Dioxygen Oxidation of a Stable 1,4-Dihydropyrazine

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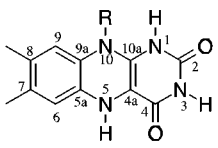
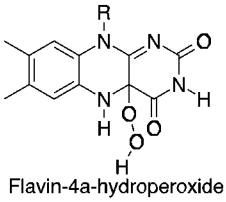
The stable 1,4-dihydropyrazine 4a,8a-diaza-2,6-dioxa-3,4,7,8-tetrahydro-4,4,8,8-tetramethylanthracene-1,5-dione (DDTTA) is oxidized by dioxygen in various organic solvents to give mixtures of 5,6-dihydro-5,5-dimethyl-3-formyl-1,4-oxazine-2-one (**1**) and a second product that was previously ascribed a dioxetane structure. The latter is now fully characterized by X-ray crystallography and found to be the diol 4a,8a-diaza-9,9a-dihydroxy-2,6-dioxa-3,4,7,8,9,9a-hexahydro-4,4,8,8-tetramethylanthracene-1,5-dione (**4**). The oxidation rate and product ratio are highly solvent dependent. Trapping experiments, reaction stoichiometry, and kinetic measurements are all consistent with a hydroperoxide intermediate that reacts with DDTTA to give the diol **4** or undergoes intramolecular fragmentation to give aldehyde **1**. Both DDTTA and the intermediate hydroperoxide are significantly less reactive than their biologically active 1,4-dihydropyrazine counterparts.

Introduction

Flavins are ubiquitous biological co-enzymes that show the unique property of one-electron redox chemistry, without the involvement of transition metals. This property arises from the pyrazine ring contained in the alloxazine heterocyclic system. Flavin analogs with N^5 replaced by carbon and consequently containing no pyrazine ring, show no one-electron redox activity.[1,2] Because of the importance of single-electron processes in the reduction and activation of triplet dioxygen, flavins are frequently involved in oxidative metabolic pathways that utilize dioxygen. In particular, many oxygenases, hydroxylases, and several biochemiluminescent pathways,[3] are believed to utilize dioxygen oxidation of dihydroflavins.[4] Initial reaction of the flavin coenzyme with dioxygen produces a 4a-flavin hydroperoxide which is the active species in oxidizing the substrate. These hydroperoxides have been directly detected in the active sites of certain enzymes and are extremely active oxidants, with the apparent ability to oxidize aromatic rings directly. A recent theoretical study attributed this activity to Coulombic effects from the alloxazine ring system;[5] however, the hydroperoxide may be a precursor to a highly reactive flavinoxy or hydroxy radical.[6]

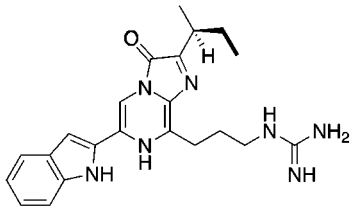


5,10-Dihydroflavin



Flavin-4a-hydroperoxide

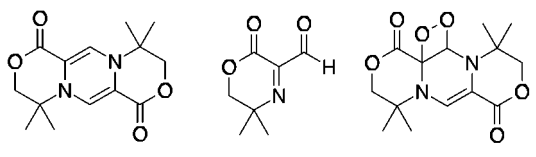
Dioxygen oxidation of 1,4-dihydropyrazines is also important in some chemiluminescent pathways. Oxidation of *Cypridina* luciferin and analogs containing the imidazo[1,2-*a*]pyrazin-3-one ring system results in chemiluminescence that is strongly dependent upon environment.[7−9] Chemiluminescence is most efficient when catalyzed by the enzyme luciferase, but in an aqueous environment (with no enzyme) chemiluminescence is not observed. In these systems both dioxetanes and hydroperoxides have been studied as possible active intermediates.[10−12]



Cypridina Luciferin

The oxidation of simple 1,4-dihydropyrazines has not been extensively investigated. Our discovery of the relatively stable 1,4-dihydropyrazine DDTTA prompted us to establish whether its dioxygen oxidation mimics that of the complex biological systems or if oxidation proceeds through significantly different pathways.

In a previous paper, we reported that the dioxygen oxidation of DDTTA was highly dependent upon environment.[13] In acetonitrile, the predominant product is an aldehyde (**1**), resulting from cleavage of the pyrazine ring, whereas in trifluoroacetic acid only the DDTTA radical cation was isolated. In acetic acid−water mixtures we reported formation of the radical cation and a species for which we postulated a dioxetane structure (**2**). The strong dependence upon environment of DDTTA oxidation may be significant with respect to oxidation of naturally occurring 1,4-dihydropyrazines. We now report further characterization of oxidation product structures and studies of the oxidation mechanism.



