

Table I. Dimensions of the Dodecahedral Coordination Polyhedron of [Ti(Et₂mtc)₄]

Bond	Length, Å	Edge type	Atoms	Length, Å
Ti-S _{1B}	2.533 (1)	a	O _{1A} -O _{1A'}	2.491 (6)
Ti-S _{2A}	2.609 (1)	a	S _{2A} -S _{2A'}	3.212 (2)
Ti-O _{1A}	2.117 (3)	b	S _{1B} -O _{2B}	3.205 (3)
Ti-O _{2B}	2.061 (3)	b	S _{1B} -O _{2B'}	3.455 (3)
		m	S _{1B} -O _{1A}	2.530 (3)
		m	S _{2A} -O _{2B}	2.540 (3)
		g	S _{1B} -S _{2A}	3.327 (2)
		g	S _{1B} -S _{2A'}	3.367 (2)
		g	O _{1A} -O _{2B}	2.662 (4)
		g	O _{1A} -O _{2B'}	2.701 (4)

The geometry of the complex (see Figure 1) is best described in terms of a slightly distorted dodecahedral coordination polyhedron with the bidentate monothiocarbamate ligands spanning *m* edges to give the *mmmm* stereoisomer.² The titanium atom is located on a crystallographic twofold axis which is coincident with the $\bar{4}$ axis of the idealized $D_{2d} - \bar{4}2m$ dodecahedron. The interpenetrating ABBA trapezoids are nearly perpendicular (dihedral angle = 87.4°) with the titanium atom and the four donor atoms which define an individual trapezoid being planar to within 0.06 Å. Other measures of the extent to which the coordination polyhedron approximates the idealized dodecahedron are the δ and ϕ shape parameters defined by Porai-Koshits and Aslanov.¹¹ The δ parameters for eight-atom geometries are 29.5° for the D_{2d} dodecahedron; 0.0, 21.8, and 48.2° for the C_{2v} biccapped trigonal prism; and 0.0 and 52.4° for the D_{4d} square antiprism; the ϕ parameters for the three idealized geometries are 0.0, 14.1, and 24.5°, respectively. For [Ti(Et₂mtc)₄] the two independent δ parameters are 26.3 and 34.8°, while the ϕ parameters are 3.6 and 3.8°, values which again indicate that the coordination polyhedron of [Ti(Et₂mtc)₄] most closely approximates a dodecahedron.

Metal-ligand bond distances and polyhedral edge lengths are listed in Table I. As is expected for molecules of this geometry,² complexing bonds to the dodecahedral B sites are shorter than bonds to the A sites (by 0.076 and 0.056 Å for Ti-S and Ti-O, respectively).

The most interesting feature of the structure is that the sulfur and oxygen atoms do *not* sort between the A and B sites as suggested by Orgel's rule, but rather partially sort so that half of the sulfur atoms are in A sites and the other half are in B sites. Thus the molecule is a pseudo- C_{2v} stereoisomer instead of one of the two possible D_{2d} stereoisomers in which the donor atoms are completely sorted. Although no one of the three stereoisomers appears to be sterically favored over the other two, the observed stereoisomer has five relatively short (3.212–3.367 Å) S··S contacts, while the D_{2d} stereoisomer with sulfur atoms in A sites would have only two short S··S contacts and the D_{2d} stereoisomer with sulfur atoms in B sites would have four long S··S contacts. This result suggests that the donor atom sorting pattern in [Ti(Et₂mtc)₄] may be determined by weakly attractive S··S interactions. Weak interligand bonding between sulfur atoms has been suggested previously for trigonal prismatic tris(1,2-dithiolene) complexes^{12,13} and may be important in the preference for cis stereochemistry in octahedral¹⁴ and square planar^{15–21} β -thioacetate complexes. Note that [Ti(Et₂dtc)₄] also has cis stereochemistry in that all four sulfur atoms are located on one side of the molecule and all four oxygen atoms are found on the other side.

