# Oxidation of tetrahydropterins by azide radical and the spectra of trihydropterin radicals[1,2]

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Spectrophotometric titration of tetrahydropterins with incremental amounts of $^{\bullet}N_3$ showed that 6,7-dimethyl-7,8-dihydropterin was an intermediate in the oxidation of 6,7-dimethyltetrahydropterin at the two electron-equivalent oxidation point. The eventual end product at four electron equivalents was 6,7-dimethylpterin. In the case of unsubstituted tetrahydropterin, the dihydro form was clearly not an exclusive product at the two electron equivalent point, and this was attributed to the disproportionation of different forms of dihydropterin to tetrahydropterin and pterin.

The azide radical oxidized tetrahydropterins to trihydropterin radicals, $^{\bullet}PnH_3$, with overall second-order rate constants at pH 7 of 4.1, 3.8, and $2.9 \times 10^9$ $M^{-1}$ $s^{-1}$ for tetrahydropterin, 6,7-dimethyltetrahydropterin, and 6-carboxylated tetrahydropterin, respectively. At pH 10 the rate constants are slightly larger due to the presence of the deprotonated enolate forms of tetrahydropterins. Spectra vary somewhat with substitution in the pterin molecule, but all species have a strong peak ($\epsilon \sim 9000$ $M^{-1}$ $cm^{-1}$) near 320 nm and lower absorption above this, with a tail extending to 580 nm.

*Key words*: tetrahydropterin oxidation, tetrahydropterin, trihydropterin radical, azide radical, trihydropterin spectrum.

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Le titrage spectrophotométrique des tétrahydroptérines avec des quantités croissantes de $^{\bullet}N_3$ a permis de démontrer que, au niveau de l'oxydation par deux équivalents d'électron, la 6,7-diméthyl-7,8-dihydroptérine est un intermédiaire dans l'oxydation de la 6,7-diméthyltétrahydroptérine. La 6,7-diméthylptérine est le produit qui se forme éventuellement au niveau de l'oxydation par quatre équivalents d'électron. Dans le cas de la tétrahydroptérine qui n'est pas substituée, il est clair que la forme dihydro n'est pas le produit exclusif au niveau de l'oxydation par deux équivalents d'électron; ce résultat est attribué à la disproportionation des diverses formes de la dihydroptérine en tétrahydroptérine et en ptérine.

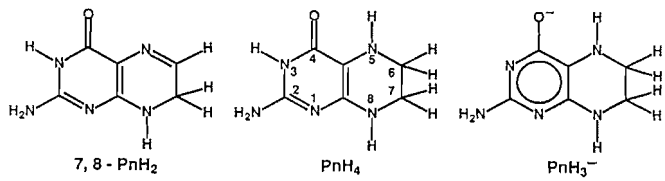
A un pH de 7, le radical azoture oxyde les tétrahydroptérines en radicaux trihydroptérines, $^{\bullet}PnH_3$; les constantes de vitesse du deuxième ordre sont respectivement 4,1, 3,8 et $2,9 \times 10^9$ $M^{-1}$ $s^{-1}$ pour la tétrahydroptérine, la 6,7-diméthyltétrahydroptérine et la tétrahydroptérine portant un groupement carboxyle en position 6. A un pH de 10, les constantes de vitesse sont légèrement supérieures à cause de la présence des formes énoliques déprotonées des tétrahydroptérines. Les spectres varient légèrement avec les substituants présents sur la molécule de ptérine; toutefois, toutes les espèces présentent une forte bande ($\epsilon = 9000$ $M^{-1}$ $cm^{-1}$) près de 320 nm et une absorption moins intense à une longueur d'onde plus grande avec une queue allant jusqu'à 580 nm.

*Mots clès* : tétrahdroptérine oxidation, tétrahydroptérine, radical trihydroptérine, radical azoture, spectre trihydroptérine.

[Traduit par la revue]

## Introduction

Reduced derivatives of folic acid are carriers of activated one-carbon units in the synthesis of nucleic acids (1), and the related unconjugated pterins act as cofactors in the enzymically-catalyzed conversion of phenylalanine to tyrosine and of tyrosine to dihydroxyphenylalanine (1–3). In addition, knowledge of the mechanisms of oxidation of reduced pterin coenzymes is important for the preservation of vitamin activity during the course of food processing (4). For these reasons, there has been extensive interest in the oxidation–reduction reactions by which the biologically relevant di- and tetrahydro forms of folic acid are interconverted. Important studies (5–9) have been conducted with the structurally simpler pterin analogues, $PnH_2$ and $PnH_4$ (as shown in Scheme 1) which still posess the heterocyclic ring system essential to the redox chemistry and biological functions.




