Inductive versus Coulombic Effects on the Barriers to Oxygen Atom Transfer from Alkyl Hydroperoxides. Model Studies on 4α-Flavin Hydroperoxide

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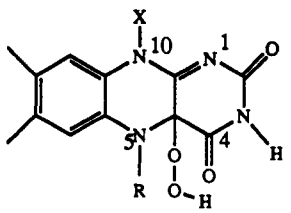
Abstract: Ab initio molecular orbital calculations have been used to study the oxidation of ammonia by a series of substituted methyl hydroperoxides XCH_2OOH (X = H, NH_2, OH, F, HC=NH, HCO). Geometries of reactants and transition states were fully optimized at the MP2/6-31G* level, and relative energies were computed at the MP4/6-31G*//MP2/6-31G* level. The barrier height for the oxidation of ammonia with CH_3OOH is predicted to be 46.6 kcal/mol. Activation energies for oxygen atom transfer from substituted CH_3OOH (XCH_2OOH) to ammonia range from 44.4 to 35.0 kcal/mol, suggesting that electronegative substituents exert a relatively small influence on the reactivity of alkyl hydroperoxides. Substituted methyl hydroperoxides $X\text{-}CH_2OOH$ (X = $-CH{=}NH_2^+$, H_3N^+) bearing a positive charge are highly efficient oxygen donors. The barrier height for the oxidation of ammonia by H_3N^+-CH_2OOH is reduced to 10.3 kcal/mol, and when a disubstituted protonated methyl hydroperoxide (H_3N^+, CHO) is used as the oxygen donor, the predicted barrier is 2.9 kcal/mol. These data suggest that through-space coulombic effects are much more effective than through-bond inductive effects in activating an alkyl hydroperoxide toward oxygen donation. Extension of these arguments to the origin of the atypical reactivity of flavin hydroperoxides suggests that protonation of 4α-flavin hydroperoxide at N_1 or N_5 would stabilize the transition state for oxygen atom transfer.

Introduction

One of the most important biochemical oxygen atom transfer reactions involves catalysis by flavoenzymes.[1-3] The flavin coenzyme functions as an electron conduit for a variety of substrates. These tricyclic isoalloxazine moieties are among the more versatile of the redox cofactors in biochemistry. When molecular oxygen is the reducible substrate for dihydroflavin reoxidation, the highly cited flavin 4α-hydroperoxide (**1a**) has



1a R=H
1b R=Et

4α-Flavin Hydroperoxide

been implicated as the key intermediate that serves either as the oxygen donor or its immediate precursor. *para*-Hydroxybenzoate hydroxylase (PHBH) has become the paradigm aromatic hydroxylase because of extensive kinetic[1b] and X-ray structural studies.[1d,4] We were particularly intrigued by the observation that a water molecule and the bihydroxide ion ($H_3O_2^-$) are present at the active site of PHBH. Native FlH(4α)OOH (**1a**) is a highly reactive oxygen donor with a half-life on the order of 2.5 ms.[2a] The importance of solvent around the flavin N_5—C_4=O region[5] and the effects of solvent access to the N_5 position[6] have been discussed. Upon substitution of normal flavin with 5-deazaflavin, the mechanism of catalysis is switched to that of pyridine nucleotides, i.e. hydride transfer. In contrast, 1-deazaflavins retain most of the chemical reactivity of normal flavins, including rapid reaction of the reduced flavin with dioxygen.[5a]

Bruice and his co-workers have shown that *N*-ethyl hydroperoxide **1b** (FlEt(4α)OOH) is an isolable alkyl peroxide that has proven to be a highly successful model for enzymatic studies.[2b,7] The N_5-ethyl substituent on **1b** prevents the elimination of H_2O_2 to afford the oxidized form of flavin. Intramolecular hydrogen bonding to N_5 has also apparently been discounted as a primary driving force for the displacement reaction since the excellent correlation with the pK_a of the departing "alcohol" for a series of ROOH oxygen donors suggested that the increased reactivity of **1b** could be attributed to inductive stabilization of the alcoholate leaving group. Model coenzyme **1b** is intermediate in reactivity between a peroxy acid and an alkyl hydrogen peroxide.[1c,2b,7] Flavin hydroperoxide **1b** has been established to be 10^4 times more reactive than H_2O_2 as an oxygen donor. However, its inductive effects are apparently not as effective at stabilizing an alkoxide anion as a *m*-chlorobenzoate anion because *m*-chloroperbenzoic acid (MCPBA) is about 10^5 more reactive than **1b** as an oxygen donor to amines and sulfides.[7c] Replacement of N_1 in **1b** with a carbon atom (C_1-4α-FlEtOOH) also results in a reduction in reactivity.[7d] A common mechanism involving nucleophilic attack on the distal oxygen with a concerted 1,2-hydrogen shift has been suggested on the basis of structure reactivity relationships among a series of nucleophiles including N, S, I^-, etc. (eq 1). The rate of oxygen donation is directly related to the acidity of the pseudobase leaving group (4α-FlEtOH), consistent with an S_N2

* Abstract published in *Advance ACS Abstracts*, May 15, 1994.

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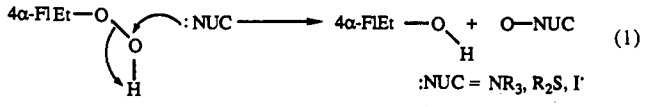
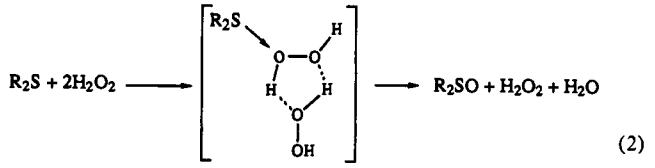


(1)

mechanism. The present study strongly suggests that a simple S_N2 displacement (eq 1) involving a 1,2-hydrogen shift in concert with oxygen atom transfer cannot account for the oxygen donation potential of native **1a**. More recently we have augmented the generally accepted mechanism involving attack by the substrate on the distal oxygen with a direct nucleophilic displacement of the β-peroxy oxygen (eq 1) to include the energetic requirements for the 1,2-hydrogen transfer to the departing alkoxide anion after the activation barrier is crossed.[8a,b]

