(17) A. W. Herriott and D. Picker, *Tetrahedron Lett.*, 4521 (1972).
(18) A. Merz, *Angew. Chem., Int. Ed. Engl.*, **12**, 846 (1973).
(19) G. M. Whitesides, J. S. Sadowski, and J. Lilburn, *J. Am. Chem. Soc.*, **96**, 2829 (1974).
(20) W. von E. Doering and W. A. Henderson, Jr., *J. Am. Chem. Soc.*, **80**, 5274 (1958).
(21) D. Seyferth and J. M. Burlitch, *J. Am. Chem. Soc.*, **86**, 2730 (1964).
(22) M. Makosza and M. Wawrzyniewicz, *Tetrahedron Lett.*, 4659 (1969).
(23) I. Tabushi, Z. Yoshida, and N. Takashi, *J. Am. Chem. Soc.*, **93**, 1820 (1971).
(24) C. A. Buehler and D. E. Pearson, "Survey of Organic Synthesis", Wiley, New York, N.Y., 1970, p 80.
(25) L. F. Fieser and M. Fieser, "Reagents for Organic Synthesis", Vol. 1, Wiley, New York, N.Y., 1967, p 1089.
(26) A. J. Solo and B. Sinigh, *J. Org. Chem.*, **30**, 1658 (1965).
(27) K. M. Ibne-Rasa, A. R. Tahir, and A. Rahman, *Chem. Ind. (London)*, 232 (1973).
(28) D. Landini, S. Quici, and F. Rolla, *Synthesis*, 397 (1975).
(29) W. K. Kwok and S. I. Miller, *J. Am. Chem. Soc.*, **92**, 4599 (1970).
(30) The use of aqueous hypochlorite in the oxidation of certain alcohols has previously been reported: G. A. Lee and H. H. Freedman, *Tetrahedron Lett.*, 1641 (1976); C. Y. Meyers, *J. Org. Chem.*, **26**, 1046 (1961).
(31) L. F. Fieser and K. L. Williamson, "Organic Experiments", 3d ed, D. C. Heath, Lexington, Mass., 1975, p 252.
(32) J. F. King, A. D. Allbutt, and R. G. Pews, *Can. J. Chem.*, **46**, 805 (1968).
(33) R. E. Buckles and N. G. Wheeler, "Organic Syntheses", Collect. Vol. IV, Wiley, New York, N.Y., 1963, p 857.
(34) R. E. Buckles, J. L. Miller, and R. J. Thurmaier, *J. Org. Chem.*, **32**, 888 (1967); R. E. Buckles, J. M. Bader, and R. J. Thurmaier, *ibid.*, **27**, 4523 (1962).

One-Electron Redox Reactions of Water-Soluble Vitamins. 4. Thiamin (Vitamin B_1), Biotin, and Pantothenic Acid

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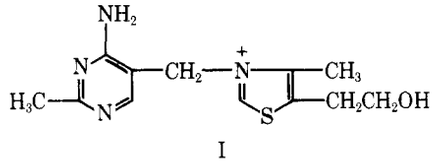
The technique of pulse radiolysis and kinetic absorption spectrophotometry was used to study the one-electron reduction of thiamin, thiazole, 4-aminopyrimidine, biotin, and pantothenic acid in aqueous solution. The acetone ketyl radical and e_{aq}^- were used as the one-electron reducing agents. The reaction rate constants of e_{aq}^- and $(CH_3)_2\dot{C}OH$ with these compounds were determined at different pH values, taking into account the dissociation constants of the substrates. The transient optical absorption spectra of the intermediates produced, their extinction coefficients, decay kinetics, and ionization constants were determined. For thiazole (Tz), the radical formed in neutral solution (·TzH) has a λ_{max} 317 nm, ϵ_{317} 3.8×10^3 M^{-1} cm^{-1}, decays with $2k \sim 2 \times 10^8$ M^{-1} s^{-1}, and has a pK_a (radical) = 3.1. This is close to that of the parent compound, pK = 2.5. For 4-aminopyrimidine (4-Am-Pm), the radicals formed have maxima at λ <260 nm, and the pK_a of the dihydro radical cation 4-Am-PmH_2·$^+$ is 6.4. Thiamin in neutral solution forms intermediates with maxima at 317 and 350 nm, and $\epsilon \sim 6.5 \times 10^3$ M^{-1} cm^{-1}. One-electron reduction of the thiazolium ring of thiamin is suggested, based on the formation of dihydrothiamin as a final product. Other assignments for these radicals are suggested and discussed. The reaction of OH radicals with biotin and pantothenic acid leads, primarily, to H atom abstraction at various sites in the molecule. The formation and ionization of the $-\dot{C}(OH)CONH-$ radical from pantothenic acid, pK_a = 6.0 ± 0.3, is proposed.

Thiamin (vitamin B_1, I), in the form of its pyrophosphate (at OH group), is the coenzyme for a number of biochemical reactions involved in carbohydrate metabolism, e.g., cleavage



I

of carbon–carbon bonds adjacent to carbonyl compounds (as in pyruvic acid).[2,3] Many common foods contain appreciable quantities of thiamin. Thiamin contains a pyrimidine and a thiazole nucleus. The activity of this vitamin is probably linked primarily to the thiazolium ring.[4] Reduction (hydrogenation) of the thiazolium ring results in a complete loss of activity. The mechanism of thiamin action has been suggested[4] to involve the ionization of the thiazolium ring, through the loss of a proton from the C_2 carbon, resulting in the formation of a zwitterion. Biotin (vitamin H) serves as an acceptor molecule for bicarbonate ion in enzymes which catalyze several biosynthetic reactions.[5] The function of the sulfur atom in biotin is still not well characterized. Protonation of biotin, $pK_2 = -1.1$ ($pK_a \sim 4.8$ for the –COOH), has been suggested[6] to occur on the ureido group, presumably on the carbonyl oxygen atom. More recent results indicate that there is no sulfur–carbonyl transannular interaction in biotin (see ref 6, 7). Pantothenic acid[2] is a component of coenzyme A, functions in acetylation reactions in amino acid, carbohydrate, and fat metabolism, and is involved in various biosynthesis with other vitamins.

The fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry was used to study the one-electron reduction of these vitamins and related compounds in water. The hydrated electron and the acetone ketyl radical were used as one-electron reducing agents. The reactions with OH radicals were also examined. The results obtained are reported below.

Experimental Section

The pulse radiolysis setup[8,9] and the experimental conditions used have been described.[10] The one-electron reduction of the compounds was brought about by reaction with e_{aq}^- and/or $(CH_3)_2\dot{C}OH$ radicals. The necessary conditions for these experiments have already been described.[10,11]

The extinction coefficients given below are based on $G(e_{aq}^-) = G(OH) = 2.8$. Dosimetry[8] was carried out using KCNS. The transient spectra presented were corrected for depletion of the ground-state absorption of the molecules.

The chemicals used were the highest purity commercially available and were obtained from Calbiochem, Cyclochemicals, and Sigma Chemicals. The reagents used were purchased from Mallinckrodt, Baker and Adamson, Aldrich, and Eastman Chemicals. Solutions were buffered using perchloric acid, potassium hydroxide, phosphate, and tetraborate.

Results and Discussion

Reactivity toward e_{aq}^-, $(CH_3)_2\dot{C}OH$, and OH Radicals. The reaction rate constants of e_{aq}^- with thiazole, 4-aminopyrimidine, and thiamin were determined at pH 6–8, taking

Table I. Reaction Rate Constants of Substrates with e_{aq}^-, $(CH_3)_2\dot{C}OH$, and OH Radicals in Water

Substrate	pK_a	e_{aq}^- [a] pH	e_{aq}^- [a] k, M^{-1} s^{-1}	$(CH_3)_2\dot{C}OH$ [b] pH	$(CH_3)_2\dot{C}OH$ [b] k, M^{-1} s^{-1}	OH [c] pH	OH [c] k, M^{-1} s^{-1}
Thiazole	2.5	8.0	2.1×10^9	0.8	6.2×10^8		
				13.3 [d]	[e]		
4-Aminopyrimidine	5.9	8.2	1.1×10^{10}	0.8–13.0	[e]		
Thiamin	4.8	6.1	3.4×10^{10}	0.5	2.2×10^8	4.7	3.2×10^9
				6.6	1.9×10^8	4.7	3.0×10^9 [c]
Biotin	−1.1, 4.8	9.0	$\leq 5 \times 10^7$			3.6	3.8×10^9
Pantothenic acid	4.4	6.6	1.2×10^8			6.6	4.5×10^9

[a] Rate determined in presence of ~0.5 M *t*-BuOH by monitoring decay kinetics of e_{aq}^- at 700 nm. [b] Rate determined by monitoring formation kinetics of transient species. [c] Rate determined by competition kinetics with KCNS, taking $k(OH + CNS^-) = 1.1 \times 10^{10}$ M^{-1} s^{-1}. [d] $(CH_3)_2\dot{C}O^-$ radical present at this pH. [e] No electron transfer at this pH.

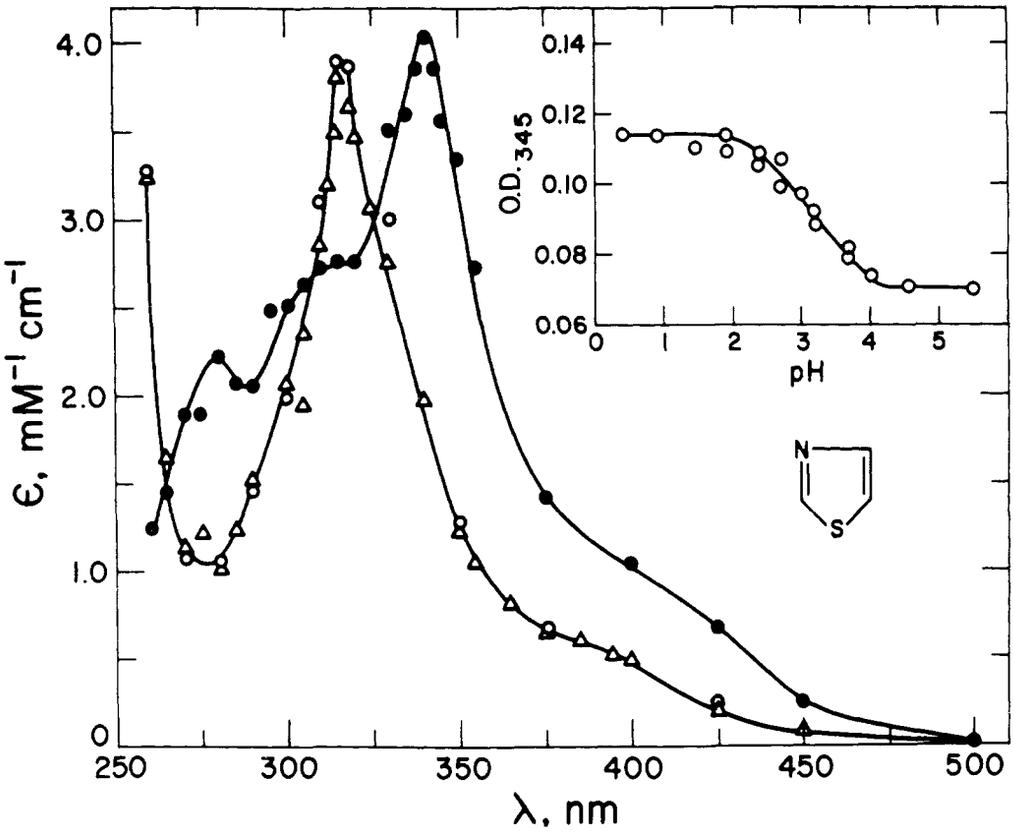


Figure 1. Adsorption spectra of intermediates produced from the one-electron reduction of thiazole (2×10^{-3} M) by (a) e_{aq}^- (in presence of 1.0 M *t*-BuOH, 1 atm argon) at pH 5.3, △, and pH 13.0, ⊙, and (b) $(CH_3)_2\dot{C}OH$ radicals (1.0 M *i*-PrOH, 1 atm argon) at pH 0.8, ●. Insert: change in absorbance at 345 nm with pH from solutions containing 2×10^{-2} M thiazole, 2.0 M *i*-PrOH, 1 atm argon (data at pH 4.0–5.5 are under same conditions as a above). Total dose ~4–8 krad/pulse.