Although Orgel's proposal may be useful in predicting the stereochemistry of some eight-coordinate dodecahedral complexes, the structure of [Ti(Et₂mtc)₄] indicates that the stereoisomer observed may be determined by factors other than

the electronic configuration of the metal atom and the π -bonding capabilities of the ligands, in this case the postulated weak, attractive S··S interactions.²²

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- (22) We intend to test this hypothesis by investigating the donor atom sorting pattern in analogous M(Et₂mtc)₄ complexes which contain larger metal atoms and perhaps longer S··S contacts.

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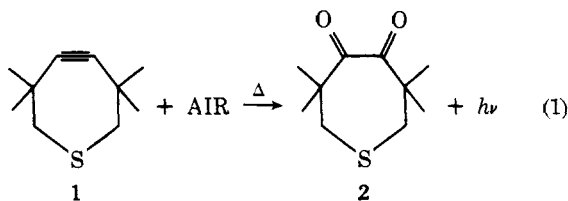
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Reaction of Strained Acetylenes with Molecular Oxygen. A Novel Chemiluminescent Reaction, Evidence for a Dioxetene, and a Mechanism for Thermal Generation of Singlet Oxygen

Sir:

The cyclic seven-membered ring acetylene **1** possesses an exceptionally small¹ C—C≡C bond angle of 146°. The two π orbitals of **1** are not degenerate, but are split into a higher energy orbital which is roughly in the molecular plane (π_y) and a lower energy orbital² which is perpendicular to the molecular plane (π_z). **1** reacts in the dark with atmospheric oxygen to produce the dione **2** (eq 1) in good yield.³ Successful cycloaddition reactions of singlet oxygen to acetylenes are rare.⁴ Importantly, reaction 1 involves triplet oxygen, so that at some stage along the pathway to **2**, a triplet-singlet intersystem crossing must occur. We report here a detailed investigation of reaction 1.

Heating (~90°) of an oxygen saturated solution of **1** in



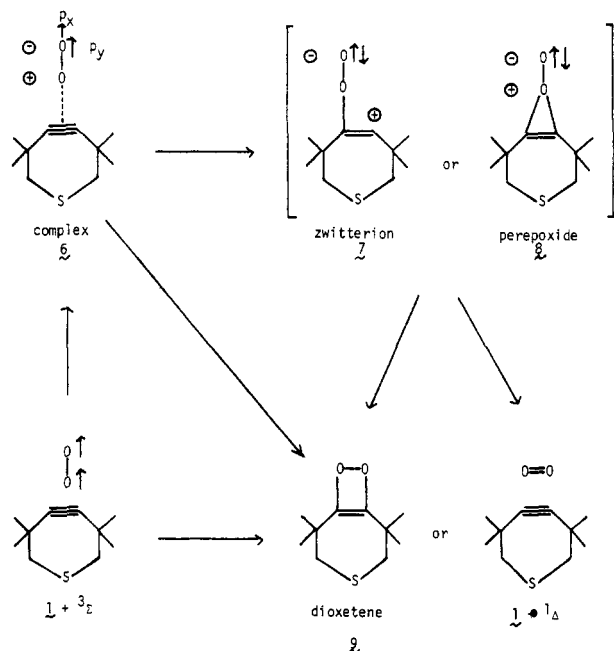
benzene or chlorobenzene results in chemiluminescence which was identified spectrophotometrically⁵ as the fluorescence of **2**. The intensity of chemiluminescence from reaction 1 is greatly enhanced by the addition of perylene. Perylene fluorescence is responsible for the enhanced chemiluminescence. The chemiluminescence from reaction 1 was measured as a function of temperature. The "step analysis" technique⁶ was used to determine an activation energy. This quantity (21 kcal/mol) represents a lower limit to the activation barrier of the rate-determining step along the reaction pathway to chemiluminescence, i.e., if an increase in temperature enhances a step which is competitive with progress toward chemiluminescence, the actual value of E_a will be greater than 21 kcal/mol. The intensity of chemiluminescence was found to be first order in both the concentration of oxygen and **1**.

