Reactions of $CO_2^{\bullet -}$ Radicals with Pterin and Pterin-6-carboxylate Ions

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Pulse radiolysis experiments showed that the reaction of pterin-6-carboxylate (Pn6CB) with $CO_2^{\bullet -}$ at pH 7 produced a monohydropterin radical similar to that observed for unsubstituted pterin (Pn), with the rate of the second order reaction for Pn6CB (k = 1.7 × 10^8 dm^3 mol^{-1} s^{-1}) being a factor of 2.5 slower than for Pn. At pH 10, where the 3,4-amide groups of the pterins were deprotonated, reactions of $CO_2^{\bullet -}$ with both pterins were too slow to be observed by this method. However, the fact that reactions occur on a slower time scale was established by the observation of products in gamma radiolysis experiments. Formation of these was followed by spectrophotometry and by measurement of CO_2 yields. Over the entire pH range 6–10, titrations of Pn6CB with $CO_2^{\bullet -}$ led to a two-electron-equivalent reduction with no incorporation of CO_2. Also the dihydro product was readily oxidised by air, a feature in which it resembled 5,8-dihydropterins reported elsewhere. With Pn at pH 7 there was again a two electron reduction with no incorporation of CO_2, but the product spectrum was completely different from that observed with Pn6CB. Furthermore, at pH 10 the stoichiometry of the titration indicated a four-electron-equivalent reduction, and a deficit in the CO_2 yields demonstrated the incorporation of at least one CO_2 per molecule. The spectrum and redox chemistry of this product strongly resembled those of tetrahydropterins. These effects of pH and the differences between Pn and Pn6CB were discussed and shown to be in accord with the known properties of di- and tetra-hydropterins.

The $CO_2^{\bullet -}$ radical anion is a relatively strong reducing agent [$E^\circ(CO_2/CO_2^{\bullet -}) = -1.9$ V][1,2] and has proven to be very useful in studies of reductions and electron transfer reactions of organic and biochemical molecules.[3–6] In the case of the pterin molecule (Pn), which is structurally related to the flavin molecule and whose derivatives also serve as cofactors in redox enzymes,[7] Moorthy and Hayon[8] demonstrated a one electron reduction to the pterin free radical PnH$^\bullet$ at pH 7, but they did not examine the final stable products. In the present study reductions of pterins to the two- and four-electron-equivalent levels were investigated with $CO_2^{\bullet -}$ radicals produced in dilute formate solutions by irradiation with a Co^{60} gamma ray source. In these solutions a series of well known reactions [eqns. (1)–(4)][9,10] take place to produce a yield of 6.0 ± 0.2 µmol of $CO_2^{\bullet -}$ per decajoule of radiation energy absorbed. [The coefficients in eqn. (1) are G values, *i.e.* µmol of product per decajoule of radiation energy absorbed.]

$$4.36\,H_2O \dashrightarrow{N} 2.90\,e_{(aq)}^- + 2.90\,H_{(aq)}^+ + 0.52\,H^\bullet + 0.47\,H_2 + 0.73\,H_2O_2 + 2.90\,OH^\bullet \quad (1)$$

$$N_2O + H_2O + e_{(aq)}^- \longrightarrow N_2 + OH^- + OH^\bullet \quad (2)$$

$$OH^\bullet + HCO_2^- \longrightarrow H_2O + CO_2^{\bullet -} \quad (3)$$

$$H^\bullet + HCO_{2-} \longrightarrow H_2 + CO_2^{\bullet -} \quad (4)$$

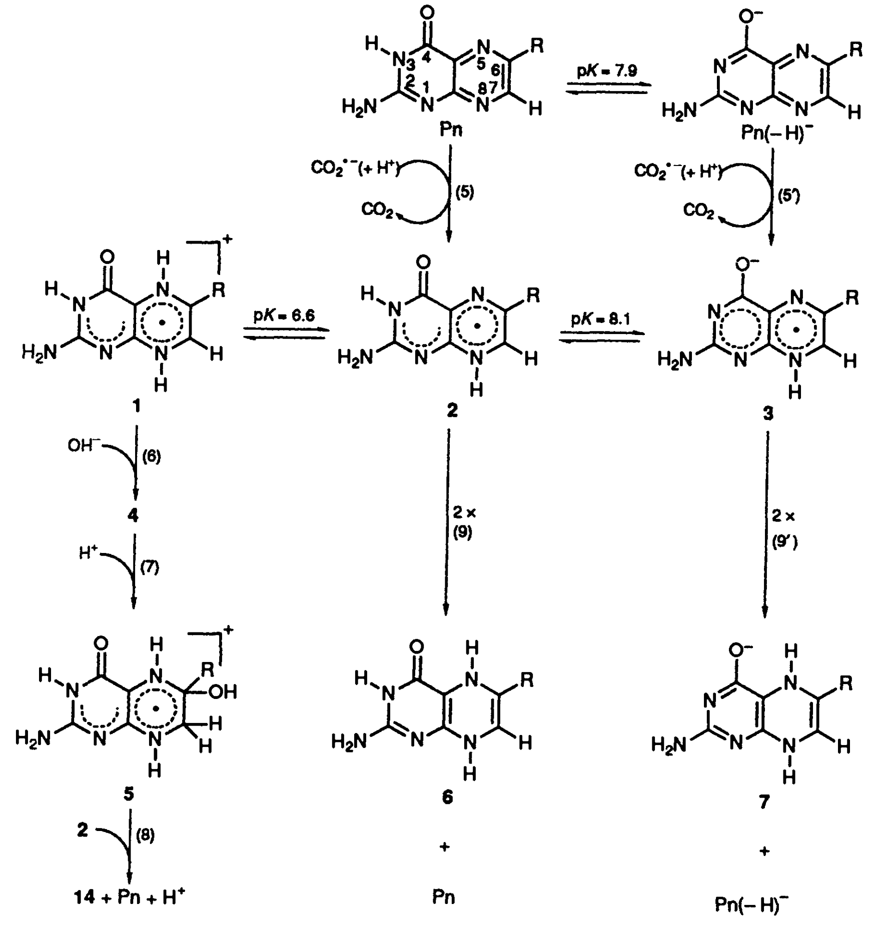
Experiments were conducted with both Pn and pterin-6-carboxylate (Pn6CB, see structures in Scheme 1), because it was known from electrochemical studies[11] that the presence of substituents at position 6 of the pyrazine ring reduced the number of products formed and simplified the chemistry. Pulse radiolysis experiments[9,10] were conducted to confirm that the PnH6CB$^\bullet$ radical, the counterpart of PnH$^\bullet$, was formed from Pn6CB. The investigation has produced new information relating to the reactivity of $CO_2^{\bullet -}$ radicals and the decay reactions of the pterin radical intermediates.