into account the dissociation constants of these molecules; see Table I. A $k = 2.1 \times 10^9$ M^{-1} s^{-1} was found for thiazole. This value is considerably higher than that for thiophene (6.5×10^7 M^{-1} s^{-1})[12] and pyrrole (6.0×10^5 M^{-1} s^{-1})[12], but agrees well with the value 2.5×10^9 M^{-1} s^{-1} given in the literature[12] for thiazole. The $k = 1.1 \times 10^{10}$ M^{-1} s^{-1} for 4-aminopyrimidine is somewhat less than that recently[13] found for pyrimidine, $k = 2.0 \times 10^{10}$ M^{-1} s^{-1}. The lower value for 4-aminopyrimidine may be connected with the presence of the electron-donating amino group. With thiamin, the value of 3.4×10^{10} M^{-1} s^{-1} can be rationalized on the basis of the expected high reactivity of the thiazolium cation.

The one-electron reduction of these compounds by $(CH_3)_2\dot{C}OH$ radicals was observed to occur with the thiazolium species, but not with 4-aminopyrimidine or thiazole; see Table I. This is in general agreement with observations[10,11,13] that the protonated forms of nitrogen heteroaromatic compounds (which have higher redox potentials than the neutral forms) are readily reduced by this donor radical. In the case of 4-aminopyrimidine, even the protonated form is not reduced by $(CH_3)_2\dot{C}OH$ radicals. This is to be compared with pyrimidine itself, whose protonated form has a much higher redox potential and is reduced by $(CH_3)_2\dot{C}OH$ radicals.[13] The difference between pyrimidine and 4-aminopyrimidine might be attributed to the strong electron-donating property of the amino group.

Table I shows the reaction rate constants of e_{aq}^- with biotin and pantothenic acid. With both vitamins the reactivity is relatively low, $k \leq 10^8$ M^{-1} s^{-1}, and it was not possible to adjust the experimental conditions in order to examine the transient species formed from the reduction of these compounds by e_{aq}^-. The reduction potentials of these vitamins are also very low (i.e., negative) to enable their reduction by electron donor radicals such as $(CH_3)_2\dot{C}OH$.

Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and Ionization Constants of Intermediates Produced from the Reaction with e_{aq}^- of Thiazole, 4-Aminopyrimidine, and Thiamin in Water

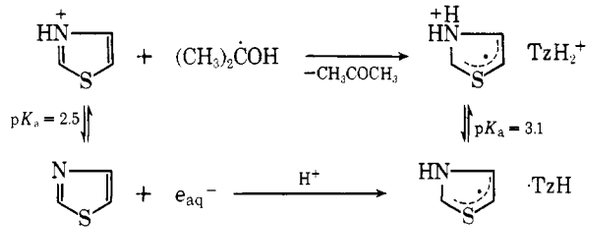
Substrate	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} s^{-1}	pK_a (radical)
Thiazole	5.3, 13.0[a]	317	3.8×10^3	2.2×10^8	3.1
	0.8[b]	340	4.1×10^3	1.5×10^8	
		280	2.2×10^3		
4-Amino-pyrimidine	3.5[a]	<300	3.1×10^3	*d*	6.4
	13.3[a]	<260	3.7×10^3	1.7×10^9 [c]	
Thiamine	4.4, 6.6[b]	317	6.5×10^3		
		350	6.6×10^3	*f*	~1.6[e]
	0.5[b]	345	6.7×10^3	*f*	
		362	6.3×10^3	*f*	

[a] Determined in presence of 1.0 M *t*-BuOH; decay kinetics may, in part, be due to reaction with β-alcohol radicals. [b] Determined in presence of 1.0 M isopropyl alcohol. [c] A very long-lived intermediate is produced from this second-order decay. [d] Mixed kinetics. [e] Estimated ±0.3; see text. [f] Decay at all λ's to an intermediate with only slightly lower ϵ than the initial transient; the intermediate decays by second-order kinetics with $2k = 7.6 \times 10^5$ M^{-1} s^{-1} at pH 4.4, and decays over a period of $t > 20$ s at pH 0.5.

The reaction rate constants of the vitamins with OH radicals were determined at appropriate pH values (see Table I), and were found to be $\sim 4 \times 10^9$ M^{-1} s^{-1}.

Thiazole. Relatively strong transient absorption spectra are observed from the one-electron reduction of thiazole (Tz, pK_a = 2.5) in water by e_{aq}^- and thiazolium ion by $(CH_3)_2\dot{C}OH$ radicals. These were determined at pH 0.8 (using acetone ketyl radicals) and at pH 5.3 and 13.0 (using e_{aq}^-) and are shown in Figure 1 and Table II.

At pH 0.8, the absorption maximum at 340 nm is red shifted compared to λ_{max} 317 nm at pH 5.3. The acid form of carbon-centered free radicals is usually[14] blue shifted compared to the basic form of the radical. A recent study[13] of nitrogen-centered heteroaromatic free radicals has shown that the absorption spectra of the radical cations are red shifted compared to those of neutral radicals. No change in transient spectrum was observed between pH 5.3 and 13.0. However, the change in acidic solutions was titrated (see insert in Figure 1), and a pK_a ~3.1 was observed. This value is close to the pK_a = 2.5 of thiazole itself. The following reaction scheme is suggested:

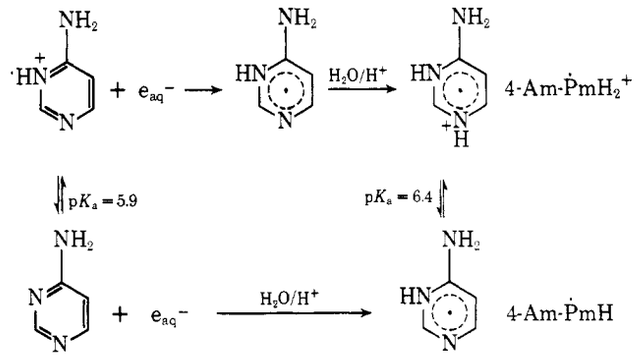


The protonation of ·TzH and ·Tz$^-$ was too fast to be observed under our experimental conditions. The reaction rate constants for the protonation step of some other N-heteroaromatic radicals are slower and have been observed.[10,11,13] The radical forms present at pH 2.0 and 5.0 could also be the neutral ·TzH and the ·Tz anion radicals. However, since the radical anions of many nitrogen heteroaromatic compounds are rapidly protonated in aqueous media (even in neutral and alkaline solutions),[13] one can expect the same to be the case for thiazole, unless the sulfur atom helps to stabilize the radical anion against protonation. The shifts in the absorption maxima are also in agreement with the above assignment.

