In summary, hydroxylating agents or products derived from addition of O_2 at any position other than the 4a-carbon are excluded.

Within the instrumental resolution (0.02 ppm) a single ^{13}C resonance is observed for **2**. Since hydroxylation at C4a generates a chiral center and racemic **1** (at C6) is employed, the absence of a second resonance is due to either magnetic equivalence at C4a in the two diastereomers or the less likely formation of a *single* geometrical isomer from *either* enantiomer. The latter hypothesis excludes nonenzymatic formation of **2**.

It has been suggested that an oxenoid species, **5**, which is formed at the 4a-carbon by addition of oxygen to the tetrahydropterin (or dihydroflavin), may be the actual hydroxylating species (Scheme II).[20] Support for this has been presented by Ayling[2] who observed that cleavage of the C4a–N5 bond occurs when pyrimidine cofactors are used, giving rise to an amine and, after reduction with β-mercaptoethanol, a 5-hydroxy pyrimidine (divicine). This raises the possibility that the resonance at 72.3 ppm might be the hydrated ketone **7** and the one at 148.0 ppm is due to **6**. However, structures **6** and **7** can be eliminated since all of the species involved must be ring closed, i.e., tetrahydropterins. Figure 2 shows the coupled and ^1H decoupled ^{13}C NMR spectra for the intermediates.[21] The observed coupling constants between C4a and H6 for the species are as follows: 6-MPH_4 (**1**), 1.8 Hz; 4a-OH (**2**), 2.3 Hz; quinonoid-6-MPH_2 (**3**), 5.2 Hz. It is clear that the long-range coupling from C4a to the proton at C6 is preserved in all the species, thus eliminating **6** and **7**[22] and supporting **2** and **3**. The tautomeric state of the quinonoid dihydropterin **3** is the subject of the following communication.

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Registry No. **1**, 942-41-6; **2**, 83387-39-7; **3**, 70786-93-5; 4, 17377-13-8; phenylalanine hydroxylase, 9029-73-6.

(20) Hamilton, G. A. *Prog. Bioorg. Chem.* **1971**, *1*, 83–157.

(21) The coupling between C4a and H6 is confirmed in the 360-MHz ^1H spectra of both **1** and **3**, when compared with those of their unlabeled counterparts.

(22) In addition the chemical shift of a ketone and its hydrate would be expected to resonate at a lower field than an imine or its hydrate. For example, the chemical shifts for the C5 of alloxan and its hydrate are 167.0 and 85.0 ppm, respectively.

