SINGLET MOLECULAR OXYGEN CHEMISTRY
AND IMPLICATIONS
FOR FLAVIN-COFACTOR HYDROXYLATIONS

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Using the results of extensive ab initio calculations on
the reaction of singlet molecular oxygen ($^1$O$_2$) with ethylene
and combining these results with thermochemical estimates of
substituent effects, we have predicted the energetics for var-
ious intermediates in the addition of $^1$O$_2$ to substituted ole-
fin. It is concluded that the major reaction pathway involves
the biradical intermediate, although certain solvents and sub-
stituents can greatly enhance the zwitterion character of this
state. Detailed comparisons of the theoretical predictions
with experimental results show that the stereospecificity and
regiospecificity of the $^1$O$_2$-ene reaction can be understood in
terms of the biradical intermediate or transition state. Using
the above results, we have made estimates of various possible
intermediates for flavin-cofactor hydroxylations. From these
estimates we propose a mechanism by which a reduced flavin in-
duces triplet dioxygen to hydroxylate phenol.

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I. INTRODUCTION

Occasionally, it has been suggested that singlet molecular oxygen (hereafter denoted as $^1O_2$) might play a role in such biological processes as photooxidation (1), bioluminescence (2a), photocarcinogenicity (2b), and biosynthesis of prostaglandins (3). In order to provide some insight into possible mechanisms by which $^1O_2$ or $^3O_2$ (normal ground state molecular O$_2$) might play a role in biochemical reactions, we will describe in some detail the microscopic mechanism for the reaction of $^1O_2$ with simple olefins (4). This mechanism is derived from theoretical studies and is compatible with current experimental observations. We will conclude with an application of these ideas to consideration of flavin-cofactor hydroxylation.

In discussing the reaction of $^1O_2$ with simple olefins, we will examine

1) the formation of dioxetane

\[
^1O_2 + \overset{\text{C-O}}{\text{\centerline{C-O}}} \rightarrow \overset{\text{C-O}}{\text{\centerline{C-O}}} \tag{1}
\]

2) the chemiluminescence arising from decomposition of dioxetane

\[
\overset{\text{C-O}}{\text{\centerline{C-O}}} \rightarrow \overset{\text{C-O}}{\text{\centerline{C-O}}} \quad \text{heat} \quad \overset{\text{\centerline{C-O}}}{\text{\centerline{\text{O}}} + (\overset{\text{\centerline{\text{C=O}}}^*}{\text{\centerline{\text{C=O}}}}) \tag{2}
\]

where the excited species may be a triplet or excited single state, and

3) the ene reaction

\[
^1O_2 + \overset{\text{D}}{\text{\centerline{\text{\text{D}}}}} \rightarrow \overset{\text{O-O-D}}{\text{\centerline{\text{O-O-D}}} \tag{3}
\]

which may compete with (1).

Before proceeding, we will briefly review the theoretical methods.

II. THEORETICAL METHODS

We will make two uses of theory; one qualitative and one quantitative. The qualitative use will be to provide a conceptual framework for discussing both theoretical and experimental results and for predicting the new reactions. The quantitative use will be to provide numerical information that is difficult or impossible to obtain experimentally, for example, the geometries and energies of the reaction intermediates and saddle point for the reaction. For these qualitative purposes the essence of the wavefunction must be simply describable and effects due to substituents and other modifications must be obvious. For the quantitative purposes it is essential for the bond energies and barrier heights to be very accurately described. There is one method, the generalized valence bond (GVB) method (5), that satisfies both requirements. Some essentials are outlined below.

A. Hartree-Fock

The most common approach to wavefunctions is the Hartree-Fock (HF) or molecular orbital method. For a simple molecule this method involves placing two electrons (one of each spin) in each molecular orbital of a Slater determinant wavefunction and optimizing the shape of these molecular orbitals to achieve the lowest energy. This method is conceptually simple and provides the basis for qualitative explanations of the spectra of atoms, molecules, inorganic complexes, and solids. The HF method is accurate for many purposes (e.g., geometries of simple molecules) but is not accurate for bond energies, as indicated in Table I (6). Table I is for nearly exact HF calculations; there are a number of approximate procedures for HF methods including:

i) STO-3G and STO-431G (7): ab initio calculations using a restricted basis set (vide infra),

ii) Xα (8): use of an approximation to certain terms (exchange integrals) and sometimes additional severe approximations (the muffin-tin assumption of spherical symmetry within a sphere about each nucleus and uniform potentials in between the spheres), and

iii) extended Hückel theory, iterative extended Hückel theory, CNDO, INDO, MNDO, MINDO (9-12): various semi-empirical versions.

None of these methods removes the basic flaw in the HF method, even when parameterized to fit experimental data.
TABLE I. Bond Energies (kcal)

<table>
<thead>
<tr>
<th>Bond</th>
<th>Hartree-Fock</th>
<th>GVB-CI</th>
<th>Experimental</th>
</tr>
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<tbody>
<tr>
<td>CH₃-CH₃</td>
<td>72.1</td>
<td>93.9</td>
<td>96.7</td>
</tr>
<tr>
<td>CH₃-OH</td>
<td>62.9</td>
<td>98.0</td>
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<tr>
<td>HO-OH</td>
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<td>H₂C=CH₂</td>
<td>123.3</td>
<td>171.6</td>
<td>180.3</td>
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<tr>
<td>H₂C=O</td>
<td>105.5</td>
<td>174.6</td>
<td>182.9</td>
</tr>
<tr>
<td>O₂</td>
<td>98.2</td>
<td>97.6</td>
<td></td>
</tr>
</tbody>
</table>

*These values are from the bottom of the potential curve \( P_e \) and hence the zero-point energy must be subtracted to yield the direct experimental value. All calculations use a good basis set, as indicated in Sec. II.D.

