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# The Reaction of Nucleophilic Species with Quinonoid 6,6,7,7-Tetramethyldihydropterin

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The products, kinetics, and mechanisms for the reaction of $HO^-$, $NH_2OCH_3$, and HOCl with 1,5-quinonoid 6,6,7,7-tetramethyldihydropterin are provided. The reaction with hydroxide leads to a rearranged imidazolone whose structure was proved by independent synthesis. With methoxylamine the product is a dihydro adduct derived from methoxylamine displacement of the 2-amino group. In this case the structure of the product was obtained via X-ray crystallography and $^{13}C$ NMR with the former providing novel information about the tautomeric form of this pterin quinonoid species. The chlorination of the quinonoid generates N2 or N3 chloroamines based on high-resolution mass spectrometric analysis. Mechanisms for these transformations are proposed. 

## INTRODUCTION

Dialkyl substitution at the 6,6-positions of 2-amino-4(3*H*)oxotetrahydropterin ($H_4$pterin) permits the generation of stable 1,5-quinonoid dihydropterins (*1*, *2*, *3*) such as $\mathbf{1}_{ox}$ that can be isolated. A 1,5-quinonoid dihydropterin species is generated transiently from the 4a-hydroxy adduct of 6-methyl $H_4$pterin that is formed during the phenylalanine hydroxylase-catalyzed hydroxylation of phenylalanine (*4*). It was of considerable interest to determine whether the quinonoid form undergoes reversible nucleophilic addition as a possible synthetic route to a putative 4a-hydroperoxytetrahydropterin that may act as the hydroxylating species (*5*). The macroscopic $pK_a$ for dissociation of the protonated 1,5-quinonoid 6,6,7,7-tetramethyltetrahydropterin (i.e., $\mathbf{1}_{ox}H^+$ of Eq. [1], text) is 5.2 (*1*) (Eq. [2]) and probably represents the distribution of species shown in Eq. [1] weighted towards protonation at N2 (*6*). A priori, nucleophilic addition to the C4a position of $\mathbf{1}_{ox}H^+$ is plausible (Eq. [3]) based on the case (*7*) of nucleophilic additions to the C4a position of N5-alkyl flavins (Eq. [4]).



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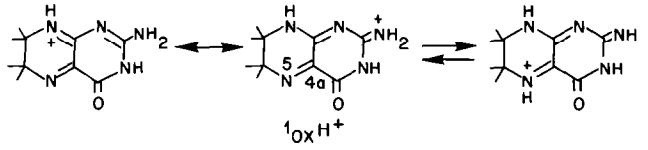
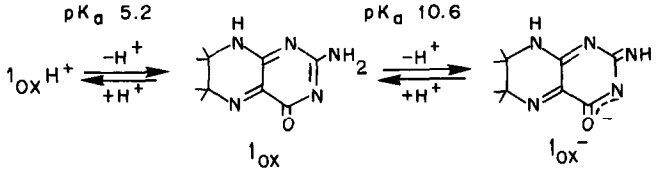



[1]

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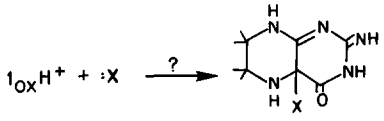


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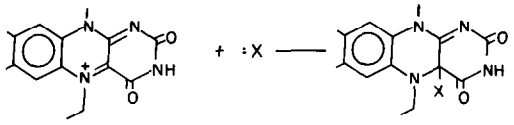



[4]

The present report details the reactions of the tetramethyldihydropterin ($\mathbf{1}_{ox}$) with hydroxide ion, methoxylamine, and peracetic acid and characterizes the unexpected products. The product of the reaction with methoxylamine has also provided important, unequivocal evidence as to the tautomeric form of the quinonoid species.