SCHEME 1. Structures of dihydro and tetrahydropterins.

Several authors have postulated the involvement of trihydropterin radicals ($^{\bullet}PnH_3$) as intermediates, for example in the autooxidation of $PnH_4$ (8, 9). Such radicals have been produced in strongly acid media by chemical oxidizing agents (10, 11) and more recently in acetonitrile by photochemical methods (12). They have been characterized in those systems by esr. However, although frequent reference has been made to the coloration of these solutions (8, 9, 11), definitive investigations of the absorption spectra of $^{\bullet}PnH_3$ radicals appear to be lacking. The main reason for this is that to date only relatively mild oxidizing agents, such as oxygen and $Fe(CN)_6^{3-}$, have been used. These react relatively slowly with $PnH_4$ and its derivatives, which

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[2]Taken in part from the M.Sc. Thesis of M. Farahani, Department of Chemistry, University of Calgary, 1987.

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means that the ambient concentrations of radicals are low and their optical absorbances cannot be properly differentiated from those of parent compounds and the $PnH_2$ and pterin (Pn) products. The object of this work was to find an alternative one-electron oxidant, which would still sequentially convert $PnH_4$ to $PnH_2$ and Pn via a radical mechanism, but at the same time could be used under conditions that would permit the absorption spectrum of $^{\cdot}PnH_3$ to be recorded.

The azide radical, $^{\cdot}N_3$, which normally reacts with aromatic systems specifically by electron transfer (13, 14), was chosen as a suitable oxidant. The radical can be generated conveniently through the oxidation of $N_3^-$ by $^{\cdot}OH$ radicals, produced in the pulse radiolysis of $N_2O$-saturated solutions. The following relevant reactions (15, 16) take place immediately after the pulse and are complete within 10 ns:

$$[1]\quad 4.36H_2O \rightarrow 2.90\, e_{aq}^- + 2.90\, H^+ + 0.52\, {}^{\cdot}H + 0.47\, H_2 + 0.73\, H_2O_2 + 2.90\, {}^{\cdot}OH$$

$$[2]\quad N_2O + H_2O + e_{aq}^- \rightarrow N_2 + OH^- + {}^{\cdot}OH$$

$$[3]\quad {}^{\cdot}OH + N_3^- \rightarrow OH^- + {}^{\cdot}N_3$$

The coefficients in reaction [1] are moles per 10 MJ of radiation energy absorbed.

Azide has the advantage over several other radicals in that it does not itself absorb significantly above 300 nm, provided complexation with $N_3^-$ is eliminated by keeping the $N_3^-$ concentration below 0.2 M. Also the $^{\cdot}H$ atoms are removed by reaction with $N_3^-$ to form $^{\cdot}HN_3^-$ (14) which is a potential reducing agent and would be inert to $PnH_4$.

This paper demonstrates that $^{\cdot}N_3$ sequentially oxidizes tetrahydropterins to their dihydro and pterin forms. It also reports the spectra of trihydropterin radicals derived from unsubstituted tetrahydropterin ($PnH_4$), 6,7-dimethyltetrahydropterin ($Pn(Me)_2H_4$), and 6-carboxylated tetrahydropterin ($Pn(CO_2^-)H_4$). The latter was produced by reaction of $^{\cdot}CO_2^-$ with pterin.[4] Experiments were carried out mainly at neutral pH and pH 10.

## Experimental

Pulse radiolysis experiments were conducted at 23 ± 2°C in 1 cm optical path length "supercil" cells on solutions, which were flushed after each radiation pulse. For some experiments, the electron beam source was the 1.5 MeV Van de Graaff accelerator at the University of Calgary, but most of the pulse radiolysis was done with the 8 MeV linear accelerator in the U.S. Department of Energy Radiation Laboratory at the University of Notre Dame. Methods of dosimetry and other experimental details for these two facilities have been given in refs. 17 and 13, respectively. γ-Radiolysis was carried out at room temperature in an Atomic Energy of Canada Gamma Cell, either at Calgary or Notre Dame. Dose rates were checked routinely with the Fricke dosimeter solution (16) with the ferric ion yield taken from the literature as 1.61 mole per MJ of radiation energy absorbed (15).