Experimental

Gamma irradiations were performed at the University of Calgary or the Hahn-Meitner Institute in standard gamma irradiation sources with dose rates of 8–15 Gy min^{-1} as determined with the Fricke dosimeter.[9,10] Pulse radiolysis experiments were conducted with the 1.5 MeV van de Graaff accelerator at the University of Calgary or the 8 MeV linear accelerator in the United States Department of Energy Laboratory at the University of Notre Dame. Details relating to those machines and the dosimetry procedures have already been given.[12,13] The formation of reduced pterin was followed by spectrophotometry, and yields of CO_2 were measured to determine whether $CO_2^{\bullet -}$ reacted purely by electron transfer or also by formation of stable adducts. Absorption spectra were measured on a Cary 219 Spectrophotometer. Carbon dioxide was determined as bicarbonate ion and quantified using a Dionex 2010 ion chromatograph equipped with a HPICE-AS1 column. The eluent was deionised water at pH 6.8–7.2 with a flow rate of 2 cm^3 min^{-1}. After irradiation solutions were brought to pH 12 by addition of CO_2-free NaOH solution (0.1 mol dm^{-3}) and injected into the chromatograph. Other details of the ion chromatography procedures have been presented elsewhere.[14]

All experiments were conducted at room temperature (23 ± 2 °C) in solutions containing 1–20 mmol dm^{-3} phosphate buffer, 1–20 mmol dm^{-3} formate ion and the desired concentration of pterin – usually 20–100 µmol dm^{-3}. All chemicals were the purest available and the solutions were made up in triply distilled or chromatographically-purified deionised water. Before irradiation they were adjusted to the desired pH by the addition of small amounts of sodium hydroxide or perchloric acid, carefully deaerated and saturated with oxygen-free nitrous oxide.

Pterin and pterin-6-carboxylic acid were obtained from Fluka or Sigma, and gave identical results. 5,6,7,8-tetrahydropterin (PnH_4) was obtained as a sulphate salt from K and K laboratories. This was made up in air-free solution at pH 7 or



Scheme 1 Pterin radical formulae of Moorthy and Hayon and decay reactions (R = H for pterin and R = CO_2^- for pterin-6-carboxylate)

10 and oxidised with stoichiometric amounts of potassium ferricyanide[15] to produce 7,8-dihydropterin (7,8-PnH_2). 7,8-Dihydropterin-5-carboxylate (7,8-$PnH_2$6CB) was obtained by a similar oxidation from the tetrahydroderivative prepared by reducing Pn at pH 10 with $CO_2^{\bullet -}$ (see Results and Discussion).

Results and Discussion

(i) *Confirmation of Radical Products by Pulse Radiolysis*.—In their research Moorthy and Hayon studied the reactions of both $CO_2^{\bullet -}$ and $e_{(aq)}^-$ with Pn, and determined the p*K* values of the pterin radicals from the pH dependences of the absorption intensities at selected wavelengths.[8] They showed that at pH 7 the primary anion radical formed by addition of $e_{(aq)}^-$ to Pn was protonated very rapidly, for example the second-order rate constant for reaction with $H_2PO_4^-$ was 3×10^8 dm^3 mol^{-1} s^{-1}. Based on that result and information from EPR experiments, they attributed the spectrum seen after 10 µs at pH 7 primarily to the 8-monohydropterin radical, PnH$^\bullet$ (**2** in Scheme 1). At that pH the same species was produced by both $e_{(aq)}^-$ and $CO_2^{\bullet -}$. The spectrum in Fig. 1(*a*) is in good agreement with their spectrum (λ_{max} 330–340 and 450–460 nm). The spectrum of the radicals formed from Pn6CB [Fig. 1(*b*)] was very similar in form to that produced from Pn, and it can therefore be taken to have the same structure. As with the parent molecule, the absorption peaks were slightly red shifted, and had slightly higher absorbance coefficients than those of the unsubstituted pterin counterpart (*e.g.* ε = 7750 dm^3 mol^{-1} cm^{-1} at 345 nm *vs.* 5530 dm^3 mol^{-1} cm^{-1} at 340 nm respectively for Pn6CB and Pn).

Since protonation of the initial intermediate was very rapid, the overall reaction in buffered solution can be written as eqn. (5). From the linear dependence of the pseudo-first-order rate

$$Pn + CO_2^{\bullet -}(+H^+) \longrightarrow PnH^\bullet + CO_2 \qquad (5)$$

constants observed in the build up of the PnH$^\bullet$ absorbance on Pn concentration the second-order rate constant for reaction (5) was found to be $4.5 \pm 0.3 \times 10^8$ dm^3 mol^{-1}, s^{-1}, in excellent agreement with the value of 4.6×10^8 dm^3 mol^{-1} s^{-1} reported by Moorthy and Hayon.[8] The value for the corresponding reaction with Pn6CB was $1.7 \pm 0.2 \times 10^8$ dm^3 mol^{-1} s^{-1}, two and a half times slower. That factor demonstrated that there was a significant steric and/or coulombic hindrance to the reaction due to the presence of the carboxylate group at position 6 in the pyrazine ring.