## EXPERIMENTAL PROCEDURES

*Physical measurements.* uv–Visible spectra were recorded on a Cary 118C or Perkin–Elmer Lambda 3 spectrophotometer. Measurements of pH were carried out using a doubly standardized Radiometer 26 or Beckman 4500 digital pH meter. The ir spectra were recorded as Nujol mulls on a Perkin–Elmer 137 spectrophotometer. Melting points were obtained in open capillary tubes on a Mel-Temp apparatus. $^1$H NMR spectra were recorded on a Nicolet NT-300 (300-MHz) spectrometer. Mass spectra were obtained on a V.G. Micromass ZAB-2F mass spectrometer operating in the positive chemical ion mode at 450 eV. Rapid-scan stopped-flow spectra were recorded on an OLIS rapid-scan spectrophotometry system.

*Preparation of methoxylamine adduct of* $\mathbf{1}_{ox}$. Twenty milligrams of $\mathbf{1}_{ox}$ was dissolved in 10 ml of 0.67 M methoxylamine/methoxylamine hydrochloride buffer (pH 4.5) and the yellow solution allowed to stand at room temperature for 2 days. The reaction mixture was poured into 100 ml of water and extracted six times with 40-ml portions of methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated down to an orange-yellow gum

which was dissolved in a little ether. Almost immediately the orange adduct crystallized out. The adduct was filtered off and recrystallized from carbon tetrachloride. Yield 7 mg (36%); mp 214–218°C (dec). High-resolution mass spectrometry (positive chemical ionization) shows M + 1 obs 252.1468 (M + 1 calcd 252.1458) fitting formula $C_{11}H_{17}O_2N_5$. The ir (Nujol mull) spectrum shows amide C=O at 1710 $cm^{-1}$ and imine at 1610 and 1650 $cm^{-1}$. $^1H$ NMR ($CDCl_3$) $\delta$ 8.99 (1H, s, $D_2O$-exchangeable), 5.59 (1H, s, $D_2O$-exchangeable), 3.93 (3H, s), 1.38 (6H, s), 1.28 (6H, s).

*Preparation of chloramine of* $\mathbf{1}_{ox}$. A solution of 8 mg of $\mathbf{1}_{ox}$ in 550 $\mu$l of methanol was added to a solution of 570 $\mu$l of 40% peracetic acid in 150 ml of 1 M acetate buffer (pH 4.5) and 150 ml of 1 M KCl. The reaction mixture was stirred in the dark at 30°C and monitored by following the change in absorbance at 370 nm of an aliquot in a cuvette. After 1 h an autocatalytic reaction set in with an increase in absorbance and after four hours it was over. The reaction mixture was extracted five times with 40-ml portions of methylene chloride. The combined organic extracts were dried over anhydrous magnesium sulfate and evaporated down to yield the chloramine as a yellow solid which was stirred in a little ether and filtered. Yield 3 mg (38%); mp > 200°C dec. The chloramine is light sensitive and must be stored in the dark. High-resolution MS (positive chemical ionization) shows M + 1 obs 256.0988 (M + 1 calcd 256.0964) fitting formula $C_{10}H_{15}N_5O^{35}Cl$ and M + 1 obs 258.0956 (M + 1 calcd 258.0935) fitting formula $C_{10}N_{15}N_5O^{37}Cl$. The ir (Nujol mull) spectrum shows amide C=O at 1720 $cm^{-1}$. UV ($CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon$ $M^{-1}$ $cm^{-1}$) 246 (7210), 318 (4000), 368 (4760) nm.

*Reduction of chloramine of* $\mathbf{1}_{ox}$. Sodium sulfite solution (5 $\mu$l, 1 M) was added to a solution of the chloramine of $\mathbf{1}_{ox}$ (0.073 mg) in 3 ml of 1 M acetate buffer (pH 4.5). The resulting uv spectrum was that of 6,6,7,7-tetramethyltetrahydropterin with $\lambda_{max}$ at 274 and 330 nm.