DDTTA 1 2

Results

As previously reported, reaction of DDTTA with extremely low concentrations of oxygen in a mixture of

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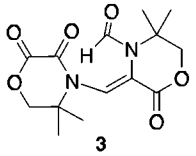
acetic acid and water yielded a species which has an ^{1}H NMR spectrum consistent with complete loss of the molecular symmetry. This is indicated by the four distinct methyl singlets and two AB patterns corresponding to two methylene groups. The chemical shifts of the two methine protons are consistent with loss of one of the two double bonds. On the basis of a peak in the mass spectrum at $m/z = 310$ corresponding to DDTTA + O_2, this product was previously assigned the dioxetane structure **2**. This structure was recently questioned by Adam and co-workers[14] on the basis of its reported relative stability and now appears to be incorrect. Addition of peroxide scavengers such as triphenylphosphine to the reaction mixture produced no perceptible change in the NMR spectrum, strongly suggesting that the product is not a peroxide. The dioxetane cleavage product **3** suggested by Adam cannot be the species giving rise to the bulk NMR signals because it would show only two methyl singlets, no AB patterns, and have a distinctive resonance as a result of the formamide function.



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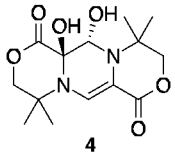

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Decreasing the concentration of acetic acid in the reaction mixture, using a considerably lower concentration of DDTTA (0.007 vs 0.035 mol/L), and use of substoichiometric quantities of O_2 yielded relatively pure samples of the oxidation product. These conditions favor the formation of **4** over the complex side reactions observed at high DDTTA concentrations. Subsequent kinetic experiments were performed at the lower DDTTA concentration. The purer product allowed for the detection of an OH stretch in the IR spectrum. In addition, ^{1}H NMR spectroscopy in dry, acid free, chloroform-*d* and in DMSO-d_6 revealed two OH protons that exchange with D_2O; one of these is coupled to a signal at 5.18 ppm. These signals were not previously observed as a result of traces of acetic acid remaining in the sample. The mass spectrum revealed a molecular ion at $m/z = 312.1307$ corresponding to a molecular formula of $C_{14}H_{20}N_2O_6$ (calculated $m/z = 312.1321$). These data collectively indicate that the substance is 4a,8a-diaza-9,9a-dihydroxy-2,6-dioxa-3,4,7,8,9,9a-hexahydro-4,4,8,8-tetramethylanthracene-1,5-dione (**4**). X-ray crystallography confirmed this hypothesis and established a trans relationship for the two hydroxy groups (Figure 1).

Diol **4** is more conveniently synthesized by reaction of DDTTA with 0.9 equiv of *tert*-butyl hydroperoxide in acetic acid/water under an inert atmosphere. It is also formed from reaction of DDTTA radical cation with potassium superoxide in wet dimethyl sulfoxide.

The difficulty observed in correctly identifying **4** resulted in part from its instability. In aqueous solution

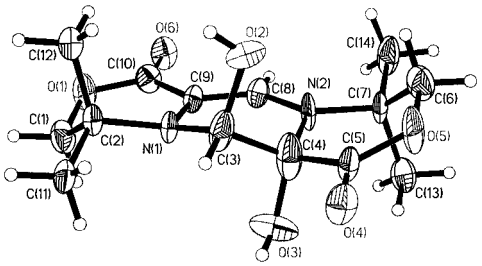
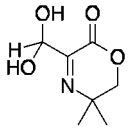


Figure 1. Thermal ellipsoid plot for diol **4** showing atomic numbering scheme. Ellipsoids are plotted at the 50% probability level.

trans diol **4** undergoes slow atmospheric oxidation to give, ultimately, the aldehyde **1** which exists in equilibrium with its hydrate (**5**). Addition of *tert*−butyl hydroperoxide to aqueous solutions of **4** produced **1** and **5** quantitatively. This susceptibility to further oxidation is a result of the electron rich diamino ene functionality of **4**. Because of the hemiaminal functionality of **4** the molecule is also susceptible to simple fragmentation reactions. In chloroform, **4** decomposes over several days at room temperature to give complex mixtures of products. These mixtures are unstable, and all attempts to isolate the components through silica gel chromatography resulted only in isolation of the aldehyde **1**.

This instability, and the observation of a molecular ion at $m/z = 310$, led to the incorrect assignment of a dioxetane structure. The $m/z = 310$ molecular ion is frequently observed in oxidized samples of DDTTA. Because this ion appears relatively stable and the diol **4** produces only a very weak molecular ion signal, unless the sample is relatively pure, the $m/z = 310$ signal can be easily mistaken for a molecular ion representing the bulk of the sample. High-resolution mass spectroscopy gives m/z=310.1174 (calcd for $C_{14}H_{18}N_2O_6$; m/z = 310.1164). This molecular formula is consistent with several possible structures including the diol oxidation product 9a-*H*-4a,8a-diaza-2,6-dioxa-9a-hydroxy-3,4,7,8-tetrahydro-4,4,8,8-tetramethylanthracene-1,5,9-trione (**6**), the originally proposed dioxetane (**2**), or the dioxetane cleavage product (**3**) suggested by Adam and co-workers. Without further spectroscopic data, these structures could not be distinguished. Since this product was only formed in trace amounts, further characterization was not pursued.