We have found that unstrained cyclic acetylenes such as 1,7-cyclododecadiyne and 1,8-cyclotetradecadiyne do not react with ground-state triplet or singlet oxygen under a variety of reaction conditions.⁴ However, acetylene **1** reacts with singlet oxygen even at -90°C .



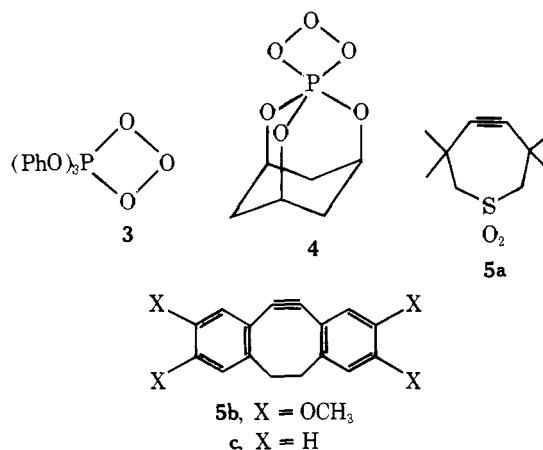
For example, irradiation of **1** in the presence of polymer based rose bengal⁷ in methylene chloride at -90°C yields **2** quantitatively.⁸ If the polymer is separated from the reaction mixture by filtration (at -90°C), the resultant solution is chemiluminescent (fluorescence of **2**) when warmed to $\sim -30^\circ\text{C}$. Thus, the reaction of **1** and singlet oxygen produces an intermediate whose decomposition results in formation of excited states of **2**. This intermediate is stable for days at -90°C . Reaction of **1** with the phosphorus ozonides **3**^{9a} and **4**^{9b} also results in chemiluminescence at temperatures below 0°C .

Scheme I. Proposed chemical mechanism for reaction 1. For an orbital description of the conversion of the triplet complex **6** into the singlet zwitterion **7** or peroxide **8**, see Figure 1.



Although the chemiluminescence produced by the reaction of **1** and singlet oxygen is spectrally identical with that produced by the reaction of **1** and triplet oxygen, the former occurs with an intensity which is at least thousands of times greater than reaction 1 at the same temperature. Thus, **1** does react with singlet oxygen and evidently a dioxetene (Scheme I, **9**) intermediate is produced, and the latter is the immediate precursor of the excited state of **2**. In contrast to reaction 1, the chemiluminescence intensity of reaction 2 is independent of the concentration of **1**, i.e., the reaction is first order in singlet oxygen, but zero order in **1**.¹⁰

Is an experimentally detectable intermediate produced in reaction 2? Analysis of the filtered reaction mixture by low-temperature (-78°C) infrared spectroscopy indicated that only a negligible amount of dione **2** is formed. However, upon warming to room temperature, both chemiluminescence (from excited **2**) and quantitative formation of **2** are observed. This intermediate was found to decompose to **2** with an activation energy of 18 kcal/mol.



It is possible that the nucleophilic sulfur¹¹ of **1** interacts with oxygen and participates in the reaction sequence. However, since the reactions of acetylenes **5a** and **5b** with oxygen (${}^3\Sigma$ and ${}^1\Delta$) parallel those of **1** and are similarly chemiluminescent, we rule out sulfur participation as a significant factor in reactions 1 and 2. Acetylene **5c** does not measurably react with ${}^3\Sigma$ oxygen under our experimental conditions and reacts very slowly with ${}^1\Delta$ oxygen. The results with acetylenes **1** and **5** indicate that reactivity of acetylenes with oxygen requires either structural strain of the acetylene and/or electron releasing substituents.⁴