Moorthy and Hayon reported second-order decays of their PnH$^\bullet$ radicals with $2k = 1.8 \times 10^8$ dm^3 mol^{-1} s^{-1} at pH 7.2. However, the doses per pulse which they used were relatively large, 10–100 fold higher than those employed here. This might have caused second-order processes to dominate and mask first-order processes. Since such processes are of importance in the interpretation of low dose rate gamma radiolysis experiments, the decay of the PnH$^\bullet$ radicals was therefore examined with our pulse sizes of typically 2 Gy. As shown in the inset of Fig. 1(*a*), at pH 7 there was indeed a first order process with a $t_{\frac{1}{2}}$ of 0.9 ms. Further reference to the decay of PnH$^\bullet$ at pH 7 will be made in the section dealing with the gamma radiolysis experiments. Unfortunately it turned out that Pn6CB was very sensitive to light, and absorbance signals became unstable, if exposure to the analysing light beam was prolonged beyond the 100 µs time scale. Regretably this precluded studies of the decay of the radicals formed from it.

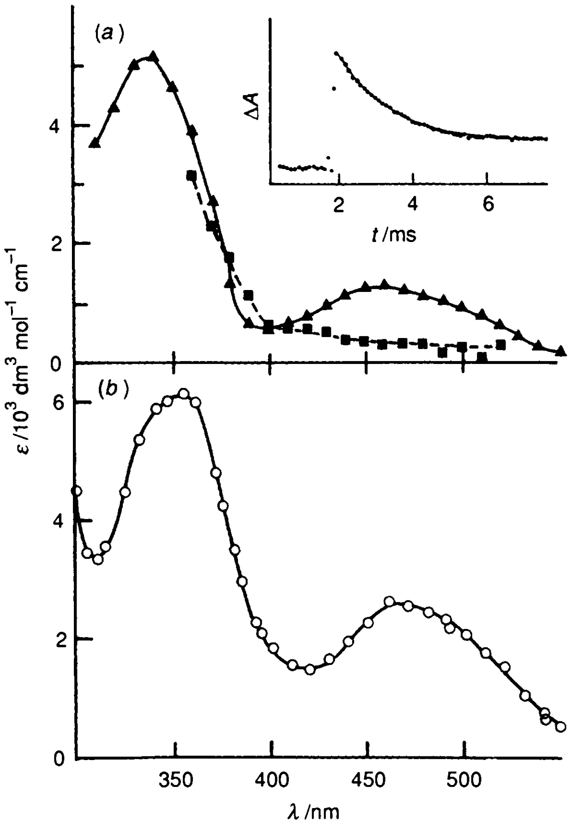


Fig. 1 Spectra of radicals produced by reaction of $CO_2^{\bullet -}$ with 50 to 100 μmol dm^{-3} pterins in 10 mmol dm^{-3} phosphate buffer and 20 mmol dm^{-3} formate at pH 7. (*a*) Pn: 200 μs (Δ) and 40 ms (■) after pulse. Inset: Example of decay at 472 nm with fitted first-order kinetic plot. (*b*) Pn6CB: 300 μs (○) after pulse.

Attempts were made to follow the reaction of $CO_2^{\bullet -}$ with Pn at pH 10. However, the change in absorbance with time was on the time scale of second-order radical decay, and spectral changes were indistinct. These observations agreed with Moorthy and Hayon's report[8] that they could see no evidence of a reaction at this pH. While no rate constant or absorbance spectrum of the product can be reported for pH 10 from the pulse radiolysis experiments, it was clear from the gamma radiolysis results (see below) that a reaction did occur on a slower time scale. Thus in Scheme 1 a reaction similar to (5) has been included for that pH. The p*K* values of the radicals and parent molecules were taken from refs. 8 and 16 respectively.

(ii) *Long-lived Products: Gamma Radiolysis Experiments.—Experiments in the absence of pterins.* Carbonate and oxalate yields were determined over the dose range 0–250 Gy in 1–3 mmol dm^{-3} formate solutions at pH 7 and 10. Concentration *vs.* dose plots were linear and gave *G* values of 2.2 ± 0.2 and 2.3 ± 0.2 μmole per decajoule, for CO_2 and oxalate respectively. These products were attributed to the occurrence of reactions (10)–(12). Reactions (12) and (3) have been shown to constitute

$$CO_2^{\bullet -} + CO_2^{\bullet -} \longrightarrow C_2O_4^{2-} \qquad (10)$$

$$CO_2^{\bullet -} + CO_2^{\bullet -}(+H^+) \longrightarrow CO_2 + HCO_2^- \qquad (11)$$

$$CO_2^{\bullet -} + N_2O(+H^+) \longrightarrow CO_2 + N_2 + OH^\bullet \qquad (12)$$

a chain which, in the absence of substrates reactive to $CO_2^{\bullet -}$, leads to the decomposition of formate.[17] The present oxalate yields indicated that reaction (10) accounted for 77% [= 2.3/(6.0 × 1/2)] of the mutual reactions of $CO_2^{\bullet -}$ at the pHs used here. The remainder of the total yield of 6.0 μmoles per decajoule of $CO_2^{\bullet -}$ must have undergone termination by disproportionation. That would have produced a yield of 0.7 [= (6.0/2) − 2.3] μmoles per decajoule of CO_2. The remaining 1.5 μmoles of CO_2 (= 2.2 − 0.7) has to be attributed to the chain reaction.