These radicals decay by second-order kinetics (Table II). The apparent slightly higher decay rate at pH 5.3–13.0 may be due, in part, to the reaction between thiazole radicals and the β-alcohol radical produced from *t*-BuOH.

4-Aminopyrimidine. 4-Aminopyrimidine (4-Am-Pm) has a pK_a = 5.9 due to protonation at the N_1 position. This is to be compared to a pK_a = 1.3 for pyrimidine itself. One-electron reduction at pH 3.5 by e_{aq}^- gives rise to a relatively weak transient absorption immediately after the 30-ns pulse; see Figure 2. This absorption increases with time, and the full spectrum ~5 μs later is also shown. At pH 13.3, the spectrum observed is the same as the initial spectrum formed at pH 3.5; see Figure 2. By monitoring the change in absorbance at 345 nm with pH,a pK_a (radical) = 6.4 was observed (insert, Figure 2).

The following reactions are suggested:



The scheme presented above is similar to that recently suggested[13] for pyrimidine (Pm) and other diazabenzenes. The pK_a of the ·PmH$_2^+$ radical to form ·PmH was found[13] to be 7.6 ± 0.1. The spectral characteristics of the 4-aminopyrimidine and the pyrimidine radicals, and their changes with pH, are very similar. This supports the assignment given above.

At pH 13.3, the radicals decay by second-order kinetics. One may conclude that unimolecular deamination is not the mode of decay of these radicals. In acidic media, however, the decay kinetics are complex. There are indications that the β-alcohol radical from *t*-BuOH present in solution interacts with the 4-Am-ṖmH$_2^+$ cation to give secondary transient species. Similar reactions were observed with other diazine radicals.[13]

Thiamin. Thiamin (Tm) has a pK_a = 4.8, which has been assigned to protonation on the pyrimidine ring. It is unstable[15] at pH ≥ 7.0 and no experiments were therefore performed in alkaline solutions. One-electron reduction of thiamin at pH 0.5 by $(CH_3)_2\dot{C}OH$ radicals gave a transient spectrum with maxima at 345 and 362 nm. At pH 6.6, reduction of thiamin gives a spectrum which is blue shifted compared to that produced at pH 0.5; see Figure 3. The relatively small change in absorbance at 365 nm was monitored as a function of pH, and a pK_a ~ 1.6 ± 0.3 could be derived. The transient spectrum remained unchanged in the pH range 3–6, i.e., over the pK_a = 4.8 of thiamin.

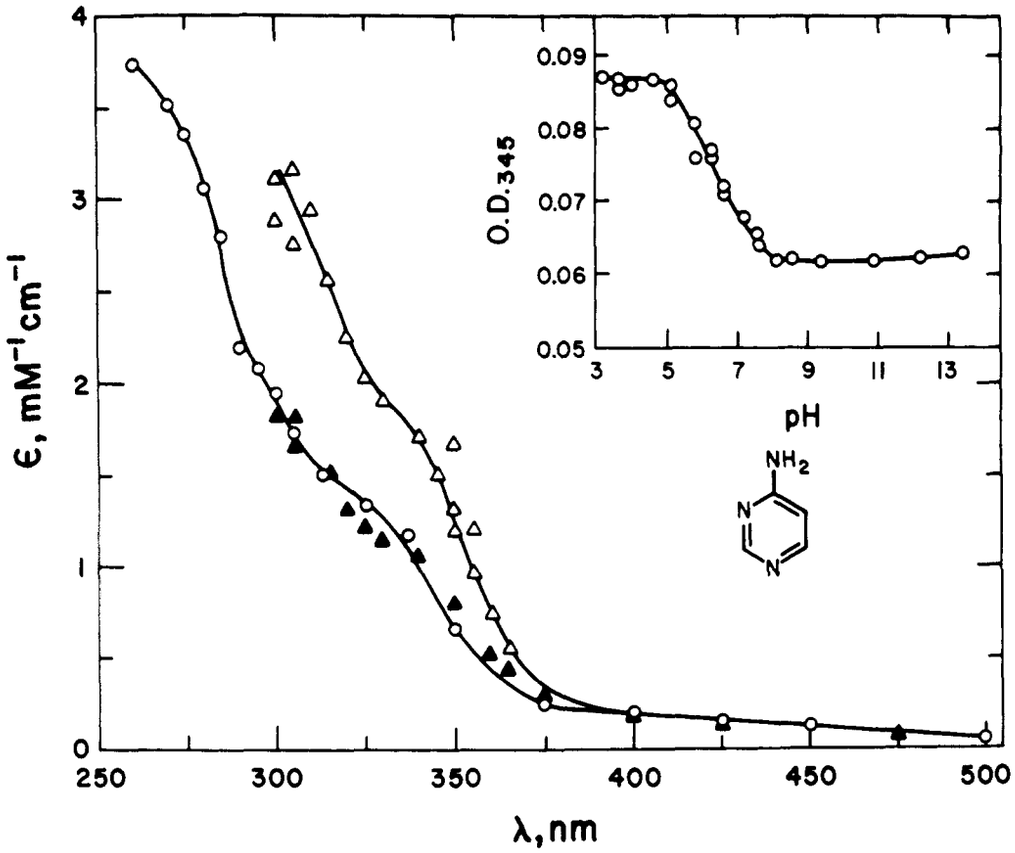


Figure 2. Absorption spectra of intermediates produced from the one-electron reduction by e_{aq}^- of 4-aminopyrimidine (in presence of 1–2 M *t*-BuOH, 1 atm argon) at pH 3.5 (10^{-2} M, transient species T_1, ▲, and T_2, △, measured at ≤0.5 and ~5.0 μs after the pulse, respectively) and pH 13.3 (10^{-3} M, ⊙) at ≤0.5 μs. Total dose ~10 krad/pulse. Insert: change in absorbance at 345 nm with pH (solutions contained 10^{-2} M 4-aminopyrimidine, 2.0 M *t*-BuOH, 1 atm argon).

