monoacetate, trihydrate) by the azide procedure was carried out as described above for 3G, Ile^8 1–20 and gave the partially protected Ala^8,Orn^{10}-S-peptide (3F, Ala^8 1–20, 0.18 g, 50%).

Treatment with anhydrous TFA, purification on Amberlite CG 50, and on Sephadex G 25, followed by lyophilization, gave the pure Ala^8, Orn^{10}-S-peptide (3G, Ala^8 1–20) (0.029 g, 20%), $[\alpha]^{22}\text{D}$ $-79.5 \pm 2°$ (*c* 0.1, water), single ninhydrin- and Pauly-positive component on paper electrophoresis at pH 1.9, 3.5, 6.4, and 9.5; amino acid ratios in acid hydrolysate: Lys$_{2.10}$, Glu$_{2.90}$, Thr$_{1.85}$, Ala$_{5.80}$,Orn$_{1.10}$, His$_{1.00}$, Met$_{0.96}$, Asp$_{1.07}$, Ser$_{2.85}$; amino acid ratios in AP-M digest: Lys$_{2.05}$, Glu$_{2.05}$, Thr$_{1.90}$, Ala$_{5.85}$, Orn$_{1.05}$, Gln$_{1.00}$, His$_{1.00}$, Met$_{0.95}$, Asp$_{1.10}$, Ser$_{2.80}$. The enzymic activity data are listed in Table I.

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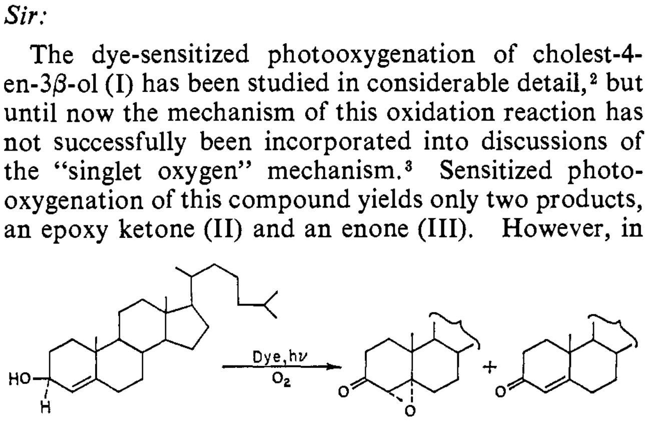
Communications to the Editor

Evidence for the Participation of $^1\Sigma_g{}^+$ and $^1\Delta_g$ Oxygen in Dye-Sensitized Photooxygenation Reactions. I[1]

Sir:

The dye-sensitized photooxygenation of cholest-4-en-3β-ol (I) has been studied in considerable detail,[2] but until now the mechanism of this oxidation reaction has not successfully been incorporated into discussions of the "singlet oxygen" mechanism.[3] Sensitized photooxygenation of this compound yields only two products, an epoxy ketone (II) and an enone (III). However, in contrast to other sensitized photooxygenations which are believed to involve singlet oxygen molecules (presumably $^1\Delta_g$),[4–8] this reaction gives an enone:epoxy ketone (III:II) product distribution ratio which varies from 30:1 to 1:5, depending upon the nature of the sensitizer used. To account for these observations, we propose *that both $^1\Sigma_g{}^+$ and $^1\Delta_g$ oxygen molecules are involved as reaction intermediates, that these two species exhibit different chemical reactivities, and that the observed variation in product distribution with sensitizer results from a variation in the relative amounts of $^1\Sigma_g{}^+$ and $^1\Delta_g$ generated.* This suggestion is prompted by the following considerations.