The basic problem with the HF description of bonds is that cleavage of a covalent bond generally leads to two radicals (with the two electrons of the bond each separating onto individual fragments), whereas the HF wavefunction, having only doubly-occupied orbitals, leads to mixtures of covalent and ionic products (5).

B. Generalized Valence Bond

In the valence bond (VB) description, one starts with the proper atomic orbitals of the fragments and pairs these orbitals on different centers to form the bonds. Although providing a correct description of the dissociated species, the use of atomic orbitals is not substantially accurate to describe the molecule with the bond fully formed. The GVB method (5) is a self-consistent field version of VB which allows each bond pair to be described in terms of two different orbitals as in VB but optimizes the shape of the orbitals as in HF. No restrictions are placed upon the shape or delocalization of the GVB orbitals, and hence HF is a special case of GVB in which the two orbitals of each bond pair are forced to be identical.

For the systems discussed in this paper, the GVB orbitals are each primarily localized on a single center and hence qualitatively the description is as in VB.

C. GVB-CI

In obtaining sufficient accuracy for assessing reaction mechanisms, it is necessary to go beyond the GVB wavefunction. In particular:

1) There are extra correlation effects involving the two electrons of a bond that are important at small R. For example, when one electron is instantaneously on the starboard side of the bond, the other prefers to be more on the portside (this is generally referred to as \( \pi \) or angular correlation). The important such correlation effects are included by allowing five orbitals to describe the two electrons of the bond pair being broken in a reaction and solving self-consistently for the optimum orbitals.

2) There are correlation effects involving simultaneous movements of the electrons in two adjacent bonds. These effects generally become small as one of the bonds is broken and hence are required for accurate bond energies. These effects are included by allowing terms in the wavefunction in which an electron of one bond pair is allowed to use GVB orbitals of the same or other bond pairs. The resulting wavefunction is referred to as GVB-CI (CI denotes configuration interaction or superposition of configurations).

The results of several GVB-CI calculations are tabulated in Table I.

D. Basis Sets

In order to solve for the optimum HF or GVB orbitals for a polyatomic molecule, the standard approach is to expand each unknown orbital \( \phi_i \) in terms of a set of basis functions \( \{ \chi_{\mu}, \mu=1,...,M \} \)

\[
\phi_i = \sum_{\mu=1}^{M} c_{\mu i} \chi_{\mu}
\]

and to solve for the expansion coefficients. This is equivalent to using Fourier series except that atomic-like \( \chi_{\mu} \) are used rather than sinusoidal functions. Practicality of this method rests upon being able to use a small number (M) of basis functions without leading to bias in the results. From numerous studies a set of principles has been established by which such a basis set may be found (13). For a first-row atom (C or O), such a basis includes at least one function to describe the 1s electron, two optimal functions for each valence (2s or 2p) atomic orbital (to allow contraction or
expansion of the orbitals upon bond formation), and a set of d-like functions (to describe polarization effects upon bond formation). Thus there is a minimum of 14 basis functions per C or O and two or five functions per H. We find that any restrictions upon this basis leads to large error in the bond energies.

**E. Example**

As an illustration, we will describe the calculation of the energy required to open the ring of dioxetane (6),

\[ \text{O}_2 + \text{H} = \text{O}_2 \]

With a basis of 64 functions, we solved for the GVB wavefunction allowing five orbitals to describe the O–O bond pair and two orbitals to describe all other valence pairs [viz., four CH bonds, one CC bond, two CO bonds, and four lone-pair orbitals on the O atoms]. This leads to a total of 27 optimized valence orbitals (plus four core orbitals), whereas there would be 12 valence orbitals in an HF calculation. Thus one could think of the GVB orbitals as 12 occupied orbitals plus 15 optimized correlating orbitals. Within this set of 27 functions we then carry out a high-order CI calculation (up to quadruple excitations) to account for various additional correlation effects.

The result of all this is a bond energy of

\[ D = 14 \text{ kcal} \]

for (4). Since a normal O–O bond is 38 kcal, another way of stating (5) is to say that the strain energy is 24 kcal. Since experimental values for the strain energies of

\[ \text{and} \]

are 26 kcal (14), our value seems reasonable [experimental values are not available for (4)].

Throughout this paper we will use kcal as an abbreviation for kcal/mole. 1 kcal/mole = 4.184 kJ/mole.

**Singlet Molecular Oxygen Chemistry**

In order to make full use of such theoretical predictions, we will need to estimate the effects of substituent effects on various bond energies. Thus, consider that we have the energy for

\[ ^1\text{O}_2 + \text{H} = ^1\text{H}_2 \]

How can we estimate the energy for

\[ ^1\text{O}_2 + \text{R} = ^1\text{R}_2 \]

or

\[ ^1\text{O}_2 + \text{R} = ^1\text{R}_2 \]

Rather than repeating the calculations with various possible \( R \), we will modify the theoretical numbers using, for example, the experimental value for

\[ \text{to correct the right-hand side of (7a). This is equivalent to using Benson's group additivity approach (14).} \]

**III. ELECTRONIC STRUCTURE OF \(^1\text{O}_2\)**

Before discussing the chemistry of \(^1\text{O}_2\), it is appropriate to review the electronic structure of \(^1\text{O}_2\) (5,15). Ignoring the O 1s and O 2s orbitals for the moment, the oxygen atom may be pictured as
where $\infty$ and $\circ$ represent the p orbitals parallel and perpendicular to the plane of the paper and the dots indicate occupation. To form the bond between two O atoms, we must bring the atoms together so that two singly-occupied orbitals overlap, leading to

$$\begin{align*}
(10a) & \\
(10b) & \\
(11a) & \\
(11b) &
\end{align*}$$

where the line indicates singlet pairing of the connected orbitals (that is, a bond). Each configuration in (10) and (11) leads to both a singlet and a triplet state, depending on the coupling of the singly-occupied $\pi$ orbitals.