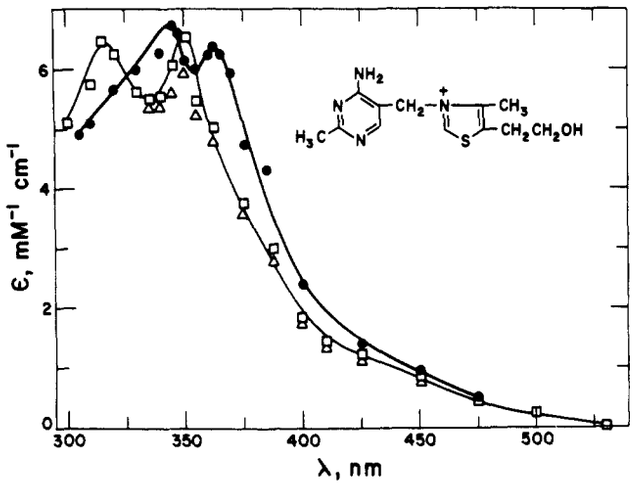
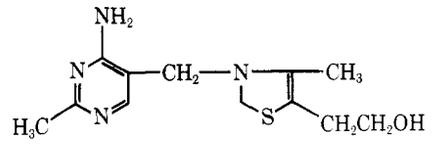


Figure 3. Absorption spectra of intermediates produced from the one-electron reduction by $(CH_3)_2\dot{C}OH$ and e_{aq}^- of thiamin (5×10^{-3} M thiamin, 1.0 M *i*-PrOH, 1 atm N_2O) at pH 6.6 (transient species T_1, □, and T_2, △, read at "zero" time and ~200 μs after the pulse, respectively) and pH 0.5, ●. Total dose ~3 krad/pulse.

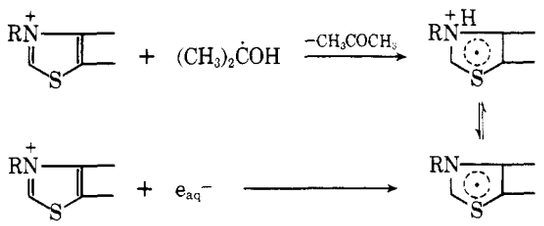
From the reactivity of thiamin toward one-electron reducing agents, and from the nature of the absorption spectrum of the transient species formed, as compared to thiazole, it would appear that the thiazolium ring is the primary site for one-electron reduction. The thiazolium ring has been found[16] to be the site of attack in the chemical, as well as electrochemical, reduction of thiamin to form dihydrothiamin (II). In steady state radiolysis experiments (solution contained 10^{-4} M thiamin, 1.0 M isopropyl alcohol, 1 atm N_2O, pH ~5.0)



II

the UV spectrum of the solution was identical with the absorption spectrum of dihydrothiamin.

Based on the above considerations, the following reaction scheme is suggested:



As suggested above for thiazole, one could also consider a different assignment for these radicals. The strong delocalization of the lone pair of electrons on the sulfur atom leaves the S atom in the thiamin with a net positive charge,[3] thus facilitating the interaction of one-electron reducing agents with the molecule.

The decay kinetics of the electron adduct to thiamin are somewhat unusual. At all pH's, the initial absorbance decreases by ~10% in ~200 μs after the 30-ns pulse at all wavelengths. The transient spectrum at ~200 μs after the pulse is essentially identical with the initial spectrum observed at ~0.1 μs after the pulse. It decays by second-order kinetics with $2k = 7.6 \times 10^5$ M^{-1} s^{-1} at pH 6.6 and 8.8×10^4 M^{-1} s^{-1} at pH 4.4

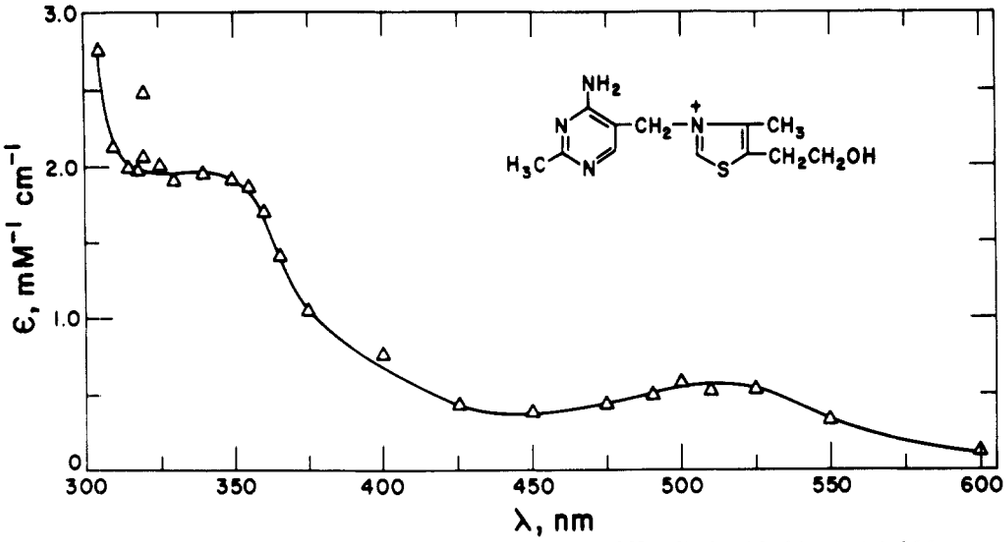


Figure 4. Absorption spectrum of intermediates produced from the reaction of OH radicals with thiamin (10^{-3} M, 1 atm N_2O, pH 4.4, ~7 krad/pulse).