*X-Ray structural determination of* $\mathbf{3}_{ox}$. Crystals were grown from a saturated solution of $CCl_4$. An orange crystal of approximate dimensions 0.28 × 0.30 × 0.50 mm was mounted on a glass fiber using epoxy cement with the longest dimension coincident with the glass fiber axis. The crystal was optically centered on an Enraf-Nonius four-circle CAD4 automated diffractometer. Enraf-Nonius programs[1] were employed to determine the space group I222 (No. 23) and the orthorhombic cell dimensions (a) = 11.464 Å (*3*), (b) = 12.392 Å (*4*), (c) = 18.831 Å (*4*), and V = 2675 $Å^3$ (*2*). The number of formula units per unit cell *Z* was found to be 8 based on a calculated density of 1.248 g $cm^{-3}$. The formula weight of 252.29 corresponds to the formula $C_{11}H_{17}N_5O_2$.

Intensity data were collected at room temperature [21°C (*1*)] using MoK radiation ($\lambda$ = 0.71073 Å) with a graphite single-crystal monochromator (take-off angle 2.8°). A 0–20 scan technique was used with a variable scan rate of 1.0–5.0° $min^{-1}$ and a scan width of (1.0 + 0.347 tan 0)° above and below the calculated $K_{a1}$ and $K_{a2}$ positions. Three standard reflections were measured every hour and were used to apply an anisotropic drift correction (0.955–1.043) to correct for slight changes due to crystal stability and orientation changes during the data collection. A total

[1] Structure determination package was provided by Molecular Structure Corp.

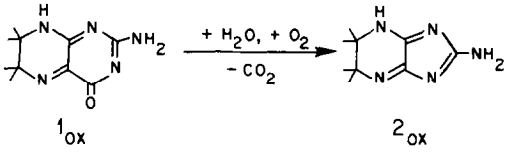
of 1905 reflections were collected in the range $3.2 < 2\theta < 44,56°$. Of these there were 1466 unique nonzero reflections. Psi scans indicated that absorption was not severe ($\mu = 0.84$ cm$^{-1}$), and no absorption corrections were applied.

The structure was solved by direct methods employing MULTAN.[1] The remaining nonhydrogen atoms were located with several Fourier syntheses. Hydrogen atoms were located from a difference Fourier map and refined with fixed isotropic temperature parameter (B = 5.0 Å$^2$). In the final cycle of least-squares refinements the structure converged (max shift $0.01\sigma$) with 976 reflections ($I < 3\sigma(I)$) on 163 variables. Variables included the overall scale factor, positional parameters for all atoms, and anisotropic thermal parameters for all nonhydrogen atoms. Convergence was achieved with final $R$ values of $R_1 = 0.064$, $R_2 = 0.058$, and an ESD of 1.954 (see footnote[2]) employing a unit weighting scheme. A final difference Fourier map showed no peaks greater than 0.24 eÅ$^3$.

*$^{13}C$ NMR.* A tabulation of the $^{13}$C NMR shifts for $\mathbf{1}_{red}$, $\mathbf{1}_{ox}$, and $\mathbf{3}_{ox}$ is included in Table 1, since no complete $^{13}$C NMR characterization of a quinonoid dihydropterin exists in the literature. The $^{13}$C NMR spectra of 0.1–0.2 M solutions of the three samples at pH 4.5 in 6% $D_2O$ were recorded broad-band decoupled at 90.56 MHz on a Bruker WH 360-MHz NMR spectrometer at 10°C. $^{13}$C chemical shifts are reported relative to the $CDCl_3$ peak of a 10% ethylbenzene/chloroform solution.

## RESULTS

*Solvolytic rearrangement of 6,6,7,7-tetramethyldihydropterin ($\mathbf{1}_{ox}$).* Solvolytic rearrangement of $\mathbf{1}_{ox}$ in aqueous solution yields, in the presence of air, 2-amino-5,5,6,6-tetramethyl-5,6-dihydroimidazo[4,5-*b*]pyrazine ($\mathbf{2}_{ox}$) (*1*). The pH dependence of the reaction was studied in buffered aqueous solutions (acetate, phosphate, and carbonate) at 30°C ($\mu = 1.0$ with KCl) by following the disappearance of $\mathbf{1}_{ox}$ at 302 mn. In separate experiments, it was shown that the rearrangement is insensitive to buffer concentrations (between total [buffer] of 0.033 and 0.33 M). The pH–log $k_{obs}$ profile is shown in Fig. 1. The open circles are experimental rate constants and the line computer generated from the equation