For the purpose of the present study it was evident that reactions (10)–(12) would have been eliminated when $CO_2^{\bullet -}$ reacted quantitatively with pterin. In particular the oxalate yield from reaction (10) would have been suppressed to zero, whether the reaction was by electron transfer or addition. For pure electron transfer, as in reaction (5), the CO_2 yield would have been increased to 6.0 μmoles per decajoule. On the other hand, if addition occurred, the CO_2 could have been suppressed or increased, depending on the proportions of electron transfer and addition. Thus, in cases where $G(CO_2)$ was less than 6.0, measurements of the yields of both CO_2 and oxalate were required.

Experiments with Pn6CB. Carbonate concentrations were determined in irradiated solutions at pH 7 and 10 for Pn6CB concentrations of 50 and 100 μmol dm^{-3}. For these conditions they increased linearly with dose and the slope yielded $G(CO_2) = 6.4 \pm 0.3$ μmoles per decajoule. The correspondence with $G(CO_2^{\bullet -})$ indicated that with this pterin under the above conditions only electron transfer occurred.

Spectral changes which occurred during a typical titration of Pn6CB with $CO_2^{\bullet -}$ radicals have been illustrated in Fig. 2. The initial spectrum of Pn6CB was suppressed by each successive addition of $CO_2^{\bullet -}$ radicals, *i.e.* each successive period of irradiation, until finally changes ceased. There were several isosbestic points, which showed that only one product was stable on the time scale of these measurements, typically 10 min per spectral point. Plots of Δ*A*, the change in absorbance at a specific wavelength, against $[CO_2^{\bullet -}]/[Pn6CB]_0$, have been given in the inset. Here $[CO_2^{\bullet -}]$ represents the total concentration of $CO_2^{\bullet -}$ generated in the solution, and $[Pn6CB]_0$ is the initial concentration of Pn6CB. Since the plots all exhibited sharp breaks at $[CO_2^{\bullet -}]/[Pn6CB]_0 = 2.0–2.2$, it was evident that each mole of Pn6CB reacted with two moles of $CO_2^{\bullet -}$. Thus it was apparent from these results that $CO_2^{\bullet -}$ reduced Pn6CB to a dihydropterin.

Several dihydroderivatives of pterin are known. However, electrochemical investigations of the reduction of Pn and its folic acid and biopterin derivatives[11,18] have established 5,8-dihydropterin (5,8-PnH_2 or **6** in Scheme 1) as the first non-radical product. Also, as shown in Scheme 1, this was a logical product of disproportionation of $PnH^\bullet$ radicals. Therefore the final spectrum of the stable product in Fig. 2 is probably that of 5,8-dihydropterin-6-carboxylate (5,8-$PnH_2$6CB). That spectrum has been reproduced in Fig. 3(*a*), along with the spectrum of the 7,8-dihydroderivatives, prepared as described in the Experimental section. Comparison demonstrated the existence of different spectral properties for these two types of dihydro compound.

It was also found that 7,8-dihydropterins were not oxidised rapidly by oxygen under the present conditions at pH 7, while admission of air to the solution containing the product of reduction by $CO_2^{\bullet -}$ reoxidised it to the original Pn6CB in 1–2 min with a yield of >90%. That point has been illustrated by the dashed line in Fig. 2, and it is in keeping with observations on other 5,8-dihydropterin derivatives.[11]

Reductive titrations of Pn6CB were carried out over the pH range 6–10 with results similar to those displayed in Fig. 2, *i.e.* two electron reduction and quantitative reoxidation by air. At pH 12 the spectrum of the product changed, with a separate peak appearing at 275 nm and the peak at 254 nm being of lesser intensity. Since the 3,4-amide group should have a p*K* above

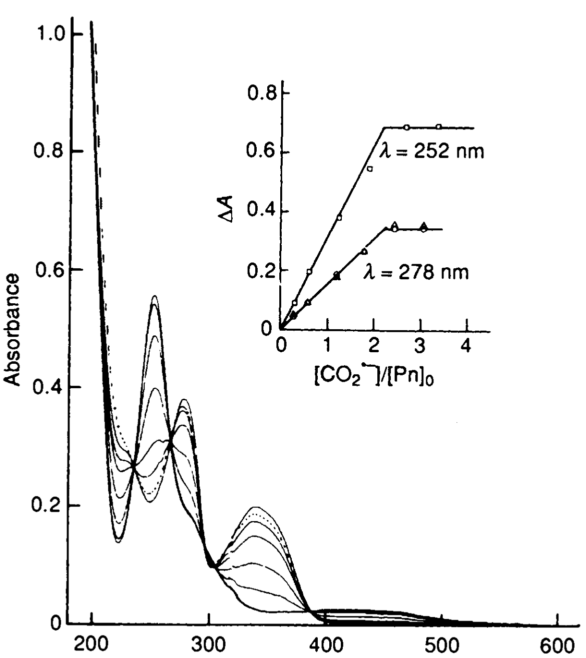


Fig. 2 Spectrophotometric titration of 50 μmol dm^{-3} Pn6CB with $CO_2^{\bullet -}$ at pH 7.2. Solid lines are spectra taken before, and after 24, 48, 95, 142, 189 and 236 Gy of irradiation; dashed line after reoxidation with air. Inset: Δ A plotted against the number of moles of $CO_2^{\bullet -}$ reacted, per mole of Pn6CB initially present.