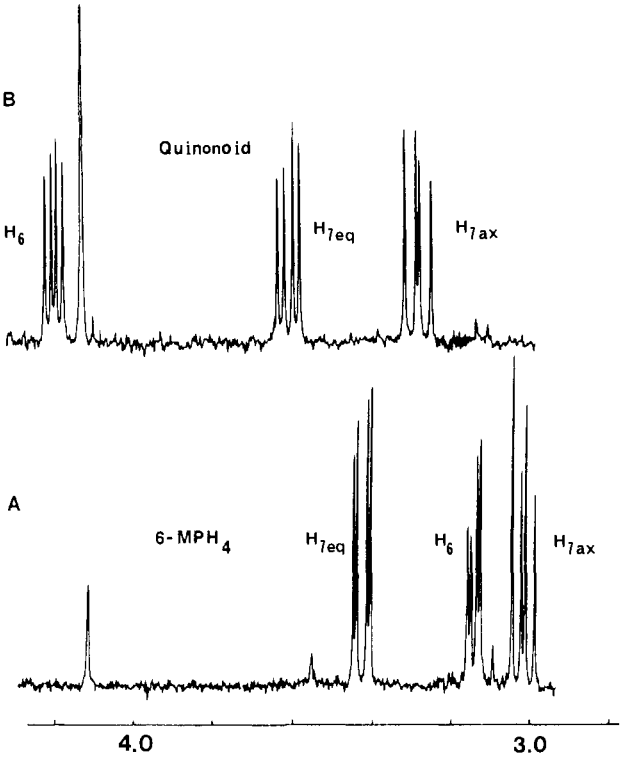

Figure 1. 360-MHz ^1H NMR spectra of 6-MPH_4 and quinonoid 6-MPH_2 at 5 °C; 32 transients were averaged per spectrum. Spectral width used; 5K, 0.305 Hz/pt resolution. The singlet at δ 4.13 is due to the protons at C7 of the rearranged 7,8-6-MPH_2 product: (A) 5 mM 6-MPH_4 in D_2O, 30 mM imidazole, 0.4 mM DSS, pD 8.4 (the 6-CH_3 at δ 1.18 was decoupled); (B) solution A, 10 min after addition of 1.1 equiv of Br_2 in D_2O, then adjusting pD to 8.4 (the 6-CH_3 at δ 1.40 was decoupled).

Structural Determination of Quinonoid Dihydropterins

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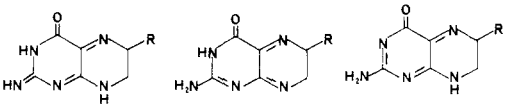
Tetrahydropterins, which serve as biological cofactors for the aromatic amino acid hydroxylases,[1,2] are oxidized during the course of enzyme turnover to quinonoid dihydropterins.[2,3] The quinonoid dihydropterins may also be generated by a wide variety of chemical oxidants including bromine, 2,6-dichloroindophenol,[4] and ferricyanide[5] as well as H_2O_2 and peroxidase.[6] The unstable quinonoid compounds can either rearrange nonenzymatically to inactive 7,8-dihydropterins[5] or be reduced back to their tetrahydro form by NADH in a reaction catalyzed by dihydropteridine reductase (E.C. 1.6.99.7).[6-8]

Postulated tautomeric structures for the quinonoid dihydropterins are shown in **1–3**. The UV spectra for quinonoid dihydropterins,[3,9] the rates for oxidation of various N-methylated tetrahydropterins,[10] and molecular orbital calculations[11] were interpreted to support the exocyclic para isomer (**1**). Support for the ortho isomer (**2**) is based on electrochemical data,[12] and arguments favoring the endocyclic para tautomer (**3**) have been derived from acid–base behavior.[13,14] The purpose of this communication is to demonstrate that the dihydropterin formed from both phenylalanine hydroxylase[15] and chemical oxidants exists as a para quinonoid isomer (**1** or **3**).



1 **2** **3**

Dehydration of 4a-hydroxy-6-methyltetrahydropterin, the initial product from phenylalanine hydroxylase,[15] gives quinonoid-6-

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(2) Kaufman, S. *Adv. Enzymol.* **1971**, *35*, 245–319.

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(9) Hemmerich, P. "Pteridine Chemistry"; Pfleiderer, W., Taylor, E. C., Eds.; Pergamon Press: London 1974; pp 143–169, 323.

(10) Viscontini, M.; Bobst, A. *Helv. Chim. Acta* **1965**, *48*, 816–822.

(11) Bobst, A. *Helv. Chim. Acta* **1967**, *50*, 1480–1491.

(12) Lund, H. "Chemistry and Biology of Pteridines"; Pfleiderer W., Ed.; Walter de Gruyter: Berlin, 1975; pp 645–670.

(13) Archer, M. C.; Vonderschmitt, D. J.; Scrimgeour, K. G. *Can. J. Biochem.* **1972**, *50*, 1174–1182.

(14) Scrimgeour, K. G. "Chemistry and Biology and Pteridines"; Pfleiderer W., Ed.; Walter de Gruyter: Berlin, 1975, pp 731–751.

(15) Lazarus, R. A.; DeBrosse, C. W.; Benkovic, S. J., previous communication in this issue.