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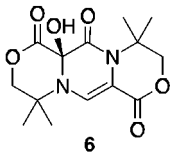

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In chloroform or dichloromethane, the diol is also produced along with several unstable products that arise from the decomposition of the diol. In acetonitrile, as previously reported, the predominant product is the aldehyde **1**. In all the solvents investigated, the rate of loss of DDTTA does not follow a simple rate law. Peroxide scavengers such as triphenylphosphine (in dichloromethane and acetonitrile) or trimethyl phosphite (in acetic acid/water) inhibit part of the oxidation pathway resulting in a strict exponential decay in [DDTTA] at constant [O_2]. In the presence of triphenylphosphine, oxidation produced triphenylphosphine oxide, diol **4**, and decomposition products arising from **4**. Oxidation of

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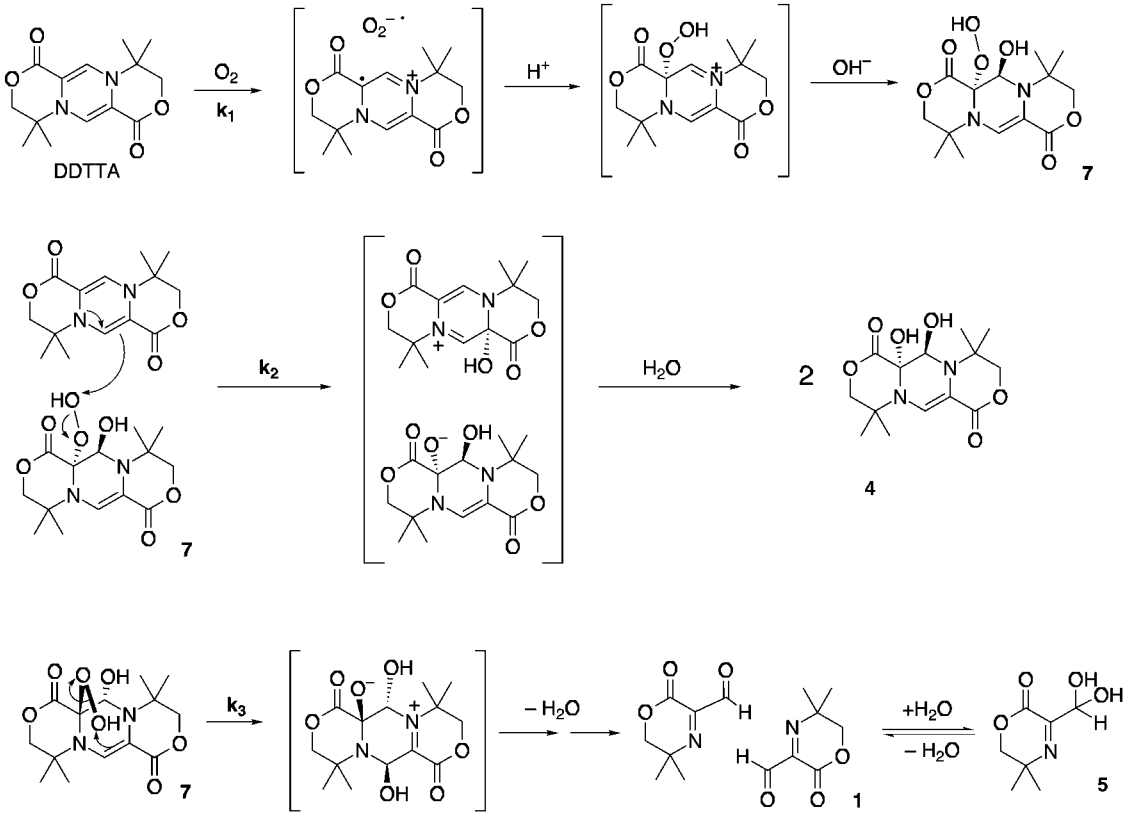
Scheme 1. Oxidation Pathway for DDTTA



DDTTA with *m*-chloroperbenzoic acid (MCPBA) in any stoichiometry yielded only aldehyde **1**. In acetic acid/water in a closed reaction vessel, addition of 1 equiv of oxygen resulted in the consumption of approximately 1.6 molar equiv of DDTTA. In a similar experiment in acetonitrile, 1 equiv of dioxygen consumed approximately 0.7 molar equiv of DDTTA.