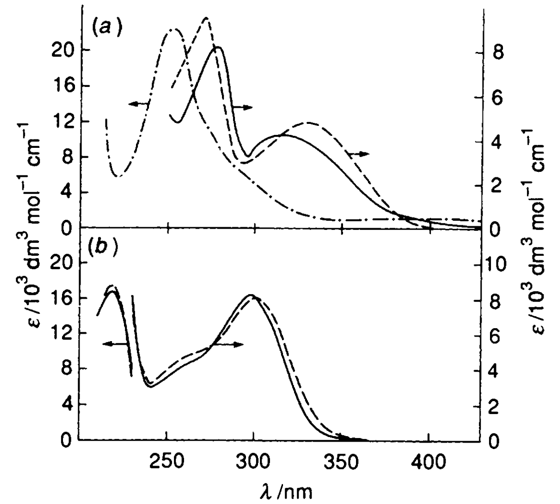


Fig. 3 Absorption spectra of reduced pterins in 10 mmol dm^{-3} phosphate buffer. (*a*) Dihydro derivatives at pH 7: (——) 7,8-PnH_2 produced by stoichiometric oxidation of PnH_4; (— — —) 7,8-dihydropterin produced by stoichiometric oxation of carboxylated tetrahydropterin (see text); (— · — · —) 5,8-dihydro derivative of Pn6CB produced by reduction with $CO_2^{\bullet -}$. (*b*) Tetrahydro derivatives at pH 10: (——) 5,6,7,8-tetrahydropterin; (— — —) carboxylated tetrahydropterin produced by reaction of Pn with $CO_2^{\bullet -}$ at pH 10 (see text).

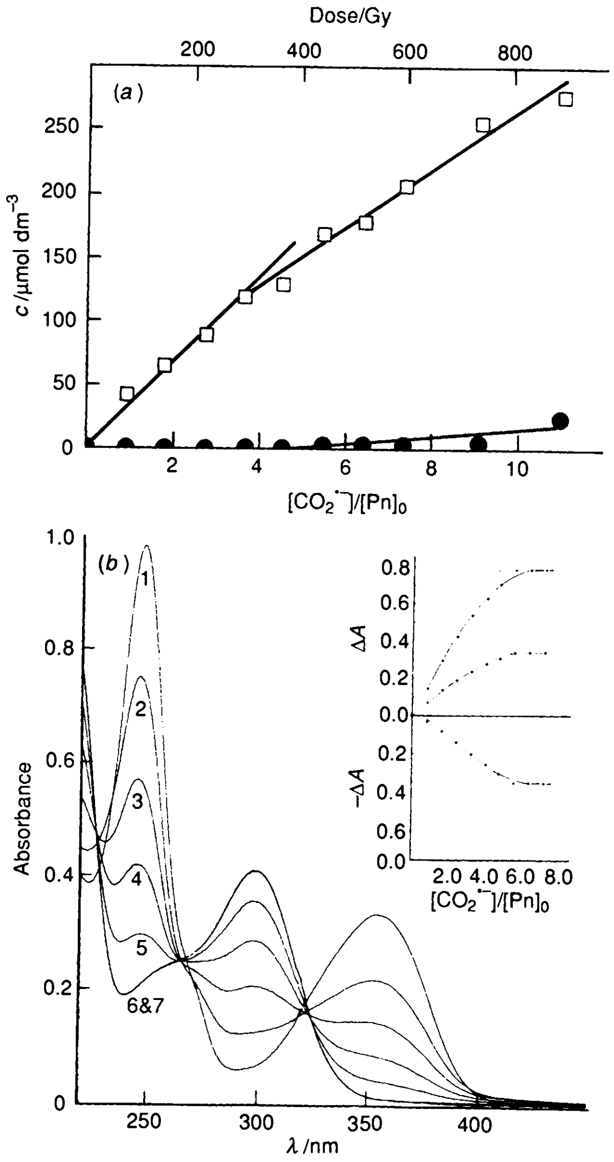
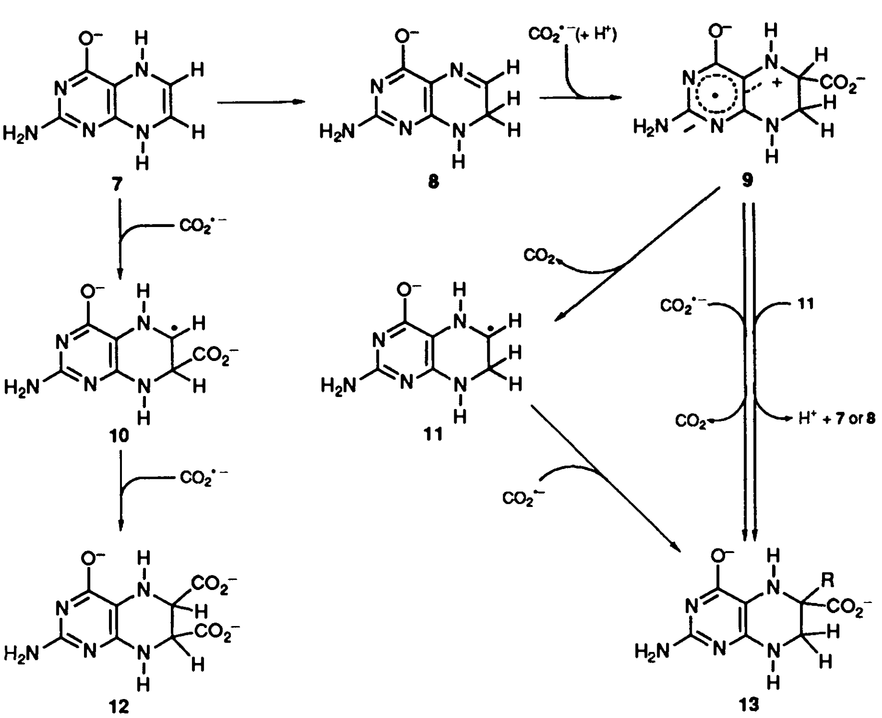


Fig. 4 Titration of 50 μmol dm^{-3} pterin with $CO_2^{\bullet -}$ at pH 10: (*a*) Oxalate (●) and CO_2 (□) concentrations *vs.* dose. (*b*) Spectra before (1), and after 120(2), 243(3), 365(4), 486(5), 608(6) and 669(7) Gy of irradiation. Inset: Δ A plotted against the number of moles of $CO_2^{\bullet -}$ reacted, per mole of pterin initially present, for (from top to bottom) 252, 359 and 302 nm respectively.