Absorption spectra of solutions were measured on a Cary 219 Spectrophotometer and absorbance coefficients were used routinely to determine concentrations of tetrahydropterins. For $PnH_4$ and $Ph(Me)_2H_4$, the first $\lambda_{max}$ was at 297 nm for both pH 7 and 10. The value of $\epsilon_{max}$ was also the same at both pH's and equal to 8200 ± 200 $M^{-1}$ $cm^{-1}$ for the former and 8500 ± 200 $M^{-1}$ $cm^{-1}$ for the latter. It may be noted that these pH coincidences were fortuitous, as some $PnH_3^-$ was present with $PnH_4$ at pH 10 (p$K$ $PnH_4$ amide ~ 10.4 (18)) and a trace of $PnH_5^+$ (p$K$ ~ 5.6 (18)) occurs at pH 7. $Pn(CO_2^-)H_4$ had $\lambda_{max}$ = 302 nm and $\epsilon_{max}$ = 8020 ± 200 $M^{-1}$ $cm^{-1}$ at pH 10.

[4]Details of $^{\cdot}CO_2^-$ reductions will be presented elsewhere (M. Farahani, P. S. Surdhar, R. Allen, C. Schoneich, K.-D. Asmus and D. A. Armstrong. To be published).

All chemicals were the purest available and solutions were made up in triply-distilled or chromatographically-purified deionized water with 10–20 mM phosphate buffer present. They were deaerated by purging with nitrous oxide, nitrogen, or argon before use. Solutions of tetrahydropterins were kept free of air to avoid oxidation by oxygen. Sources of pterin derivatives were tetrahydropterin sulphate, K and K Laboratories; 6,7-dimethyl-5,6,7,8-tetrahydropterin, pterin-6-carboxylic acid, and pterin, Fluka. Pterin obtained from the Sigma Chemical Company gave identical results.

## Results and discussion

*Two and four electron-equivalent oxidation by $^{\cdot}N_3$*

Tetrahydropterin derivatives in $N_2O$-saturated solutions, containing 0.02 to 0.05 M sodium azide, were subjected to oxidation by $^{\cdot}N_3$ produced by γ radiolysis over measured periods of time (5–15 min). Absorption spectra were taken several minutes after each successive addition of this oxidant, when the radical reactions had ceased. An illustration of one of these spectrophotometric titrations has been given in Fig. 1*a* for 6,7-dimethyltetrahydropterin at pH 10. The observations at pH 7 were similar. The numbers on the curves correspond to the number of minutes of exposure to radiation, and each ten minutes corresponds to the addition of 32 μmol of $^{\cdot}N_3$ per litre. The initial spectrum, very similar to the "ten minute spectrum", was omitted.

At the start of the oxidation, there were isobestic points at 318 and 286 nm, and, as illustrated for 318 nm in the inset, the absorbances at these wavelengths did not change until two equivalents of $^{\cdot}N_3$ had reacted per mole of $Pn(Me)_2H_4$. Beyond this point, two new isobestic points appeared at 267 and 332 nm. Finally there was no change in absorbance at any wavelength for oxidizing equivalents in excess of 4.1 per mole of $Pn(Me)H_4$ initially present, and at that point the spectrum corresponded to that of 6,7-dimethylpterin. The solubility of this compound in 0.02 M phosphate buffer at pH 7 and 10 and 23°C was found to be about 20 μM. A fine precipitate appeared in the latter stages of the oxidative titration, if the initial $Pn(Me)_2H_4$ concentration exceeded this. In separate experiments with unsubstituted pterin, no oxidation by $^{\cdot}N_3$ was seen, and this appeared true for all pterin derivatives examined here.

When $Pn(Me)_2H_4$ was allowed to react with oxygen in an aerated solution and the conversion to 6,7-dimethyl-7,8-dihydro-pterin ($Pn(Me)_2H_2$) was followed spectrophotometrically, as in earlier work (2), isobestic points were observed at the same wavelengths as those seen initially in Fig. 1*a*. Also, the spectrum at the two electron equivalence point in Fig. 1*a* ($e_{oxd}/Pn(Me)_2H_4$ = 2.0) was found to be within experimental error the same as that of $Pn(Me)_2H_2$, produced by oxidation of $Pn(Me)_2H_4$ with two equivalents of $Fe(CN)_6^{3-}$ (6, 7) or by oxidation with air. The results in Fig. 1*a* can therefore be explained on the basis of three major species being present during the titration: (*a*) $Pn(Me)_2H_4$ and $Pn(Me)_2H_2$ from $e_{oxd}/(Pn(Me)_2H_4)_0$ = 0 to 2.1; and (*b*) $Pn(Me)_2H_2$ and $Pn(Me)_2$ from $e_{oxd}/(Pn(Me)_2H_4)_0$ = 2.1 to 4.1. The fact that the titration requires a slight excess of $^{\cdot}N_3$ can be explained by the presence of the reducing entity $^{\cdot}HN_3^-$, which is formed with one tenth the yield of $^{\cdot}N_3$ (see Introduction) and could reconvert some Pn and $PnH_2$ to their reduced forms.