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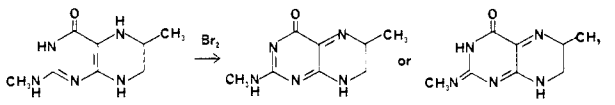
MPH_2, which has been characterized by (1) the resonance of its 4a-carbon in the ^{13}C NMR spectrum (148.0 ppm),[15] (2) its UV spectrum at pH 8.2 (λ_{max} 242 nm (ϵ 6500) 303 (ϵ 8300) 365, sh (ϵ 2800); see also ref 5), and (3) its ability to serve as a cofactor for dihydropteridine reductase (K_m = 35 μM).[6] Chemical oxidation (vide supra) of 6-MPH_4 gives quinonoid-6-MPH_2 with identical properties, indicating that the same species is formed in all cases.

The tautomeric structure of quinonoid-6-MPH_2 was determined by analysis of its 360-MHz NMR spectrum. Figure 1 contrasts the ^1H spectra for the pyrazine ring protons in 6-MPH_4 and quinonoid-6-MPH_2 (generated by Br_2 oxidation) at pD 8.4[16] and 5 °C.[17] The 6-CH_3 resonance has been decoupled from H6 in each case, to facilitate extraction of the apparent first-order coupling constants.

The most striking observation is the downfield shift of 1.08 ppm shown by H6 upon oxidation, while the H7 axial and H7 equatorial protons are shifted downfield by only 0.27 and 0.18 ppm, respectively. Our interpretation of this result is that H6 in quinonoid 6-MPH_2 is adjacent to an imine nitrogen,[25] whereas the protons at C7 are not; that is the para quinonoid (**1** or **3**) is implied, and the ortho tautomer (**2**) is excluded. These chemical shifts do not vary upon changing pD to 7.5 or 6.8, obviating protonation at N5 of quinonoid-6-MPH_2 as the source of this shift.[26] Bromine oxidation of *cis*-6,7-dimethyltetrahydropterin (6,7-$DMPH_4$) and 6-methyltetrahydrolumazine (6-MLH_4)[27] to their quinonoid forms induces very similar shifts at H6 ($\Delta\delta$ = +1.11 and +1.06), $H7_{eq}$ ($\Delta\delta$ = +0.25 and +0.15), and $H7_{ax}$ ($\Delta\delta$ = +0.27).

Bromine oxidation of 2-methylamino-6-MPH_4 (**4**) also produces a quinonoid intermediate (**5**) having similar chemical shifts for the pyrazine ring protons (δ 3.26, $H7_{ax}$, 3.60, $H7_{eq}$; 4.18, H6) but



which shows a pronounced difference in its UV absorption spectrum (λ_{max} 245 nm (ϵ 7100), 323 (ϵ 10 800)) from that of quinonoid-6-MPH_2, -6,7-$DMPH_2$, or -3,6-$DMPH_2$,[28] all of which have a λ_{max} of 303 nm. This bathochromic shift of 20 nm was first explained by the fact that the amino group is part of an extended conjugation system,[3] i.e., the exocyclic para tautomer (**6**). However, the failure to observe a large chemical shift change for the 2-methylamino protons (δ 2.82 to 2.96) upon oxidation to quinonoid as expected for the exocyclic form (**6**) implies that the endocyclic tautomer (**5**) is formed in this case. However, it is questionable whether extrapolation of the UV data from **5** to the parent pterin is permissible owing to the perturbation of the tautomeric equilibrium by methyl substitution.[29]

Acknowledgment. We thank Dr. Lloyd Jackman for helpful discussions. This research was supported by the National Science Foundation (PCM 81-03670) and the National Institutes of Health, NIH (GM-27525).

Registry No. **1**, 83650-46-8; **3**, 83650-48-0; **4**, 67129-03-7; **5**, 83650-47-9; 6-MPH_4, 942-41-6; *cis*-6,7-$DMPH_4$, 60378-42-9; 6-MLH_4, 83650-49-1; *cis*-6,7-$DMPH_2$, 83650-50-4; 4a-hydroxy-6-methyltetrahydropterin, 83387-39-7.