[5]

$$k_{obs} = k_1 \frac{K_{app\ 1}}{K_{app\ 1} + a_H} + k_2 \frac{K_{app\ 2}}{K_{app\ 2} + a_H} \qquad [6]$$

by employing the constants $k_1 = 4.94 \times 10^{-5}$ M$^{-1}$ s$^{-1}$, $k_2 = 4.20 \times 10^{-4}$ M$^{-1}$ s$^{-1}$,

[2] $2R_1 = \Sigma\ 1Fo1 - 1Fe1/\Sigma\ 1Fo1$

$R_2 = [\Sigma w(1Fo1 - 1Fe1)^2/\Sigma wFo^2]^{1/2}$, $w = 1/(\sigma(Fo))^2$.

ESD is the estimated standard deviation of an observation of unit weight.

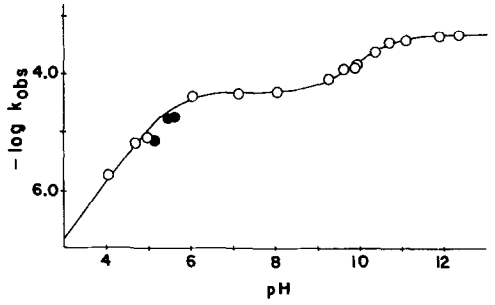
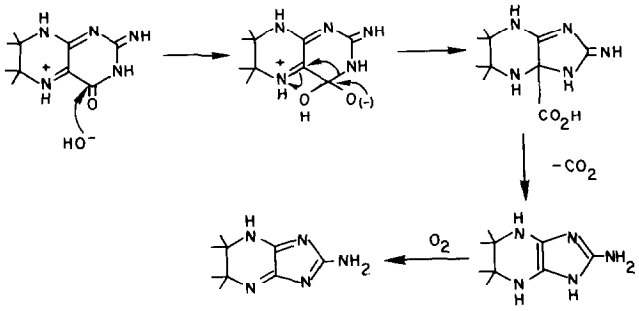



FIG. 1. pH vs the logarithms of the observed pseudo-first-order rate constants for solvolytic ring contraction of 6,6,7,7-tetramethyl-1,5-dihydropterin at constant pH values.

$pK_{app\,1}$ = 5.56 and $pK_{app\,2}$ = 10.43. The kinetically determined constants $pK_{app\,1}$ and $pK_{app\,2}$ agree closely with determined $pK_a$ values of $\mathbf{1}_{ox}$ (*1*). Two mechanisms will provide the kinetic Eq. [6]. These involve attack of $H_2O$ upon $\mathbf{1}_{ox}$ and $\mathbf{1}_{ox}^-$ or, alternatively $HO^-$ attack upon $\mathbf{1}_{ox}H^+$ and $\mathbf{1}_{ox}$. The latter is the most sensible, as shown for $\mathbf{1}_{ox}H^+$ in the equation




[7]

Similar ring contractions to yield imidazolones have been recorded in alloxazine (*9*) and flavin (*8*) chemistry.

*Methoxylamine reaction with* $\mathbf{1}_{ox}$. Methoxylamine reacts with $\mathbf{1}_{ox}$ (aqueous soln, $\mu$ = 1.0, 30°C) in the pH range of 3.82 to 5.61 to provide a product with $\lambda_{max}$ at 407 nm (Fig. 2). Plots of ([$NH_2OMe$] + [$^+NH_3OMe$]) vs the pseudo-first-order rate constant (determined at 407 nm) for product formation are linear with positive intercept, suggestive of a two-term kinetic equation as shown in the equation

$$k_{obs} = k_1'[NH_2OMe] + k_2' \qquad [8]$$

where $k_1'$ and $k_2'$ are pH-dependent constants. The intercept values ($k_2'$) are included in Fig. 1 as shaded circles. Inspection of Fig. 1 shows that these points fit the pH–log $k_{obs}$ profile for solvolytic rearrangement of $\mathbf{1}_{ox}$. Thus, Eq. [8] results from the competitive reaction of methoxylamine with $\mathbf{1}_{ox}$ ($k_1'$) and solvolytic rearrangement of $\mathbf{1}_{ox}$ ($k_2'$).