10,[16] this observation may have been due to ionisation of that group. At pH 5 the product was no longer reoxidised quantitatively by oxygen, a feature which resembled behaviour seen earlier in $CO_2^{\bullet -}$ reactions with flavins at low pH.[5]

Experiments with Pn. (*a*) pH 10: As shown in Fig. 4(*a*), for the titration of 50 μmol dm^{-3} Pn with $CO_2^{\bullet -}$ at pH 10 trace concentrations of oxalate were observed beyond $[CO_2^{\bullet -}]/[Pn]_0 = 4.0$, but up to that point the formation of that product was completely suppressed. This feature was a clear demonstration that $CO_2^{\bullet -}$ was reacting quantitatively with the Pn. At the same time, $G(CO_2)$, determined from the slope of the plot in the region between $[CO_2^{\bullet -}]/[Pn] = 0$ and 4 in Fig. 4(*a*) was 4.2 ± 0.2 μmole per decajoule. This indicated that *ca.* 70% (= 4.2/6.0) of the $CO_2^{\bullet -}$ radicals were reacting by electron transfer, while the remaining 30% must have undergone addition to the pterin. The changes in absorbance during a reductive titration with the same pterin concentration have been presented in Fig. 4(*b*). The ΔA *vs.* $[CO_2^{\bullet -}]/[Pn]_0$ plots in the inset all indicated cessation of the reduction near the four $CO_2^{\bullet -}$ equivalent point. The plot for 302 nm, the wavelength of maximum absorption of the product, was displaced by *ca.* one equivalent, which suggested that traces of an intermediate dihydropterin were present during the course of the titration. However, spectral changes ceased at $[CO_2^{\bullet -}]/[Pn]_0 = 5.0$. The spectrum at that point has been reproduced in Fig. 3(*b*), where it can be seen to resemble very closely the spectrum of 5,6,7,8-tetrahydropterin. Therefore the results strongly implied that at pH 10 $CO_2^{\bullet -}$ radicals reduced pterin to the tetrahydro level. The $G(CO_2)$ values, however, demonstrated that the products contained at least one carboxylate group per molecule,



Scheme 2 Mechanisms of formation of carboxylated tetrahydropterins at pH 10

which must result from the irreversible addition of $CO_2^{\bullet -}$ to the pyrazine ring. These products have been referred to below as PnH_4CB.

An interesting feature of Fig. 4(*a*) was that, after the four electron equivalent point, *G*(oxalate) increased but remained rather small (0.3 μmole per decajoule), which demonstrated that $CO_2^{\bullet -}$ radicals continued to react with the pterin molecules. At the same time $G(CO_2)$ became equal to 2.8 ± 0.3 μmole per decajoule. These observations could be explained if the tetrahydroderivative PnH_46CB catalysed the disproportionation of $CO_2^{\bullet -}$, as in reactions (13) and (14).

$$CO_2^{\bullet -} + PnH_46CB \longrightarrow HCO_2^{\bullet -} + PnH_36CB^{\bullet} \quad (13)$$

$$CO_2^{\bullet -} + PnH_36CB^{\bullet}(+H^+) \longrightarrow CO_2 + PnH_46CB \quad (14)$$

Earlier investigations of the reduction of pterins, made with conventional chemical reagents[19] or electrochemical techniques,[11] showed that, in the absence of substituents at positions 6 and/or 7 of the pyrazine ring, 5,8-PnH_2 was unstable and rearranged spontaneously to 7,8-dihydropterin (7,8-PnH_2). This rearrangement has been shown in Scheme 2 for the deprotonated forms of 5,8-PnH_2 (7) and 7,8-PnH_2 (**8**). The remainder of Scheme 2 shows mechanisms for the reduction of 7 or **8** to the four-electron-equivalent level at pH 10. Addition of $CO_2^{\bullet -}$ to the only remaining double bond in the pyrazine ring of **8** to form radical **9** is a key reaction. Since radical **9** contains a partially positive nitrogen at position 5 with a carboxylate on the alpha carbon at position 6, one might expect it to form **11** by elimination of CO_2, as has been shown to occur in the case of alphatic radicals of that type.[14] However, due to the resonance stabilisation in radical **9**, this process appears to be relatively slow.[20] That would make it possible for both **9** and **11** to accumulate, and permit a reaction between them to occur as shown. An alternative mechanism in the left hand column of Scheme 2 involves successive additions of $CO_2^{\bullet -}$ to 5,8-PnH_2, and produces a dicarboxylated product. That would explain the fact that the $G(CO_2)$ values implied that slightly more than one in four of the $CO_2^{\bullet -}$ radicals underwent addition, and therefore that some tetrahydro-derivatives could have more than one carboxylate group per molecule. However, the dominant feature was apparently addition of one $CO_2^{\bullet -}$.

Stoichiometric oxidation of the PnH_4CB products with two moles of ferricyanide gave rise to 7,8-dihydroderivatives with the spectrum shown by the dashed line in Fig. 3(*a*). Comparison with the spectrum of unsubstituted 7,8-PnH_4 shows that, as with the parent Pn and Pn6CB pterins and the $PnH^{\bullet}$ radicals, the absorption spectra of the carboxylated derivatives were significantly different.