Since (10) has two singly-occupied orbitals, we can spin-pair these orbitals to obtain a singlet state ($S=0$) and a triplet state ($S=1$). Since the orbitals are orthogonal, the triplet is lower by about 1 eV (twice the exchange integral between the singly-occupied orbitals). Combining the configurations as in (10) leads to a resonant and an antiresonant combination for both the triplet and singlet states. The separation between the resonant and antiresonant states is several eV, and we will consider only the resonant states; these have the symmetry designations $3\pi_g^-$ and $1\pi_g^+$. The states in (11) have singly-occupied $\pi$ orbitals that overlap. As a result, the triplet state is much higher than the singlet state, and only the singlet states are low-lying. There are two singlet states: $1\Delta_g$ [resonance combination of (11)] and $1\Sigma_g^+$ (antiresonance combination). However, the resonance interactions here are small and the final ordering of energies is (16)

$3\pi_g^-$ ground state
$1\Delta_g$ and $1\Sigma_g^+$ at 0.984 eV = 22.7 kcal
$1\Sigma_g^+$ at 1.650 eV = 38.0 kcal.

FIGURE 1. Simple conceptual model of the lowest triplet and singlet states of $O_2$.

The forms (10b) for $1\Delta_g^-$ and (11a) for $1\Sigma_g^+$ appear quite different; however, expressing the $1\Delta_g^-$ wavefunction (11a) in terms of orbitals rotated by 45° about the axis leads to a form equivalent to (10a).

Thus, in discussing the $3\pi_g^-$ and $1\Delta_g$ states, we may use the orbital diagram from (10a). Generally we will simplify this, as shown in Fig. 1.

An analysis of the bond energy of $O_2$ indicates that

$$\begin{align*}
\sigma \text{ bond} &= 47 \text{ kcal} \\
\pi \text{ bonds} &= 71 \text{ kcal} \\
\text{total bond} &= 118 \text{ kcal}
\end{align*}$$

That is, the $\pi$ system dominates the bonding, mainly due to the resonance stabilization.

Now consider bonding $O_2$ to a simple atom, H. Clearly we want the (singly-occupied) orbital of H to overlap a singly-occupied orbital of $O_2$ to form a $\sigma$ bond as in Fig. 2a. However, in the resonant configuration of $O_2$, the corresponding $\pi$ orbital is doubly-occupied, leading to a repulsive interaction. Consequently, in bonding the H to $O_2$ we lose most of the resonance interaction in the $\pi$ system of $O_2$. The result is a much weaker H-O bond than normal,

$$\begin{align*}
\text{normal H-O bond} &= 104 \text{ kcal} \\
\text{loss in resonance energy} &= 57 \text{ kcal} \\
\text{H-O$_2$ bond} &= 47 \text{ kcal}
\end{align*}$$

FIGURE 2. Bonding of $O_2$ to H.
IV. FORMATION OF DIOXETANE

Now consider attack of $^3\text{O}_2$ or $^1\text{O}_2$ on a simple olefin. Recoupling orbitals to yield a single new C-O bond leads to the configuration

$$
(12)
$$

where the two unpaired orbitals must be triplet-coupled if we start with $^3\text{O}_2$ and singlet-coupled if we start with $^1\text{O}_2$. Now we examine the interactions between the second C and second O to see whether the molecule might close in one step. Since the occupied orbital on the oxygen is perpendicular to the COO plane, the p orbital of the terminal carbon in (6) overlaps the doubly-occupied p orbital of the terminal oxygen, leading to repulsive interactions. Hence, the molecule cannot close in one step for the planar COOO orientation shown in (12).

As mentioned above, (12) leads to both a singlet spin state and a triplet state. However, since these orbitals are separated by a large distance, the exchange integral determining the separation of orbitals is small, and hence the singlet and triplet states are nearly degenerate (separation <0.5 kcal, with the triplet lower). Thus the potential curves for the (planar) addition of $^1\text{O}_2 + \text{C}_2\text{H}_4$ and $^3\text{O}_2 + \text{C}_2\text{H}_4$ are as in Fig. 3.

Before discussing closure, we should examine the energetics. Starting with ethylene and $^3\text{O}_2$, formation of (12) leads to a new C-O bond worth ~85 kcal (based on the C-O bond of Et-OMe), but we lose the ethylene π bond (~61 kcal) and the O$_2$ resonance (~57 kcal). Thus, formation of (12) is uphill about 33 kcal. Since the $^1\text{O}_2$ state starts at an energy 22.5 kcal higher, it is only ~10.5 kcal uphill to form the intermediate (12).

Indeed, our best estimates for these energies are about 1.5 kcal lower, as indicated in Fig. 3.

Now consider closure of (12) to form dioxetane,

$$
(13)
$$

The final geometry of the dioxetane is essentially planar [that is, puckering of the ring leads to very little (~1 kcal) extra stability] with geometries as shown in Fig. 4. However, (12) cannot close without non-coplanar orientation of the CC and CO units. Rotating the CC bond out of the COO plane and twisting the terminal CH$_2$ group to start overlapping the singly-occupied oxygen orbital to form the bond leads first to additional repulsive interactions between the non-bonded orbitals

$$
\begin{align*}
1.537 \text{Å} & \rightarrow [\begin{array}{c}
\text{H}_2\text{C} \rightarrow \text{O} \\
\text{H}_2\text{C} \leftarrow \text{O}
\end{array}] & \mu = 3.625 \text{D} \\
1.462 \text{Å} \\
\text{H}_2\text{C} \rightarrow \text{O} & \rightarrow 1.514 \text{Å}\\n\text{H}_2\text{C} \leftarrow \text{O} & \rightarrow 1.503 \text{Å}
\end{align*}
$$

\[ \mu = 5.591 \text{D} \]

\[ \text{H}_2\text{C} \rightarrow \text{O} \]
which has two nonbonding electron pairs on the O, as illustrated in (15b). Adding an O atom with the configuration $(1s)^2(2s)^2(p_x)^2(p_y)^2(p_z)^0$ (taking z as the OO axis) leads then to a bonding structure

which is close to the calculated wavefunction and geometry. We find that the peroxide is 54 kcal above dioxetane and indeed opening the CO bond of the peroxide is downhill about 8 kcal. Consequently, we do not believe that the peroxide plays a role in formation of the dioxetane.