These observations suggest the reaction pathway depicted in Scheme 1 for the reaction in aqueous acetic acid. Reaction of DDTTA with dioxygen and water gives the hydroperoxy alcohol **7**. This hydroperoxide reacts with a further equivalent of DDTTA and water to give two molecules of diol **4**. The observed *trans* stereochemistry is consistent with the addition of water to the least hindered face of the cationic intermediate. The observed stoichiometry (1 mol of O_2 consumes 1.6 mol of DDTTA) is less than the expected 1:2 ratio and indicates that other reactions are consuming hydroperoxide **7**. Aldehyde **1** was initially proposed to arise from direct addition of dioxygen to DDTTA followed by fragmentation.[13] Kinetic measurements disprove this hypothesis, and alternate mechanisms must be proposed. Aldehyde **1** could conceivably arise from the unimolecular decomposition of the hydroperoxide **7**, reaction of **7** with the diol **4**, or further oxidation of **4** with molecular oxygen. Of these pathways, the last can be discounted since complete oxidation to **1** takes ~1 week at room temperature. The second pathway results in regeneration of a molecule of diol, and this and the first pathway cannot be distinguished on the basis of stoichiometry; however, the second pathway is inconsistent with kinetic measurements (*vide infra*). Inspection of models indicates that the axial hydroperoxy group in **7** is ideally situated to oxidize the remaining double bond. The resulting intermediate could rapidly fragment and lose water to give **1**. Scheme 1 is also consistent with the observations in aprotic solvents, since water was not rigorously excluded from the reaction vessel.

The differential rate equations for this mechanism are given below.

$$\frac{[\mathrm{DDTTA}]}{\mathrm{d}t} = -k_1[\mathrm{DDTTA}][\mathrm{O_2}] - k_2[\mathrm{DDTTA}][\mathbf{7}]$$

$$\frac{\mathrm{d}[\mathbf{7}]}{\mathrm{d}t} = k_1[\mathrm{DDTTA}][\mathrm{O_2}] - {}_2[\mathrm{DDTTA}][\mathbf{7}] - k_3[\mathbf{7}]$$

$$\frac{\mathrm{d}[\mathbf{4}]}{\mathrm{d}t} = 2k_2[\mathrm{DDTTA}][\mathbf{7}]$$

$$\frac{\mathrm{d}[\mathbf{1}]}{\mathrm{d}t} = 2k_3[\mathbf{7}]$$

$$\frac{\mathrm{d}[\mathrm{O_2}]}{\mathrm{d}t} = -k_1[\mathrm{DDTTA}][\mathrm{O_2}]$$

In a stirred solution, equilibration of dissolved oxygen with the atmosphere is rapid and $[O_2]$ is effectively constant. With addition of an efficient peroxide scavenger, the reaction becomes pseudo first order with rate equation

$$\frac{\mathrm{d}[\mathrm{DDTTA}]}{\mathrm{d}t} = -k'_1[\mathrm{DDTTA}]$$

where $k_1' = k_1\,[O_2]_0$. Rate constant k_1 can then be determined from the rate of loss of DDTTA and the concentration of dissolved oxygen. In a solution saturated with DDTTA and containing excess undissolved DDTTA, provided that the rate of solution of DDTTA is

Table 1. Rate Constants for Dioxygen Oxidation of DDTTA (Scheme 1)

solvent	$k_1/M^{-1}\ s^{-1}$	$k_2/M^{-1}\ s^{-1}$	k_3/s^{-1}
acetic acid/water (2:3 v/v)	0.060(2)	0.13(5)	
acetonitrile	0.131(2)	0.20(4)	0.0019(4)
dichloromethane	0.006(2)	0.004(5)	

fast compared with the rate of oxidation, [DDTTA] is effectively constant. Under these conditions, if no additional oxygen enters the system, the rate equations reduce to

$$\frac{d[\mathbf{7}]}{dt} = k_1''[O_2] - (k_2' + k_3)[\mathbf{7}]$$

$$\frac{d[\mathbf{4}]}{dt} = 2k_2'[\mathbf{7}]$$

$$\frac{d[\mathbf{q}]}{dt} = 2k_3[\mathbf{7}]$$

$$\frac{d[O_2]}{dt} = -k_1''[O_2]$$

where $k_1'' = k_1[\text{DDTTA}]_0$ and $k_2' = k_2[\text{DDTTA}]_0$. The problem thus becomes a combination of consecutive and competing pseudo-first order reactions. This system has an analytical solution with the concentrations of peroxide **7**, diol **4**, and aldehyde **1** given by

$$[\mathbf{7}] = k_1''[O_2]_0\left\{\frac{e^{-k_1''t} - e^{-(k_2'+k_3)t}}{k_2' + k_3 - k_1''}\right\}$$

$$[\mathbf{4}] = 2k_2'[O_2]_0 \left\{\frac{k_1''(e^{-(k_2'+k_3)t} - 1) + e^{-k_1''t}(e^{-k_1''t} - 1)(k_2' + k_3)}{(k_2' + k_3)(k_2' + k_3 - k_1'')}\right\}$$

$$[\mathbf{1}] = 2k_3[O_2]_0 \left\{\frac{k_1''(e^{-(k_2'+k_3)t} - 1) + e^{-k_1''t}(e^{-k_1''t} - 1)(k_2' + k_3)}{(k_2' + k_3)(k_2' + k_3 - k_1'')}\right\}$$