The effects observed with $PnH_4$ and $Pn(CO_2^-)H_4$ were different to the extent that there was no clearcut two-electron equivalent end point, although oxidation again ceased at the four-electron equivalent point. This has been illustrated for the case of the carboxylated tetrahydropterin at pH 10 in Fig. 1*b*. The presence of the isobestic point between the absorbances of the

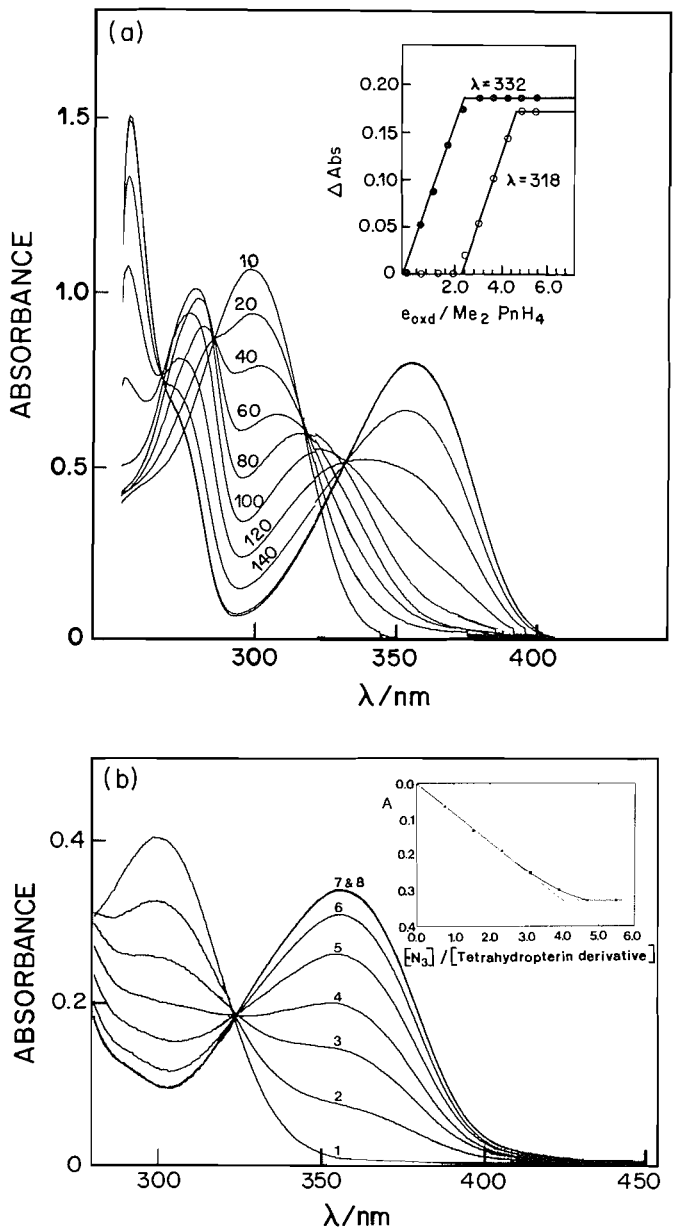



FIG. 1. Spectrophotometric titrations of tetrahydropterins in $N_2O$-saturated 40 mM $NaN_3$ solutions at pH 10. (*a*) 62 μM 6,7-dimethyltetrahydropterin. Numbers on curves correspond to minutes of exposure to γ radiation; (*b*) 50 μM 6-carboxylated tetrahydropterin. Numbered curves correspond to 0 (*1*), 8 (*2*), 16 (*3*), 24 (*4*), 32 (*5*), 40 (*6*), 48 (*7*) and 56 min (*8*) of γ radiation. Insets: Changes in absorbance versus moles of oxidant $^{\bullet}N_3$ per mole of initial tetrahydropterin.