(16) pD = pH meter reading + 0.40: Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188-190.

(17) Chemical shifts and coupling constants (CH_3 decoupled) for Figure 1. 6-MPH_4: δ 1.19 (d, J = 6.4 Hz, CH_3), 3.01 (d of d, $H7_{ax}$, 3.12, (d of d, H6), 3.43 (d of d, $H7_{eq}$, $J_{6,7_{ax}}$ = 8.6 Hz, $J_{6,7_{eq}}$ = 2.8 Hz, $J_{7_{eq},7_{ax}}$ = 12.2 Hz). Quinonoid-6-MPH_2: δ 1.40 (d, J = 6.4 Hz, CH_3), 3.28 (d of d, $H_{7_{ax}}$, 3.61 (d of d, $H7_{eq}$), 4.20 (d of d, H6) $J_{6,7_{ax}}$ = 10.6 Hz, $J_{6,7_{eq}}$ = 6.0 Hz, $J_{7_{eq},7_{ax}}$ = 13.8 Hz). The resonance assigned to H6 in both cases is coupled to C4a in the 4a-^{13}C enriched compound.[15] It is also split by the methyl group at C6 if a coupled spectrum is run. In addition this resonance disappears when the proton at C6 is replaced by deuterium.

(18) Conformational studies on various tetrahydropterins[19-21] and tetrahydrofolates[22,23] have demonstrated that the tetrahydropyrazine ring is in a half-chair conformation. The values of the vicinal coupling constants between the pyrazine protons in quinonoid-6-MPH_2 suggest that the conformation in this ring deviates slightly from the pseudochair structure observed for the tetrahydropterins,[19-21] giving a structure which is flattened at N5[24] and shows an increased preference for the pseudochair in which the 6-CH_3 is equatorial. The same trends in $^3J_{6,7_{ax,eq}}$ are evident in the quinonoids of 6,7-$DMPH_2$ and 6-MLH_2.

(19) Ganguly, A. N.; Bieri, J. H.; Viscontini, M. *Helv. Chim. Acta* **1981**, *64*, 367-372.

(20) Webber, R.; Viscontini, M. *Helv. Chim. Acta* **1975**, *58*, 1772-1780.

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(22) Poe, M.; Jackman, L. M.; Benkovic, S. J. *Biochemistry* **1979**, *18*, 5527-5530.

(23) Poe, M.; Benkovic, S. J. *Biochemistry* **1980**, *19*, 4576-4582.

(24) Lambert, J. B. *J. Am. Chem. Soc.* **1967**, *89*, 1836-1840.

(25) For example, the chemical shifts for the methyl protons of *N*-benzylmethylamine and *N*-benzylidinemethylamine are 2.36 and 3.41, respectively. Similarly, the chemical shifts for the methylene protons of *N*-benzyl-*N'*,*N'*-dimethylethylenediamine and *N*,*N'*-dibenzylidineethylenediamine are 2.54 and 3.96, respectively: Sadtler Standard Spectra, Sadtler Research Laboratories, Inc., 1978.

(26) This has been confirmed for quinonoid-6,6,7,7-tetramethylpterin, whose N5 $pK_a \simeq 5.2$ (T. C. Bruice, personal communication).

