Ozone Model for Bonding of an O_2 to Heme in Oxyhemoglobin

(electronic structure/generalized valence bond/configuration interaction/excited electronic states/electric field gradient)

WILLIAM A. GODDARD, III* AND BARRY D. OLAFSON

Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91125

ABSTRACT   Several rather different models of the Fe—O₂ bond in oxyhemoglobin have previously been proposed, none of which provide a satisfactory explanation of several properties. We propose a new model for the bonding of an O₂ to the Fe of myoglobin and hemoglobin and report ab initio generalized valence bond and configuration interaction calculations on FeO₂ that corroborate this model. Our model is based closely upon the bonding in ozone which recent theoretical studies have shown to be basically a biradical with a singlet state stabilized by a three-center four-electron pi bond. In this model, the facile formation and dissociation of the Fe—O₂ bond is easily rationalized since the O₂ always retains its triplet ground state character. The ozone model leads naturally to a large negative electric field gradient (in agreement with Mössbauer studies) and to z-polarized (perpendicular to the heme) charge transfer transitions. It also suggests that the 1.3 eV transition, present in HbO₂ and absent in HbCO, is due to a porphyrin-to-Fe transition, analogous to that of ferric hemoglobins (e.g., HbCN).
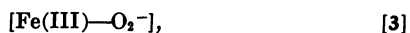
I. Introduction

There have been a number of formulations of the bonding of the O₂ to the heme in oxyhemoglobin (HbO₂); examples are the linear model [Fe=O—O and Fe—O=O] of Pauling and Coryell (1), the bent model

$$\text{Fe}=\ddot{\text{O}} \diagdown \qquad\qquad [1]$$
$$:\ddot{\text{O}}:$$

of Pauling (2), the ring model

$$\text{Fe} \leftarrow \| \begin{smallmatrix} \text{O} \\ \\ \text{O} \end{smallmatrix} \qquad\qquad [2]$$

of Griffith (3), the met-superoxide model of Weiss (4),

$$[\text{Fe(III)}\!-\!\text{O}_2{}^-], \qquad\qquad [3]$$

and the two-electron oxidative addition model of Gray (5)

$$\text{Fe(IV)} \diagup\!\!\diagdown \begin{smallmatrix} \text{O} \\ \\ \text{O} \end{smallmatrix} \Big]^{2-}. \qquad\qquad [4]$$

Recent structural studies by Collman and co-workers (6) on a model Fe—O₂ complex strongly support the bent geometry [1], but none of these structures provides a ready explanation for the following experimental properties of oxyhemoglobin:
1. The ease of forming and breaking the bond between the

Fe and O₂ (all models [1–4] suggest a drastic change in the O₂ bond upon addition to the heme);
2. The large asymmetry in the charge distribution about the Fe (as measured in the Mössbauer studies, refs. 7 and 8);
3. The low-lying 1.3 eV electronic transition (9) present in HbO₂ but absent in HbCO;
4. The strong z-polarized (perpendicular to the heme), visible and ultraviolet electronic transitions (9) present in HbO₂ but absent in HbCO.

We propose a new model for the bonding of O₂ to the heme based closely upon the bonding of the O₂ to the O in ozone (10–13). This model is corroborated by extensive theoretical studies of FeO₂ and is consistent with the above experimental observations.

II. The bonding in ozone

Recent ab initio studies (10–13) have shown that the ground state of ozone is basically biradical in character. Ignoring the 1s and 2s electrons, the ground state of an O atom can be viewed as in Fig. 1a, where p-orbitals in the plane are indicated by two-lobed figures and the p-orbital out of the plane is indicated by a circle; the dots indicate how many electrons are in each orbital. Generalized valence bond (GVB) calcula-
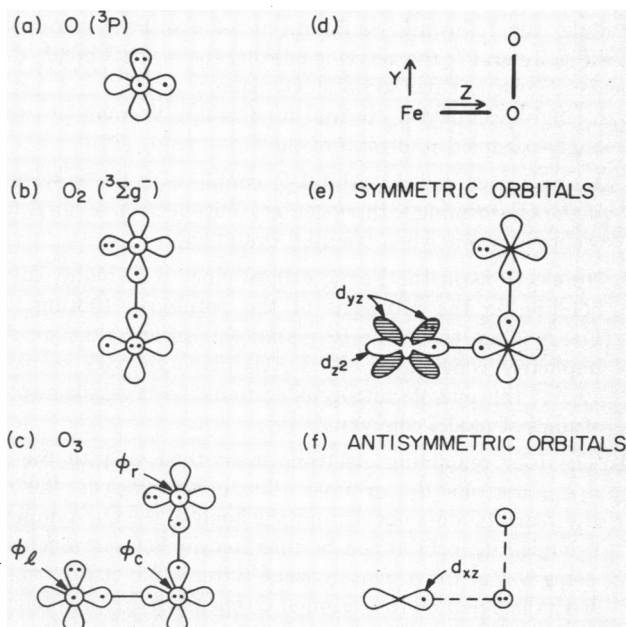