V. Decomposition of the Dioxetane

Turro and co-workers (19) have shown that pyrolytic decomposition of dioxetane leads to chemiluminescence,

Indeed, in some cases the yield of triplet products is as high as 50%. Since dioxetane is a singlet, this implies a spin change during the chemical reaction -- quite unexpected for a molecule without heavy atoms. This leads to the major question: What kind of mechanism could account for such high efficiencies of spin changes in a chemical reaction?

We find that opening of the O-O bond leads to an intermediate of the form

$\begin{align*}
\text{(17a)} \\
\text{(17b)} \\
\text{(18)}
\end{align*}$
which still has a barrier to decomposition. However, (18) is really a number of states (eight). That is, the radical (singly-occupied) orbital on each oxygen can be either \( \sigma \) or \( \pi \) (in the plane or perpendicular), leading to a total of four spatial configurations, as indicated in Figs. 6 and 7. In addition, each configuration leads to a spin singlet and a triplet and hence a total of eight states. The energies of these states are indicated in Fig. 6. Now consider decomposition to two formaldehydes by stretching the C-C bond\(^5\) of (18). It is the seventh state, \( ^14\pi \), that dissociates to yield ground state products,

\[
S_0 + S_0,
\]

while lower states of the intermediate, \( ^3\pi \) and \( ^1\pi \), yield excited states of the products

\[
S_0 + T_1 \text{ or } S_0 + S_1.
\]

\(^5\)This is oversimplified somewhat but suffices to illustrate the major points.

For the unsubstituted case, the energy barrier going to \( S_0 + T_1 \) is slightly lower than that going to \( S_0 + S_0 \), suggesting the following mechanism for producing large amounts of triplet products.

As the energized dioxetane opens up to form (18), we produce various distributions over the singlet states \( ^12\pi, ^13\pi, \) and \( ^14\pi \). Considering (18) with the large amount of vibrational energy needed to decompose (but only a small excess since we examine the pyrolysis case), imagine large oscillations in the singlet levels of Fig. 6. There are four cases in which a singlet state crosses a triplet state for which there should be significant singlet-triplet coupling,

\[
\begin{align*}
^12\pi & = ^33\pi \\
^13\pi & = ^32\pi \\
^13\pi & = ^34\pi \\
^14\pi & = ^33\pi,
\end{align*}
\]

as indicated in Fig. 6. For hydrocarbons, the probability of
a spin-flip upon traversing such a region is not large; however, these crossings are considerably below the energy barriers for decomposition, and hence there may be thousands of vibrations through such points prior to dissociation to \( S_0 + S_0 \). Thus, even though a spin-flip is unlikely for any one vibration across such a crossing, we may in this way build up a concentration of triplet species before the molecule dissociates. Since the activation barrier for the triplet species is lower than for the singlet, the triplet states will decompose more rapidly than the triplet. Depending upon the relative energy barriers for singlet and triplet decompositions and upon the positions of the singlet-triplet crossings relative to these energy barriers, there could be large variations in the yield of triplet products. Indeed, with an understanding of the mechanism and of substituent effects upon these barriers and crossing points, one could probably design the molecule to yield very high fractions of either triplet or singlet excited products. The overall process for decomposition of dioxetane is illustrated in Fig. 7.

VI. THE ENE REACTION

A. The Biradical Mechanism

The ene reaction (19) generally predominates for \( \text{^1O}_2 \) attack on olefins containing an abstractable \( H \) on a carbon adjacent to the double bond. There is no general agreement on the mechanism, with concerted pathways (20)

\[
\text{O} \quad \text{D}
\]

(19)

peroxide intermediates (17,18)

\[
\text{O} \quad \text{D}
\]

(20)

and zwitterion intermediates (20,21)

\[
\text{O} \quad \text{D}
\]

having been suggested at various times (here D denotes the hydrogen to be transferred).

Since the product ratios of liquid phase \( \text{^1O}_2 \) reactions are generally similar, whether the solvent is low polarity (benzene) or high polarity (ether), the use of gas phase numbers may be satisfactory also for liquid phase reactions. Considering ethylene and \( \text{^1O}_2 \) in the gas phase, our calculations yield the energies tabulated in Fig. 8 for the biradical, peroxide, and zwitterion species. As shown below, the \&H to form the biradical is comparable with experimental activation energies (4 to 7 kcal for two to four substituents) (22). Thus the initial step of \( \text{^1O}_2 \) attack on an olefin yields the 1-4 biradical as indicated in Fig. 9. As discussed in Sec. III, this intermediate may close to dioxetane, but the process involves a large extra energy barrier. If there is an abstractable \( H \) on one of the carbons adjacent to the double bond, the \&H for abstracting this \( H \) to form the peroxide is -50 to -55 kcal.

\[
\text{^1O}_2 + \text{OLEFIN} \quad \Delta H \quad \text{KCAL}
\]

\[
\text{H}_2\text{C} - \text{O} \quad +9
\]

BIRADICAL

\[
\text{H}_2\text{C} - \text{O} \quad +17
\]

PEROXIDE

\[
\text{H}_2\text{C} \quad +30
\]

ZWITTERION

FIGURE 8. Possible intermediates in \( \text{^1O}_2 \)-olefin reactions.
(depending upon the exact pattern of substituents). If there are no steric constraints, this CH bond can be well aligned with the radical orbital of the terminal O and only a small energy barrier is expected in the 1-5 transfer of the H atom to form the peroxy. Our best estimate is that this energy barrier does not exceed 1 kcal and it may be zero. Thus the presence of an abstracted H with no steric constraints should lead to ene products rather than dioxetane.