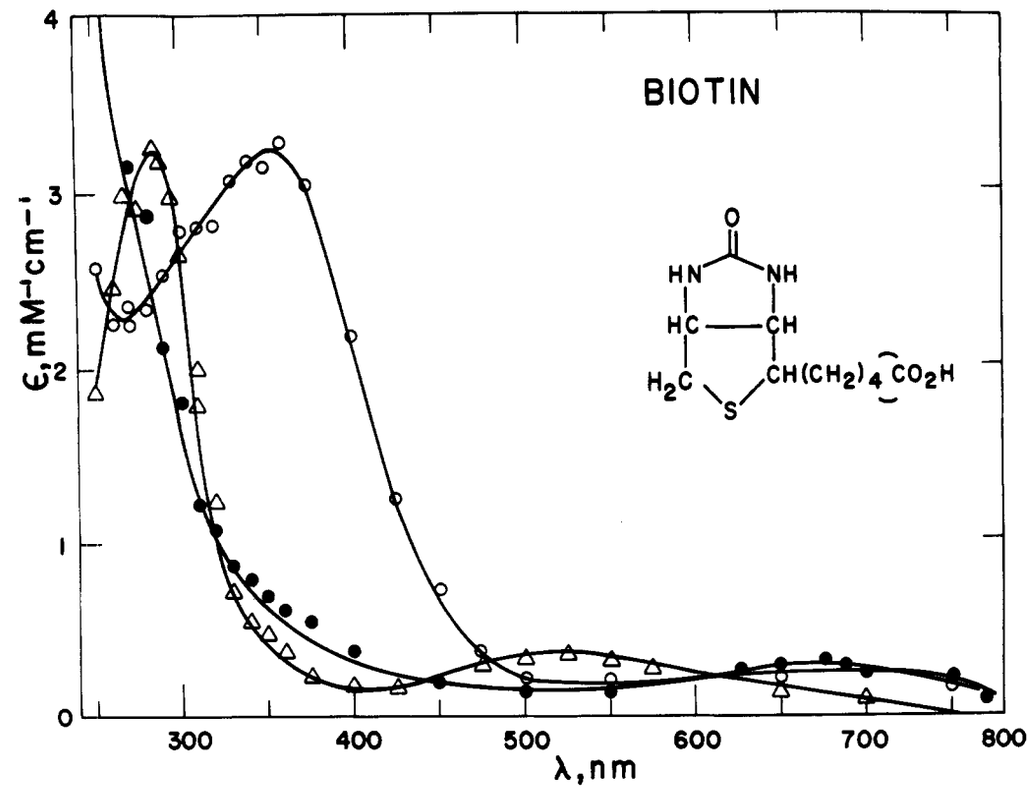


Figure 5. Transient species produced from the reaction of OH radicals with biotin (10^{-3} M, in presence of 1 atm N_2O) at pH 10.4 (T_1, ○), pH 3.7 (T_2, △), and pH 13.3 (T_3, ●). Total dose ~10 krad/pulse (see text).

(see Table II). At pH 0.5 it has a lifetime >20 s. The small initial decay may be due to reaction with the β-hydroxy radical formed (~15%) from isopropyl alcohol on reaction with OH radicals. Similar reactions were observed[13] between nitrogen heterocyclic radicals and the β-hydroxy radical of *t*-BuOH. The pH dependence of the bimolecular decay may be attributed to the overall charge of +1 on the transient species (due to protonation of the pyrimidine ring, $pK_a = 4.8$). At still lower pH's, viz., below the pK_a (radical) = 1.6, the species bears two positive charges, which could explain its relatively slow decay.

An experiment was carried out to observe the transient species produced from the reaction of OH radicals with thiamin. The spectrum observed is shown in Figure 4. Absorption maxima at 515, ~340, and <305 nm can be seen. The OH radicals are expected to add primarily to the pyrimidine ring[18] and to the thiazolium ring (possibly at the C_2 position). In addition, H-atom abstraction by OH from the side chains may occur. The radical produced decays by second-order kinetics with $2k = 1.6 \times 10^8$ M^{-1} s^{-1} at pH 4.7.

Biotin. The reaction of OH radicals with biotin produces a transient optical absorption spectrum T_1 at pH 10.4 with absorption maxima at ~350 and ~680 nm and ϵ_{350} 3.2×10^3 and ϵ_{680} 250 M^{-1} cm^{-1}; see Figure 5. This intermediate decays relatively fast in a pH-dependent manner. In acidic solutions it decays by reaction with H^+ with a $k = 7.4 \times 10^4$ M^{-1} s^{-1}.

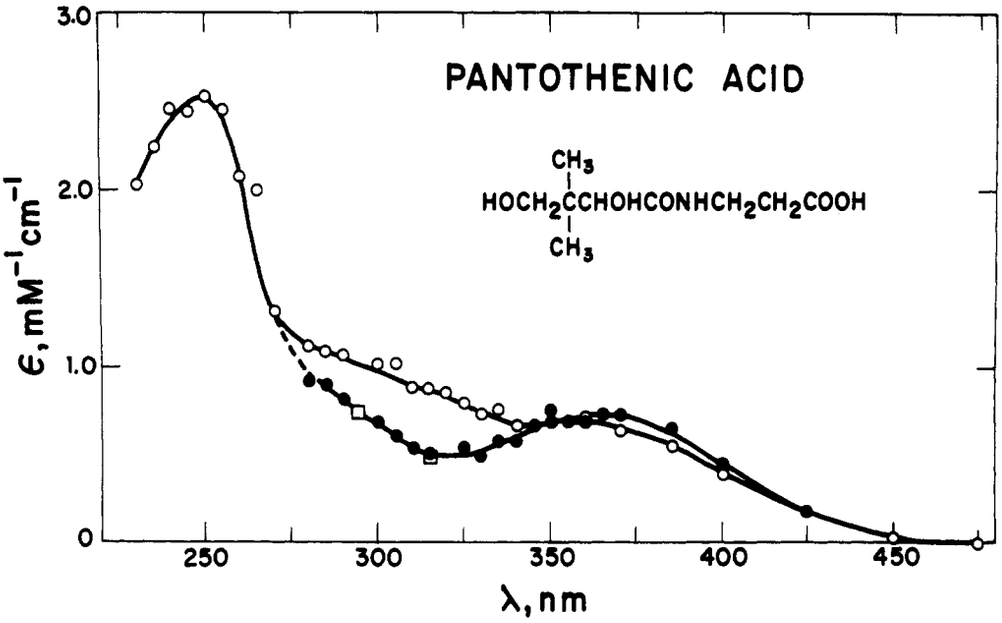
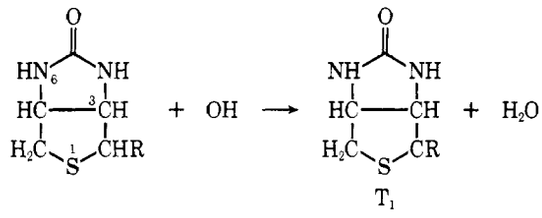


Figure 6. Transient species produced from the reaction of OH radicals with pantothenic acid (2×10^{-3} M, 1 atm N_2O) at pH 6.7. Dark symbols, ●, observed immediately after the pulse and open symbols, ○, ~2 μs later. At pH 4.7, □, only one transient spectrum is observed in the wavelength range 270–340 nm. Total dose ~20 krad/pulse.