In the pH range of investigation the pterin substrate exists as both protonated and neutral species (Eq. [2]). The reaction of methoxylamine with the pterin can be discussed in terms of the equation

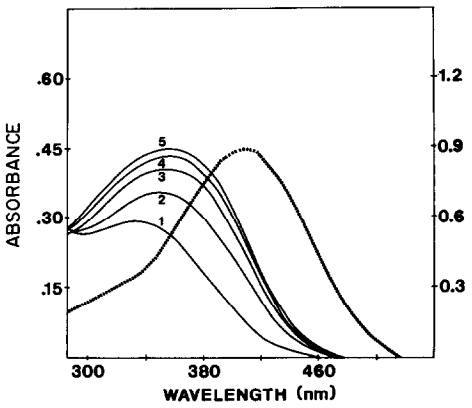
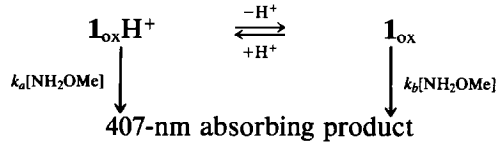



FIG. 2. Solid lines: kinetic run of 6,6,7,7-tetramethyl-1,5-dihydropterin ($1.67 \times 10^{-4}$ M) with *m*-chloroperbenzoic acid ($2.42 \times 10^{-2}$ M) in 0.33 M acetate buffer of pH 4.5 ($\mu$ = 1.0 with KCl) at 30°C; spectra were recorded by rapid-scan stopped flow after curve 1, 1; curve 2, 26; curve 3, 51; curve 4, 101; and curve 5, 201 ms. Dotted line: spectrum of $\mathbf{3}_{ox}$ adduct from reaction of 6,6,7,7-tetramethyl-1,5-dihydropterin ($8.4 \times 10^{-5}$ M) in 1 M methoxylamine buffer at pH 3.8 after 24 h.



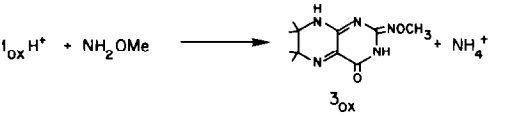

[9]

In Fig. 3 there is plotted the experimental pseudo-first-order rate constant vs methoxylamine free base at five pH values. The slopes of these plots equal $k_1'$ (Eq. [8]). The equation

$$k_1' = \frac{k_a a_H + k_b K_{a1}}{K_{a1} + a_H} \qquad [10]$$

follows from Eq. [9] and from a plot of $k_1'(K_{a1} + a_H)$ vs $a_H$ (Fig. 4) there is obtained as slope $k_a$ ($= 8.0 \times 10^{-4}$ M$^{-1}$ s$^{-1}$). The value of $k_b$ is imperceptible as shown by the lack of an intercept in the plot. Thus, the product is formed by nucleophilic attack of methoxylamine free base upon $\mathbf{1}_{ox}H^+$.

The product formed on reaction of methoxylamine was isolated from a preparative-scale reaction (Experimental Procedures) and crystallized from carbon tetrachloride. High-resolution mass spectral analysis (positive chemical ionization) established the structural formula of the adduct as $C_{11}H_{17}O_2N_5$. This result suggested the reaction




[11]

*X-Ray crystallographic verification of* $\mathbf{3}_{ox}$ *structure.* The structure of $\mathbf{3}_{ox}$ was verified by X-ray crystallography and $^{13}C$ NMR spectrometry. A three-dimen-