According to the correlation diagrams,^{12,13} for addition of oxygen to ethylenes the electronic behavior of the state arising from the initial interaction of ${}^3\Sigma$ oxygen with a ground-state olefin is exactly parallel to that of the interaction of the corresponding state arising from the interaction of ${}^1\Delta$ oxygen with ground-state olefin, except that the latter state lies 22 kcal higher in energy and possesses a different spin multiplicity. Thus, the conversion of the triplet collision complex **6** to a weakly bound singlet collision complex is expected to require an activation of ~ 22 kcal/mol. Since the minimum value of E_a for the chemiluminescent reaction is 21 kcal/mol, the energetic requirement to produce ${}^1\Delta$ oxygen (or its chemical equivalent) is met. However, in addition to the energetic requirement, a mechanism for spin inversion must be available and must operate effectively during the lifetime of the complex.

As an activation energy of 18 kcal/mol is found for the decomposition of the chemiluminescent intermediate produced in reaction 2; the 21 kcal/mol of activation required for reaction 1 cannot be ascribed to decomposition of the intermediate. This requires that some step prior to formation of the intermediate is rate determining.

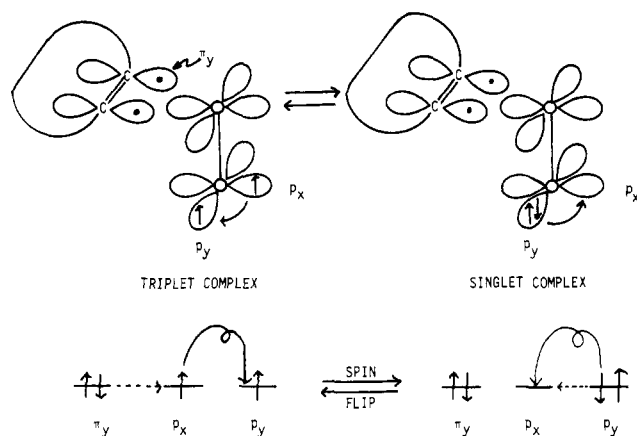


Figure 1. Schematic representation of the initial interactions of **1** and $^3\Sigma$ oxygen. Top: Orbital interactions show how a $p_x \rightarrow p_y$ jump occurs in a charge-transfer complex of **1** + $^3\Sigma$ oxygen. Bottom: Simplified orbital level scheme indicating the coupled nature of the $p_x \rightarrow p_y$ jump induced by charge-transfer interaction of **1** and $^3\Sigma$. According to this mechanism, $^1\Delta$ oxygen should be produced selectively.

We propose that the addition of $^3\Sigma$ oxygen to **1** probably occurs via a similar geometry to that for addition of $^1\Delta$ to **1** (Figure 1). The latter allows the most effective charge transfer from the π_y orbital of the acetylene to a π^* orbital of oxygen. A weak complex of the acetylene and ground-state oxygen would result from this charge-transfer interaction as the system approaches the transition state for reaction. The electronic structure of the complex may be qualitatively represented as one in which an electron of the π_y orbital of the acetylene is transferred to an oxygen π^* orbital. This geometry has been nominated as the most^{12,13} probable one for the addition of $^1\Delta$ to ethylenes. With closer proximity of the molecular components, a tendency toward full bond formation from carbon to oxygen occurs. This bond formation has the effect of producing two orbitals of comparable energy on the second oxygen atom. Such a situation is precisely what is needed to promote a favorable situation for spin-orbit coupling,^{14,15} i.e., a one-center $p_x \rightarrow p_y$ jump on an oxygen atom (Figure 1). Furthermore, the state produced by such a $p_x \rightarrow p_y$ jump may possess substantial ionic character, a situation which further favors a rapid rate of spin inversion.^{15,16}

Once the spin inversion has occurred, either an open zwitterion (**7**) or peroxide (**8**) may result as the initial product.¹⁷ Whichever species is formed, generation of a dioxetene (**9**) or fragmentation to singlet oxygen and **1** are plausible reaction pathways (Scheme I). This mechanism provides a resolution to the problem of spin inversion and suggests that the reactive oxidizing intermediate which is produced during reaction **1** is probably an ionic oxygen-acetylene adduct or is free singlet oxygen generated by fragmentation of the ionic adduct.¹⁸