Similarly, our best estimate of the barrier to abstracting the O_2 of the biradical intermediate to reform reactants is less than 1 kcal and it may be zero. Thus we envision only shallow barriers (or no barriers) separating the biradical

\[
\begin{align*}
\text{O}_2 \text{ BIRADICAL MECHANISM}
\end{align*}
\]

(22)

from reactants and products, and we will ignore these small barriers in the subsequent discussion.

There are accurate kinetic data over a range of temperatures for the \(^1\text{O}_2\) reactions with many olefins, as listed in Table II. In each case we compare the calculated (4) \(\Delta H\) to

| Olefin | Olefin | Zwitterion | Peroxide | Biradical | Expt.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td></td>
<td>(\Delta H_f)</td>
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<td>(\Delta H(\beta))</td>
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<td>-0.8</td>
<td>31</td>
<td>5.5</td>
<td>&gt;8.2</td>
<td></td>
</tr>
<tr>
<td>(\Delta\lambda)</td>
<td>-9.2</td>
<td>30</td>
<td>15</td>
<td>4.3</td>
<td>5.0</td>
</tr>
<tr>
<td>(\Delta\mu)</td>
<td>-17.0</td>
<td>13</td>
<td>2.8</td>
<td>4.0</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Enthalpy of forming olefin-dioxygen adducts from olefin and \(^1\text{O}_2\) is listed for zwitterion, peroxy and biradical intermediates. \(\Delta H_f(a)\) and \(\Delta H(\beta)\) are estimated enthalpies (kcal) of addition to \(\alpha\) and \(\beta\) sides of olefin, respectively.
form the biradical intermediate with the observed activation energy. The agreement is quite good, with the trends and magnitudes of the substituent effects all explained. Although strongly supportive, such comparisons do not prove a mechanism; for this we need stereoselective or regioselective reactions.

B. Regioselectivity

Pitts and co-workers (23) examined the gas phase reaction

\[
\begin{align*}
1^1O_2 + &\quad\text{(23a)} \\
\text{MeO} &\quad\text{MeO}
\end{align*}
\]

(23a)

\[
\begin{align*}
\text{MeO} &\quad\text{MeO}
\end{align*}
\]

(23b)

and found products in the ratio of

\[
\frac{(23a)}{(23b)} = \frac{29}{71}. 
\]

We find that the \(\Delta H\) for the biradical precursor of (23a) is 5.7 kcal as compared with 5.2 kcal for (23b), leading to a predicted product ratio of 30:70. Although in agreement with experiment, we would not want to rely on such small effects in establishing a mechanism.

More dramatic effects occur for cases with an MeO substituent. As an example, consider \(1^1O_2\) attack on

\[
\begin{align*}
\text{MeO} &\quad\text{MeO}
\end{align*}
\]

(24)

This leads to two possible biradical intermediates

\[
\begin{align*}
\text{MeO} &\quad\text{MeO}
\end{align*}
\]

(25)

which we will denote as the \(\alpha\) and \(\beta\) species, respectively. Now we must ask which of these intermediates is more stable, and by how much. It is well-known that a carbon radical center adjacent to an oxygen lone pair as in \(\beta\) is stabilized by 4 kcal

\[\text{Estimating substituent effects is indicated in Sec. II.E.}\]

\[\text{(vide infra) and consequently, one might expect \(\beta\) to be significantly below \(\alpha\). However, there is a second, even larger factor favoring \(\alpha\) and we calculate}\]

\[
\begin{align*}
\Delta H_\alpha &= 0.0 \text{ kcal} \\
\Delta H_\beta &= 2.0 \text{ kcal.}
\end{align*}
\]

This additional factor is referred to as \textit{lone-pair stabilization} or the \textit{anomeric effect}; since this interaction is of some importance here, we will outline the essence of it.

C. Lone-Pair Stabilization

Consider two oxygen atoms bonded to the same carbon

\[
\begin{align*}
\text{R} &\quad\text{O} \\
\text{C} &\quad\text{Me}
\end{align*}
\]

(26)

where \(X\) is also an OR group. If the lone pair of the oxygen overlaps the ionic \(\sigma\) bond (C-X), as shown in (26), there is an extra stabilization of 3 to 5 kcal. By similarly aligning \(X\) so that its lone pair overlaps the left ionic CO bond, we obtained an additional 3 to 5 kcal of stabilization. The net effect for two OR groups on the same C is 6 to 10 kcal of stabilization.

As an example of this, consider the following sequence of bond energies. First (14)

\[
\begin{align*}
\text{D(H-CH}_2\text{CH}_3) &= 98.3 \text{ kcal} \\
\text{D(H-CH}_2\text{OH)} &= 94 \pm 2 \text{ kcal} \\
\text{D(H-CH}_2\text{OMe)} &= 94.1 \text{ kcal.}
\end{align*}
\]

Thus breaking the CH bond is 4.2 kcal weaker if there is an OR group also bonded to the carbon. We interpret this as a weak three-electron \(\pi\) bond stabilizing the radical. Second (14)

\[
\begin{align*}
\text{D(OH-Me)} &= 91.4 \text{ kcal} \\
\text{D(OH-Et)} &= 91.6 \text{ kcal} \\
\text{D(OH-CH}_2\text{OH)} &= Y.
\end{align*}
\]

Due to the 4.2 kcal stabilization of the \(\cdot\text{CH}_2\text{OH}\) radical by the three-electron \(\pi\) bond, we would have expected \(Y = 91.5 - 4.2 = 87.3 \text{ kcal. In fact (14), } Y = 96.9 \text{ kcal. Thus the C-O bond is}\)
9.6 kcal stronger than expected due to the second O substituent on the C. We conclude that lone-pair stabilization dominates this effect.