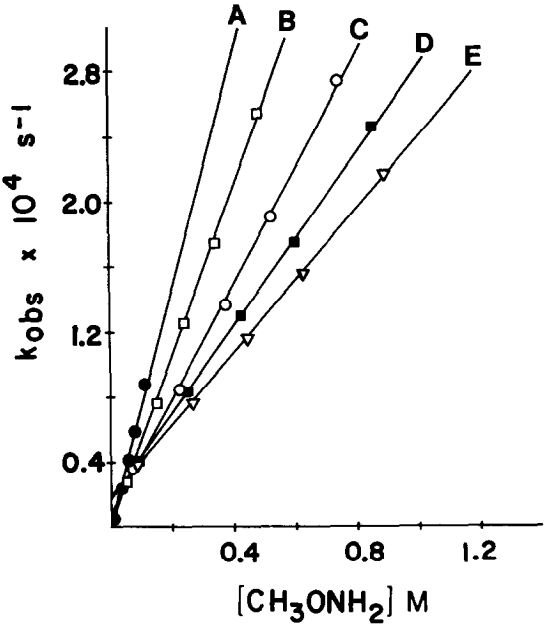



FIG. 3. Plot of $k_{obs}$ vs free methoxylamine concentration for the reaction of 6,6,7,7-tetramethyl-1,5-dihydropterin with methoxylamine at varying pH: (A) 3.82; (B) 4.66; (C) 5.16; (D) 5.43; (E) 5.61.

sional illustration of its structure is shown in Fig. 5, and Tables 2 and 3 contain corresponding selected bond lengths and bond angles.

*The reactions of m-chloroperbenzoic acid and peracetic acid with* $\mathbf{1}_{ox}$. These reactions in aqueous solutions at 30°C between pH 4.0 and 5.5 (acetic/sodium acetate buffer, 0.33 M) with $\mu$ = 1.0 (KCl) were followed by monitoring the

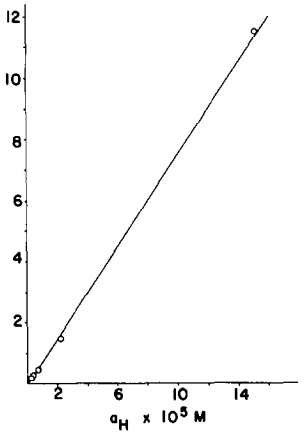



FIG. 4. Plot of the apparent second-order rate constant ($k_1'$) times the sum of the acid dissociation constant of $\mathbf{1}_{ox}H^+$ plus the activity of hydrogen ion ($K_{a1} + a_H$) vs the activity of hydrogen ion. The slope of the line equals the second-order rate constant for reaction of $NH_2OMe$ with $\mathbf{1}_{ox}H^+$.

TABLE 1

$^{13}$C CHEMICAL SHIFTS (ppm) FOR $\mathbf{1}_{red}$, $\mathbf{1}_{ox}$, AND $\mathbf{3}_{ox}$

| | C4 | C2 | C8A | C4A | C6 | C7 | C11, 13 | C10, 12 | C9 |
|---|---|---|---|---|---|---|---|---|---|
| $\mathbf{1}_{red}$ | 161.52 | 156.88[a] | 156.79[a] | 86.46 | 61.86 | 57.13 | 24.97 | 21.88 | |
| $\mathbf{1}_{ox}$ | 160.43 | 160.25[a] | 153.64 | 145.82 | 66.07 | 58.55 | 24.98 | 24.02 | |
| $\mathbf{3}_{ox}$ | 159.65 | 150.18[a] | 149.88[a] | 146.17 | 65.62 | 57.11 | 25.63 | 24.39 | 63.93[b] |

[a] Peaks separated by less than 0.5 ppm are assigned arbitrarily.
[b] Verified by recording a coupled spectrum.