On the basis of ab initio calculations, we have suggested[8a,b] that the reactive form of hydrogen peroxide in the gas phase is its high-energy isomeric form water oxide (H_2OO).[9] The potential energy surface for oxygen donation from H_2O_2 to amines and alkenes in the gas phase is almost entirely dominated by the energetic requirements (56.0 kcal/mol) for the accompanying 1,2-hydrogen migration to afford water oxide.[8a] The origin of the barrier for a 1,2-hydrogen shift across an O–O bond may be attributed in part to the fact that the rearrangement is a 4-electron process involving a filled in-plane σ-type O–O bond at the migration terminus that has pseudo π* symmetry and the concerted process is formally forbidden on the basis of symmetry arguments. Although the barrier for a concerted S_N2 displacement by ammonia on water oxide is surprisingly high (51.3 kcal/mol when measured relative to hydrogen peroxide), a direct displacement of OH^- from H_2O_2 without a 1,2-hydrogen shift would afford OH^- and H_3NOH^+ which is 198.2 kcal/mol (HF/6-31G*) above the energy of the reactants in the gas phase. Solvation of a hydroxide ion by one water molecule affording bihydroxide ion ($H_3O_2^-$) is accompanied by only 35.2 kcal/mol (HF/6-31G*) of stabilization. Consequently, oxygen transfer from a hydroperoxide to a nucleophilic amine can in principle be accompanied by (a) a 1,2-hydrogen migration that takes place prior to rate-limiting O–O bond rupture, (b) hydrogen migration in concert with the oxidation step, or (c) proton transfer in a separate step after N–O bond making in the transition state. In discussions of this mechanism, provisions must be made for the timing of the hydrogen transfer that accompanies oxygen atom transfer from a hydroperoxide. In principle, a water molecule serving as a catalyst could circumvent this problem in an alkyl hydrogen peroxide like **1a** by accepting a proton from the distal peroxide oxygen and transferring one of its hydrogens to the proximal oxygen (α-oxygen of the ROOH), effecting a formal 1,4-hydrogen shift.[8b] For example, when oxygen transfer was computed relative to hydrogen peroxide and two isolated water molecules, the barrier height was reduced to 11.2 kcal/mol for an overall decrease in activation energy of 40.2 kcal/mol. We were not particularly surprised about the nucleophilic attack being facilitated by solvent-assisted proton transfer, since this is a well-established precedent for both hydrogen peroxide and alkyl hydrogen peroxides.[10] In aprotic solvent, sulfide oxidation is second order in hydrogen peroxide and the second molecule of peroxide plays the role of the protic solvent (HA) in the proton shift (eq 2).[10a] Although we have predicted that a remarkable



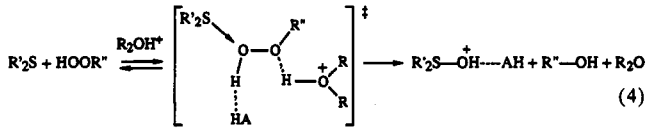
(2)

decrease in the $\Delta E^{\ddagger}$ results from this water catalysis with oxidations involving hydroperoxide,[8a] the barriers for these oxygen-transfer reactions when measured from gas-phase reactant clusters are still much too high to be commensurate with the oxygen donor potentials of hydroperoxides such as native **1a**.

We have recently addressed two fundamental questions concerning the transition state for oxygen donation from hydroperoxides: (a) the position of the hydrogen of the –OOH moiety in the TS and (b) solvation requirements for facile O–O bond cleavage.[8c] These ab initio calculations strongly support a two-step oxidative process where a proton transfer takes place after nucleophilic attack at the distal oxygen of ROOH and transfer of HO^+ to the nucleophile (eq 3). When the oxidation step is general acid catalyzed by a second molecule of ROOH (eq 2), calculated activation barriers range between 35 and 50

$$RO\text{–}OH + :NUC \rightarrow [RO^- HO\text{—}NUC^+] \rightarrow R\text{–}OH + O\text{–}NUC \quad (3)$$

kcal/mol. Although kinetic evidence is consistent with the bimolecularity of H_2O_2 in anhydrous dioxane solvent, theoretical calculations suggest that some additional type of catalysis must be required in order to bring both theoretical and experimental evidence into agreement. When proton transfer from the solvent is combined with protic solvent (HA) stabilization of an ionic transition state, the calculated activation barriers (MP4//MP2/6-31G*) are reduced to 5–15 kcal/mol. An overall suggested mechanism for the oxidation of a sulfide involves a protonated solvent molecule (R_2OH^+) transferring a proton to the distal oxygen of the hydroperoxide in concert with a second molecule of solvent (HA) stabilizing the formal transfer of HO^+ from the peroxide to the nucleophile (eq 4).[8c] By analogy, a comparable



(4)

reaction occurring in a flavin-mediated oxygen atom donation could also involve a similar proton transfer to the α-oxygen after displacement of the pseudobase leaving group 4α-FlO^-. However, no evidence for general acid catalysis in S-oxidation and N-oxidation by flavin hydroperoxide **1b** was observed.[7a] Given the observed reactivity of native **1a**, a process involving a simple 1,2-hydrogen migration in concert with oxygen atom transfer (eq 1) would exhibit an energy barrier that is far in excess of that of an oxygen donor that is capable of achieving aromatic hydroxylation, a feat that unactivated model systems have not yet been found competent to achieve. The atypical reactivity of this rather complex but short-lived hydroperoxide demands a unique explanation. In an earlier report we suggested that hydrogen bonding by the N_5 hydrogen on one side[8b] and one (or two) water molecule on the other would afford a stabilized transition structure for oxygen transfer from native flavin **1a**, providing a novel explanation for the unusual monooxygenase reactivity of flavins. The data in the present study strongly suggest that Coulombic effects can make a more substantial contribution to the unusual oxygen donor capacity of 4α-flavin hydroperoxide (**1a**) than has been recognized previously.

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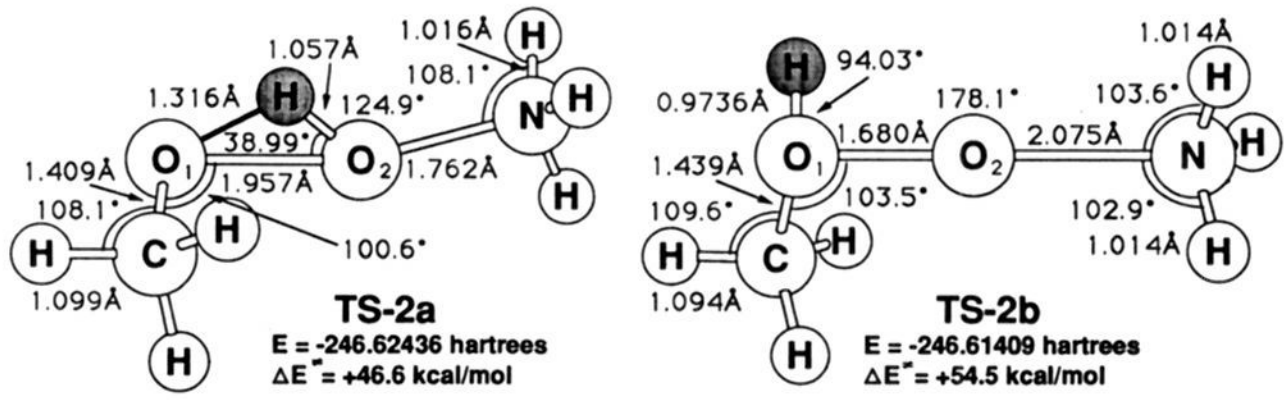


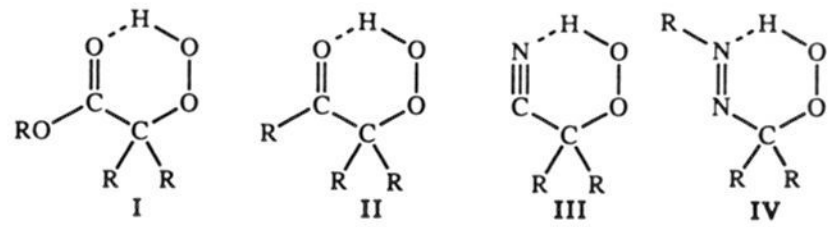
Figure 1. Transition structures and activation barriers for the oxidation of ammonia by methyl hydroperoxide: the position of the hydrogen in the transition state (MP4//MP2/6-31G*). Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

Method of Calculation

Molecular orbital calculations were carried out using the Gaussian 92 program system[11a] utilizing gradient geometry optimization.[11b] The geometries of the reactants and transition structures were first determined at the MP2/3-21G level of theory. All geometries were then fully optimized without geometry constraints using second-order Møller–Plesset perturbation theory (MP2/6-31G*). Relevant energies and barrier heights were computed with the 6-31G* basis set using fourth-order Møller–Plesset perturbation theory (frozen core, MP4SDTQ/6-31G*//MP2/6-31G*). Vibrational frequency calculations at the MP2/6-31G* level were used to characterize all stationary points as either minima (zero imaginary frequencies), first-order transition states (a single imaginary frequency), or second-order saddle points (SOSP, two imaginary frequencies). All activation barriers reported are with zero point energy corrections, and relative energies cited are at the MP4//MP2/6-31G* level unless noted otherwise.

