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Radical Intermediates Produced from the One-Electron Reduction of Lumazine in Water

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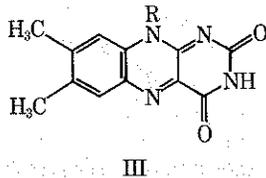
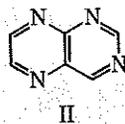
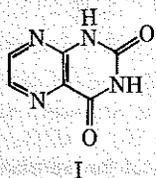
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The one-electron reduction of lumazine (LH₂) in aqueous solutions was brought about using e_{aq}⁻ and (CH₃)₂COH radicals as the reducing agents. The fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry was used to monitor and determine the spectral characteristics and acid-base properties of the free-radical intermediates produced. The reaction rate constants of e_{aq}⁻ and (CH₃)₂COH radicals with the neutral and ionic forms LH₂, LH⁻, and L²⁻ of lumazine were determined. The reactivity toward e_{aq}⁻ is 2–3 × 10¹⁰ M⁻¹ sec⁻¹. At pH 5.2, the reaction of e_{aq}⁻ or (CH₃)₂COH with LH₂ gives rise to an initial transient (T₁) which changes with time to another transient species (T₂). It is shown that proton donors lead to the change T₁ → T₂. The rate constants for the reaction of T₁ with H₂O⁺, LH₂, and H₂PO₄⁻ are 1.6 × 10¹⁰, 2.7 × 10⁸, and 3.6 × 10⁸ M⁻¹ sec⁻¹, respectively. The -N₃H in lumazine is suggested to act as the proton donor for this reaction. Based on the transient spectra and ionization constants of the free radicals formed at pH 0.8, 5.2, 10.2, and 14.0, a tentative scheme of reactions is suggested for the formation of the LH₄·⁺, (LH₂)·⁻, LH₃·, LH₂·⁻, and LH·²⁻ radicals.

Lumazine (I) is a substituted pteridine (II) and forms part of the skeleton of flavins, such as riboflavin (III).

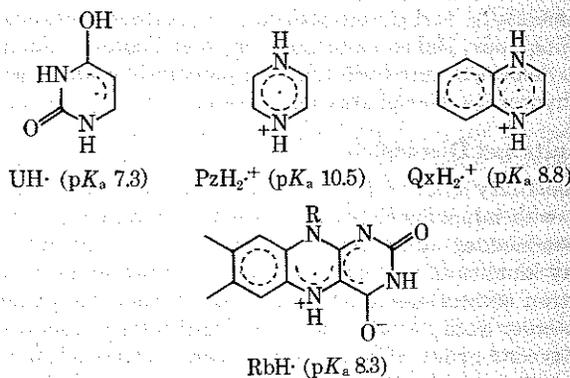


These molecules can be readily reduced, and the intermediate free radicals formed are considered to play a role in biochemical redox reactions. Various aspects of the physicochemical properties of these free radicals, including acid-base properties,² are under study. The one-electron reduction of lumazine has been examined in CF₃COOH by ESR.³ A radical cation has been observed, with the odd electron localized on the pyrazine ring.

The radicals produced from the reduction by e_{aq}⁻ of the uracil⁴ (U) and the pyrazine⁵ (Pz) rings in lumazine have been studied, as well as the related molecules pterin,⁶ folic acid,⁶ quinoxaline⁵ (Qx), and riboflavin^{3,7} (Rb). The site of reduction and the pK_a values of the radicals produced vary considerably.

No work appears to have been carried out to determine the optical spectra, ionization constants, reactivity, and decay kinetics of the lumazine free radicals. Using the fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry, the one-electron reduction of lumazine by e_{aq}⁻, (CH₃)₂COH, and (CH₃)₂CO⁻ radicals in

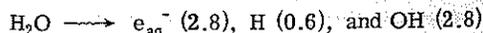
water has been studied. The results obtained are presented below.



Experimental Section

The pulse radiolysis experimental set-up used has been described.^{8,9} Single pulses of 2.3-MeV electrons and ~30 nsec duration (Febetron 705 machine) were used.