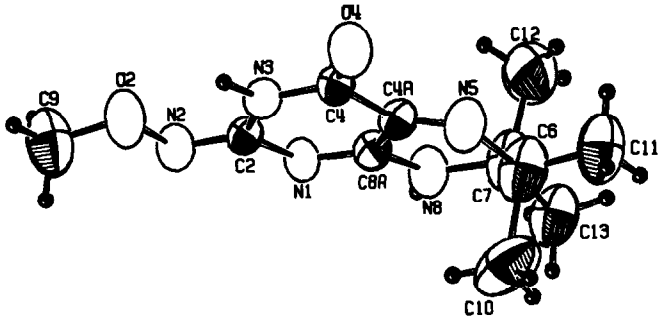



FIG 5. An ORTEP representation of $\mathbf{3}_{ox}$ giving the crystallographic numbering system used.

TABLE 2

SELECTED BOND LENGTHS (Å) FOR $\mathbf{3}_{ox}$

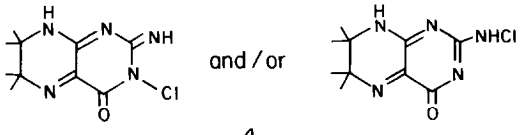
| | | | |
|---|---|---|---|
| O2—N2 | 1.411(5) | N5—C6 | 1.499(7) |
| N2—C2 | 1.293(5) | C6—C7 | 1.482(8) |
| C2—N3 | 1.414(6) | C7—N8 | 1.470(7) |
| N3—C4 | 1.360(7) | N8—C8A | 1.338(6) |
| C4—O4 | 1.200(6) | C8A—C4A | 1.480(7) |
| C4—C4A | 1.510(6) | C8A—N1 | 1.288(6) |
| C4A—N5 | 1.258(5) | N1—C2 | 1.362(6) |

TABLE 3

SELECTED BOND ANGLES (deg) FOR $\mathbf{3}_{ox}$

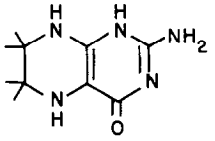
| | | | |
|---|---|---|---|
| O2—N2—C2 | 109.3(4) | N5—C6—C7 | 112.4(5) |
| N2—C2—N3 | 121.0(5) | C6—C7—N8 | 111.6(5) |
| C2—N3—C4 | 125.0(4) | C7—N8—C8A | 121.6(4) |
| N3—C4—C4A | 113.5(4) | N8—C8A—C4A | 115.3(4) |
| N3—C4—O4 | 123.6(5) | N8—C8A—N1 | 120.5(5) |
| O4—C4—C4A | 122.9(5) | N1—C8A—C4A | 124.1(5) |
| C4—C4A—N5 | 117.6(5) | C8A—N1—C2 | 119.2(4) |
| C4—C4A—C8A | 116.4(4) | N1—C2—N3 | 121.0(5) |
| N5—C4A—C8A | 125.7(5) | N1—C2—N2 | 118.0(5) |
| C4A—N5—C6 | 117.4(5) | | |

increase in absorbance at 370–375 nm. For both *m*-chloroperbenzoic acid and peracetic acid the reactions are characterized by a rather nonreproducible lag phase and a sigmoidal increase in absorbance with time. The reaction using *m*-chloroperbenzoic acid is particularly rapid, occurring in the millisecond time range (see Fig. 2). In the course of the investigation of these reactions it was found that in the absence of KCl (employed to maintain constant ionic strength) there was no reaction of $\mathbf{1}_{ox}$ with either acid and that HOCl ($10^{-3}$ mM, pH 4.5) reacts with $\mathbf{1}_{ox}$ to provide the same product ($\lambda_{max}$ 368 nm, $\varepsilon_{9150}$) obtained with peracids in the presence of KCl. The reaction with HOCl was found to be very rapid and not characterized by a lag phase. The product was isolated from a preparative experiment (Experimental Procedures) and from high-resolution mass spectroscopy (positive chemical ionization) the structural formula was found to be $C_{10}H_{14}N_5OCl$, suggesting the following structures:




$\mathbf{4}_{ox}$

That chlorination has occurred upon N2 and the structure is the endocyclic form is supported by the presence of strong M + 1 peaks in the mass spectra (positive chemical ionization) at 206 and 208 corresponding to a loss of $NH^{35}Cl$ and $NH^{37}Cl$. However, $^1H$ NMR would suggest a mixture of N2 and N3 chlorinated, especially since the 6,6,7,7-methyl pattern is complex. It is not clear whether the two species are formed via competing pathways or through interconversion. Chlorinations of pyrimidine derivatives at N3 have been reported for thymine and uracil but not the corresponding pterins (*10, 11*). The chlorinated product(s) are reduced in aqueous solution by sodium sulfite to yield $\mathbf{1}_{red}$.