FIG. 1. Orbital occupation diagrams e and f show the σ and π orbitals of FeO₂, respectively. Note that the $d_{x^2-y^2}$ orbital (lying in the porphyrin plane) is not shown.

---

tions (13) on O_2 show that the ground state has the form in Fig. 1b that corresponds to two ground state oxygen atoms coupled together (the line indicates bond formation—singlet pairing of electrons).

Generalized valence bond calculations on ozone (10–13) show that the ground state has the form in Fig. 1c. Comparing a, b, and c of Fig. 1, we see that the bond of O and O_2 to form ozone is made without a drastic effect upon the bonding in the O_2.

The doubly-occupied π-orbital (denoted as ϕ_c) in Fig. 1c is shown as localized. In fact, ϕ_c delocalizes somewhat onto both terminal atoms, resulting in a significant contribution to the bonding. The configuration in Fig. 1c has two singly-occupied orbitals (denoted as ϕ_l and ϕ_r) and, hence, leads to both a singlet state and a triplet state. The requirement (Pauli principle) that both ϕ_l and ϕ_r be orthogonal to the delocalized ϕ_c leads to an increased overlap between ϕ_l and ϕ_r and stabilizes the singlet state by about 1 eV with respect to the triplet state (comparing adiabatic energies).

Thus, we can view ozone as a biradical formed by coupling ground state O to ground state O_2. The singlet state of the product is stabilized by a special three-center, four-electron π-bond (10–13).

The bonding in Fig. 1c would suggest a 90° bond angle. The actual bond angle is larger (117°) due primarily to repulsive interaction between the doubly-occupied p-orbitals on the terminal atoms.

III. The bonding in FeO₂

Recent theoretical studies have shown that, just as in ozone, bonding of O_2 to H (14), to CH_2 (15, 16), and to CHO (17) does not lead to drastic modifications of the bonding within the O_2. We propose that the bonding of the O_2 to Fe in oxy-hemoglobin (HbO_2) and oxymyoglobin (MbO_2) is basically similar to the bonding of O and O_2 in ozone. In order for O_2 to bond to Hb without a drastic modification of the O_2 orbitals, the following configuration of the Fe is required (see Fig. 1e and f; the z-axis is parallel to the FeO bond; the x-axis is perpendicular to the FeO_2 plane):

1. A singly-occupied d_{z^2} orbital for forming a σ-bond to the singly-occupied p_z orbital of the O_2;

2. A singly-occupied d_{xz} orbital to couple with the π system of the O_2, forming a three-center, four-electron π-bond (as occurs in ozone when bonding O and O_2);

3. No electrons in the Fe d_{xy} orbital since it is highly anti-bonding to the nitrogens of the porphyrin (taking the Fe–O_2 plane to be the yz plane; this means that we are implicitly considering the Fe–O_2 plane to pass between two of the pyrrole nitrogens as found in crystallographic studies of model compounds (6);

4. The four remaining electrons must then go into the Fe $d_{x^2-y^2}$ and Fe d_{yz} orbitals. The occupation of the Fe $d_{x^2-y^2}$ orbital does not disrupt the bond to the O_2. A doubly-occupied d_{yz} orbital can be tolerated in FeO_2 in much the same way as the p_y pair in ozone (interaction of this orbital with the p pair on the terminal O_2 opens up the bond angle).

We refer to this configuration of FeO_2 (Fig. 1e and f) as 4π to indicate that there are four electrons in π orbitals (Fig. 1f). Other low-lying states have electrons excited from a σ orbital (Fig. 1e) to a π orbital, leading to 5π and 6π states.