The lone-pair stabilization demands specific conformations of the OR groups. Thus, to maximize this interaction for methanediol suggests

\[ (27) \]

The conformational effects associated with lone-pair stabilization are referred to as the anomic effect (24).

**D. Methoxy-Substituted Olefins**

1. Theory. The predicted energies of \( ^1\text{O}_2 \) attack on various methoxy-substituted cases are shown in Table III (4). In all cases the methoxy has the effect of directing the attack to the carbon with the methoxy group

\[ (28) \]

however, in general, two possible ene products arise from (28). Namely, the H can be abstracted from either of the groups attached to the \( \beta \) carbon. However, the lone-pair stabilization selectively enhances one of these. Requiring the oxygen lonepair from the \( ^1\text{O}_2 \) to overlap the CO bond of the methoxy group leads to two possible configurations,

\[ (29) \]

\[ (30) \]

Of these, only (29) has the O radical center adjacent to an abstractable hydrogen and this leads to abstraction from the substituent cis with respect to the MeO group. Since barriers to product formation are small compared with the rotational barriers\(^7\), we expect

\[ (31) \]

2. Experiment. Experimental studies of methoxy-substituted systems are in agreement with the above theoretical predictions. In order to test our predictions, Hammond (25) recently carried out gas phase experiments yielding the following results,

\[ (32) \]

\[ (33) \]

In reaction (32) the only observed product results from initial \( \alpha \) addition followed by cis abstraction, consistent with both predictions (i) and (ii). In reaction (33) there are no abstractable hydrogens cis to the methoxy group and, therefore, the preferred mode is not available. The result is that now two competing modes are detectable, one being \( \alpha \) addition (followed by trans abstraction) and the second being \( \beta \) addition.

\(^7\)The barrier for rotation of the \( \cdot\text{OO} \) about the CO axis is expected to be \( \sim 3 \) kcal (the anomic interaction), while the barrier for rotation of the C radical center is expected to be \( \sim 3.5 \) kcal (14).
Related work by Cross (26), using crossed molecular beams, has shown that gas phase addition of $^{1}$O$_{2}$ to 2-methoxypropane leads to luminescence, presumably from decomposition of a dioxetane. Cross notes that this is an unexpected result since activation energies for 2+2 cycloaddition (forming dioxetane) are typically much larger than those for the ene process. However, the results in Table III indicate the preferred biradical is the $\alpha$ adduct,

$$\text{MeO} + \text{H} + ^{1}\text{O}_{2} \rightarrow \text{MeO} \text{OO}+ \text{H} \text{Me} \text{H}$$  \hspace{1cm} (34)

which cannot undergo the ene reaction.

Additional support for this interpretation comes from work by Conia et al. (27), summarized in Eqs. (35) and (36). Again the products show a clear bias toward H-abstraction from the substituent cis to the methoxy group. In the latter reaction, (36), the dominant (cis abstraction) product is 9 kcal less stable than the minor product (trans abstraction) indicating that the effect is indeed an important one. Conia also notes that replacement of the methoxy group by methyl removes the directing effect. In this case, products resulting from abstraction of the cyclopropyl hydrogen are not observed (from either isomer).

Finally, recent studies by Foote et al. (28) also show the importance of this directing effect. In this work, the observed reactions are

$$\text{MeO} + \text{Me} + ^{1}\text{O}_{2} \rightarrow \text{MeO} \text{OOH} \text{Me} \text{H} \text{Me}$$  \hspace{1cm} (37)

$$\text{MeO} + \text{Me} + ^{1}\text{O}_{2} \rightarrow \text{MeO} \text{OO} \text{Me} \text{Me}$$  \hspace{1cm} (38)

In both cases, the only observed products are those resulting from attack of the oxygen radical center on the group cis to the methoxy.
VII. SUMMARY

From the above studies, we conclude that attack of ¹⁰₂ on an olefin leads to a 1-4 biradical (39) which subsequently forms the ene product (40) or closes to the dioxetane (41) if the ene product is not allowed. The dioxetanes can subsequently decompose to yield chemiluminescence.

Triplet dioxygen, ³⁰₂, can also lead to a similar 1-4 biradical intermediate (39), but formation of products (40) and (41) would require a spin-flip. In addition, the activation barrier for forming (39) from ³⁰₂ is an extra 22 kcal higher than from ¹⁰₂, making such reactions quite slow at room temperature.

We should emphasize that the above analysis is for gas phase processes. A major effect of a polar solvent would be to stabilize the zwitterion intermediate with respect to the biradical species. We have included estimates (4) of the relative energies for these species in Tables II and III. In some cases quite different product distributions could be obtained if the solvent is sufficiently polar to stabilize the zwitterion. Current experimental results do not indicate great differences in product distribution for polar and nonpolar solvents, suggesting that the biradical pathway dominates.

VIII. FLAVIN-COFACtor HYDROXYLATIONS

We will now illustrate how the insights from the ¹⁰₂ studies may be applied to qualitative and semi-quantitative discussions of other molecular oxygen reactions.

For example, flavin is the cofactor for hydroxylation of phenol by molecular oxygen (29,30)
aromatic molecule. The initial step leading to a triplet biradical is uphill 29 kcal for a simple olefin\(^8\)

\[
\text{\(3^\circ \text{O}_2 \rightarrow \)} \begin{array}{c}
\text{\(\text{Me}\)} \\
\text{\(\text{Me}\)}
\end{array} \quad \Delta H = 29 \text{ kcal}
\]

and 44 kcal for benzene (the increase results from loss of resonance energy in the intermediate)

\[
\text{\(3^\circ \text{O}_2 \rightarrow \)} \begin{array}{c}
\text{\(\text{H}\)} \\
\text{\(\text{H}\)} \\
\text{\(\text{H}\)}
\end{array} \quad \Delta H = 44 \text{ kcal}
\]

For phenol, there are several possible processes\(^9\)

\[
\text{\(3^\circ \text{O}_2 \rightarrow \)} \begin{array}{c}
\text{\(\text{OH}\)} \\
\text{\(\text{H}\)}
\end{array} \quad \Delta H = 46 \text{ kcal}
\]

\[
\text{\(3^\circ \text{O}_2 \rightarrow \)} \begin{array}{c}
\text{\(\text{OH}\)} \\
\text{\(\text{H}\)}
\end{array} \quad \Delta H = 35 \text{ kcal}
\]

where (47b) is favored due to lone-pair stabilization. Clearly, in order for \(3^\circ \text{O}_2\) to attack such substrates, it needs to be activated.