Observation of the generation of aldehyde **1** by ^{1}H NMR then allows the determination of rate constants k_2 and k_3. In acetonitrile, rate constants k_1, k_2, and k_3 were determined by nonlinear least-squares fitting to the respective integrated rate laws. Numerical integration of the differential rate laws was used to refine these rate constants, using additional data from oxidation of DDTTA under conditions of constant $[O_2]$. The resulting rate constants were within 10% of the analytically determined values and are reported in Table 1. In acetic acid/water and in dichloromethane only small traces of **1** are formed. Because of this, meaningful values of k_3 could not be determined in these solvents. In these cases, k_2 was determined by numerical integration and fitting to DDTTA concentration data using previously determined values of k_1. Plots of observed and calculated concentrations are shown in Figure 2. Inclusion of the bimolecular reaction of **7** with **4** in the mechanistic scheme does not produce improved curve fits. Furthermore, replacement of the unimolecular fragmentation of **7** with the reaction of **7** and **4** reduces the quality of the curve fit and results in unreasonably large values for k_2. We conclude that the reaction of **7** with **4**, though a feasible reaction, does not form a significant part of the reaction pathway.

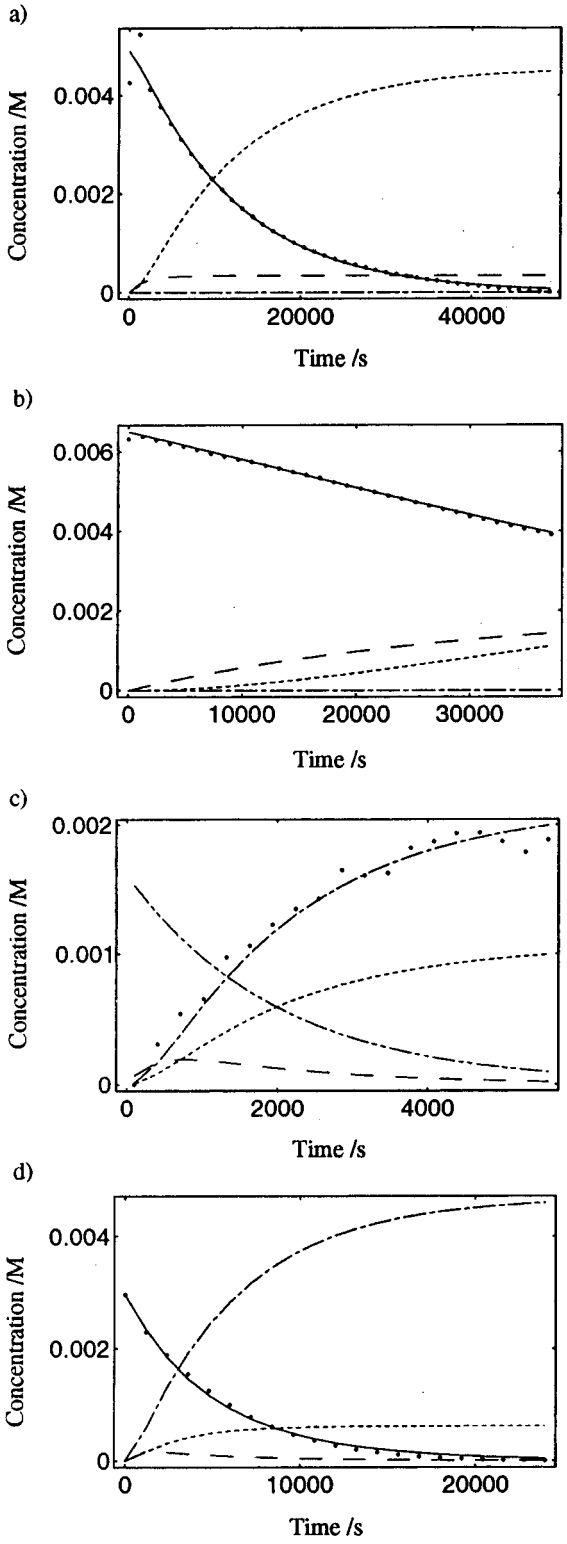


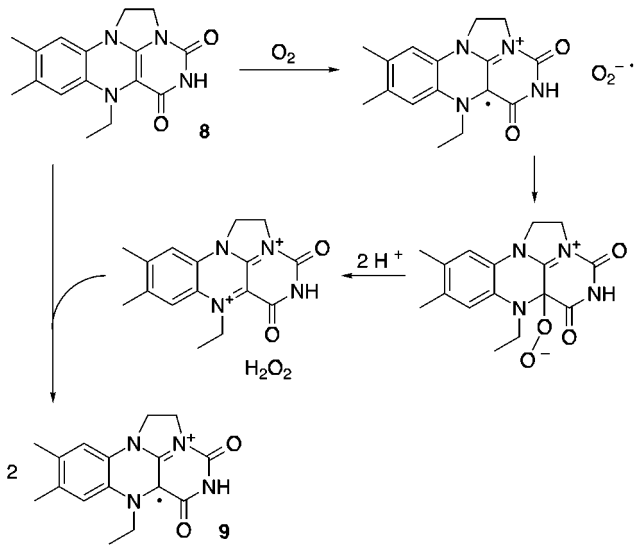
Figure 2. Oxidation of DDTTA vs time. (a) In acetic acid/water (3:2 v/v) with constant $[O_2]$. (b) In dichloromethane with constant $[O_2]$. (c) In acetonitrile with constant [DDTTA]. (d) In acetonitrile with constant $[O_2]$. Observed data points are represented by small circles (·). The lines are calculated concentrations of DDTTA (—), **7** (— —), **4** (- - -), **1** (— - —), and dioxygen (— - - —) using the kinetic scheme and rate constants described in the text.

Discussion

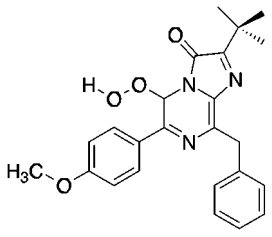
The kinetic data are consistent with the proposed reaction scheme and confirm that the oxidation is subject to pronounced solvent effects. The initial reaction of dioxygen with dihydroflavins or dihydroflavin analogs is generally assumed to proceed through outer-sphere electron transfer[15] to give a radical cation and superoxide, followed by spin inversion and radical combination to give the hydroperoxide. Presumably, oxidation of imidazo[1,2-*a*]pyrazines is similar. Available evidence suggests that dioxygen oxidation of DDTTA initially follows the same pathway. $DDTTA^{+\bullet}$ reacts directly with $O_2^{-\bullet}$ giving, ultimately, diol **4**. Trapping experiments with triphenylphosphine strongly indicate the existence of a peroxide intermediate. The coupling reaction between various oxygen radical species and flavin semiquinone radicals has been measured and is fast ($k \approx 10^8-10^9$ M^{-1} s^{-1}).[16] This accounts for the predominance of radical combination rather than escape from the solvent cage in these reactions. In all cases the rate of reaction of dioxygen with DDTTA is considerably slower than reported rates for dihydroflavins or alkylated dihydroflavins which range from 17 to 1500 M^{-1} s^{-1}.[17,18] This likely results from differences in the dihydropyrazine substitution; DDTTA bears two directly bonded electron-withdrawing carbonyl groups and is thus less electron rich.