The radiolysis of water produces e_{aq}⁻, H, and OH radi-



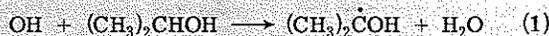
cals, where the numbers in parentheses are the G values (number of free radicals formed per 100 eV of energy absorbed). One-electron reduction of lumazine (LH₂) by e_{aq}⁻ was carried out in solutions containing ~1.0 M *tert*-butyl alcohol (to scavenge the OH radicals) and saturated with argon. One-electron reduction by (CH₃)₂COH radicals was

TABLE I: Reaction Rate Constants of e_{aq}^- and $(CH_3)_2COH$ Radicals with Lumazine (LH_2) in Aqueous Solution at Different pH Values

Ionic form ^a	pH	$k(e_{aq}^- + S)$, $M^{-1} sec^{-1}$ ^b	pH	$k[(CH_3)_2COH + S]$, $M^{-1} sec^{-1}$ ^b	% efficiency ^c
LH_2	6.3	2.9×10^{10}	0.8, 5.1	1.3×10^9	100
LH^-	9.9	2.1×10^{10}	9.5	1.7×10^8	70
L^{2-}	14.0	1.9×10^{10}	14.0	1.0×10^9 ^d	85

^a See text. ^b Values to $\pm 10\%$. ^c Efficiency of formation of the reduced lumazine radical by one-electron reduction. ^d At this pH the donor radical is present as $(CH_3)_2CO^-$.

carried out in solutions containing 1–2 M isopropyl alcohol saturated with N_2O :



The concentrations of lumazine, *t*-BuOH, *i*-PrOH, and N_2O were chosen based on the known^{10,11} rate constants for their reaction with e_{aq}^- and OH radicals in order to obtain the appropriate experimental conditions.

Extinction coefficients were derived based on the *G* values of the primary radicals from water and the KCNS dosimetry.⁸ The transient absorption spectra observed were corrected for depletion of lumazine at the appropriate pH values and wavelengths.

The chemicals used were the highest purity available commercially, and were obtained from Aldrich Chemicals, Mallinckrodt, Baker and Adamson, and Eastman Chemicals. Solutions were buffered with perchloric acid, potassium hydroxide, phosphates, and borate.

Results and Discussion

Reactivity toward e_{aq}^- . The reaction rate constant of e_{aq}^- with lumazine (LH_2 , $pK_a \sim -3, 7.95$, and 12.64) was determined at different pH values, by monitoring the decay kinetics of e_{aq}^- at 700 nm. At this wavelength, the electron adduct species do not absorb. From the pseudo-first-order decay at two–three concentrations of lumazine, the second-order rate constants were derived. Table I shows these values for the acid–base forms LH_2 , LH^- , and L^{2-} . At pH 6.3, $k = 2.9 \times 10^{10} M^{-1} sec^{-1}$ for LH_2 and can be compared to $k = 2.3 \times 10^{10} M^{-1} sec^{-1}$ for riboflavin¹⁰ at pH 5.9, $2.1 \times 10^{10} M^{-1} sec^{-1}$ for pyrazine⁵ at pH 7.0, and $2.5 \times 10^{10} M^{-1} sec^{-1}$ for pterin⁶ at pH 6.5.

The small decrease in the rate constant with increase in pH (Table I) may be the result of the keto–enol tautomerism of lumazine and/or to the negative charge carried by the LH^- and L^{2-} forms. At pH 14.0, the ionic strength of the solution is also affecting the observed reaction rate constant (the actual *k* values would be lower).

Reactivity toward the Acetone Ketyl Radical. The $(CH_3)_2COH$ radical was produced in situ and used to reduce lumazine at various pH values. Table I shows the efficiency of the electron transfer reaction 4, as well as the rate

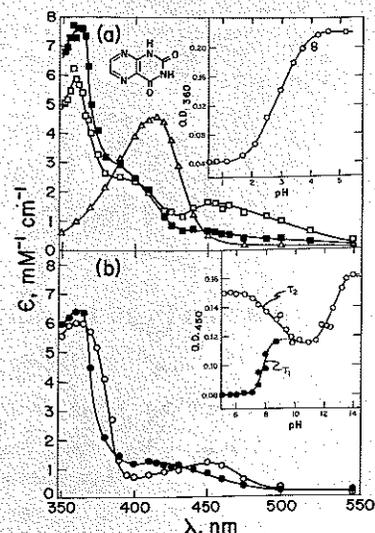


Figure 1. Optical spectra of intermediates produced from the one-electron reduction of lumazine ($2.5 \times 10^{-4} M$) in water by (a) $(CH_3)_2COH$ radicals (in 1.0 M *i*-PrOH) at pH 0.8, Δ , and by e_{aq}^- (in 1.0 M *t*-BuOH) at pH 5.2 (T_1 species, \blacksquare , read at $\sim 0.5 \mu sec$ after the pulse; T_2 species, \square , read at $\sim 5 \mu sec$). Inset shows change in absorbance at 360 nm with pH; (b) e_{aq}^- at pH 10.2, \bullet , and pH 14.0, \circ . Inset shows change in absorbance at 450 nm with pH for transient species T_1 and T_2 . Total dose ~ 5 –10 krads/pulse. The spectra have been corrected for depletion of the solute.