Summarizing, in order to make a strong bond between Hb and O_2, the configuration on the Fe must be

$$(d^i_{x^2-y^2})^2(d_{yz})^2(d_{xz})^1(d_{z^2})^1 \doteq (t_{2g})^5(e_g)^1$$

in terms of octahedral symmetry functions (note with our coordinate system $d_{x^2-y^2}$ is of t_{2g} type). This configuration leads to one component of the ^3E state (C_{4v} symmetry group) of Hb. Although not the ground state of deoxyhemoglobin, there is some evidence (18) that the ^3E state becomes the lowest state of Hb when the Fe is in the plane of the heme.

Most descriptions (2, 3, 7–9) of HbO_2 have used crystal field or ligand field concepts in which the ligand is assumed to have a closed shell configuration. Thus, it has been assumed that the O_2 is a singlet state and that the ferrous iron is low spin ($S = 0$)

$$(dxy)^2(dxz)^2(dyz)^2 = (t_{2g})^6.$$

In the GVB description, the configuration of the O_2 and of the iron are basically triplet states; there is then a σ bond between the Fe and one O and a four-electron three-center π bond, leading to a ground singlet state despite the essentially triplet character of the Fe and of the O_2. Thus, the ozone model of HbO_2 suggests that the Fe is essentially of intermediate spin ($S = 1$), whereas ligand field descriptions usually assume either high spin ($S = 2$) or low spin ($S = 0$) ferrous iron. As shown below, only $S = 1$ iron is consistent with the Mössbauer studies.

IV. FeO₂ calculations

The most questionable aspect of our ozone model of the bonding in HbO_2 is that replacement of an O with the Fe could well lead to significant changes due to the differing ionic character and overlap of various orbitals. To test the applicability of these ideas to the FeO_2 unit, we carried out extensive *ab initio* studies of the FeO_2 system, applying the generalized valence bond (19) and configuration interaction (CI) methods to the various possible electronic configurations ($4\pi,5\pi,6\pi$) of FeO_2, using both the open geometry ($\measuredangle$ FeOO $= 136°$) and the ring (Griffith) geometry.† As discussed in Section V the results are in good agreement with the model. Although the FeO_2 unit was treated quite well, there were serious approximations in the treatment of the rest of the protein. The porphyrin was not included explicitly; we merely excluded the d_{xy} orbital (which would be highly antibonding to the pyrrole nitrogens) from occupation. The two charges formally assigned to the porphyrin ring when describing the

---

iron as ferrous are included by placing them in the 4s orbital of the iron.‡ We totally ignored the proximal imidazole; this is an approximation with serious consequences since it could no doubt have a strong effect upon the calculated bond energy of the Fe to the O_2 and on the oscillator strengths for transitions to the excited states. These approximations may also lead to reordering of some of the electronic states, but should not cause gross changes in the overall character of these states.

Bear in mind that our basic *model* of the Fe—O_2 bond rests on *qualitative* concepts, based on the electronic structure of ozone and of peroxyradicals. The calculations reported here serve as a first test of the model, particularly in the use of these concepts for describing the bond to a transition metal. However, the calculations do not prove the model (due to the very approximate treatment of the other ligands) nor is the model solely based on the calculations. In comparing our model with experiment, we quote quantities such as excitation energies; however, it is to be understood that these numbers are yet of only qualitative significance insofar as correspondence to oxymyoglobin and oxyhemoglobin is concerned.

On the other hand, the FeO_2 part of the system is treated quite accurately with very extensive configuration interaction of fully *ab initio* wavefunctions. Hence, we believe that they are quite adequate for testing the ozone model of the Fe—O_2 bond in oxyhemoglobin.

V. Ground state of FeO_2 from GVB calculations

The GVB-CI calculations lead to a singlet ground state with a 4π configuration and open (Pauling) geometry, in agreement with the ozone model. The GVB orbitals† (bottom of p. 2336) for the singlet 4π open geometry ground state are shown in Fig. 2. We see that these orbitals correspond closely to the schematic description in Fig. 1e and f. The O_2 orbitals of Fig. 2 are very similar to the O_2 orbitals of free O_2, much more so than for ozone and other peroxy radicals that have been studied.