Results and Discussion

In model studies involving 4α-hydroperoxy-5-alkylflavins, Bruice[7e] convincingly demonstrated that the rate of oxygen atom transfer to sulfides, amines, and iodide ion by a series of hydroperoxides (ROOH) is dependent upon the ability of leaving group RO^- to support a negative charge. An excellent correlation was observed between the second-order rate constants for sulfide oxidation and the pK_a of the alcohol (ROH) corresponding to the departing alkoxide. The high reactivity of 4α-hydroperoxyflavins (Fl–OOH) was attributed to the electronegativity of the 4α-position and hence the good leaving group ability of Fl–O^-. The pK_a of Fl–OH (ca. 9.4) is markedly lower than the pK_a of water, the reduced form of H_2O_2, and based upon their relative pK_a's, Fl–O^- anion is considerably more stable than the anion of *tert*-butyl alcohol. The ability of Fl–O^- to support a developing negative charge in the TS for oxygen transfer has been ascribed to the electronegativity of the ring nitrogens at positions 1, 5, and 10, as well as to the carbonyl group at position 4. It has also been speculated that the 4α-flavins have structural features that allow cyclic proton transfer in the TS which would facilitate oxygen transfer.[12a,b] The same internal proton transfer has been invoked for oxidations with α-hydroperoxy esters **I**, α-hydroperoxy ketones **II**, α-hydroperoxy nitriles **III**, and α-azohydroperoxides **IV**.[12,13] However, any contribution of internal hydrogen bonding of this type to the driving force for epoxidation has been questioned.[7c]



Our approach to the origin of the reactivity of **1a** in the present work has been to examine the inductive and hydrogen bonding effects of the various functionalities in flavin hydroperoxide **1a**. The substituents on C_4 in **1a** may be abbreviated by considering the series of compounds below that we have examined as derivatives of the parent alkyl hydroperoxide CH_3OOH.

The position of the migrating hydrogen in the transition state for the oxidation of ammonia with methyl hydroperoxide has now been firmly established.[8c] The activation energy for oxygen transfer to ammonia in concert with a 1,2-hydrogen shift (TS-**2a**, Figure 1) is 7.9 kcal/mol lower in energy than the two-step process involving a 1,2-hydrogen shift affording methanol oxide prior to rate-limiting O–O bond rupture (TS-**2b**). Both of these stationary points exhibit a single imaginary frequency at the MP2/6-31G* level. We attribute the change in mechanism for CH_3OOH relative to oxidation with H_2O_2[8a] to the enhanced stabilization of leaving group CH_3O^- relative to HO^- in the gas phase. The normal mode for the single imaginary frequency for TS-**2a** is comprised largely of the hydrogen migration.[8c]

To examine the effects of the electronegativity of an α-substituent on the stabilization of the alkoxide leaving group, we have employed X–CH_2OOH as the oxidant (X = NH_2, OH, F). Surprisingly, α-aminomethyl hydroperoxide (**3**) (Figure 2) does not exist as a minimum in a conformation where intramolecular hydrogen bonding to the amine lone pair is observed. The transition state ($\Delta E^\ddagger$ = 44.4 kcal/mol) for the oxidation of ammonia to H_3N–O (TS-**4a**) was only 2.2 kcal/mol lower than that for the reduction of the parent hydroperoxide CH_3OOH (Figure 1). When the nitrogen lone pair of the NH_2 group is involved in hydrogen bonding to the migrating hydrogen (N_5–H_3, TS-**4b**), the activation barrier is substantially increased ($\Delta E^\ddagger$ = 50.0 kcal/mol). Both TS-**4a** and TS-**4b** are first-order saddle points as established by an analytical frequency calculation at both MP2/3-21G and MP2/6-31G* levels. The increase in the barrier height possibly reflects a higher energy conformation of the oxidant. This result would tend to exclude a meaningful contribution from hydrogen bonding to N_5 in flavin hydroperoxide **1a**.

When the electronegativity effect of a hydroxyl substituent was examined, the TS for the oxidation of ammonia with **5** (Figure 3) was stabilized by 4.3 kcal/mol relative to TS-**2a** (Figure 1). This reduction in barrier could be due in part to the hydrogen bonding of the hydroxyl group with the developing alkoxide ion (H_6–O_1) as evidenced in TS-**6**. When fluoro-substituted hydroperoxide **7** was employed as the oxygen donor, the barrier height ($\Delta E^\ddagger$ = 35.0 kcal/mol) was reduced by 11.6 kcal/mol relative to unsubstituted CH_3OOH (TS-**2a**). The trends in geometry changes noted for the transition structures as the electronegativity

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(13) It has been suggested that five-membered intramolecular hydrogen bonding in α-azo hydroperoxides plays a major role in oxygen atom transfer: Baumstark, A. L.; Vasquez, P. C. *J. Org. Chem.* **1987**, *52*, 1939.