The initial T_1 transient produces in acid solutions a spectrum (T_2) with $\lambda_{max} \sim 285$ and 520 nm and ϵ_{285} 3.2×10^3 and ϵ_{520} 380 M^{-1} cm^{-1} (Figure 5). The change of T_1 to T_2 can also be brought about by $H_2PO_4^-$ ions with $k = 4.4 \times 10^8$ M^{-1} s^{-1}. Protonation by water occurs with $k \sim 6.0 \times 10^3$ M^{-1} s^{-1}.

Transient T_1 decays in alkaline solution by reaction with OH^- with $k = 2.7 \times 10^7$ M^{-1} s^{-1} to give an intermediate (T_3) with maxima at ~680 and <250 nm, with ϵ_{680} 300 and ϵ_{250} 4.6 $\times 10^3$ M^{-1} cm^{-1}; see Figure 5. Both T_2 and T_3 species decay by second-order kinetics. For T_2, $2k = 6.4 \times 10^8$ M^{-1} s^{-1} at pH 3.6, monitored at 290 nm. For T_3, $2k = 4.2 \pm 2 \times 10^8$ M^{-1} s^{-1} at pH 13.3, monitored at 300, 360, and 675 nm.

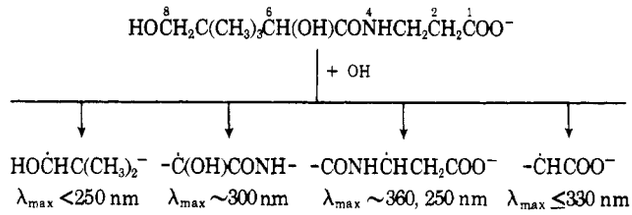
The attack on biotin by OH radicals can occur at various sites: H atom abstraction at C_2, C_3, C_7, and C_8 positions, as well as abstraction from the side chain. It is suggested that one of the more important sites is at the C_2 position:



The protonation reaction of T_1 with H^+ or $H_2PO_4^-$ is suggested to occur at the sulfur atom, with the formation of $>^+SH$. The reaction T_1 with OH^- ions is less clear, and may involve ring opening of biotin. Alternatively, some H-atom abstraction at the C_3 position may also be occurring, resulting in ionization of the $-N_4H-$ group in alkaline solution.

Pantothenic Acid. The reaction of OH radicals with pantothenic acid can result in the formation of a number of free radicals, as a result of attack at various sites in the molecule. Figure 6 shows the transient absorption spectrum observed. In the wavelength region ~270–340 nm, the initial absorption at pH 6.7 increased with time, as shown, whereas at pH 4.7 there was no such change—the spectrum was identical with the initial spectrum at pH 6.7. This change in absorbance at 315 nm was monitored as a function of pH. A titration curve was found with a pK_a ~6.0 ± 0.3.

Four major sites for H-atom abstraction from pantothenic acid can be considered:



(a) At the C_2 position. Such a radical is expected[19] to absorb weakly at λ below ~330 nm with a relatively low extinction coefficient. (b) At the C_3 position. Such a radical, $-CONH\dot{C}HCH_2COO^-$, is expected[20] to have two absorption maxima at ~360 and ~250 nm. These bands can be observed in Figure 6. (c) At the C_6 position. Such a radical, $-\dot{C}(OH)CONH-$, is expected[21] to absorb at λ ~300 nm. Such an α-diketone ketyl radical should have[14] a relatively low pK_a (radical) value. The observed $pK_a = 6.0 \pm 0.3$ from pantothenic acid can be assigned to this radical:

$$-\dot{C}(OH)CONH- \underset{pK_a = 6.0 \pm 0.3}{\rightleftharpoons} -\dot{C}(O^-)CONH- + H^+$$

(d) At the C_8 position. With the formation of an α-alkylhydroxy radical $HO\dot{C}HCH(CH_3)_2$. Such a radical would have[14] a $pK_a \geq 11.0$. Because of the instability of pantothenic acid at pH ≥ 7.0, this region has not been investigated.

The presence of the $-\dot{C}(O^-)CONH-$ radical anion in neutral solution of pantothenic acid would indicate that it is a good reducing agent[22] for one-electron transfer reactions. Such a radical is expected to have a low (i.e., negative) oxidation potential.[22]

Registry No.—Thiazole, 288-47-1; 4-aminopyrimidine, 591-54-8; thiamin, 59-43-8; biotin, 58-85-5; pantothenic acid, 79-83-4; $(CH_3)_2\dot{C}OH$, 5131-95-3; OH, 3352-57-6.

References and Notes

(1) Visiting Scientist from Bhabha Atomic Research Center, Chemistry Division, Trombay, Bombay, 400085, India.
(2) M. Florkin and E. H. Stotz, Ed., "Comprehensive Biochemistry", Vol. 11, American Elsevier, New York, N.Y., 1963.