To test for the possible formation of singlet oxygen we measured the quenching of chemiluminescence (perylene) of reaction **1** by tetramethylethylene (TME), a known singlet oxygen acceptor. The chemiluminescence was quenched and a Stern-Volmer analysis of the quenching data yields a value of $k_{q\tau} \sim 5 \text{ M}^{-1}$. Neither the photoexcited fluorescence of **2** nor of perylene is quenched by TME under the conditions employed to measure $k_{q\tau}$. Thus, TME is trapping some intermediate along the chemiluminescence pathway, but the intermediate is not S_1 of **2** nor S_1 of perylene. A quantitative identification of the trapped intermediate is not possible because neither the lifetime of $^1\Delta$ oxygen nor T_1 of **2** is known under the reaction conditions. The oxidation of TME occurs under the reaction conditions.¹⁹ Similarly, 1,2-dimethylcyclohexane, 1,2-diethoxyethylene, and adamantylidene adamantane are oxidized under the reaction conditions. Finally, DABCO (1,4-diazabicyclo[2.2.2]octane) quenches the

chemiluminescence of reaction **1**, but not the fluorescence of **2** or perylene. Since photoexcitation of **2**, under the reaction conditions in the presence of TME and oxygen, does not produce the same oxidation product, we conclude that T_1 of **2** is not responsible for the oxidation of TME. Our quenching and trapping results, however, do not clearly discriminate between "free" singlet oxygen and an oxygen-acetylene complex.

In summary, the oxidation of strained acetylenes with triplet or singlet molecular oxygen are examples of chemiluminescent organic reactions. Both a dioxetene²⁰ and an oxidizing intermediate are produced along the chemiluminescence pathway. The possibility exists that free singlet oxygen may be generated by decomposition of an intermediate oxygen-acetylene complex. The latter process, if it occurs, would regenerate the starting material and, therefore, would represent a catalytic thermal generation of $^1\Delta$ oxygen from $^3\Sigma$ oxygen. Such a catalysis has far reaching implications in many oxidation reactions which involve $^3\Sigma$ as a reagent.

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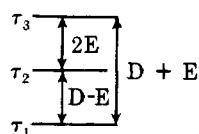
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Optical Detection of Magnetic Resonance of Glucagon and Glucagon Peptides in Solution. A Conformational Change Related to Critical Length of the Peptide Chain

Sir:

The x-ray crystal structure of glucagon, a 29 residue¹ adenylate-cyclase-stimulating hormone,²⁻⁴ recently reported by Blundell and co-workers,⁵ shows that the polypeptide chain is largely helical. By contrast, circular dichroism studies^{6,7} on dilute solutions of glucagon indicate that, near neutral pH, there is 15-20% α -helical structure in the polypeptide. This is consistent with the theoretical predictions (31% α -helix) of Chou and Fasman,⁸ made on the basis of a set of empirical rules deduced from examination of a number of protein crystal structure models. We wish to report the first evidence that there is specific nonrandom structure in glucagon, in dilute solid solution at neutral pH, in the region of the single tryptophan residue (Trp-25). Further, our investigation of glucagon peptide fragments suggests that a distinct conformational transition occurs in peptide fragments containing Trp-25, which are longer than some minimum critical length.

Glucagon was obtained from Elanco Products and was purified by ion-exchange chromatography.⁹ Glucagon peptides were generously donated by Dr. W. W. Bromer of Eli Lilly and Dr. A. Fontana of the Institute for Organic Chemistry, Padova. The polypeptides were dissolved in 1:1 ethylene glycol/water (EGW) buffered with 0.1 M K_xPO_4 at pH 7.4, at final polypeptide concentrations of less than 10^{-5} M. The samples were investigated using optical detection of magnetic resonance (ODMR)¹⁰⁻¹² which provides a sensitive means for determining the zero-field splittings (zfs)¹³ in the lowest excited triplet state of phosphorescent molecules. The zero-field splitting parameters which characterize the disposition of the triplet sublevels are defined as shown below.¹⁴ The triplet states



were populated by optical excitation at 297 nm at temperatures around 1.3 K. The zfs parameters and the sublevel decay and spin-lattice relaxation rate constants in tryptophan have been reported by Zuclich et al.¹⁵ and by Rousslang and Kwiram.^{16,17} All three of the zero-field transitions of tryptophan were observed in the *N*-acetyl derivative and the peptides. The D - E and the 2E transitions were readily detected, but the D + E transition could only be detected by a double resonance