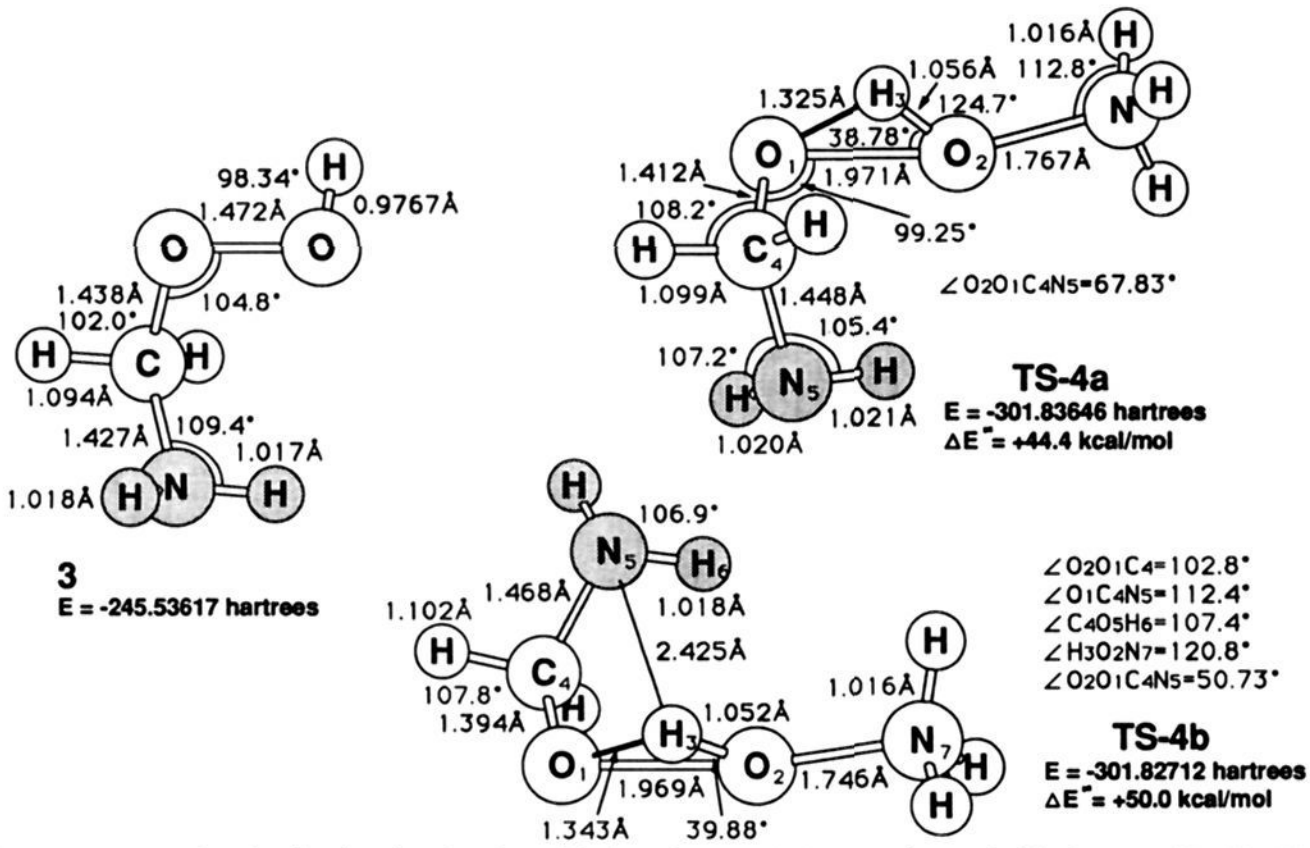


Figure 2. Transition structures and activation barriers for the oxidation of ammonia by α-aminomethyl hydroperoxide (**3**) without (TS-**4a**) and with (TS-**4b**) hydrogen bonding of the amino substituent to the migrating hydrogen: the effect of the electronegativity of the substituent on the activation barrier (MP4//MP2/6-31G*). The total energy of NH_3 at this level is −56.371 26 au. Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

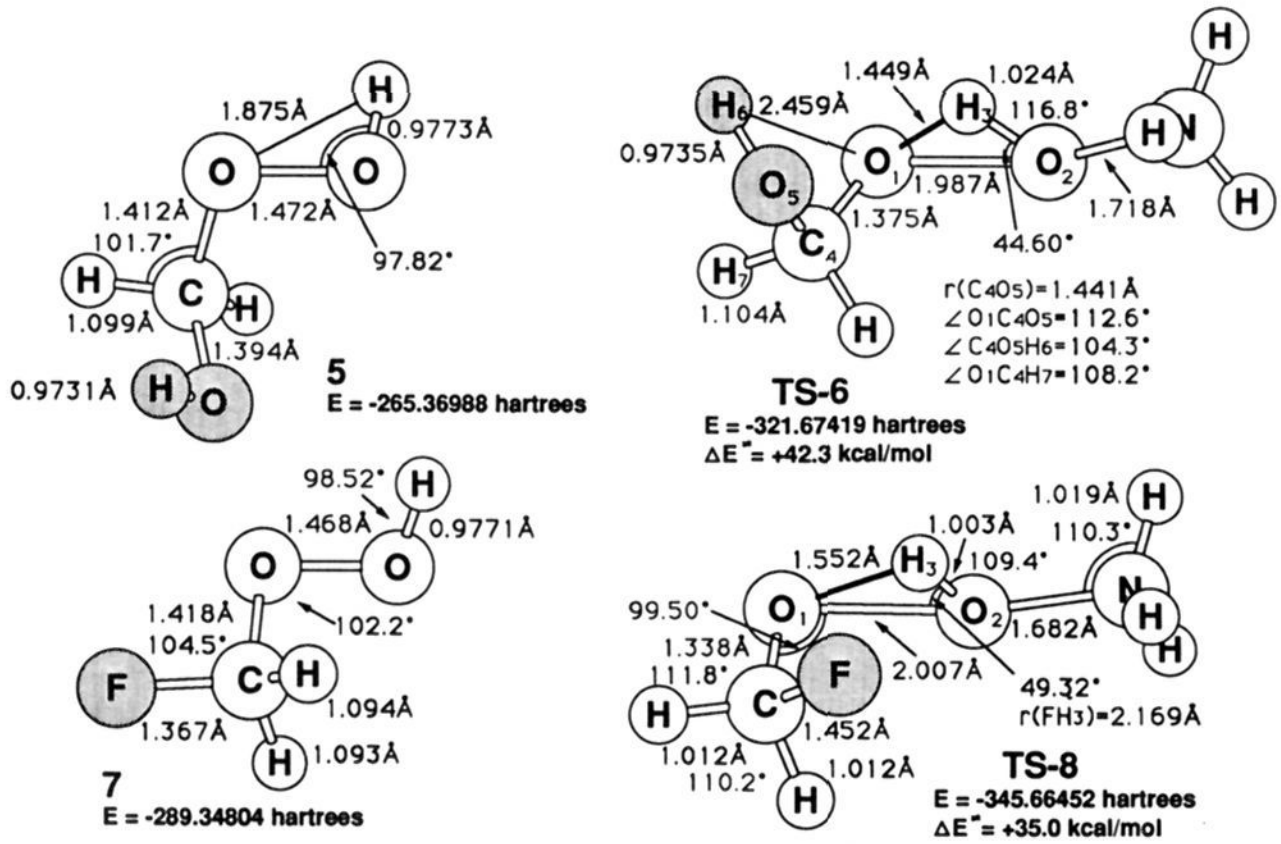


Figure 3. Transition structures and activation barriers for the oxidation of ammonia by α-hydroxy- and α-fluoromethyl hydroperoxide: the effect of electronegativity on the activation barrier (MP4//MP2/6-31G*). Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

of X was varied from H to F showed an increase in the O–O bond distance reflecting the enhanced stability of the alkoxide leaving group and a shortening of the N–O_2 bond. The increasing bond length of the hydrogen bridging to oxygen (H_3–O_1) is also consistent with the stabilizing influence of the substituent X on the leaving group.

Despite the systematic reduction in the activation energy for oxygen atom transfer with an increase in electronegativity of substituent X, the barrier height of 35.0 kcal/mol for α-fluoromethyl hydroperoxide is still too high to make this a viable oxygen donor. Inductive effects are known to increase the oxygen-transfer propensity of an oxidant as evidenced by the reactivity of the H_2O_2 adduct of hexafluoroacetone. The hydroperoxide formed when H_2O_2 adds to the carbonyl group of hexafluoroacetone is capable of alkene epoxidation under mild reaction conditions.[14] In an effort to mimic this inductive effect, we have examined the reactivity of the H_2O_2 adduct of carbonyl difluoride **9** toward ammonia oxidation (Figure 4). The minimum structure of **9** exhibits a strong hydrogen bond (H_6–O_2) and two relatively short C–O bonds. A reduction in barrier height of 17.7 kcal/mol relative to TS-**2a** has been realized by substitution of all three methyl group hydrogens of CH_3OOH with electronegative substituents. However, the activation energy still remains relatively high at 28.9 kcal/mol. By comparison, the activation energy required for the oxidation of ammonia with the paradigm oxidant peroxyformic acid is 18.4 kcal/mol at the same level of theory.

We next addressed the inductive effect of an imine substituent (X = –C=NH) on the efficacy of oxygen transfer from a

(14) Heggs, R. P.; Ganem, B. *J. Am. Chem. Soc.* **1979**, *101*, 2484.

