then oxidize uric acid:

$$Br_2^{\bullet -} + UH_2^- \rightarrow {}^{\bullet}UH^- + 2Br^- + H^+ \quad (9)$$

The measured value for oxygen consumption, $G(-O_2)$ = 0.3, compared to the value expected when solute peroxy radicals are formed and decay either by the Russel-type mechanism or by an electron-transfer to oxygen, $G(-O_2)$ = 3.1,[30] indicates that only H atoms (G(H) = 0.6), which are not scavenged by Br$^-$, contribute to oxygen consumption. Hence, we conclude that in neutral solutions the urate radical does not react with oxygen, i.e.

$$^{\bullet}UH^- + O_2 \rightarrow \text{no measurable reaction} \quad (10)$$

$$k < 10^{-2} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

Halogenated peroxy radicals are known to react with electron donors much faster than unhalogenated peroxy radicals.[31] The $Cl_3COO^{\bullet}$ radical was generated in the 2-propanol (35%)/acetone (>1 mol dm^{-3})/CCl_4 (0.05 mol dm^{-3}) O_2-saturated aqueous solutions.[32] The reaction rate constant of the trichloromethylperoxy radical with uric acid was 2 orders of magnitude higher than

$$Cl_3COO^{\bullet} + UH_2^- \rightarrow Cl_3COO^- + {}^{\bullet}UH^- + H^+ \quad (11)$$

$$k = (3.2 \pm 0.3) \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

corresponding k value for an unhalogenated peroxy radical (reaction 8). This rate is comparable to $k = 5 \times 10^8$ dm^3 mol^{-1} s^{-1} for α-tocopherol[27] and $k = 2 \times 10^8$ dm^3 mol^{-1} s^{-1} for ascorbate.[27] Consequently, the inactivation of the $Cl_3COO^{\bullet}$ radical by uric acid may be also expected in liver in vivo.

Uric acid reacts quite efficiently with the nitrite radical, $NO_2^{\bullet}$. The $NO_2^{\bullet}$ radical was generated in the following system: 0.1 mol dm^{-3} NO_2^-, N_2O-saturated aqueous solution at pH 7, 20 °C, by the $^{\bullet}$OH radical reaction:[33]

$$^{\bullet}OH + NO_2^- \rightarrow NO_2^{\bullet} + OH^- \quad (12)$$

$$k = 1.1 \times 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

Under these conditions, the nitrite radical was found to be rapidly inactivated by uric acid:

$$NO_2^{\bullet} + UH_2^- \rightarrow NO_2^- + {}^{\bullet}UH^- + H^+ \quad (13)$$

$$k = (1.8 \pm 0.2) \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

Repair of Oxidative Damage to DNA Bases. Guanine, one of the four DNA bases, was selected as the most appropriate model of oxidative damage to DNA. Guanine has the lowest redox potential,[34] E_7 = 0.95 V, and should be at the bottom of the redox cascade in which initial oxidative damage to the other bases is transferred to guanine. Such a scheme has been already discussed for direct ionization of DNA[35] in which guanine acts as a charge sink. In both instances, one would expect formation of the guanyl radical. It is interesting to note that 50% of $^{\bullet}$OH radical adducts of guanine would also yield a guanyl radical upon water elimination.[34]

Guanyl radical can be easily generated by pulse radiolysis from guanosine, R–G:[34,36]

$$Tl(II) + R\text{–}G \rightarrow Tl(I) + R\text{–}G^{\bullet +} \quad (14)$$

$$k = 1.3 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

In neutral solutions the guanyl radical cation rapidly loses a proton:[36]

$$R\text{–}G^{\bullet +} \rightleftarrows R\text{–}\dot{G}(-H) + H^+ \quad (15)$$

$$pK_a = 3.9 \quad \text{(ref 37)}$$

The guanyl radical, R–Ġ(–H), is a resonant[34,36,38] and moderately strong transient oxidant, as indicated by its redox potential. Its interaction with uric acid was studied in the following system: 2 mmol dm^{-3} Tl_2SO_4 and 0.4 mmol dm^{-3} guanosine in an N_2O-saturated aqueous solution. The following repair reaction was found to take place at a diffusion-controlled rate:

$$R\text{–}\dot{G}(-H) + UH_2^- \rightarrow R\text{–}G + {}^{\bullet}UH^- \quad (16)$$

$$k = (1.2 \pm 0.1) \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

Conclusions

The physicochemical properties of uric acid as related to free radical and one-electron-redox processes and its redox potential, $E_7(^{\bullet}UH^-/UH_2^-)$ = 0.59 V, confirm the premise[12] that uric acid may be an important physiological antioxidant.

The unpaired electron in the urate radical is highly delocalized, resulting in the resonance-stabilized radical.[36,38] Spectral features and the unreactivity of guanyl with oxygen resemble other resonance-stabilized purinyl radicals.[38]

The urate radical may be repaired by ascorbate (reaction 6). Hence, the suggested damage to enzymes by the urate radical[13] may be prevented in vivo by ascorbate, as shown here and recently by Maples and Mason.[39]

Antioxidant properties of uric acid and its ability to inactivate peroxy radicals is another confirmation of the general rule[40] for antioxidant interaction with peroxy radicals[41] via one-electron donation rather than an H atom abstraction,[40,42] i.e.:

$$ZAH + XROO^{\bullet} \rightarrow Z\dot{A}H^+ + XROO^- \quad (17)$$

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in which AH is an antioxidant (e.g., phenolic), ROO• is a peroxy radical, Z is an electron-donating substituent, and X is an electron-withdrawing substituent. In general, as Z is added to an antioxidant or becomes a better electron donor and as the electron affinity of X increases (e.g., by introduction of halogens into α position), a faster reaction rate is observed for inactivation of peroxy radicals, e.g., reaction 6 vs reaction 11.