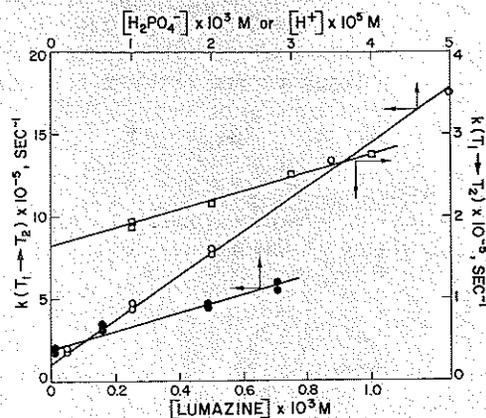
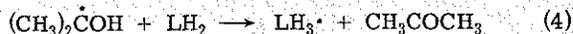


Figure 2. Dependence of the rate of protonation of the transient species T_1 and T_2 produced from the reduction of lumazine by e_{aq}^- upon the concentration of (a) lumazine, \square ; (b) H^+ , \oplus ; and (c) $H_2PO_4^-$, \circ . The differences in the intercept values are due to changes in the initial concentrations of lumazine, H^+ , or $H_2PO_4^-$ used in each case.



constants for reaction 4. The values for k_4 were determined by monitoring the formation kinetics of the lumazine radicals produced at the appropriate wavelength (see below). The efficiencies were derived by comparison with the absorbance of the lumazine radicals produced directly by reaction with e_{aq}^- (*t*-BuOH system), under otherwise identical experimental conditions.

The redox potential of lumazine does not seem to have been determined. The kinetic potential¹² of the $(CH_3)_2COH$ radical is $E_k^{01} = -0.82 V$. The efficiency of electron transfer to LH_2 is 100% suggesting that $E^{01}(LH_2) > -0.82 V$. A 70% efficiency for LH^- (Table I) suggests that $E^{01}(LH^-) \leq -0.82 V$. The $(CH_3)_2CO^-$ radical has a $E_k^{01} < -1.6 V$ (ref 12), and since only 85% transfer to form

TABLE II: Absorption Spectra, Extinction Coefficients, Ionization Constants, and Decay Kinetics of the Radicals Produced from the One-Electron Reduction of Lumazine in Water

pH	λ_{\max} , nm	ϵ , $M^{-1} \text{ cm}^{-1}$	$2k$, $M^{-1} \text{ sec}^{-1a}$	pK_a (radical)	Suggested radical
0.8 ^a	415	4.6×10^3	1.7×10^8	2.9	$\text{LH}_4^{\cdot+}$
5.2 ^{b,c}	(T ₁) 362; 450 (T ₂) 360; 455	7.8×10^3 ; 7.0×10^2 5.8×10^3 ; 1.7×10^3	d 2.6×10^8	e	$(\text{LH}_2)^{\cdot-}$ LH_3^{\cdot}
10.2 ^b	362; ~415	6.4×10^3 ; 1.2×10^3	1.2×10^8	~8.6	$\text{LH}_2^{\cdot-}$
14.0 ^b	365; 450	6.1×10^3 ; 1.2×10^3	1.1×10^8	~12.6	$\text{LH}^{\cdot 2-}$

^a Radical produced by reaction with $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$. ^b Radical produced by reaction with e_{aq}^- . ^c Transient species T₁ and T₂ observed at this pH. ^d T₁ decays by first-order kinetics to give transient T₂ (see text). ^e T₁ species has an apparent $pK_a \sim 7.9$ (see text).