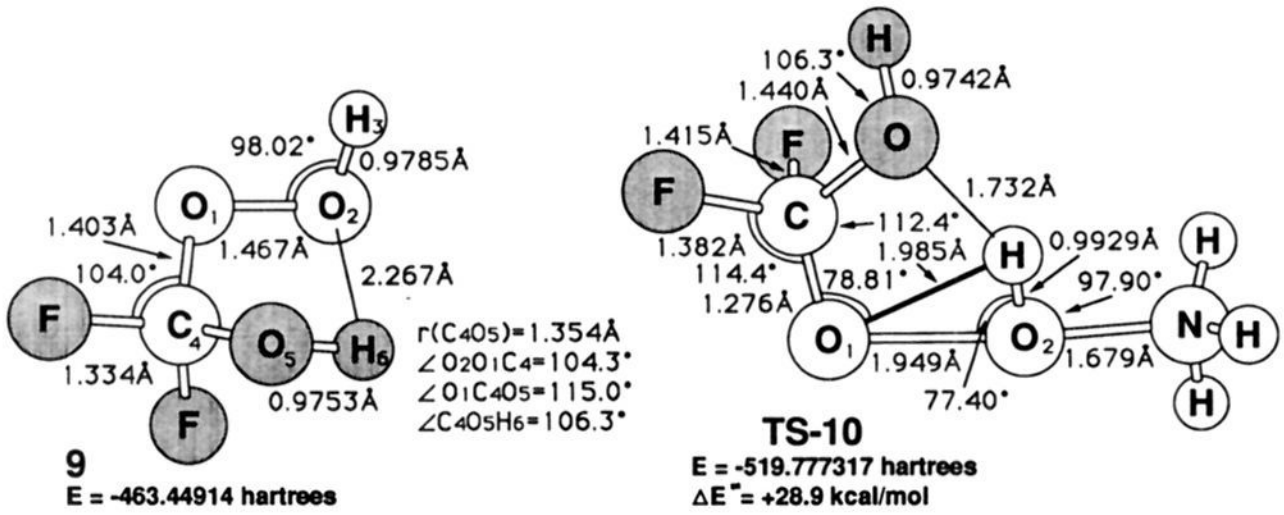


Figure 4. Optimized structure of the minimum of the H_2O_2 adduct of carbonyl difluoride (**9**) and transition structure for the oxidation of ammonia (TS-**10**). Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

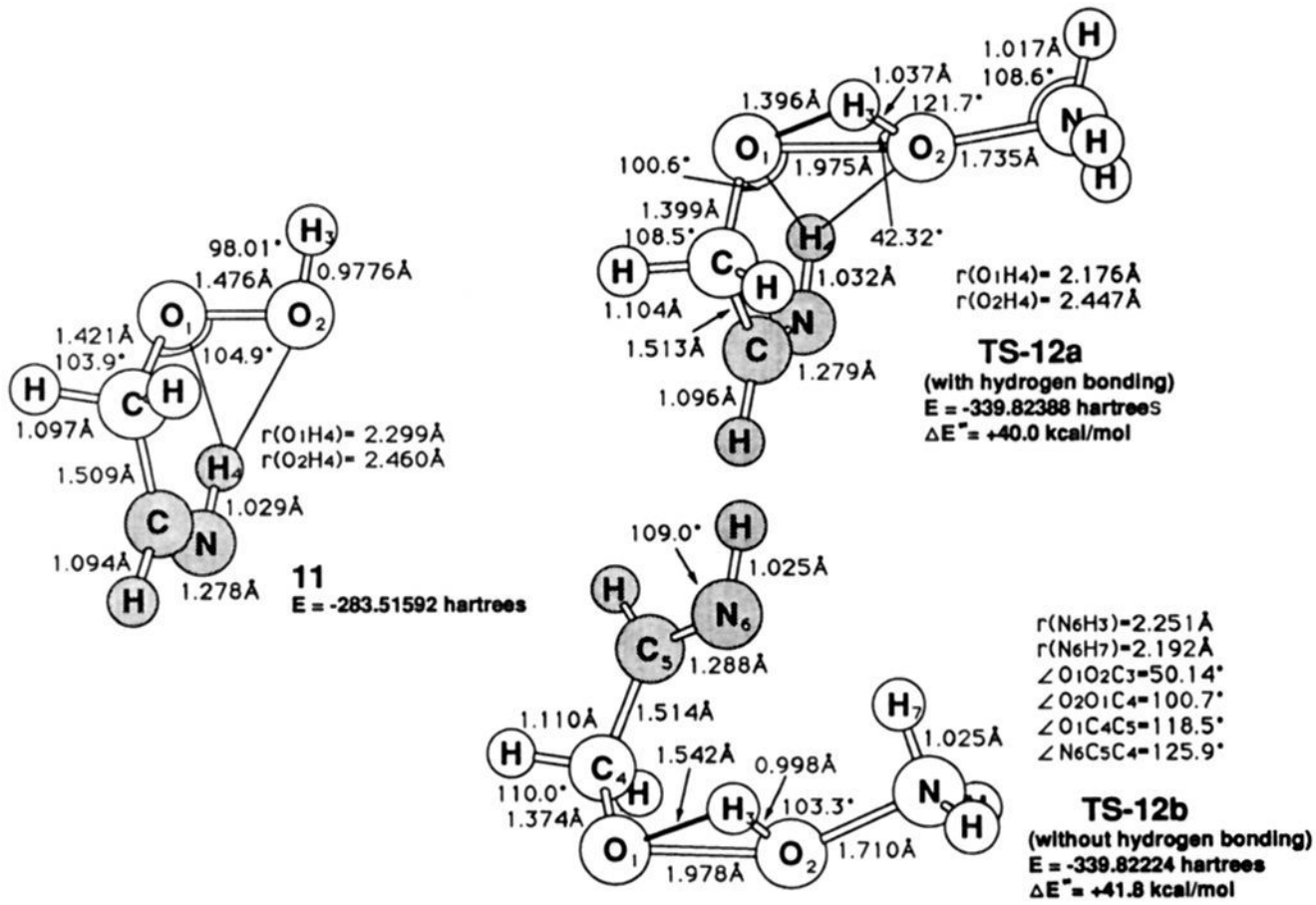


Figure 5. Optimized structure for imine-substituted methyl hydroperoxide **11** and transition structure for the oxidation of ammonia with (TS-**12a**) and without (TS-**12b**) hydrogen bonding (H_4–O_1). Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

substituted methyl hydroperoxide. The minimum energy conformation of imine-substituted hydroperoxide **11** (Figure 5) has an intramolecular hydrogen bond forming a five-membered ring structure (H_4–O_1 = 2.3 Å). The imine functionality exerts a very modest inductive effect on the oxygen-transfer process as evidenced by an activation barrier for H_3N–O formation of 40.0 kcal/mol (TS-**12a**). This is a surprisingly high activation barrier since the imine hydrogen is in close proximity to the developing alkoxide leaving group (O_1–H_4 = 2.18 Å). When hydroperoxide **11** is in a conformation where hydrogen bonding is absent (TS-**12b**), the barrier is only 1.8 kcal/mol higher in energy. Both transition structures exhibit a single imaginary frequency at the MP2/6-31G* level.