(3) B. Pullman and A. Pullman, "Quantum Biochemistry", Interscience, New York, N.Y., 1963.
(4) R. Breslow, *Ann. N.Y. Acad. Sci.*, **98**, 445 (1962).
(5) F. Lynen, *Biochem. J.*, **102**, 381 (1967).
(6) C. E. Bowen, E. Rauscher, and L. L. Ingraham, *Arch. Biochem. Biophys.*, **125**, 865 (1968); M. Caplow, *Biochemistry*, **8**, 2656 (1969).
(7) J. A. Glasel, *Biochemistry*, **5**, 1851 (1966); A. C. Mildvan and M. C. Scrutton, *ibid.*, **6**, 2978 (1967).
(8) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969).
(9) J. P. Keene, E. D. Black, and E. Hayon, *Rev. Sci. Instrum.*, **40**, 1199 (1969).
(10) P. N. Moorthy and E. Hayon, *J. Org. Chem.*, **41**, 1607 (1976).
(11) P. N. Moorthy and E. Hayon, *J. Am. Chem. Soc.*, **97**, 2048 (1975).
(12) M. Anbar, M. Bambenek, and A. B. Ross, *Nat. Stand. Ref. Data Ser., Nat. Bur. Stand.*, **No. 43**, 1 (1973).
(13) P. N. Moorthy and E. Hayon, *J. Phys. Chem.*, **78**, 2615 (1974); P. N. Moorthy and E. Hayon, *Indian J. Chem.*, **14B**, 206 (1976).
(14) See E. Hayon and M. Simic, *Acc. Chem. Res.*, **7**, 114 (1974), and references cited therein.
(15) G. D. Maier and D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 4385 (1957).
(16) G. P. Tikhomirova, *Ukr. Khim. Zh.*, **28**, 1048 (1962).
(17) K. K. Bhanot and P. N. Moorthy, private communication.
(18) See, e.g., E. Hayon and M. Simic, *J. Am. Chem. Soc.*, **95**, 1029 (1973).
(19) P. Neta, M. Simic, and E. Hayon, *J. Phys. Chem.*, **73**, 4207 (1969).
(20) E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, *J. Am. Chem. Soc.*, **92**, 3898 (1970); **93**, 5388 (1971).
(21) J. Lillie, G. Beck, and A. Henglein, *Ber. Bunsenges. Phys. Chem.*, **72**, 529 (1968).
(22) P. S. Rao and E. Hayon, *J. Am. Chem. Soc.*, **96**, 1287 (1974); **97**, 2986 (1975).

Stable Heptamethine Pyrylium Dyes That Absorb in the Infrared

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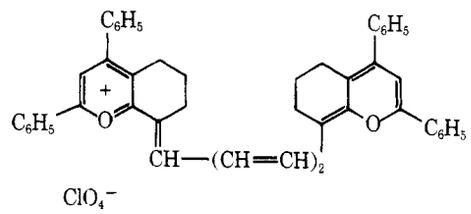
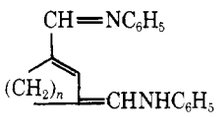
The stability of heptamethine pyrylium dyes has been greatly increased by incorporating all but two of the methine groups in rings. The effect of ring size and a chlorine atom in the center of the methine chain on the absorption maxima of the dyes is described. By the suitable choice of these factors, a dye which absorbs at about 1.2 μ was obtained. Heptamethine pyrylium dyes were prepared from an indene-1,3-dialdehyde derivative as well as the corresponding 3-chloro derivative. These dyes were not as stable nor did they absorb at as long wavelengths as other similar dyes containing five- and six-membered rings at the center of the methine chain. It has been shown that heptamethine pyrylium dyes containing only the three central methine groups in a ring are as stable as the dyes that contain all but two of the methine groups in rings, and that a thiopyrylium homologue is the most stable heptamethine dye that we have prepared.

For many years, chemists have been interested in preparing dyes that show electronic absorptions further into the infrared region of the spectrum than existing dyes, and cyanine dyes have proved to be exceptionally useful in this respect. The work with cyanine dyes has been reviewed.[1] A particularly useful route to infrared-absorbing dyes has taken advantage of the fact that the pyrylium nucleus, compared to other heterocyclic nuclei, gives large bathochromic shifts when incorporated in polymethine dyes.[2] For example, the relatively simple dye **1** (in methylene chloride) shows absorption at 1040 nm with ϵ 125 000.



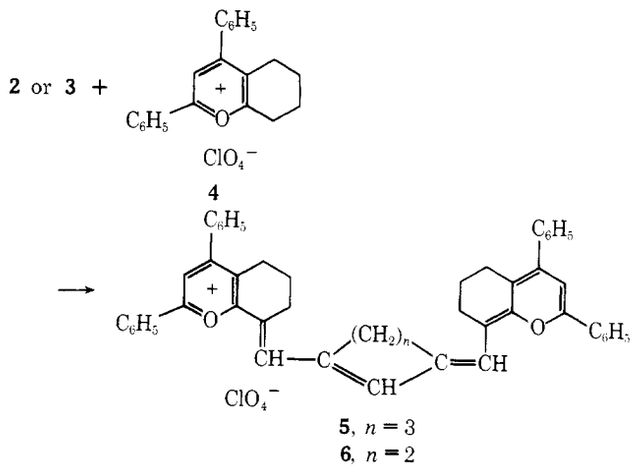
1

The present paper describes more recent work on preparing polymethine pyrylium dyes that absorb in the infrared. The successful preparation of **1** depended on partially rigidizing the polymethine chain by incorporating two of the methine groups in rings. Attempts to prepare the pyryloheptamethine dye without these rings were unsuccessful. Evidently, we have reached the limits of stability with **1**, since the next higher vinylog could not be prepared. This result was not surprising because the stability of **1**, although much better than that of other polymethine dyes absorbing in this region of the spectrum, is still not good. It was apparent that the most obvious way to increase the stability of **1** or prepare higher vinylogs would be to incorporate more methine groups in rings. Unfortunately, the reported methods[1] for doing this failed with



2, $n = 3$
3, $n = 2$

pyrylium dyes. It was, therefore, with interest that we noted the recent preparation[3] of **2** and **3**, which have been used by Russian workers to prepare some pyrylium, thiopyrylium, and selenopyrylium dyes.[4] We have used these bisaldehyde derivatives to prepare heptamethine dyes **5** and **6** which contain all but two of the methine groups in rings.



5, $n = 3$
6, $n = 2$

The λ_{max} of **5** and **6**, 1090 nm (ϵ 140 000) and 1138 (70 000), respectively, illustrate two characteristics that are common to dyes of this type, namely, that the dyes containing five-