The GVB-CI wavefunction leads to Mulliken populations (on the Fe, central O, and terminal O, respectively) of 25.91, 8.09, and 8.00 electrons of which 1.14, 1.57, and 1.29 are in the π system. Thus, there is a net transfer of 0.23 σ electrons from the Fe toward the O_2 (as expected from the ionic character of the FeO σ bond), but this is partly counterbalanced by back donation in the π system of 0.14 electrons from the O_2 toward the Fe, leading to an overall neutral system. This is

---

‡ (*a*) We also carried out calculations with the two Fe(4s) electrons deleted. Although the character of the ground state is qualitatively unchanged, there are larger effects on the character of some excited states, especially those involving charge transfer character. (*b*) Most previous theoretical discussions have been in the context of molecular orbital or Hartree–Fock (HF) wavefunctions. It is particularly interesting to note that the HF method leads to a *quintet* ground state for FeO_2 with the singlet state over 4 eV higher. This results from intrinsic bias in the molecular orbital or HF method for high-spin states (for example, the HF method incorrectly leads to a triplet ground state for ozone with the singlet state over 2 eV higher) (10–12). It is likely that the HF method would lead to a quintet ground state of HbO_2, perhaps explaining the inability of Zerner *et al.* (18) in converging their approximate calculations on the axial configuration of HbO_2.
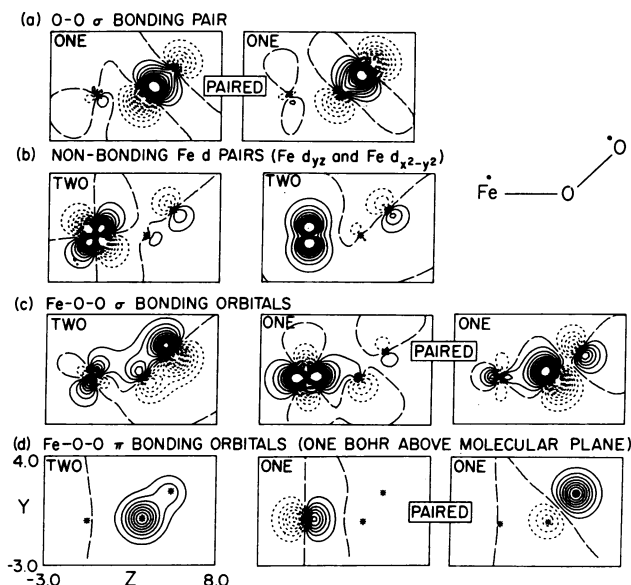

FIG. 2. The GVB orbitals of FeO_2. Plotted in the FeO_2 plane for a-c and one bohr (0.0529 nm) above the molecular plane for d. Long dashes indicate zero amplitude; the increment between contours is 0.05 atomic units. The label ONE or TWO indicates the number of electrons in the orbital. A pair of orbitals connected by the label PAIRED is coupled into a singlet bond pair (just as in a valence bond wavefunction).

contrary to the suggestion by Weiss (4) that the Fe—O_2 bond is more like $Fe^{+++}O_2^-$.

These results indicate that one can quite accurately view the ground state of HbO_2 as one component of the ^3E state of Hb coupled with the $^3\Sigma_g^-$ state of O_2 (in the yz plane) to form a singlet state.

The ozone model of HbO_2 leads to an electric field gradient (q) on the Fe that is large and negative, in agreement with the Mössbauer studies (7). In fact, when atomic orbitals (8) are used, the electric field gradient parameters are [q_D and q_O for Hb and HbO_2, respectively; electronic charge e set to 1]

$$q_D \equiv V_{zz} = +\frac{4}{7}\langle 1/r^3\rangle_d$$

$$\eta q_D \equiv (V_{xx} - V_{yy}) = 0$$

for Hb and

$$q_O = -\frac{2}{7}\langle 1/r^3\rangle_d$$

$$rq_O = \frac{6}{7}\langle 1/r^3\rangle_d$$

for HbO_2. Thus, the quadrupole splitting energy [$\Delta E = (Qq/2)\sqrt{1 + 1/3\eta^2}$] is $\Delta E_D = \Delta E_O|(4/7)(Q/2)\langle 1/r^3\rangle_d|$ for both Hb and HbO_2. This is in excellent agreement with the results of Mössbauer experiments (7): $\Delta E(\text{Hb}) = 2.40$ mm/sec and $\Delta E(\text{HbO})_2 = 2.24$ mm/sec. In addition, from application of magnetic fields, it is known that the q of HbO_2 is negative (7) in agreement with our model. We consider this to be strong evidence for the ozone model of HbO_2. None of the previous models of the electronic structure of HbO_2 has been

used to explain the Mössbauer results§ [low spin Fe leads to $q = 0$; the approximate molecular orbital calculations of Zerner et al. (18) lead to the correct magnitude (8) for q but the wrong sign (7).]

VI. Excited states of FeO₂ from GVB calculations

We find that the lowest state of the ring (Griffith) geometry is a 6π state (as expected) but that it lies 1.45 eV above the lowest state of the open (Pauling) geometry. This is about the same separation as found for ozone (8–13).