I II III

We recently showed, on theoretical grounds, that quenching of excited triplet-state molecules by molecular oxygen occurs primarily by transfer of electronic excitation energy to the molecular oxygen.[9] With high-energy triplet-state sensitizers ($E_t > 50$ kcal) our calculations indicated that in the quenching process about 10 $^1\Sigma_g{}^+$ molecules are generated for every $^1\Delta_g$. With low-energy sensitizers ($E_t < 38$ kcal) energy conservation permits only $^1\Delta_g$ oxygen to be generated. Energy transfer from sensitizers with intermediate triplet-state energies ($50 > E_t > 38$ kcal) was not adequately treated by our theory and, in order to predict the $^1\Sigma_g{}^+$:$^1\Delta_g$ ratio expected from these sensitizers, we make use of experimental observations on triplet–triplet energy transfer.[10] Porter and Wilkinson found that, as the triplet-state energy of the sensitizer approaches (*e.g.*, within 5 kcal) the triplet state of the acceptor, the transfer probability is significantly reduced.[11] Assuming that similar results are obtained when oxygen is the acceptor, we expect the $^1\Sigma_g{}^+$:$^1\Delta_g$ ratio to smoothly decrease to zero as E_t approaches 37.7 kcal, the energy of the $^1\Sigma_g{}^+$ state. These considerations allow us to construct the curve given in Figure 1a, showing the expected variation in the $^1\Sigma_g{}^+$:$^1\Delta_g$ ratio for a wide range of sensitizer triplet-state energies. If $^1\Sigma_g{}^+$ and $^1\Delta_g$ oxygen were to exhibit different chemical reactivities, this curve suggests that the product distribution would vary with triplet-state energy of the sensitizer in a predictable way.

To test for the possible involvement of $^1\Sigma_g{}^+$ and $^1\Delta_g$ oxygen as intermediates, we have measured the triplet-state energies of a number of the sensitizers which were used in the sensitized photooxygenation of I.[12] In Figure 1b we have plotted the enone:epoxy ketone (III:II) ratio obtained from the sensitized photooxygenation of I as a function of the triplet-state energy of the sensitizers.[2] It is apparent from a comparison of the curves in Figures 1a and 1b that the variation in product distribution with E_t is well accounted for in terms of the predicted variation of the $^1\Sigma_g{}^+$:$^1\Delta_g$ ratio and the proposed involvement of both $^1\Sigma_g{}^+$ and $^1\Delta_g$ molecular oxygen as reaction intermediates. Low-energy sensitizers, which produce only $^1\Delta_g$, give a III:II ratio of

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[1] Portions of this work were presented at the 153rd National Meeting of the American Chemical Society, Miami, Fla., April 15, 1967. This work was supported by Public Health Service Grant GM-10449, and by the U. S. Army Research Office (Durham) Grant No. G804. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society (No. 1956-A1), for partial support of this research.

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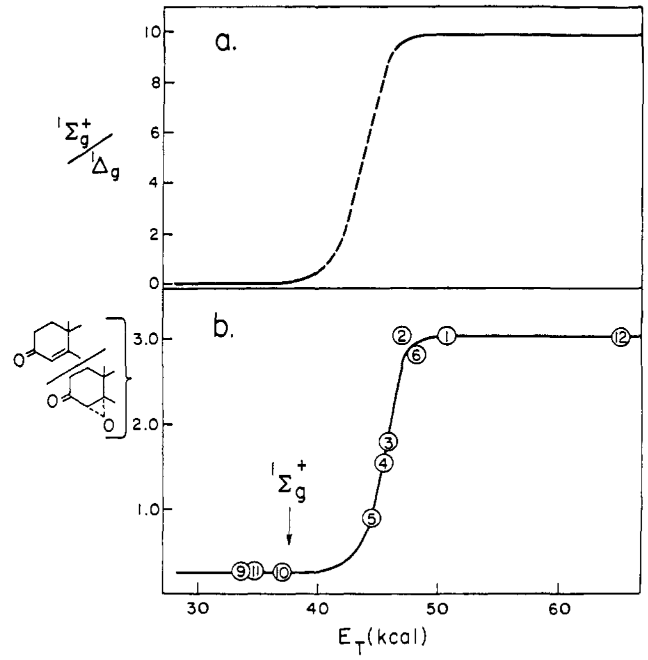

Figure 1. (a) Predicted variation in the $^1\Sigma_g^+ : ^1\Delta_g$ ratio with sensitizer triplet-state energy E_t. (b) Observed variation in the product distribution from the dye-sensitized photooxygenation of cholest-4-en-3β-ol with the triplet-state energy of the sensitizer:[2] (1) fluorescein; (2) eosin Y; (3) sulforhodamin B; (4) erythrosin B; (5) rose bengal; (6) acridine orange; (9) methylene blue; (10) hematoporphyrin; (11) chlorin e_6; and (12) triphenylene.

about 1:4. The dramatic change in the III:II ratio with intermediate energy sensitizers is attributed to the increase in the $^1\Sigma_g^+ : ^1\Delta_g$ ratio as E_t is increased from 38 to 50 kcal. High-energy sensitizers generate mainly $^1\Sigma_g^+$ oxygen, and it appears that this species reacts primarily to give the enone. While the exact nature of the reactivity of $^1\Sigma_g^+$ with I remains to be determined, it is possible that the enone arises from a hydrogen-abstraction-type mechanism involving $^1\Sigma_g^+$.