Now consider \(3^\circ \text{O}_2\) attack on the flavin. We estimate\(^{10}\) the following energetics

\(^8\)Abstraction of an H to form H\(\text{O}_2\) would have \(\Delta H \approx 60 \text{ kcal}\).

\(^9\)Abstraction of the acidic H to form H\(\text{O}_2\) would have \(\Delta H = 41 \text{ kcal}\).

\(^{10}\)In making such estimates, we make use of the best available information on substituent effects on various bonds (14, 31-33). Unfortunately, there is considerable uncertainty due to lack of reliable experimental information on relevant model systems. The relative \(\Delta H\)'s for various alternative processes should be more accurate than the absolute values.
where attack at 5a is equivalent to 9a, (49b), and attack at 6, 7, 8, 9 leads to even higher $\Delta H$. The nitrogen lone pairs adjacent to the 4a-10a double bond of FH$_2$ weaken this bond sufficiently that $^{3}$O$_2$ attack on this olefinic bond involves an activation barrier comparable to that of $^{1}$O$_2$ on a simple olefin.$^{11}$

Summarizing, because of the groups surrounding the olefinic part of the flavin (4a-10a), the flavin can bond $^{3}$O$_2$ to form a triplet biradical. There should be a preference for position 4a with respect to 10a (96% to 4% based on the $\Delta H$ of 3 and 5, respectively).

$^{11}$The 5 and 10 nitrogens also serve to direct the $^{3}$O$_2$ to prefer positions 5a and 4a with respect to 6, 7, 8, 9.

Next we consider attack of the O$_2$-FH$_2$ complex on phenol,

Our estimate is that $\Delta H = -9$ kcal for this process. This should be compared with $\Delta H = +35$ kcal for the analogous process with $^{3}$O$_2$. Thus bonding the $^{3}$O$_2$ to the flavin has had the effect of activating the $^{3}$O$_2$ so that attack on a phenol substrate should be facile.$^{12}$ There are also other products higher in energy than (50) but with $\Delta H \approx 0$, namely, attack at another carbon of the phenol ($\Delta H = +2$ kcal) or abstraction of the acid hydrogen ($\Delta H = -2$ kcal). We will consider just the lowest energy product here.

C. Decomposition of the Flavin-O$_2$-Substrate Complex

An O-O bond as in (50) would normally be worth 38 kcal, leading to two biradicals as in (51).$^{13}$ However, we estimate that even for the gas phase species the O-FH$_2$ zwitterion in (52) is $\nabla 29$ kcal more stable than the biradical depicted in (51). Thus we estimate that the activation energy for breaking the O-O bond of (50) is only 9, leading to the products in (52).

$^{12}$Note that although $\Delta H$ is negative, there may be an activation energy for the reaction.

$^{13}$These biradicals can rearrange to lower energy states but the activation energy would be more closely related to the bond energy without rearrangement.
The reason for this tremendous stabilization of the zwitterion is the presence of the nitrogen lone pairs at 1 and 10, which stabilize a positive change at 10a.

The above analysis was for the complex involving $^3\text{O}_2$ attack at position 4a. Attack of $^3\text{O}_2$ at position 10a was estimated to be only about 2 kcal higher, and the subsequent chemistry for this species should also be considered. As indicated in (53), we find that the O-O bond of the flavin-O$_2$-substrate complex is significantly stronger, $\Delta H = 15$ kcal. The reason is that stabilization of the positive charge at position 4a is significantly less, so that the zwitterion O-FH$_2$ complex (53) is not as stable.

Thus we conclude that it is the 4a O$_2$-FH$_2$ complex that dominates substrate oxidation.

After step (52), there are two fragments which may carry out their subsequent chemistry and rearrangements independently.

In an aqueous environment, a species such as (54) would deprotonate at the 1 and 5 nitrogens and protonate at the oxy-anion position to form oxidized flavin (55) plus water (34). This process might possibly go through a process such as

in which case some of the oxygen of labeled O$_2$ would end up in the flavin rather than in the H$_2$O.

Next we consider the likely rearrangements of the oxy-substrate complex.

Since we started with triplet O$_2$, (57) will be a triplet state unless there has been a spin-flip in one of the earlier steps. Some of the possible intermediates are shown in Fig. 11 along with estimates of $\Delta H$'s. There are at least two plausible
E. Summary and Solvent Effects

1. Summary. Summarizing, the above gas-phase analysis leads to the mechanism,

\[ \text{FH}_2 + ^3\text{O}_2 \leftrightarrow \text{product} \]

\[ \text{substrate} \]

Now we should address some of the likely changes resulting from the solvent.

2. The Initial FH\textsubscript{2}-O\textsubscript{2} Complex. For a sufficiently polar medium, the singlet zwitterion form of (47) is significantly stabilized over the triplet form; thus, depending upon the rate of substrate attack and the rate of dissociation of (47) to FH\textsubscript{2} + ^3O\textsubscript{2}, there could be conversion to the singlet zwitterion, which would probably deprotonate to yield the peroxide anion (60).
Indeed, using flavins blocked at the 5 position, Bruice and co-workers (35) observe a chemistry interpretable in terms of (60) as the first intermediate. In addition, from spectroscopic studies of such systems, Massey and co-workers (36) find evidence of a species of the form (60) as the first intermediate.