As would be expected from the GVB description of the ground state, we find a number of low-lying excited states of FeO₂. There is a low-lying 4π triplet state at 0.37 eV corresponding to a triplet coupling of the singly-occupied orbitals of gFi. le and f (analogous to the low-lying triplet state of ozone). The 5π configurations lead to a triplet state at 0.33 eV (20) and a singlet state at 0.36 eV; these states involve basically an Fe $d_{zy} \rightarrow$ Fe d_{xz} transition (very weak). The lowest quintet state has a 5π configuration and lies at 0.87 eV.

All calculated transitions of the FeO₂ unit were found to be weak. Most calculated transitions are expected to be weak because they correspond to $d \rightarrow d$ transitions on the Fe or to triplet $\rightarrow$ singlet transitions on the O₂.

In ozone, the lowest strong electronic transition has a peak at 4.9 eV (the Hartley band) with an oscillator strength of $f = 0.1$. This transition corresponds essentially to excitation of an electron from the double-occupied O pπ orbital on the center O to the single-occupied terminal O pπ orbitals (see Fig. 1c), i.e., π-charge transfer (11, 12). For FeO₂, we expect two analogous π-charge transfer transitions, each of which should have a large-z-polarized component. Experimentally (9), two z-polarized transitions have been observed in oxyhemoglobin at 3.9 eV with $f = 0.1$, and at 2.6 eV with $f = 0.02$ (whereas such transitions are not found HbCO). We believe that the higher transition corresponds to the π-charge transfer (Hartley) transition of ozone; it is not clear from the model that the second expected transition would be as low as 2.6 eV. However, the calculations lead to three z-polarized transitions with components of charge transfer from the oxygen to the Fe in the π system lying at 1.8 eV, 2.0 eV, and 3.4 eV. There is also a z-polarized transition at 4.7 eV with a shift of π electron density to the outer (terminal) oxygen. In each case, we find that the sigma system readjusts so as to keep the molecule essentially neutral, and the calculated oscillator strengths are all very small. As discussed earlier, a better treatment of the other ligands could lead to large effects upon properties such as the oscillator strength.

VII. CoO₂

From the above model of the Fe—O₂ bond, we expect analogous Co systems to involve essentially the same O₂ configuration but with an additional electron in the Co d_{xz} orbital [Co configuration $(d_{x^2-y^2})^2(d_{yx})^2(d_{xz})^2(d_{z^2})^1$]. Thus, the deoxy case has a singly-occupied d_{z^2} orbital on the Co, whereas the CoO₂ has all orbitals paired except for the single-occupied

(antibonding) π orbital on the O₂ (the d_{xz} orbital of Fig. 2d is doubly-occupied, leaving the antibonding orbital on the O₂ as singly-occupied).

Electron spin resonance studies (20) of Co(II) Schiff base compounds (exhibiting reversible O₂ bonding) show that the five-coordinate deoxy complex is low-spin with the unpaired electron in a d_{z^2} orbital, whereas the CoO₂ compound has spin ¹/₂ with the spin density on the O₂. This is in excellent agreement with the GVB model. One would expect a comparable but slightly smaller bond angle for CoO₂ than for FeO₂ (due to the loss of the three-center π bond) as observed (21) ($\measuredangle$ CoOO $= 126°$, $\measuredangle$ FeOO $= 136°$).

In the usual ligand field models, it is assumed that an O₂ ligand is in its singlet state, in disagreement with the electron spin resonance data for Co(II) O₂ (20). As a result it was concluded (20) that the CoO₂ bond is Co(III) O₂⁻ [analogous to the Weiss formulation (4) of FeO₂]. The GVB model leads to a simple explanation of the experimental results for Co(II), without such assumptions.

VIII. Other comments

Stephens and Eaton (22) studied ferric hemoglobins with magnetic circular dichroism and concluded that the 1.3 eV absorption corresponds to a porphyrin $\rightarrow$ Fe t_{2g} transition (there is at least one t_{2g} hole for ferric compounds). In the ozone model of HbO₂, there is also one t_{2g} hole on the Fe so that a similar transition is expected, as observed (9). In HbCO where all the t_{2g} orbitals are double-occupied, no corresponding transition is found. Also, for CoO₂ such a transition would not be expected.