$\mathbf{1}_{red}$

## DISCUSSION

In this study we set out to investigate the reaction of $\mathbf{1}_{ox}H^+$ with nucleophiles whose conjugate acids possess p$K_a$ values below that of $\mathbf{1}_{ox}H^+$. Under these conditions considerable concentrations of nucleophile basic species would exist in the presence of $\mathbf{1}_{ox}H^+$ favoring nucleophilic addition, possibly to the C4a position of $\mathbf{1}_{ox}H^+$ (Eq. [3]). Methoxylamine, however, reacts with $\mathbf{1}_{ox}H^+$ at the C2 position to displace the amino function (Eq. [11]) yielding $\mathbf{3}_{ox}$. Water does not exhibit a propensity to act as a nucleophile but $HO^-$ adds to the C4 position of $\mathbf{1}_{ox}H^+$ to yield, via the mechanism of Eq. [7], the imidazo pyrazine $\mathbf{2}_{ox}$. The reactions of *m*-

chloroperbenzoic acid and peracetic acid with $\mathbf{1}_{ox}H^+$ were investigated in order to ascertain if the nature of the products would support an initial C4a adduct. Both peracids were found to be unreactive with $\mathbf{1}_{ox}H^+$ but in the presence of the KCl employed to maintain ionic strength there was generated the chloramine $\mathbf{4}_{ox}$. The very rapid formation of $\mathbf{4}_{ox}$ was shown to occur from the peracid oxidation of $Cl^-$ to yield the chlorinating agent HOCl that reacted at N2 and/or N3. Thus, nonenzymatic addition to the 4a carbon is not a normal reaction course.

The X-ray structure of $\mathbf{3}_{ox}$ provides unequivocal evidence for the tautomeric form of this pterin quinonoid species (Fig. 5 and Tables 2 and 3). Of particular significance are the N2—C2, N1—C8a, and N5—C4a bonds which have lengths typical of C=N bonds (1.28 Å). On the other hand the N3—C2 bond length (1.41 Å) is (slightly) longer than that expected for a C—N bond (1.36 Å). These data conclusively support an *exo para* tautomer for this particular quinonoid species with a methoxyl group at N2 whereas the quinonoid form of 4-methyltetrahydropterin is an *endo para* tautomer (*2*). The $^{13}C$ chemical shifts for the C2, C8a, and C4a carbons also are in accord with sp2 hybridization. Significantly, the N8—C8a bond length (1.34 Å) has double-bond character similar to the corresponding linkage in 5,6,7-trimethyltetrahydropterin (1.32 Å) (*14*). However, the conjugation in $\mathbf{3}_{ox}$ appears to involve a 1,4-diimine moiety (N8—C8a—C4a—N5) whereas in the trimethyltetrahydropterin the reasonance is that anticipated for the vinylogous amide portion of the pterin ring (N8—C8a—C4a—C4) since the C4a—C4 bond is significantly longer in $\mathbf{3}_{ox}$ (1.51 Å vs 1.40 Å).

The conformation of $\mathbf{3}_{ox}$ is similar to that of 5,6,7-trimethyltetrahydropterin. The pyrimidine ring adopts a slightly less planar conformation, while the tetrahydropyrazine ring is similarly puckered. In both molecules, C6 lies significantly above the plane of the pyrimidine ring (by −0.595 Å in $\mathbf{3}_{ox}$), while C7 lies below this plane (0.166 Å in $\mathbf{3}_{ox}$). Surprisingly, N5 (−0.309 Å) and N8 (0.110 Å) also lie significantly above and below the pyrmidine plane. This may be due to the rather large steric interactions of the four methyl groups on C6 and C7.

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