A formyl-substituted hydroperoxide does exhibit the expected[12] relatively strong hydrogen bond between the carbonyl carbon and the hydrogen of the –OOH moiety (O_3–H = 2.15 Å) as shown in minimum **13** (Figure 6). However, this cyclic hydrogen bonding interaction does not appear to facilitate oxygen transfer since the barrier height for TS-**14a** ($\Delta E^{\ddagger}$ = 40.5 kcal/mol) is quite comparable to those noted above for electron withdrawing substituents in the absence of this potential internal proton transfer (H_3–O_6). A direct measure of the effect of proton transfer to the carbonyl oxygen on the barrier height is possible since a first-order saddle point was also found for the reduction of hydroperoxide **13** by ammonia in a conformation without hydrogen bonding (TS-**14b**). These data suggest that intramolecular hydrogen bonding in this six-membered cyclic array reduces the activation barrier by only 1.5 kcal/mol and that the inductive effect of an adjacent carbonyl group exerts a surprisingly small effect upon the reactivity of an alkyl hydroperoxide. Since proton transfer to the departing alkoxide oxygen (O_1) takes place after the barrier is crossed, it exerts little influence upon the barrier height. This observation is consistent with earlier calculations[8a] that also suggest that a 1,2-hydrogen transfer from alkyl hydroperoxides in concert with O–O bond cleavage has a prohibitively high activation barrier.

We have also reported that alkene epoxidation with peroxyformic acid involves an intramolecular proton shift from the –OOH moiety to the carbonyl oxygen of the peroxy acid after the barrier is crossed.[8d] However, the activation energy for oxygen transfer from peroxyformic acid to ethylene is relatively low ($\Delta E^{\ddagger}$ = 16.5 kcal/mol), reflecting the activating effect of an α-carbonyl group on O–O bond cleavage. This activation barrier is in excellent agreement with experiment.[15] The above conclusions concerning the inability of a carbonyl group that is separated from the –OOH functionality by a carbon atom to activate the hydroperoxide is further buttressed by model studies on disubstituted methyl hydroperoxide **15** (X = NH_2, HCO, Figure 7). The carbonyl

(15) Plesnicar, B. In *The Chemistry of Peroxides*; Patai, S., Ed.; John Wiley and Sons: New York, 1971; Vol. 2, p 355.

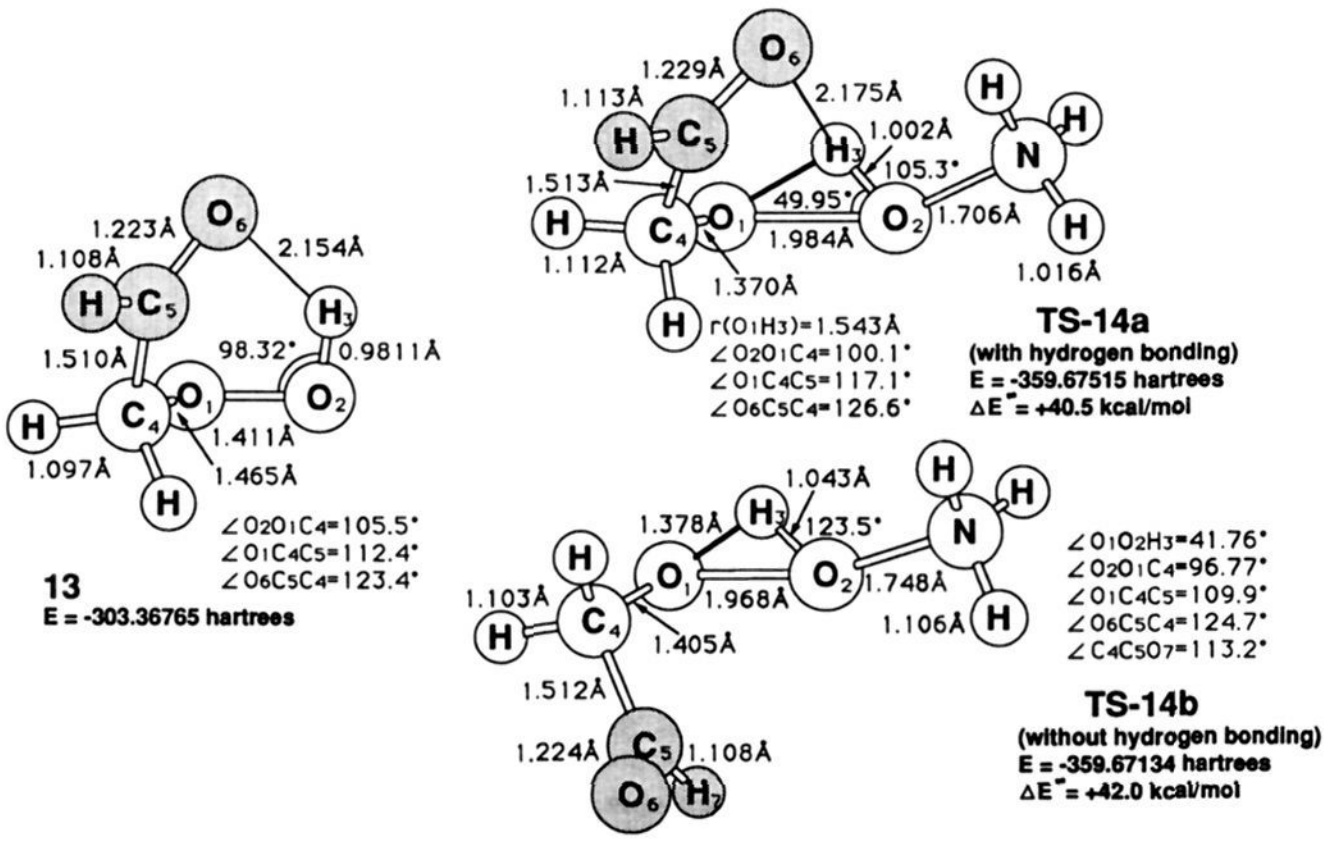


Figure 6. Optimized structure for formyl-substituted methyl hydroperoxide **13** and transition structure for the oxidation of ammonia with (TS-**14a**) and without (TS-**14b**) hydrogen bonding of the carbonyl oxygen to the migrating hydrogen (O_6–H_3). Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