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Evidence for the Participation of $^1\Sigma_g^+$ and $^1\Delta_g$ Oxygen in Dye-Sensitized Photooxygenation Reactions. II[1]

Sir:

In the accompanying communication we proposed that both $^1\Sigma_g^+$ and $^1\Delta_g$ singlet oxygen molecules are involved as intermediates in the dye-sensitized photooxygenation of cholest-4-en-3β-ol (I) and that these species exhibit different chemical reactivity toward I.[2] Since $^1\Sigma_g^+$ is known to have a much shorter lifetime in solutions than $^1\Delta_g$,[3,4] a necessary consequence of our "two singlet oxygen hypothesis" is that a reduction in substrate (I) concentration should favor reactions involving $^1\Delta_g$ oxygen. In fact, with sufficiently low concentrations of substrate, only products characteristic of reactions of $^1\Delta_g$ oxygen should be produced (primarily the epoxy ketone II). We examined the effect of cholestenol (I) concentration on the product distribution with five different sensitizers, and the results are presented in Table I. As required by the "two singlet oxygen hypothesis," these data demonstrate the enone: epoxy ketone ratio is very sensitive to the concentration of I when high-energy sensitizers such as fluorescein or eosin are used. A particularly important point is that with sufficiently low acceptor concentrations (10^{-4} *M*) the enone:epoxy ketone ratio is the same as that produced using a low-energy sensitizer, that is, sensitizers which generate only $^1\Delta_g$ oxygen.[2] Furthermore, with the low-energy sensitizer methylene blue, the concentration of I has little effect on the enone:epoxy ketone ratio.

Table I. Product Variation with Concentration of Substrate in the Photosensitized Photooxygenation of Cholest-4-en-3β-ol (I)[a]

Sensitizer	Concn of I[b]	III:II[c]
Methylene blue	1.3×10^{-2}	1:3[d]
	1.0×10^{-4}	1:4–1:5
Fluorescein	1.3×10^{-2}	3:1[e]
	1.0×10^{-4}	1:3
Eosin Y	1.3×10^{-2}	3:1[d]
	8.2×10^{-4}	1:1
	4.0×10^{-4}	1:4
	2.5×10^{-4}	1:4
	1.6×10^{-4}	1:4
	8.2×10^{-5}	1:5
Lumichrome[f]	1.0×10^{-2}	6:1
Riboflavin	1.3×10^{-2}	30:1[d]
	5.0×10^{-4}	6:1
	1.0×10^{-4}	1:2
	7.5×10^{-5}	1:5

[a] The photooxygenation conditions were essentially the same as those used by Nickon,[5] with the exception that a 500-w tungsten lamp was used and typical runs were 24 hr long. [b] Concentration in moles/liter. [c] Ratios determined by chromatography and infrared spectroscopy. [d] This value was also reported by Nickon.[5] [e] Data of Nickon.[5] [f] Irradiation for 3 days.

When riboflavin was used as a sensitizer, an unexpectedly high enone:epoxy ketone ratio of 30:1 was obtained.[5] Since riboflavin is known to participate in photoinduced hydrogen-abstraction reactions,[6,7] we were inclined to attribute the large enone production to riboflavin-abstraction reactions rather than to reactions of $^1\Sigma_g^+$ and $^1\Delta_g$ oxygen. We anticipated, therefore, that reduction of substrate (I) concentration would both reduce the importance of direct reaction of riboflavin with I, as compared with energy transfer to oxygen, and favor reactions of $^1\Delta_g$ oxygen over $^1\Sigma_g^+$. We found that with sufficient dilution of enol I (see Table I) we obtained the expected 150-fold decrease in the enone: epoxy ketone ratio from 30:1 to 1:5, the product distribution produced by $^1\Delta_g$ oxygen reactions with I.

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