The antioxidation activity of uric acid may be extended to chemical repair of oxidative damage to DNA. Uric acid repairs efficiently the guanyl radical, reaction 16, in simple guanine derivatives and DNA.[34] This type of reaction may be critical in the repair of direct effects of ionizing radiation[2] in cells.

(43) Commercial products are identified here only for technical purposes and are not meant to be an endorsement by the National Institute of Standards and Technology.

Photosensitive Cyclomer Formation of 1,1′-(1,2-Ethanediyl)bis(pyridinyl) Diradical and Its Derivatives

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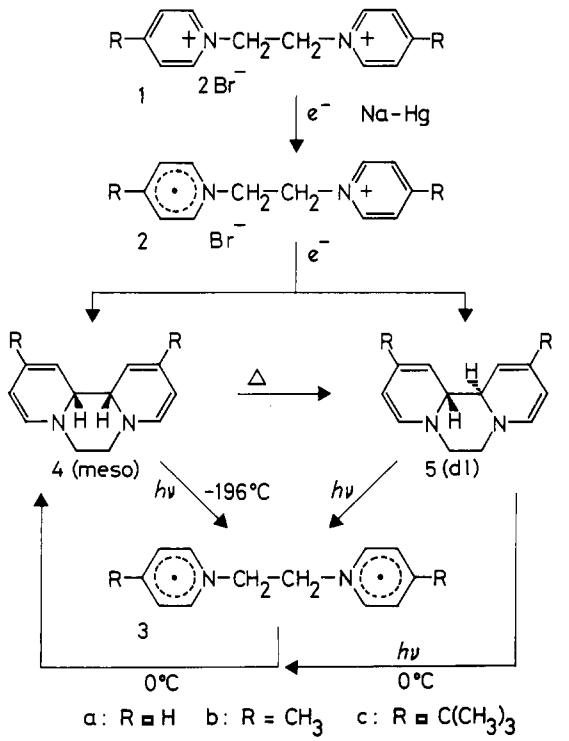
Abstract: Two-electron reduction of 1,1′-(1,2-ethanediyl)bis(pyridinium) dibromide (**1a**) with sodium amalgam affords the meso and *dl* cyclomers formed by intramolecular cyclization of the diradical (**3a**). The meso cyclomer (**4a**) is thermally converted into the *dl* cyclomer (**5a**), while inversely **5a** is photolytically converted into **4a**. Reduction of the 4,4′-dimethyl and 4,4′-di-*tert*-butyl derivatives (**1b** and **1c**, respectively) of **1a** similarly affords the corresponding meso (**4b** and **4c**) and *dl* (**5b** and **5c**) cyclomers, which are convertible to each other. One-electron reduction products (**2b** and **2c**) of **1b** and **1c** are detected at room temperature as shown by the ESR spectra in acetonitrile, which exhibited intramolecular rapid spin exchange between the two pyridine rings in the cation radical. The activation energy for the meso to *dl* conversion was obtained by NMR spectroscopy to be 22.4, 26.9, and 28.8 kcal·mol^{-1} for **4a–c**, respectively. Photodissociation of the cyclomers gives the diradicals, which were characterized by the ESR triplet spectra at −196 °C with the zero-field parameters: $|D| = 0.0222$ and $|E| = 0.0012$ cm^{-1} for **3a**, $|D| = 0.0210$ and $|E| = 0.0008$ cm^{-1} for **3b**, and $|D| = 0.0219$ and $|E| = 0.0016$ cm^{-1} for **3c**. Calculations of the D value suggest that the dihedral angle of the NCH_2CH_2N group of the diradical in a rigid glass is about 95° for these three diradicals.

Our recent study revealed that the 1,1′-(1,2-ethanediyl)bis-[4-(methoxycarbonyl)pyridinyl] diradical is substantially in equilibrium with the cyclomers formed by intramolecular cyclization of the diradical.[2] The equilibrium tends overwhelmingly toward the cyclomers in the dark, and the cyclomers photodissociate to generate the diradical, which is characterized by an ESR triplet spectrum at −196 °C. These results strongly implied a possibility that similar diradicals can be generated photochemically from the corresponding cyclomers. Since photolytic C–C bond cleavage of the dimers of alkylpyridinyl radicals has been demonstrated,[3,4] various pyridinyl diradicals without any electron-withdrawing and electron-delocalizing groups in the pyridine rings might be generated. We therefore examined the properties of the cyclomers of 1,1′-(1,2-ethanediyl)bis(pyridinyl) diradical and its 4,4′-dimethyl and 4,4′-di-*tert*-butyl derivatives and report the structural assignments to the cyclomers in their meso and *dl* forms, the conversion of both forms to each other, and the photochemical generation of the diradicals from the cyclomers. These reactions are summarized in Scheme I.

Scheme I



Results and Discussion

Reduction of Bis(pyridinium) Salts and Conversion of Two Isomeric Products. Reduction of 1,1′-(1,2-ethanediyl)bis(pyridinium) dibromide (**1a**) with sodium amalgam was carried out by two procedures: (P-1) When standard vacuum-line techniques are used, **1a** (100 mg) and 3% sodium amalgam (450 mg) in degassed acetonitrile (30 mL) were stirred in a flask at 0 °C for 4 h. After the amalgam changed to a liquid state the solvent was removed, the residue was extracted with 2-methyltetrahydrofuran (MTHF), and the solvent was replaced by CD_3CN or CH_3CN.

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