TABLE III: Rate Constants for Protonation of the Lumazine Radical Anion (T₁ Species) by Proton Donors in Water^a

Proton donor, HA	$k(\text{T}_1 \xrightarrow{\text{HA}} \text{T}_2)$, $M^{-1} \text{ sec}^{-1a}$
H_3O^+	1.6×10^{10}
Lumazine, LH_2	2.7×10^8
H_2PO_4^-	3.6×10^8
H_2O	$\sim 10^5 \text{ sec}^{-1b}$

^a See text. ^b Intercept value in plot of the multilinear regression analysis of the kinetic data, assumed to represent protonation by the solvent.

L^{2-} was observed, it follows that the redox potential of L^{2-} is very negative. These observations indicate that the dike-to form of lumazine is much more easily reduced than the mono-enolate (or dienolate) form. Similar differences between the reducibilities of the keto and enol forms of other compounds have been observed.⁶ The more powerful reducing agent e_{aq}^- ($E^0 \sim -2.8 \text{ V}$) does not probe into such differences in reactivity.

Formation of Free Radical Intermediates

The intermediates produced from the reaction of e_{aq}^- with lumazine in aqueous solution (in the presence of 1.0 M t -BuOH) at pH 5.2 are shown in Figure 1a. The transient absorption spectrum and extinction coefficient observed at $\sim 0.5 \mu\text{sec}$ after the electron pulse (transient T₁) changes with time to give another transient species (T₂) whose spectrum was read at $\sim 5 \mu\text{sec}$ after the pulse (Figure 1a and Table II).

The change from T₁ to T₂ was found to require a proton, reaction 5. Various proton donors were found to bring



about this reaction, albeit at different rates. Figure 2 shows the dependence of k_5 upon the concentrations of H^+ , lumazine, and H_2PO_4^- ions (used as buffer). The rate constants for protonation of the T₁ radical intermediate by various proton donors are given in Table III.

Protonation of T₁ by H_3O^+ is very fast, $k = 1.6 \times 10^{10} M^{-1} \text{ sec}^{-1}$, whereas lumazine and H_2PO_4^- protonate T₁ with rate constant values of 2.7×10^8 and $3.6 \times 10^8 M^{-1} \text{ sec}^{-1}$, respectively.

Similar intermediate species T₁ and T₂ were observed on one-electron reduction of various aromatic nitrogen heterocyclic compounds, including pyrazine,^{5,13} pterin,⁶ folic

acid,⁶ pyridoxin,¹⁴ and pyridoxal phosphate.¹⁴ In the case of protonation of the pterin T₁ species by pterin itself, it was clearly shown⁶ that the proton comes from the $-\text{N}_3\text{H}$ position since no protonation was observed from 3-methylpterin. Based on these results, it is suggested that the protonation of the T₁ species by lumazine may also be due primarily to the $-\text{N}_3\text{H}$ group in lumazine.

At pH 0.8, the reaction of $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$ radicals with LH_2 produced a completely different transient spectrum with λ_{\max} 415 nm, see Figure 1a. Although there is no way of comparing this spectrum with that formed by reaction of e_{aq}^- with lumazine, the observed spectrum can nevertheless be attributed to such a species for the following reasons: such an identity was established at pH 5, and neither lumazine nor the acetone ketyl radical have a pK_a in the pH range 0.8–5.0. Since their redox potentials remain unchanged, it is reasonable to assume that one-electron reduction of lumazine by $(\text{CH}_3)_2\dot{\text{C}}\text{OH}$ at pH 0.8 occurs via an electron transfer mechanism.