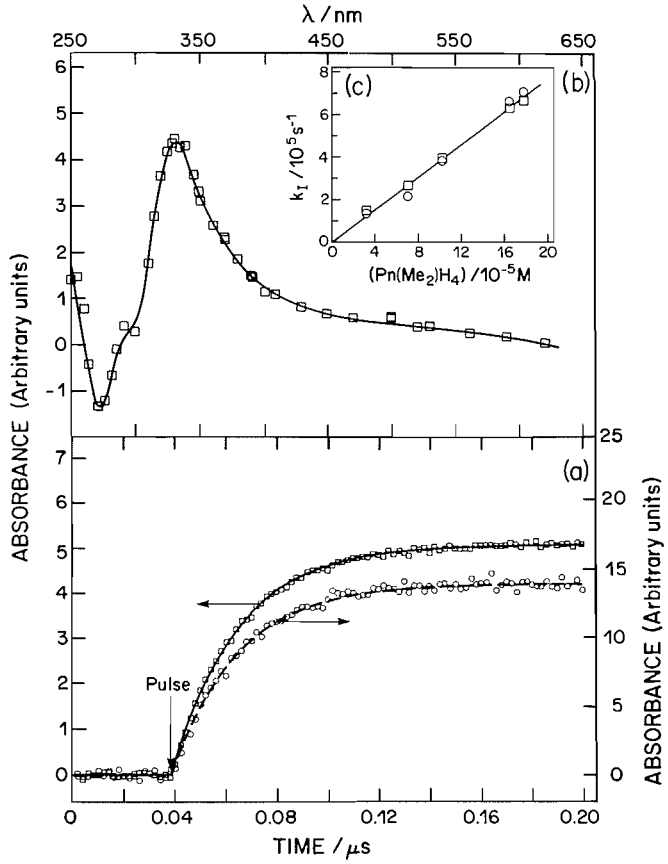



FIG. 2. Growth of absorbance of radicals produced by $^{\bullet}N_3$ oxidation illustrated for 6,7-dimethyltetrahydropterin at pH 7 in 0.05 M $NaN_3$ solutions. (*a*) Kinetics of growth at 330 nm (□) and 400 nm (○) for 103 μM solutions. Lines are fitted first-order growth curves. (*b*) Differential spectrum—changes in absorbance when radical formation was complete plotted against wavelength—for a 32 μM solution. (*c*) Pseudo first order rate constants for growth at 330 nm (□) and 400 nm (○) plotted against concentration of the tetrahydropterin.

tetrahydropterin and pterin implies that pterin is present right from the start of the titration and that the 7,8-dihydropterin never builds up to a significant concentration.

The earlier research with $Fe(CN)_6^{3-}$ (6, 7) as oxidant showed that the first non-radical product of one electron oxidation was a quinonoid dihydropterin (6, 7). This rearranged to 7,8-dihydropterin in a process, which was on a sub-second time scale for "unhindered" tetrahydropterins, like $PnH_4$, but could be observed by spectrophotometry for the 6,7-dimethyl derivative. Also, with the exception of this derivative, the quinonoid dihydropterins disproportionated quite rapidly with their 7,8-isomers to yield the $PnH_4$ and Pn forms. The present observations for unsubstituted $PnH_4$ are in agreement with this. The finding that $Pn(CO_2^-)H_4$ behaved similarly is consistent with the fact that blocking groups were required at both C-6 and C-7 to fully inhibit the disproportionation. In summary, therefore, the products and stoichiometry observed on the oxidation of tetrahydropterins with $^{\bullet}N_3$ are similar to those seen with $Fe(CN)_6^{3-}$. However, as will be appreciated from the next section, the primary one-electron oxidation step is much faster.

*Pulse radiolysis observation of one electron oxidation*

Kinetics of $^{\bullet}PnH_3$ Growth:

The reaction of $^{\bullet}N_3$ radicals with tetrahydropterin derivatives was followed by observing the changes in absorption ($\Delta A$) at wavelengths in the region $\lambda$ = 250 to 650 nm. Examples of the growth of $\Delta A$ *with time at* $\lambda$ = 330 and 400 nm are shown in Fig. 2*a* for 103 μM 6,7-dimethyltetrahydropterin at pH 7. For solutions of a particular tetrahydropterin at a given pH, the growth obeyed pseudo first-order kinetics at all wavelengths with similar rates. Plots of the pseudo first-order rate constants versus concentrations of the tetrahydropterin were linear, as illustrated by the example in Fig. 2*c*. This is consistent with reaction [4].