The foregoing observations have demonstrated a marked difference in the reactivity of Pn and Pn6CB with $CO_2^{\bullet -}$ at pH 10. As stated earlier, the substituted 5,8-dihydro species is less likely to rearrange to the 7,8-form. Thus it appears that 5,8-PnH_26CB, the probable two-electron-equivalent reduction product for Pn6CB, was much more inert to reaction with $CO_2^{\bullet -}$ than 7,8-PnH_2. However, it may be noted that for Pn6CB a slow very inefficient reduction beyond the two-electron-equivalent point could be forced at pH 10 with a 10–20 fold excess of $CO_2^{\bullet -}$. This also produced a product with absorbance bands typical of a tetrahydropterin, *i.e.* absorbance maxima of 300 nm (ε = 7100 dm^3 mol^{-1} cm^{-1}) and 221 nm (ε = 15 050 dm^3 mol^{-1} cm^{-1}).

(*b*) pH 7: In addition to the rapid rearrangement of the 5,8-dihydroderivative, a further complication with unsubstituted pterins is that at low pH the 7,8-PnH_2 derivative is susceptible to the addition of water,[11,18] which results in the formation of 6-hydroxy-5,6,7,8-tetrahydropterin [(6OH)PnH_4], [reaction (15)]. Thus one may expect that the reductive titration of Pn

$$7,8\text{-}PnH_2 + HO_2 \rightleftharpoons \underset{\mathbf{14}}{(6OH)PnH_4} \quad (15)$$

with $CO_2^{\bullet -}$ at this pH may yield products which are different from those at pH 10. Reaction of $CO_2^{\bullet -}$ with Pn was again confirmed by the fact that oxalate production was completely suppressed up to $[CO_2^{\bullet -}]/[Pn]_0$ = 4.0 [see Fig. 5(*a*)]. The initial slope of the curved CO_2 concentration *vs.* dose plot in Fig. 5(*a*) corresponded to $G(CO_2^{\bullet -})$ = 6.0, which showed that every $CO_2^{\bullet -}$ underwent electron transfer over the initial region from $[CO_2^{\bullet -}]/[Pn]_0$ = 0 to 2.0. Also, as shown in Fig. 5(*b*) and inset,

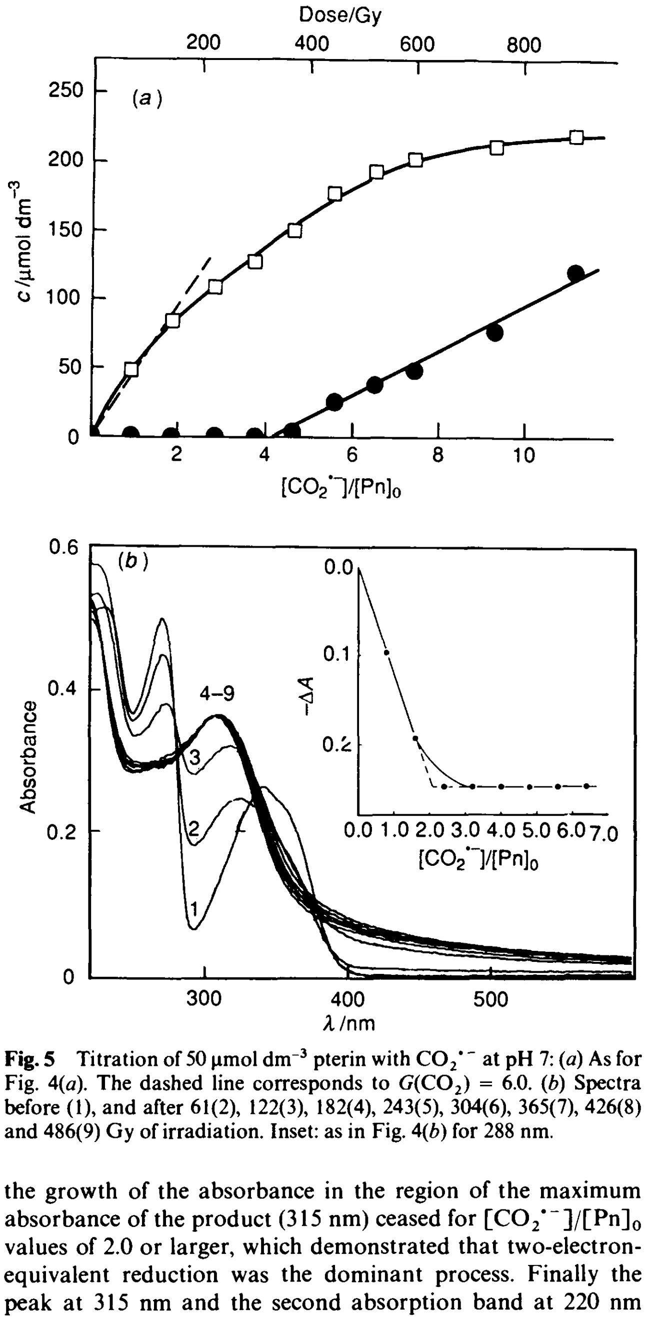


Fig. 5 Titration of 50 μmol dm^{-3} pterin with $CO_2^{\bullet -}$ at pH 7: (*a*) As for Fig. 4(*a*). The dashed line corresponds to $G(CO_2)$ = 6.0. (*b*) Spectra before (1), and after 61(2), 122(3), 182(4), 243(5), 304(6), 365(7), 426(8) and 486(9) Gy of irradiation. Inset: as in Fig. 4(*b*) for 288 nm.

the growth of the absorbance in the region of the maximum absorbance of the product (315 nm) ceased for $[CO_2^{\bullet -}]/[Pn]_0$ values of 2.0 or larger, which demonstrated that two-electron-equivalent reduction was the dominant process. Finally the peak at 315 nm and the second absorption band at 220 nm exhibited by the major product are characteristic of a tetrahydropterin [see Fig. 3(*b*)]. This is what would be expected for $(6OH)PnH_4$. Also air oxidation of that product produced Pn. This is in contrast to PnH_4 which forms 7,8-PnH_2.