(27) The chemical shifts and coupling constants at pD 8.4. 6,7-$DMPH_4$: δ 1.08 (d, $CH_3(6)$), 1.09 (d, $CH_3(7)$), 3.19 (d of q, H6), 3.58 (d of q, H7, $J_{6,7}$ = 3.3 Hz, $J_{7,CH_3(7)}$ = 6.7 Hz, $J_{6,CH_3(6)}$ = 6.7 Hz). Quinonoid-6,7-$DMPH_2$: δ 1.21 (d, $CH_3(6)$), 1.24 (d, $CH_3(7)$), 3.87 (d of q, H7), 4.30 (d of q, H6, $J_{7,CH_3(7)}$ = 6.7 Hz, $J_{6,CH3(6)}$ = 6.7 Hz, $J_{6,7}$ = 5.5 Hz). The assignment of the resonances was confirmed by replacing the proton at C6 with a deuterium. 6-MLH_4: δ 1.19 (d, J = 6.4 Hz, CH_3), 2.99 (d of d, $H7_{ax}$), 3.09 (d of d (CH_3 decoupled), H6), 3.46, (d of d, H7, $J_{6,7_{eq}}$ = 2.7 Hz, $J_{6,7_{ax}}$ = 8.6 Hz, $J_{7_{eq},7_{ax}}$ = 12.2 Hz). Quinonoid 6-MLH_2: δ 1.39 (d, J = 6.4 Hz, CH_3), 3.26 (d of d, $H7_{ax}$), 3.61 (d of d, $H7_{eq}$), 4.15 (d of d (CH_3 decoupled), H6, $J_{6,7_{ax}}$ = 10.7 Hz, $J_{6,7_{eq}}$ = 6.3 Hz, $J_{7_{ax},7_{eq}}$ = 14.3 Hz).

(28) Quinonoid-3,6-$DMPH_2$ was too unstable for a ^1H NMR spectrum to be obtained.

(29) Jackman, L. M.; Jen, T. *J. Am. Chem. Soc.* **1975**, *97*, 2811-2818.

H-D Kinetic Isotope Effects of 16 and 22 in the Oxidation of H_2O_2

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In a recent communication we reported a D_2O/H_2O isotope effect of 16.1 for the comproportionation reaction in eq 1.[1] It

$$(bpy)_2(py)Ru^{IV}O^{2+} + (bpy)_2(py)Ru^{II}OH_2^{2+} \rightarrow 2(bpy)_2(py)Ru^{III}OH^{2+} \quad (1)$$

was argued that the origin of the effect was in the proton content of the reagents, which creates a proton demand for the reaction. The direct, outer-sphere electron transfer pathway shown in eq 2 is accessible, but slower (k(25 °C) < 3 × 10^3 M^{-1} s^{-1}) because

$$(bpy)_2(py)Ru^{IV}O^{2+} + (bpy)_2(py)Ru^{II}OH_2^{2+} \rightarrow (bpy)_2(py)Ru^{III}O^{+} + (bpy)_2(py)Ru^{III}OH_2^{3+} \quad (2)$$

of the high-energy nature of the initial products with regard to their proton compositions.[2] Rather, it was argued, a more facile pathway for the system exists in which proton and electron transfer are coupled (eq 3) in a mechanism that could be described as involving "hydrogen atom transfer".

$$(bpy)_2(py)Ru^{IV}{=}O^{2+}, H_2ORu^{II}(py)(bpy)_2^{2+} \rightarrow (bpy)_2(py)Ru^{III}OH^{2+}, HORu^{III}(py)(bpy)_2^{2+} \quad (3)$$

We report here a similar effect in the oxidation of H_2O_2 by both $(bpy)_2(py)Ru^{IV}O^{2+}$ and $(bpy)_2(py)Ru^{III}OH^{2+}$. The reactions occur with the stoichiometries shown in eq 4 and 5. Over the pH range

$$(bpy)_2(py)RuO^{2+} + H_2O_2 \rightarrow (bpy)_2(py)RuOH_2^{2+} + O_2 \quad (4)$$

$$2(bpy)_2(py)RuOH^{2+} + H_2O_2 \rightarrow 2(bpy)_2(py)RuOH^{2+} + O_2 \quad (5)$$

(1) Binstead, R. A.; Moyer, B. A.; Samuels, G. J.; Meyer, T. J. *J. Am. Chem. Soc.* **1981**, *103*, 2897-2899.

(2) Moyer, B. A.; Meyer, T. J. *Inorg. Chem.* **1981**, *20*, 436-444.

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