One should keep in mind here that even if the flavin complex (47) does convert to the peroxide in polar media, this does not prove that this conversion takes place in the operational enzyme. The interaction between enzyme and flavin and the polarity of the medium in this region could both serve to prevent (or enhance) the transformation from (47) to (60).

Since the initial O₂-FH₂ species is a triplet state, one might hope to use electron spin resonance (ESR) to detect this species and thereby determine the kinetics of some of the processes.\(^{15}\) In the absence of substrate, this (triplet) complex can redissociate to products and the concentration should yield the ΔG for forming the complex. Although the rate of triplet → singlet spin-flip is small, if the solvent is sufficiently polar, the spin-flip may irreversibly lead to the zwitterion (59) and subsequently the peroxide complex (60). In the absence of substrate, (60) would probably lead to production of peroxide and oxidized flavin,\(^{16}\)

(60) → Fox + HOO⁻.

This would probably increase the ΔG for forming the complex analogous to (50) and would probably decrease the propensity to attack the 1 position of the substrate. However, formation of the (ionized)

FH₂-O₂ - substrate

complex corresponding to (50) would still be plausible (but not necessarily with attack on the 1 position).

After the triplet O₂-FH₂ complex (47) has converted to peroxide (60), attack on the ionized substrate would seem even less favorable and attack on the 1 position unfavorable. Indeed, in this situation formation of the complex might be acid-catalyzed (one species unionized).

An alternative reaction for (60) would be the decomposition

(60) → Fox + H⁺ + O₂

with attack of superoxide (O₂⁻) or peroxide (HOO⁻) on the substrate. We believe that this is not energetically favorable.
but cannot provide quantitative estimates. An experimental test would be to deliberately generate superoxide and examine the reactions with the substrate.17

4. Decomposition of the Complex. If a flavin-O₂-substrate complex is formed in solution, the decomposition to oxy-flavin and oxy-substrate complexes should be favorable, just as in our gas-phase analysis.

Using spectroscopic techniques, Massey and co-workers (36) observed three intermediates with intermediates I and III having very similar spectra. If we identify

\[ \text{(63)} \]

as species III (quite reasonable in terms of our model), then we would have to assign the peroxide (60) as species I (but with the caveat in Sec. E.2).

5. Comments. The analysis in this section of flavin-cofactor hydroylations must be considered as highly speculative. We have no information about enzyme-flavin interactions and have made only very qualitative estimates on the effect of the solvent or medium. Indeed, even the gas-phase estimates are in some cases uncertain due to a lack of solid experimental (or theoretical) data on substituent effects and resonance energies on similar systems. However, even with such uncertainties, we believe that such a semi-quantitative analysis helps focus attention on the plausible reaction mechanisms and upon the intermediates most likely to be important. This certainty helps the theoretician in directing his attention toward the places where further study would be most effective in answering mechanistic questions. We hope that it is of similar use to experimentalists in designing experiments to give yes and no answers about these mechanistic questions.

17 At this meeting there has been some discussion of possible effects of O₂ on cells. A point to be noted is that although HO₂ is 99.2% ionized in aqueous solutions at pH 7, it may be nearly totally unionized in hydrophobic, nonpolar media. Thus, for example, in diffusing through cell walls, the O₂ may first be protonated, diffuse through the hydrophobic regions as peroxy radical, and then be reionized. Thus, in discussing effects of O₂ on hydrophobic regions of cells, it may be the chemistry of peroxy radicals that is relevant, not the chemistry of superoxide anions.

References


DISCUSSION

GROVES: If one accepts, for the moment, that the initial oxygenated flavin triplet diradical adds to the substrate phenol in the manner you have depicted, the resulting peroxy diradical (i) should have no barrier to triplet-singlet interconversion. Accordingly, singlet (i) could simply decompose directly to singlet products, without worrying about triplet catechol precursors.

GODDARD: I agree that the triplet and singlet biradicals would be very close (perhaps singlet is lower) and a priori one would expect that the flavin-O2-substrate species may convert to singlet before decomposing. [The rate is determined by the spin-orbit coupling between triplet and singlet and may be slow even without a barrier.] If it did so, formation of

would probably occur. This would probably lead to an NIH shift (not observed) which was why I assumed that the decomposition had occurred before spin flip.

TRAYLOR: You write as intermediates, the diradical 'C-\(\cdot\)C\(=\)O\(\cdot\)' or zwitterion \(\cdot\)C-\(\cdot\)C\(=\)O\(\cdot\)' which seem to me to have no lifetime. Should these not give the epoxide directly?

GODDARD: I would expect the singlet to quickly form the epoxide in the absence of bimolecular processes. However, the triplet would have different chemistry. I assumed that the triplet product (i) might lead to a 1-3 \(\cdot\)H shift to give (ii):
before spin flip. After spin-flip to singlet, OH migration would lead to catechol (and no NIH shift).

FOOTE: If the internal H transfer doesn't have a substantial energy barrier (25 kcal), the flavin diradical will unimolecularly transfer a hydrogen (to give the hydroperoxide) and will not have time to undergo a bimolecular reaction with phenol.

GODDARD: I have no direct information (yet) on the barrier for this process, but I believe that it would greatly exceed that for the chemistry of the triplet state. I expect that proton transfer would be fastest for the singlet state (which would be a zwitterion) and could possibly lead to a facile reaction, e.g.

MASSEY: I would like to know whether you consider that any of the radical intermediates which you postulate would be detectable by EPR.

GODDARD: Some of the biradical intermediates are triplet and may be EPR-detectable if the lines are not too broad and if the concentration is sufficiently high. Perhaps with sufficiently fast quenching in an inert material at low temperature one could see a signal.