Above $[CO_2^{\bullet -}]/[Pn]_0$ = 4.0 $G(CO_2)$ and *G*(oxalate) approached the values observed in the absence of pterin, which indicated that at that point the products were inert to $CO_2^{\bullet -}$. However, there were still reactions in the region between the ratios of 2.0 and 4.0, because $G(CO_2)$ in that region was about 3.0 and *G*(oxalate) remained zero. In addition small changes in the absorbance spectra continued [see Fig. 5(*b*)]. Thus it appears that the product tentatively identified as $(6OH)PnH_4$ reacted with *ca.* two equivalents of $CO_2^{\bullet -}$, possibly undergoing hydrogen abstraction as proposed above for PnH_46CB in reaction (13), followed by $CO_2^{\bullet -}$ additions or other reactions of the resulting radicals. The nature of these reactions was not pursued. However, one may note that $CO_2^{\bullet -}$ does not react readily with pyrimidine ring systems. That point has been demonstrated previously by experimentally determined rate constants.[21] It was demonstrated more forcefully above for the pyrimidine rings of pterin systems by the fact that the two-electron-reduction product of Pn6CB did not react readily with $CO_2^{\bullet -}$. Therefore in $(6OH)PnH_4$ the pyrazine ring would be the likely site of attack.

There are a number of mechanisms by which $(6OH)PnH_4$ can be formed. An obvious one is *via* the disproportionation of the $PnH^{\bullet}$ radicals to 5,8-dihydropterin, followed by its rearrangement to the 7,8-form, followed by addition of water. However, Dryhurst and co-workers[18] have pointed out that at pH 7 equilibrium (15) lies toward the left, and there is very little $(6OH)PnH_4$ present. That was confirmed here with 7,8-PnH_2, prepared as described in the Experimental section. Thus the present results favoured a mechanism in which water was added to an intermediate before the 7,8-PnH_2 species was formed, and equilibrium (15) was not established.

The mechanism in Scheme 1 involves the addition of OH^- to the radical **1** as a rate-controlling step, and assumes that an equilibrium exists between radicals **1** and **2**. Based on the p*K* of 6.6, it can be seen that the concentration of radical **1** at pH 10 would have been negligible, which would explain why hydration was not important there. However, at pH 7 radicals **1** and **2** would constitute 28 and 72% of the total radicals respectively. The OH^- addition is followed by a rapid protonation and a reaction between radicals of type **5** and type **2**. The complex rate expression for the decay of $PnH^{\bullet}$ derived on the basis of the steady state approximation is then given by eqn. (16).

$$\begin{aligned} -d[PnH^{\bullet}]/[dt &= K_a^1 \times k_6 \times [H^+][OH^-][PnH^{\bullet}] \\ &= k_{obs}[PnH^{\bullet}] \quad (16) \end{aligned}$$

This predicted that the decay would be first order in radical concentration as required by the present observations [section (i) of Results and Discussion]. Experimentally the rate was also found to be independent of pH over the range 6.2–7.0, for which the ratio [**2**]/[**1**] would have changed from 0.4 to 2.5. That observation was also in agreement with the above expression, since $[H^+][OH^-] = K_w$. From the experimental values of k_{obs} (700 s^{-1}), K_a^1 and K_w,k_6 was found to be 1.7 × 10^{10} dm^3 mol^{-1} s^{-1}. This large value is in keeping with the strong susceptibility of position 6 to addition of nucleophiles.[22]

Other evidence in favour of the above mechanism was that the mutual reaction of the type **5** and type **2** radicals would directly form the $(6OH)PnH_4$ product with the spectrum observed at the two-electron-equivalent point in Fig. 5(*b*). This would explain the intensity of the absorbance observed above 350 nm after the first order decay of the radicals in the pulse radiolysis experiments at 40 ms [see Fig. 1(*a*)]. Based on the known absorbance coefficients, that absorbance cannot, however, be explained by a decay of radicals **1** and/or **2** to Pn + 5,8-PnH_2 or 7,8-PnH_2.

Conclusions

The present study has demonstrated that reactions of $CO_2^{\bullet -}$ with pterins occur over the pH range 6–12, even though at pHs above the p*K* of the 3,4-amide group they were too slow to be observed by pulse radiolysis. The presence of the 6-carboxylate group of Pn6CB had a profound effect on the products observed after the decay of the $PnH^{\bullet}$ radicals, and this was probably due to its stabilisation of the 5,8-dihydro form against rearrangement to the 7,8-dihydro form. When the above rearrangement could occur, as with Pn at pH 10, reactions with $CO_2^{\bullet -}$ continued beyond $[CO_2^{\bullet -}]/[Pn]_0$ = 2.0 to yield stable products with spectral characteristics of tetrahydropterin derivatives. A very important result was the deficit in the CO_2 yields, which demonstrated that at least one $CO_2^{\bullet -}$ per molecule was incorporated in the products formed at pH 10. That finding was consistent with results from an investigation of the spectra of radicals produced in the one-electron oxidation of those products and unsubstituted 5,6,7,8-tetrahydropterin.[20]

This investigation has added to the growing body of evidence that, as well as undergoing electron transfer reactions, $CO_2^{\bullet -}$ radicals can form adducts with heterocyclic systems and other unsaturated species.[23,24] Clearly the applications of $CO_2^{\bullet -}$ as a reducing agent and as an agent for initiating electron transfer reactions in biological or inorganic systems depend on knowing when addition occurs, as opposed to electron transfer. The utility of CO_2 yield determinations in demonstrating addition is obvious. Similar studies of flavins have already been made, and electron transfer was observed to be the major process. Details of the mechanisms of the two types of reaction and what molecular structures lead preferentially to addition remain to be determined.

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