Significantly, there is no evidence for autocatalysis in the oxidation of DDTTA, except in unpurified solvents. Autocatalysis is typically observed in flavin oxidations and results from the formation and rapid oxidation of flavin semiquinone radical. While DDTTA radical cation is formed in DDTTA oxidations (as a minor product in $HOAc/H_2O$ and the major product in trifluoroacetic acid), it is relatively inert to oxygen. In this respect, the oxidation of DDTTA is similar to the oxidation of 1,5-dihydro-1,10-ethano-5-ethyllumiflavin (**8**).[19] Flavin **8** is oxidized cleanly in aqueous solution (with a second order rate constant $k = 17$ M^{-1} s^{-1})[19] to give only the corresponding radical cation (Scheme 2). As discussed by Eberlein and co-workers, the rate-limiting step in this reaction is oxidation of the dihydroflavin by dioxygen to give the 4a-hydroperoxide.[19] The latter rapidly eliminates H_2O_2 and is reduced by a further equivalent of dihydroflavin to give two molecules of radical cation **9**. As in DDTTA, radical cation **9** is air stable and oxidation proceeds no further. In the oxidation of DDTTA, elimination of water from **4**, and by inference hydrogen peroxide from **7** to form DDTTA dication, only occurs under highly acidic conditions. Instead, the hydroperoxide **7** undergoes an intramolecular redox reaction to give the aldehyde **1**. This fragmentation is unique among reported pyrazine hydroperoxides, and the availability of this mechanism may account for the low reactivity of the hydroperoxide toward other substrates. Flavin hydroperoxides, in addition to eliminating hydrogen peroxide as in **8**, are also powerful oxidants.[20,21] They readily oxidize amines to amine oxides and are the active oxidant

Scheme 2. Oxidation Pathway for Flavin 8, As Determined by Eberlein and Co-workers



in the enzymatic hydroxylation of aromatic rings.[2] In contrast, hydroperoxide **7** only oxidizes more reactive species such as phosphines or DDTTA; presumably simple amines are not active enough to compete with the intramolecular pathway. Reaction of **7** contrasts with reaction of the hydroperoxy pyrazine **10** which was recently synthesized as part of a study of coelenterate luciferin.[11] This molecule has a suitably positioned double bond to fragment analogously to **7**. It also has an imine adjacent to the hydroperoxide which allows cyclization to give a dioxetane. Surprisingly the latter reaction appears to dominate the decomposition of **10**. Hydroperoxide **10** decomposes with a half-life of 1.3 h in methanol. Weak chemiluminescence is observed, and the products are consistent with formation and cleavage of a dioxetane. The difference in reactivity of **7** and **10** probably results from a more electron rich diamino ene functionality and the absence of an imino group in **7**.