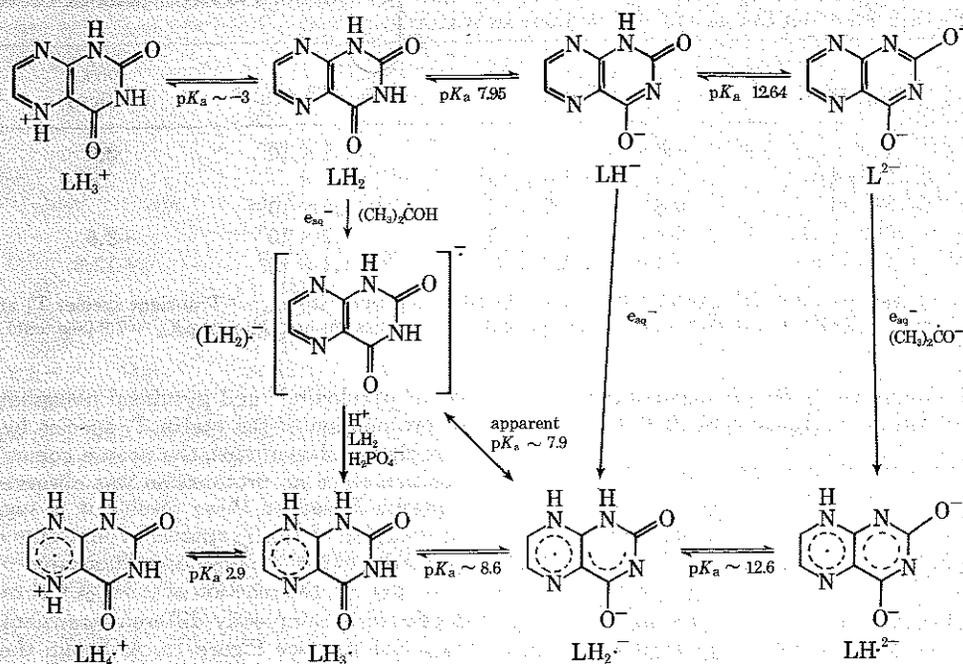
On pulse radiolysis of lumazine at pH 10.2 (ionic form LH^-), a different transient absorption spectrum is formed (Figure 1b). Only one intermediate was observed (i.e., there is no change in the spectrum with time). Another transient spectrum is formed when e_{aq}^- reacts with L^{2-} at pH 14.0. The changes in absorbance of the intermediates with pH at fixed wavelengths are shown in the inserts to Figure 1 (see also Table II).

Scheme I is tentative. Other configurations for the radical intermediates are not presented. This scheme was chosen for the following reasons: (a) due to its similarity to the radicals identified^{3,7} from riboflavin, (b) the strong electron affinity of the pyrazine ring and the great ease for protonation of pyrazinyl radicals,^{5,13,15} and (c) similar mechanisms have been suggested⁶ for pterin and folic acid.

From ESR data, Ehrenberg et al.³ inferred the formation of the radical cation $\text{LH}_4^{\cdot+}$ produced from the one-electron reduction of lumazine in acid media, with the odd electron localized on the pyrazine ring. The $pK_a = 2.9$ for this species (see Scheme I) appears reasonable, if one considers the base weakening effect of the benzene ring. Thus the pK_a of the lumazine cation LH_3^+ is -3 as compared to 0.65 for the pyrazine cation $\text{PzH}^{\cdot+}$. On this basis one can expect that the lumazine cation radical $\text{LH}_4^{\cdot+}$ should have a much lower pK_a (observed value is ~ 2.9) than that of the pyrazine cation radical $\text{PzH}_2^{\cdot+}$ ($pK_a = 10.5^5$).

The subsequent ionization constants of the lumazine radicals are close enough to those of lumazine itself. What calls for comment is the assignment of the T₁ species. In this connection it may be noted that although the transformation from T₁ to T₂ is brought about by proton donors, these two species are not related to each other as conjugate

Scheme I



acid-base pairs. Thus the T_2 spectrum at pH 10.2 (at which pH T_2 would be present in the deprotonated basic form) is not the same as the T_1 spectrum at pH 5.2. We suggest that at, e.g., pH 5.2, T_1 is a radical anion, $(LH_2) \cdot^-$ (possibly a ketyl radical anion at the C_4 position, similar to pyrimidines⁴), which undergoes an irreversible protonation reaction to give the radical $LH_3 \cdot$. This radical has a $pK_a \sim 8.6$ for the ionization of N_1H . The $pK_a \sim 7.9$ for the T_1 species is only an apparent one: with increasing pH, reaction 4 becomes progressively slower. The change in absorbance of T_1 with pH reflects the difference in the rates of protonation of N_{10} and deprotonation of N_3H .

The $LH_2 \cdot^-$ and $LH \cdot^{2-}$ radicals appear to be reasonably good reducing agents. They were both able to reduce anthraquinone-2,6-disulfonate (AQ, $E^{01} = -0.184$ V) to the radical anion $\cdot AQ^-$ with $\sim 100\%$ efficiency and a rate constant for electron transfer of $\sim 1.2 \times 10^9 M^{-1} sec^{-1}$. Acetophenone ($E^{01} = -1.3$ V) could not be reduced by either $LH_2 \cdot^-$ or $LH \cdot^{2-}$ radicals.

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