The upper state of the π-charge transfer transition in ozone dissociates to O(¹D) plus O₂(¹Δ$_g$) with a quantum yield of unity. Similar photodissociation may occur for the π-charge transfer states of HbO₂ leading then to either Hb in a singlet state plus O₂(¹Δ$_g$) or to Hb=O in a singlet state plus O(¹D). In addition to the unusual state of the Hb, this photodissociation leads to a highly reactive state of the dissociated species [O(¹D) or O₂(¹Δ$_g$)] which may well attack molecules of the protein defining the cavity around the heme. The interesting possibility here is that particular photon energies would lead to characteristic damage of the protein due to the specific chemistry of the excited dissociation products.

IX. Conclusions

We believe that the ozone model for the bonding of the O₂ to a heme provides a useful model for understanding the properties of oxyhemoglobin. The validity of this model is corroborated by ab initio studies of the FeO₂ system and by the agreement with experimental properties.

§ Eicher, H. & Trautwein, A. [(1969) *J. Chem. Phys.* 50, 2540–2551] attempted to explain the Mössbauer data of Hb and HbO₂ in terms of interactions between the ¹A₁, ³E, ⁵B₂, and ⁵E states of Fe(d^6) induced by spin-orbit coupling. They concluded that only ³E could explain the Mössbauer data for HbO₂. (Their calculated ΔE due to ³E was a factor of 50% too large.)

1. Pauling, L. & Coryell, C. D. (1936) *Proc. Nat. Acad. Sci. USA* 22, 210–216.

2. Pauling, L. (1949) in *Haemoglobin* (Butterworths Scientific Publications, London), pp. 57–65.
3. Griffith, J. S. (1956) *Proc. Roy. Soc. Ser. A* 235, 23–36.
4. Weiss, J. J. (1964) *Nature* 202, 83–84.
5. Gray, H. B. (1971) *Adv. Chem. Ser.* 100, 365–389.
6. Collman, J. P., Gagne, R. R., Reed, C. A., Robinson, W. T. & Rodley, G. S. (1974) *Proc. Nat. Acad. Sci. USA* 71, 1326–1329.
7. Lang, G. & Marshall, W. (1966) *J. Mol. Biol.* 18, 385–404.
8. Weissbluth, M. & Maling, J. E. (1967) *J. Chem. Phys.* 47, 4166–4172.
9. Makinen, M. & Eaton, W. A. (1973) *Ann. N.Y. Acad. Sci.* 206, 210–222.
10. Goddard, W. A., III, Dunning, T. H., Jr., Hunt, W. J. & Hay, P. J. (1973) *Accts. Chem. Res.* 6, 368–376.
11. Hay, P. J. & Goddard, W. A., III (1972) *Chem. Phys. Lett.* 14, 46–48.
12. Hay, P. J., Dunning, T. H., Jr. & Goddard, W. A., III (1973) *Chem. Phys. Lett.* 23, 457–462.
13. Hay, P. J., Dunning, T. H., Jr., & Goddard, W. A., III (1975) "Configuration interaction studies of O_3 and O_3^+. I Ground and excited states," *J. Chem. Phys.*, in press.
14. Goddard, W. A., III (1973) in *Lecture Notes, School on the Fundamental Chemical Basis of Reactions in the Polluted Atmosphere,* ed. Kern, C. W. (Battelle Research Center, Seattle, Wash.), pp. 254–297.
15. Wadt, W. R. & Goddard, W. A., III (1975) "The electronic structure of the criegee intermediate: ramifications for the mechanism of ozonolysis," *J. Am. Chem. Soc.*, in press.
16. Wadt, W. R. (1974) Ph.D. Dissertation, California Institute of Technology.
17. Winter, N. W., Goddard, W. A., III & Bender, C. F. (1975) "Theoretical description of the $^3A''$ and $^3A'$ states of the peroxyformyl radical," *Chem. Phys. Lett.*, in press.
18. Zerner, M., Gouterman, M. & Kobayashi, H. (1966) *Theor. Chim. Acta* 6, 363–400.
19. Hunt, W. J., Hay, P. J. & Goddard, W. A., III (1972) *J. Chem. Phys.* 57, 738–748.
20. Hoffman, B. M., Diemente, D. L. & Basolo, F. (1970) *J. Am. Chem. Soc.* 92, 61–65.
21. Rodley, G. A. & Robinson, W. R. (1972) *Nature* 235, 438–439.
22. Cheng, J. C., Osborne, G. A., Stephens, P. J. & Eaton, W. A (1973) *Nature* 241, 193–194.