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Conclusion. In acetic acid/water mixtures and in dichloromethane, dioxygen oxidation of DDTTA produces an unstable diol. The oxidation is proposed to occur through a hydroperoxide intermediate which reacts further with DDTTA. Peroxide scavengers effectively compete for this hydroperoxide resulting in pseudo-first-order kinetics of disappearance of DDTTA. Both the initial oxidation rate and reactivity of the intermediate hydroperoxide show significant solvent dependence. In acetonitrile, a unique, unimolecular fragmentation of the hydroperoxide is the major reaction pathway and results in the formation of an aldehyde as the major reaction product.

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Experimental Section

General. Synthesis of DDTTA, DDTTA radical cation trifluoroacetate, and spectroscopic measurements were as previously described.[13] Oxygen concentrations were determined by application of the Henry's law constant to literature values.[22] Deionized water with resistivity of 18.2 MΩ cm was obtained from a Millipore Milli-Q UF-Plus water purification system. Analysis of rate equations, numerical integration, and curve fitting were performed with Mathematica.[23]

Reaction of DDTTA with Dioxygen in Acetic Acid/Water. Formation of 4a,8a-Diaza-9,9a-dihydroxy-2,6-dioxa-3,4,7,8,9,9a-hexahydro-4,4,8,8-tetramethylanthracene-1,5-dione. (4) A mixture of 3.0 mL of distilled, de-ionized water and 2.0 mL of glacial acetic acid was purged with nitrogen for 10 min. DDTTA (10 mg) was added with stirring and the solution purged a further 5 min. Air (0.6 mL) was added with a gas tight syringe and the solution stirred for 12 h. Evaporation of the solvent left a greenish residue that was redissolved in deuteriochloroform. ^{1}H NMR showed the presence of only unreacted DDTTA and 4a,8a-diaza-9,9a-dihydroxy-2,6-dioxa-3,4,7,8,9,9a-hexahydro-4,4,8,8-tetramethylanthracene-1,5-dione (**4**). The latter had the following: ^{1}H NMR ($CDCl_3$) δ 7.15 (s,1H), 5.18 (s, 1H), 4.68 (d, 1H, J = 11.5 Hz) 4.15 (d, 1H, J = 11.0 Hz), 4.09 (d, 1H, J = 11.5 Hz), 4.00 (d, 1H, 11.0 Hz), 1.42 (s, 3H), 1.41 (s, 3H), 1.39 (s, 3H), 1.31 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.5, 162.9, 121.8, 105.8, 80.5, 77.20, 75.9, 73.6, 54.4, 52.3, 25.3, 25.0, 23.4, 22.9 The concentration of DDTTA radical cation was minimal as indicated by the sharp DDTTA resonances.

Layering of the chloroform solution with heptane and diffusion of the layers at −20 °C resulted in the formation of a white precipitate of **4**. This showed IR (KBr) bands at 3448 (OH), 1734 (C=O), 1617 cm^{-1} (C=C); exact mass (CI$^+$) m/z = 312.1307, calcd for $C_{14}H_{20}N_2O_6$ m/z = 312.1321. Crystallization from dichloromethane/heptane with trace water provided orthorhombic crystals suitable for X-ray analysis.[24]

Stoichiometry of the Reaction of DDTTA with Dioxygen. Representative techniques used for measurement in acetic acid/water are described. Similar techniques were used for stoichiometry measurements in other solvents.

a. By NMR Spectroscopy. DDTTA (10 mg, 36 μmol) was dissolved in a mixture of 2.0 mL of acetic acid and 3.0 mL of distilled, deionized water, previously purged with nitrogen. The flask was sealed with a septum and further purged with N_2 (U.S.P grade) for 5 min. Oxygen (0.30 mL at 630 mmHg, 10 μmol) was added to the flask using a gas tight syringe and the solution stirred for 24 h. Evaporation gave a green solid. Integration of the NMR spectrum in chloroform gave a ratio of DDTTA to **4** of 5:4 indicating consumption of 1.6 mol of DDTTA per mol of O_2. Aldehyde **1** was not detectable in the NMR spectrum.

b. By UV−Visible Spectrophotometry. A 1 cm path length cuvette, previously attached to the base of a 25 mL round bottomed flask and equipped with a rubber septum and magnetic stir bar, was filled with a mixture of distilled, deionized water (3.0 mL) and glacial acetic acid (2.0 mL) and purged with N_2. DDTTA (10 mg, 36 μmol) was added and the solution purged for a further 15 min. Monitoring the UV−vis absorbance of DDTTA at 605 nm over a 24 h period showed negligible oxidation of DDTTA resulting from diffusion of air through the septum. Air (2.0 mL, 14 μM O_2 at 640 mmHg) was injected into the flask with a syringe and the absorbance at 605 nm followed over a 24 h period. After 24 h, the absorbance had dropped from 1.3 to 0.5, and no further decay was observed. Using an extinction coefficient of 180 (previously measured for DDTTA in this solvent mixture), this corresponds to oxidation of 1.6 mol of DDTTA for each mole of oxygen.