[4] $\quad ^{\bullet}N_3 + PnH_4 \rightarrow N_3^- + \cdot PnH_4^+$

as the rate-controlling process, the values of $k_4$ calculated from the slopes of such plots have been summarized in Table 1.

Reference to Table 1 shows that, while the differences are comparable to the ±15% experimental uncertainty, there is a consistent pH dependence of $k_4$ for each derivative. For the case

TABLE 1. Rate constants for oxidation by $^{\bullet}N_3$

| Tetrahydropterin | Rate constants* pH 10 | pH 7 |
|---|---|---|
| $PnH_4$ | 5.7 | 4.1 |
| $Pn(Me)_2H_4$ | 4.3 | 3.8 |
| $Pn(CO_2^-)H_4$ | 4.2 | 2.9 |

*Units: $10^9$ $M^{-1}$ $s^{-1}$; uncertainties ±15%.

of $Pn(Me)_2H_4$, Kallen and Jencks (18) have determined the p$K$ of the 3,4-amide group to be 10.4. This means that at pH 10 about 26% of the compound will be in the deprotonated enolate form (see structures in Introduction, Scheme 1). Based on $^{\bullet}N_3$ oxidation rates for model aromatic compounds (13), one would expect a slightly higher rate constant for this species. Allowing for the fractions of enolate (0.26) and amide (0.74) forms and assuming $k_4 = 3.8 \times 10^9$ $M^{-1}$ $s^{-1}$ for the latter, one finds from the pH 10 rate constant $k_{4e} = 5.7 \times 10^9$ $M^{-1}$ $s^{-1}$

[4e] $^{\bullet}N_3 + PnH_3^- \rightarrow N_3^- + \cdot PnH_3$

for the $PnH_3^-$ form of $Pn(Me)_2H_4$. In accord with this, when the rate constant was determined at pH 11.5 where 95% of the $Pn(Me)_2H_4$ would have been in the $PnH_3^-$ form, the rate constant was found to be $5.8 \times 10^9$ $M^{-1}$ $s^{-1}$. Since the amide ionization constants for the other tetrahydropterins are probably similar to that of $Pn(Me)_2H_4$, the trends with pH for the other compounds in Table 1 can be rationalized on a similar basis. The lower rate constants for the carboxylated derivative suggest that the presence of the $CO_2^-$ group exerts a small inhibitory effect, perhaps by partially blocking access to the N-5 position. The rates at both pH's are also largest for the unsubstituted $PnH_4$.

The rate constants for the oxidation of the $PnH_4$ derivatives at pH 7 and for $Pn(Me)_2H_3^-$ are comparable to those for the $^{\bullet}N_3$ oxidation of phenolates (13) and of the dihydroflavin molecule (19), which has a number of structural similarities to tetrahydropterin. They are several orders of magnitude larger than the rate constants for oxidation of tetrahydropterins by $Fe(CN)_6^{3-}$ (~ $10^6$ $M^{-1}$ $s^{-1}$ (7)) and oxygen (~ 1 $M^{-1}$ $s^{-1}$ (8)) at the same pH's. This can be attributed primarily to the much higher reduction potential of $^{\bullet}N_3$ (20) ($E^0(^{\bullet}N_3/N_3^-)$ = 1.33 V (21); $E^0(Fe(CN)_6^{3-}/Fe(CN)_6^{4-})$ = 0.36 V (22); $E^0$ $(O_2/O_2^-)$ = −0.16 (21)).

As shown in Fig. 2*a*, for typical conditions, growth of the radical absorbance was complete at a microsecond or less. The change in absorbance at that point and before significant radical decay has been plotted against λ in Fig. 2*b* for the case of a 32 μM solution of $PnMe_2H_4$ at pH 7. Similar data were obtained for each $PnH_4$ derivative at neutral pH and pH 10. These "differential spectra" were independent of $PnH_4$ concentration, apart from expected minor variations in intensity due to the greater losses of $^{\bullet}N_3$ by combination at lower $PnH_4$ concentration (15).

The lifetimes of the present pterin radicals were surprisingly long and their decays could not be followed quantitatively with present apparatus. However, first half lives were in the region of 0.1 s. This means that rate constants for self reaction are in the region of $10^6$ $M^{-1}$ $s^{-1}$ or less, and that $^{\bullet}PnH_3$ radicals are much more stable kinetically than many other biological radicals, such as flavin and nicotinamide radicals.