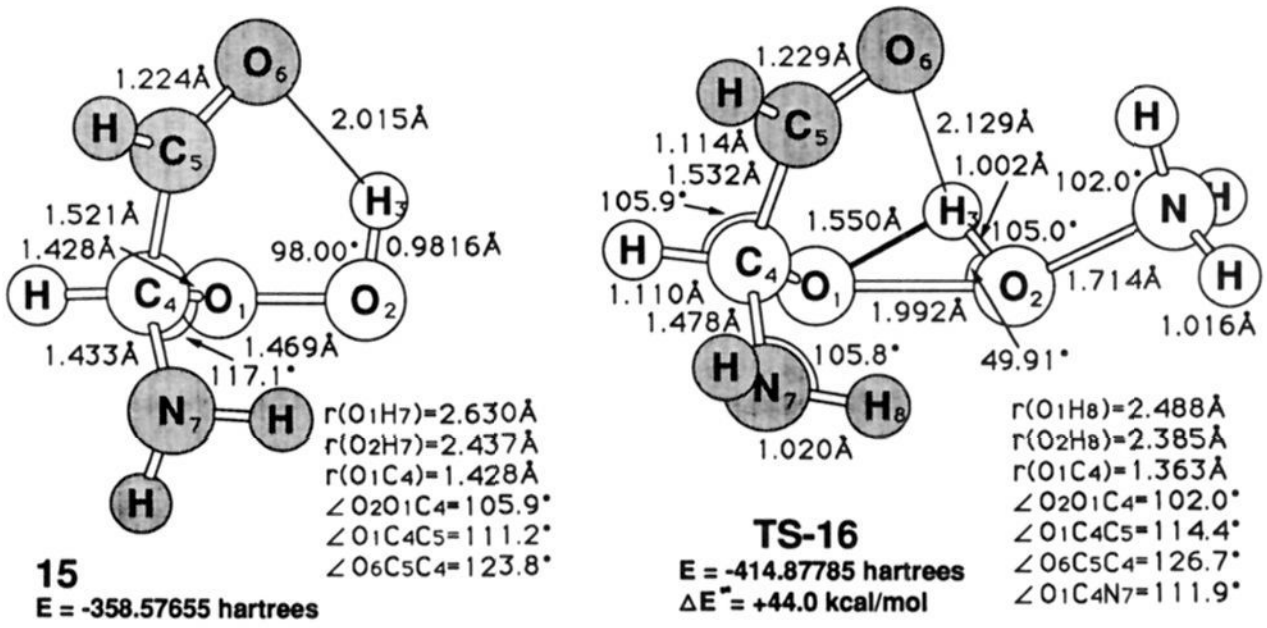
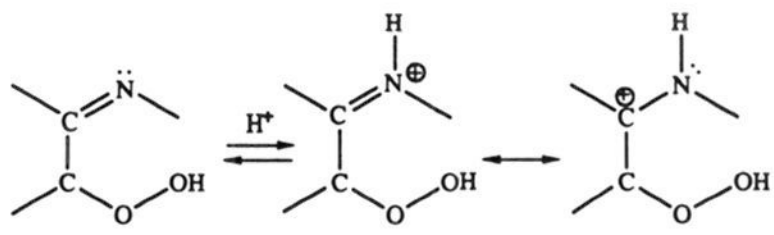


Figure 7. Transition structure (TS-**16**) for the oxidation of ammonia by disubstituted methyl hydroperoxide **15** (X = HCO, NH_2). Total energies (MP4//MP2/6-31G*) are in au, and activation barriers are in kcal/mol (with ZPE).

oxygen in **15** is sufficiently close (O_3–H_3 = 2.015 Å) to engage in relatively strong hydrogen bonding. However, the predicted activation barrier for this oxidative reaction with ammonia is 44.0 kcal/mol (TS-**16**). Thus, a synergistic effect of two electronegative substituents present on C_4 in flavin hydroperoxide **1a** is clearly not in evidence. Indeed, the presence of the two substituents appears to impede oxygen transfer since TS-**16** is higher in energy than TS-**14a** (X = –CHO).

The above data make it readily apparent that the high reactivity of flavin hydroperoxides like **1a** cannot be attributed primarily to inductive effects of the substituents at C_4 or to hydrogen bonding. Self-consistent reaction field (SCRF) calculations[8c] on transition structures involving comparable oxidations of NH_3 and H_2S by hydrogen peroxide suggest that these relatively high barrier heights are reduced very little by inclusion of solvation effects. The suggestion has been made that hydrogen bonding at the imine nitrogen (N_1) in **1a** could also enhance its reactivity. We therefore examined the reactivity of protonated hydroperoxide **17** (Figure 8) in an effort to mimic the effects of protonation at N_1 in flavin hydroperoxide **1a**. Hydrogen bonding between the protonated imine and both oxygens in **17** (Figure 8) is clearly in evidence. Significantly, the activation barrier for oxygen atom transfer to ammonia is markedly reduced in TS-**18** ($\Delta E^{\ddagger}$ = 12.9 kcal/mol). The imine proton in TS-**18** is almost fully transferred (O_1–H_7 = 1.2 Å) to the leaving group and is also engaged in strong hydrogen bonding to the lone pair on the imine nitrogen (H_7–N_6). It should be emphasized that the molecular architecture in flavin hydroperoxide **1a** would not permit proton transfer from N_1 to the developing Fl–O^- and any rate enhancement would have to derive from inductive and coulombic effects as depicted in eq 5.



(5)

This observation prompted us to examine also the potential for stabilizing the TS by protonation at N_5 in flavin hydroperoxide **1a**. We have modeled this effect by using a protonated amine substituent (X = NH_3^+) as shown in **19** (Figure 9). The barrier height (TS-**20**) relative to the unprotonated hydroperoxide (X = NH_2, TS-**4a**) where the activation energy was predicted to be 44.4 kcal/mol for oxygen transfer is now reduced dramatically

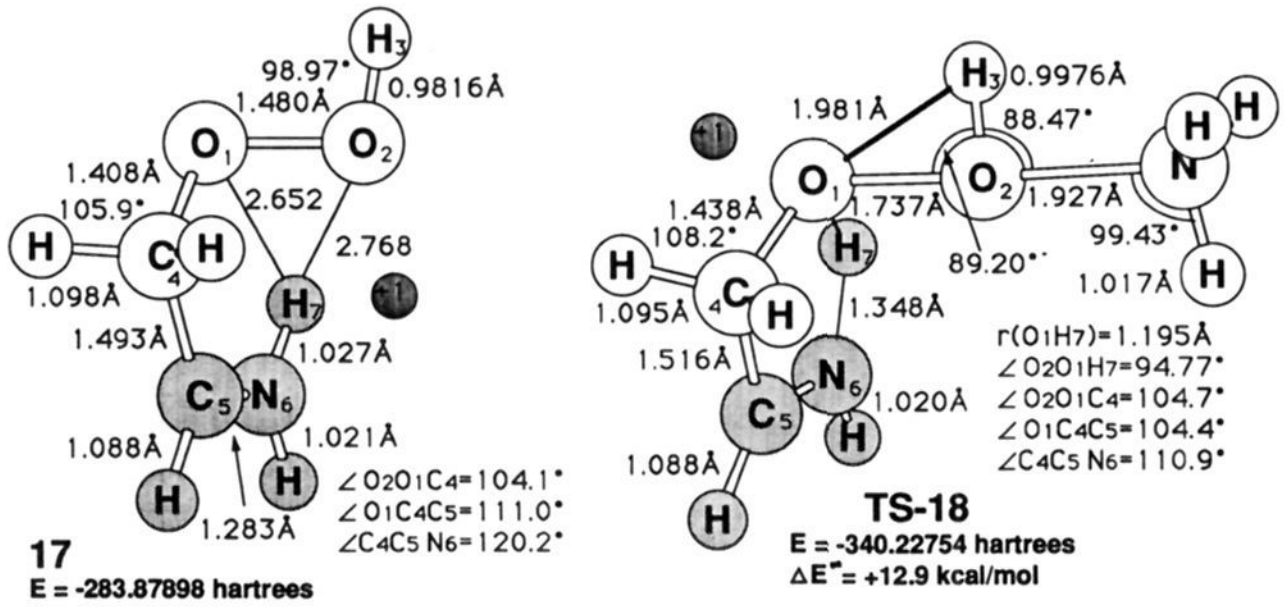
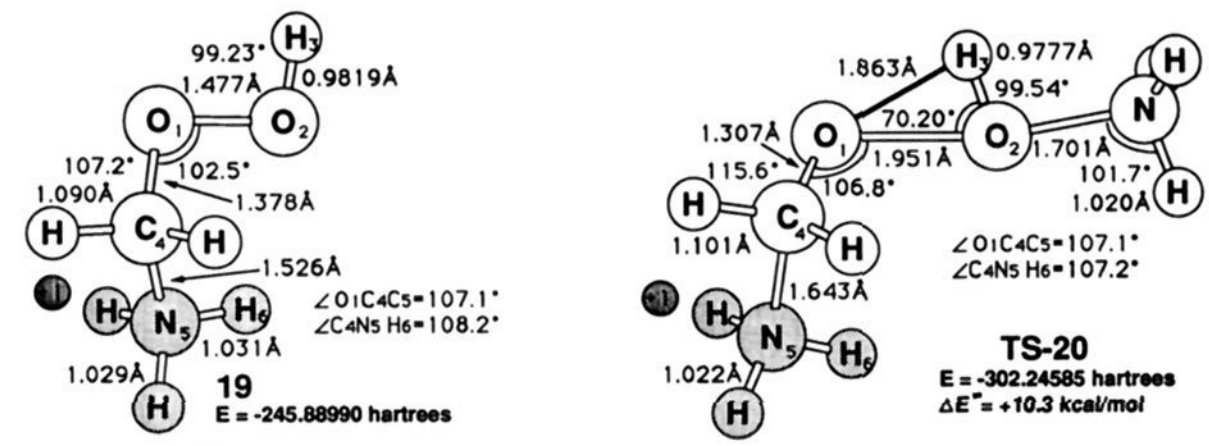