Reaction of DDTTA with *tert*-Butyl Hydroperoxide in Acetic Acid/Water. Water (15 mL) and acetic acid (5 mL) were combined and purged with nitrogen for 5 min. DDTTA (51.3 mg, 0.18 mmol) was added and purging continued for 5 min. *tert*-Butyl hydroperoxide (15 μL of a 90% solution) was added and the solution stirred under nitrogen for 40 min. Evaporation of the solvent left a blue oil to which 1 mL of D_2O was added. ^{1}H NMR indicated one major product, the diol **4**, with the following spectral properties: ^{1}H NMR (D_2O) δ 7.19 (s, 1H), 5.24 (s, 1H), 4.55 (d, 1H, J = 11.4), 4.27 (d, 1H, J = 11.4), 4.19 (d, 1H, J = 11.4), 4.10 (d, 1H, J = 11.4), 1.42 (s, 3H), 1.36 (s, 3H), 1.29 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (D_2O) δ 167.8, 166.1, 122.0, 104.9, 80.1, 76.1, 73.9, 72.3, 53.6, 50.8, 23.2, 23.0, 21.3, 21.2. Excess DDTTA precipitated as a green solid and was removed by filtration. The filtrate was evaporated to give 17 mg of diol **4** (36% based on peroxide). In deuteriochloroform this species gave an ^{1}H NMR spectrum indistinguishable from the product obtained from dioxygen oxidation. ^{1}H NMR indicated that the diol was contaminated with aldehyde **1** (as the hydrate) with resonances (in D_2O) at δ 5.09 (s, 1H), 4.26 (s, 2H), 1.38 (s, 6H). The concentration of **1** increased slowly when an aqueous acetic acid solution of **4** was allowed to stand in air.

Oxidation of DDTTA with 3-Chloroperbenzoic Acid. To a solution of 5 mg DDTTA in 1 mL of N_2 purged $CDCl_3$ was added 2.3 mg (0.5 equiv, 67% peracid by titration) of 3-chloroperbenzoic acid. After shaking to dissolve the peracid, the blue color of DDTTA was still apparent. NMR indicated the presence of only 3-chlorobenzoic acid and aldehyde (**1**) and unreacted DDTTA in a 2:1 ratio.

Oxidation of Diol 4 with *tert*-Butyl Hydroperoxide. One drop of 90% *tert*-butyl hydroperoxide (excess) was added to a solution of diol **4** in D_2O. ^{1}H NMR indicated a rapid and quantitative conversion to the aldehyde hydrate 5,6-dihydro-3-(dihydroxymethyl)-5,5-dimethyl-1,4-oxazine-2-one, **5**.

Reaction of DDTTA Radical Cation with Potassium Superoxide in DMSO. DDTTA radical cation trifluoroacetate (14.7 mg), sodium bicarbonate (2.0 mg), and potassium superoxide (1.0 mg) were combined in 1 mL of dimethyl sulfoxide-d_6. Slight effervescence was observed. ^{1}H NMR revealed one product with resonances at δ 6.85 (s, 1H), 4.97 (s, 1H), 4.40 (d, 1H, J = 11.4), 4.162 (d, 1H, J = 11.4), 4.06 (d, 1H, J = 10.4), 4.918 (d, 1H, J = 10.4), 1.33 (s, 3H), 1.25 (s, 3H), 1.22 (s, 3H), 1.18 (s, 3H). Comparison with the ^{1}H NMR spectrum of an authentic sample in DMSO indicated that the product was the diol **4**.

Oxidation of DDTTA in the Presence of Ph_3P. DDTTA (10 mg, 36 μmol) was dissolved in 1 mL of CH_2Cl_2 with 20 mg (76 mmol) of triphenylphosphine. The solution was stirred vigorously in air and the absorbance at 596 nm recorded vs time. The absorbance decayed exponentially with time constant, k_{obs}=2 × 10^{-5} s^{-1}. After evaporation of the solvent, ^{1}H NMR in $CDCl_3$ revealed the presence of triphenylphosphine, triphenylphosphine oxide, unreacted DDTTA, **4**, and decomposition products of **4**.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for diol **4** (2 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(22) Murov, S. L.; Carmicheal, I.; Hug, G. L. *Handbook of Photochemistry*; Marcel Dekker: New York, 1993.

(23) Wolfram Resarch, Inc., Mathematica v. 2.2, Champaign, IL, 1995.

(24) The authors have deposited details of the data collection and refinement, and tables of atomic coordinates with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.