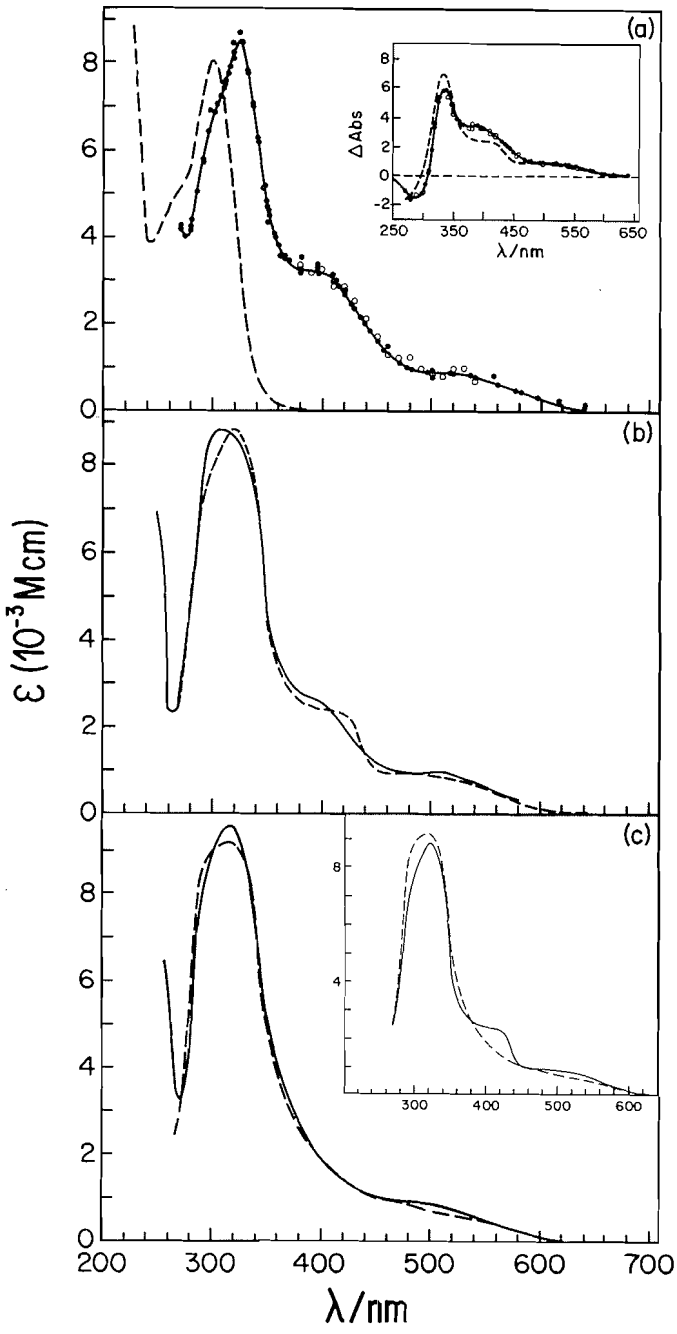


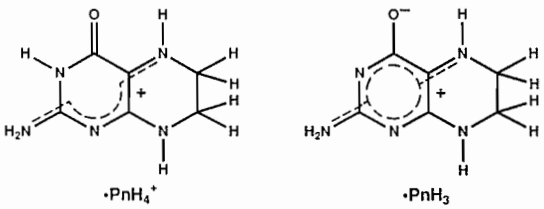

FIG. 3. Absorption spectra of radicals formed by $^{\bullet}N_3$ oxidation of tetrahydropterins. (*a*) 6-Carboxylated tetrahydropterin radical at pH 10 – solid line (filled and open circles were obtained at Notre Dame and Calgary, respectively). The dashed line is the spectrum of the parent compound. Inset: Differential spectra of radicals from: 6-carboxylated tetrahydropterin (solid line and circles) and tetrahydropterin (dashed line). (*b*) Radicals from 6,7-dimethyltetrahydropterin (solid line) and tetrahydropterin (dashed line) at pH 10. (*c*) As for (*b*) at pH 7. Inset: Comparison of the spectra of the radicals formed from $PnH_4$ at pH 10 (solid line) and pH 7 (dashed line).