Table I. Zero-Field Transition Frequencies, ν (GHz)^a

Sample	D - E	2E	D + E
Tryptophan	1.82	2.61	4.43
<i>N</i> -Acetyltryptophanamide	1.79	2.62	4.41
Glucagon (22-26)	1.79	2.62	4.41
Glucagon (22-29)	1.79	2.62	4.41
Glucagon (18-29)	1.72	2.68	4.40
Glucagon	1.72	2.68	4.40

^aZero-field splittings of samples at 1.3 K, dissolved in pH 7.4 EGW, and excited at 297 nm. The frequency values are usually averages of more than three observations (± 0.01 GHz).

experiment. Since both the 2E and D - E transitions have the principal radiative level, τ_2 , in common, the D + E transition can be observed by saturating either the 2E or D - E transitions, and simultaneously sweeping through the frequency range corresponding to D + E. The same values for D + E were obtained in both cases, providing a check on the accuracy of the 2E and D - E transitions. To ensure the greatest accuracy for extracting the ODMR transition frequencies, microwaves were swept slowly through resonance such that the line shape of the response was unaltered by the lifetimes of the tryptophan triplet sublevels.

The zfs of the hormone, peptides, tryptophan, and *N*-acetyltryptophanamide as measured by ODMR are shown in Table I. There is a small shift in the zfs in going from the zwitterion form of tryptophan to that of *N*-acetyltryptophanamide. However, the latter, because of the presence of amino- and carboxy-terminal peptide groups, is a more suitable model for tryptophan incorporated in a polypeptide chain. The ODMR results demonstrate that the zfs of tryptophan in glucagon are significantly different than those of *N*-acetyltryptophanamide alone.

Moreover, this change in the tryptophan zero-field transition frequencies takes place as one goes from the eight residue fragment (22-29) to the 12 residue fragment (18-29). Whereas the zfs of fragments 22-26 and 22-29 are identical with those of *N*-acetyltryptophanamide, fragment 18-29 apparently furnishes an environment for tryptophan equivalent to that found in glucagon.

Two possibilities were considered responsible for the large change in the zfs. A careful examination of residues adjacent to tryptophan in the hormone and peptide fragments suggested that perhaps the sulfur-containing methionine residue (Met-27) could be perturbing Trp-25 through increased spin-orbit interaction with the heavy sulfur atom. However, no shift is observed for fragment 22-29 which contains the methionine residue. Moreover, preliminary calculations suggest that this effect is too small to account for the observed 70-MHz shift in D - E.

An alternate explanation may be found by assuming an altered solvent accessibility of Trp-25 or, in more general terms, a change in the configuration of localized charges. If the 18-29 peptide forms an α -helical structure, clustering of hydrophobic residues and/or binding of the side chain to the helix backbone could alter the environment of the tryptophan. Chou and Fasman predict that residues 19-27 fulfill the necessary requirements for helical folding in glucagon, while residues 28 and 29 are either indifferent or inhibit α -helix formation. Consistent with this prediction and with the general theory^{18,19} for random coil-helix transitions in polypeptides, our data suggest that whereas fragments 22-26 and 22-29 are probably not structured, it is possible that fragment 18-29 is folded into an α -helix. Although we have no classical proof that helices per se are involved we believe that it is the formation of secondary structure in the 18-29 peptide and in glucagon that is responsible for the dramatic shift in the zfs of Trp-25. Our