Figure 8. Optimized structure for protonated imine-substituted methyl hydroperoxide (**17**), and transition structure for the oxidation of ammonia (TS-**18**). Total energies (MP4//MP2/6-31G*) are in au, and activation barriers are in kcal/mol (with ZPE).



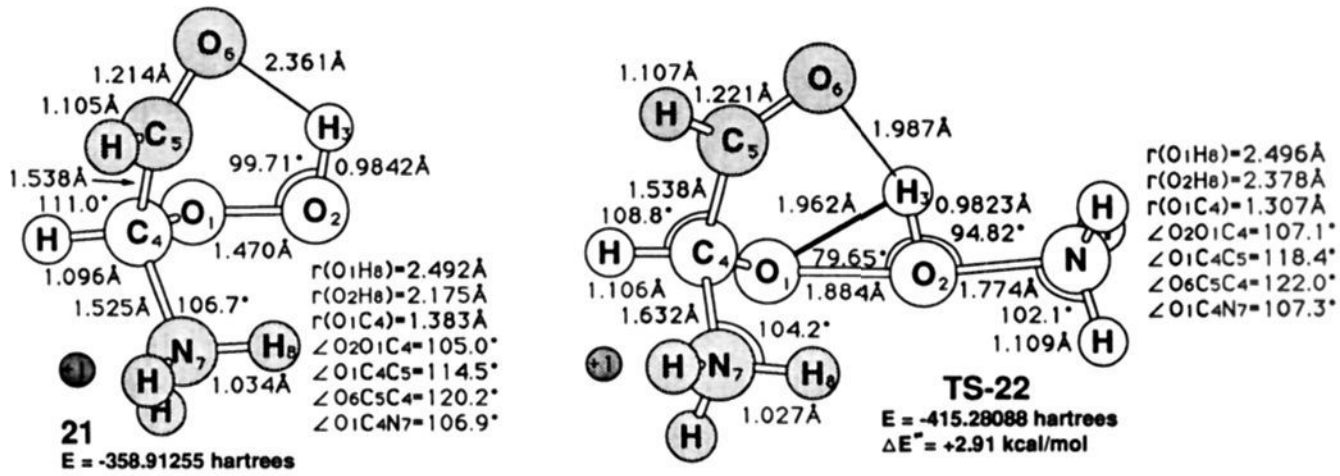


Figure 9. Transition structures (TS-**20**, TS-**22**) and activation barriers for oxygen atom transfer from protonated α-aminomethyl hydroperoxides **19** and **20** to ammonia. Total energies are in au, and activation barriers are in kcal/mol (with ZPE).

($\Delta E^{\ddagger}$ = 10.3 kcal/mol). Since hydrogen migration to O_1 takes place *after the barrier is crossed* (O_2–H_3 = 0.98 Å), the stabilizing effect of the H_3N^+ substituent must be attributed largely to through-bond Coulombic effects. The O–O and N–O_2 bond lengths in TS-**20** do not differ significantly from those in the transition state for reduction of the parent methyl hydroperoxide (TS-**2a**).

This dramatic through-space stabilizing electrostatic interaction of a positively charged substituent (H_3N^+) just one carbon removed from the oxygen of the alkoxide ion leaving group is further enhanced when a carbonyl group is also brought into play. The activation barrier for the oxidation of ammonia with cationic hydroperoxide **21** (X = H_3N^+, HCO) is now reduced to 2.9 kcal/mol (TS-**22**, Figure 9). It should be noted that the calculated Mulliken charges on N_1 and H_8 of the H_3N^+ substituent in TS-**22** are –0.84 and +0.44, respectively. The H_8–O_1 bond distance in TS-**22** is 2.49 Å. Thus, the H_3N^+ substituent has a formal charge of +1 on the nitrogen while the actual Coulombic stabilization is due to the positive charges on the hydrogen atoms.

It should be emphasized that all of the barrier heights reported herein are computed relative to isolated reactants, i.e. ammonia and the oxygen donor. This protocol was followed so that the relative reduction in barrier heights could be ascertained in the absence of the influence of stabilization energies due to the formation of gas-phase clusters between the two reactants. Based upon related calculations involving hydrogen peroxide,[8c] we estimate that the activation barrier for TS-**22** would be increased by about 20 kcal/mol if measured from a gas-phase cluster between ammonia and **21**. A stabilization energy of 20 kcal/mol is typical of ion–molecule binding energies. Barrier heights computed from reactant clusters involving neutral species are much closer to activation barriers relative to isolated reactants.

Because of the inherent difficulty in treating O–O bonds rich in electron lone pairs, the question of SCF wave function stability was also examined. For those transition states where a significant stabilizing influence of the departing alkoxide group was not observed (TS-**2**, TS-**4**, and TS-**6**), the wave function was RHF to UHF unstable with roots of the RHF → UHF stability analysis as high as –0.033. Release of the RHF constraints (HF/6-31G*) and reoptimization of the molecular orbitals (MP2/6-31G* geometry) affording a UHF stable wave function resulted in decreases in total energy for TS-**4a**, TS-**4b** and TS-**6** of 7.95, 5.18, and 3.94 kcal/mol, respectively. The remaining transition states had wave functions that were real RHF to real UHF stable.

In summary, through-bond inductive effects due to electron withdrawing substituents at C4 in 4α-flavin hydroperoxide (**1a**) should have only a modest effect on the barrier heights for oxygen atom transfer. These theoretical data on model systems strongly suggest that hydrogen bonding in the transition state or proton transfer to N_1 or N_5 could be responsible for the atypical oxygen donor propensity of flavin hydroperoxide **1a**. For example, *p*-hydroxybenzoate hydrolylase is a flavin monooxygenase where N_5 has no interaction with enzyme or substrate atom (in the oxidized enzyme/substrate complex). Protonation at N_5 could result from bulk solvent or a proton relay with Tyr 201 and Tyr 385. A protonated flavin could also serve as the positively charged counterion for the bihydroxide ion ($H_3O_2^-$) present at the active site. Stabilization of the developing positive charge on the oxidized nucleophilic center accompanying HO^+ transfer (eq 3) by a water molecule present at the active site could also result in a lowering of the activation barrier.[8a]

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