## Spectra of the radicals

Spectra of the radicals, derived by adding the values of $\Delta A$ (divided by the concentration of radicals produced per pulse) to the absorbance coefficient of the $PnH_4$ derivative determined at each given wavelenth on a Cary 219 spectrophotometer at the same pH, are shown in Fig. 3*a*, *b*, and *c*. The data for the carboxylated derivative, prepared by $^{\bullet}CO_2^-$ reduction of Pn, has been presented in more detail in Fig. 3*a*. The spectrum of this

tetrahydropterin was typical of those of the tetrahydropterins studied here, which all exhibited peaks near 300 nm and stronger absorption below 240 nm. Further discussion of $Pn(CO_2^-)H_4$ will be given elsewhere,[4] but it may be noted here that no evidence for the previously reported (23) absorbance near 380 nm was seen. It appears that this may have been due to experimental artifacts.

The isobestic point at 318 nm in Fig. 3*a* corresponds with the cross over from gain in absorbance to loss in the differential spectrum in the inset of the same Figure. It is important to note that these $\Delta A$ vs. $\lambda$ plots are characteristic "signatures" of the parent compound and its daughter radical. When a comparison of the data points for the carboxylated tetrahydropterin (the solid line and points) with the corresponding $\Delta A$ vs. $\lambda$ plot for tetrahydropterin, shown by the dashed line, was made on an enhanced scale, the differences of up to 1000 $M^{-1}$ $cm^{-1}$ units at several $\lambda$ indicated that the radicals were different.[4] This is also shown to some extent by a comparison of the absolute spectra at pH 10 in Fig. 3*a* and *b*.




SCHEME 2. Two forms of "PnH" radical.

The structures of the two $^{\bullet}PnH_3$ species formed by removal of electrons from $PnH_4$ and $PnH_3^-$ in Scheme 1 are shown in Scheme 2. Molecular orbital calculations (9, 11) on these predicted high spin densities on N-5 in both $^{\bullet}PnH_4^+$ and $^{\bullet}PnH_3$, but it was clear that other canonical forms would also contribute significantly, especially in $^{\bullet}PnH_3$ where a relatively large spin density was found (9) on C-4a. For the following reasons the data of the present study also demonstrate that there is significant delocalization of the unpaired electron from N-5 in the $^{\bullet}PnH_3$ radicals. First, recent research (24) has shown that aminyl cation radicals with α-hydrogen are susceptible to deprotonation, viz:

[5] $\dot{N}\overset{+}{H}\text{—CH(R)—} \rightarrow H^+ + \text{—NH—}\dot{C}\text{(R)—}$

with p$K$ in the region of 8. Had this occurred here the spectrum seen would have been that of an α-carbon radical localized on C-6, and such a species does not absorb strongly above 300 nm (24, 25). Second, the 6-carboxylated tetrahydropterin would be susceptible to reaction [6], which has been shown to proceed on a microsecond or shorter time scale with α-carboxylated aminyl radicals (26). Again this would give a C-6 localized α-carbon radical and an absorption very different from that observed here. It is therefore evident that the radicals are stabilized by electron delocalization to the extent that these processes do not occur or take place on a 0.1 s or longer time scale. This delocalization is clearly reflected in the wavelength at which the absorption occurs and its intensity.

[6[ $\dot{N}\overset{+}{H}\text{—CH}(CO_2^-)\text{—} \rightarrow CO_2 + \text{—NH—}\dot{C}\text{H—}$

The exact structures to be associated with the spectra in Fig. 3 remain to be determined. However, the p$K$ of the amide group in the parent molecules is ~ 10.4 (18). In the radical the p$K$ of this group should be the same or less. Since further experiments at pH 11.5 showed no significant change in absorbance from that of the radical form at pH 10, it appears that loss of the amide proton has occurred at that pH. At pH 7 there are some small differences in the absorption spectra, which suggest protonation may take place in that region. These are illustrated for the radicals of unsubstituted $PnH_4$ in the inset of Fig. 3*c*.

Finally, although this appears to be the first report of a UV/visible spectrum of $^{\bullet}PnH_3$ type radicals, the "tailing" absorbances in Fig. 3*a*, *b* and *c* at wavelengths above 400 nm are consistent with earlier reports of coloration of oxidized solutions of $PnH_4$ and its derivatives (9–11). The results reported here should stimulate further research on $^{\bullet}PnH_3$ radicals and assist in quantifying data relating to the kinetics of their reactions.

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