The Entropy-Controlled Reactivity of Singlet Oxygen ($^1\Delta_g$) toward Furans and Indoles in Toluene. A Variable-Temperature Study by Pulse Radiolysis

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Abstract: The pulse radiolysis technique has been used to produce singlet oxygen O_2^* ($^1\Delta_g$), the subsequent reactivity of which has been followed by the time-resolved bleaching of diphenylisobenzofuran (DPBF). The rate constants for reaction of O_2^* ($^1\Delta_g$) with a series of furans, cyclopentadiene, and some indoles have been determined. These constants are temperature independent, and it is concluded that the reactions involve either (a) a concerted step leading directly to endoperoxide or dioxetane which is purely entropy controlled or (b) production in a *rate-determining* entropy-controlled step of an intermediate which rapidly rearranges to products.

Introduction

The increasing evidence of the biological importance of singlet oxygen O_2^* ($^1\Delta_g$) with respect to photodynamic action and related phenomena[2,3] has stimulated attempts to understand the factors which influence its physical properties and its reactivity toward organic substrates.[4] Definitive data concerning rate parameters have come from time-resolved work using pulsed lasers to produce O_2^* ($^1\Delta_g$) via triplet sensitization[5-7] and via direct excitation of the $^1\Delta_g \leftarrow {}^3\Sigma_g$ transition.[8] In addition the products of reactions of this species with numerous types of organic molecules have been analyzed with regard to mechanistic interpretation. Nevertheless, considerable controversy exists concerning the nature of the processes whereby oxidation takes place, and this situation has been compounded by conflicting proposals based on molecular orbital calculations (vide infra). Despite the wealth of experimental results, surprisingly little data has been concerned with the effect of temperature on reaction rate parameters. Only Koch[9] and Long and Kearns[10] have reported such data for reactions in solution and gross discrepancies exist between the former's steady-state results and more recent time-resolved work.[5-7] Diphenylisobenzofuran (DPBF) reacts reasonably efficiently with O_2^* ($^1\Delta_g$) and is to date the best monitor for this species. Long and Kearns[10] investigated the effect of temperature on the rate constant for reaction of DPBF with O_2^* ($^1\Delta_g$) in chloroform; they obtained an activation energy of 1 kcal mol^{-1} and a 50% decrease in O_2^* ($^1\Delta_g$) lifetime over the range -50 to 25 °C. No mechanistic conclusions were drawn. We have recently established a method for the investigation of O_2^* ($^1\Delta_g$) reactivity based on the pulse radiolysis technique,[11] and have now examined the temperature dependence of the reactivity of this species toward a number of organic molecules. In this paper we describe the experimental approach employed and the results for two series of compounds, namely, furans and indoles.

Determination of Rate Parameters by Pulse Radiolysis

The triplet-sensitized production of O_2^* ($^1\Delta_g$), using the pulse radiolysis technique, has been described[11] and only a brief summary is given here. When a high-energy beam of electrons is incident on a target of liquid benzene (B) or toluene (T) a high yield of short-lived bound excited states of the matrix molecules is produced.[12] In the presence of a suitable energy interceptor such as naphthalene (N, 10^{-2} mol L^{-1}) a large fraction of the initially absorbed energy can be used to sensitize the production of O_2^* ($^1\Delta_g$) in aerated or oxygenated solution according to the equations

$$\mathrm{T} \xrightarrow{e^-\ \text{fast}} {}^1\mathrm{T}^*,\ {}^3\mathrm{T}^* \tag{1}$$

$${}^1\mathrm{T}^* + \mathrm{N} \rightarrow \mathrm{T} + {}^1\mathrm{N}^* \tag{2}$$

$${}^1\mathrm{N}^* \rightarrow {}^3\mathrm{N}^*,\ \mathrm{N}(\phi_\mathrm{T}) \tag{3}$$

$${}^3\mathrm{T}^* + \mathrm{N} \rightarrow \mathrm{T} + {}^3\mathrm{N}^* \tag{4}$$

$${}^3\mathrm{N}^* + \mathrm{O}_2\,({}^3\Sigma_g^-) \rightarrow \mathrm{O}_2^*\,({}^1\Delta_g) + \mathrm{N} \tag{5}$$

The final step has an efficiency which depends on the sensitizer employed and may involve the intermediacy of O_2^* ($^1\Sigma_g^+$). In the presence of the monitor DPBF and an additional quencher molecule (Q), the O_2^* ($^1\Delta_g$) produced can decay via channels (6)-(8), the rate parameters for which may depend to a greater or lesser extent on the medium.

$$\mathrm{O}_2^*\,({}^1\Delta_g) \xrightarrow{k_\mathrm{d}} \mathrm{O}_2\,({}^3\Sigma_g^-) \tag{6}$$

$$\mathrm{O}_2^*\,({}^1\Delta_g) + \mathrm{Q} \xrightarrow{k_\mathrm{q}} \text{loss of } \mathrm{O}_2^*\,({}^1\Delta_g) \tag{7}$$

$$\mathrm{O}_2^*\,({}^1\Delta_g) + \mathrm{DPBF} \xrightarrow{k_\mathrm{r}} \text{loss of DPBF} \tag{8}$$

When the initial concentration of O_2^* ($^1\Delta_g$) is small compared to the initial concentrations of DPBF and Q, the rate of loss of DPBF is given by

$$\frac{d(-[\mathrm{DPBF}])}{dt} = k_\mathrm{r}[\mathrm{DPBF}][\mathrm{O}_2^*]_0 \times \exp[-\{k_\mathrm{d} + k_\mathrm{r}[\mathrm{DPBF}] + k_\mathrm{q}[\mathrm{Q}]\}t] \tag{9}$$

where Q, the quencher molecule, may act via chemical or physical action, and $[O_2^*]_0$ is the concentration of singlet oxygen at the end of the electron pulse. It is readily shown that a plot of ln ($D_t - D_\infty$) against time will be linear with a slope, k', given by

$$k' = k_\mathrm{d} + k_\mathrm{r}[\mathrm{DPBF}] + k_\mathrm{q}[\mathrm{Q}] \tag{10}$$

where D_t and D_∞ are respectively the DPBF optical densities at time t and at completion of the bleaching event. The rate parameters k_d, k_r, and k_q can be evaluated from plots of k' against (1) DPBF concentration for k_d and k_r and (2) quencher concentration (at fixed DPBF) for k_q. In this work we have been concerned with temperature effects and thus all experiments have been carried out in toluene which has a much larger liquid range, -95 to 110 °C, than does benzene, 5-80 °C.

Experimental Section

1. **Pulse Radiolysis.** Experiments were performed at the Christie Hospital and Holt Radium Institute, Manchester, using the Vickers

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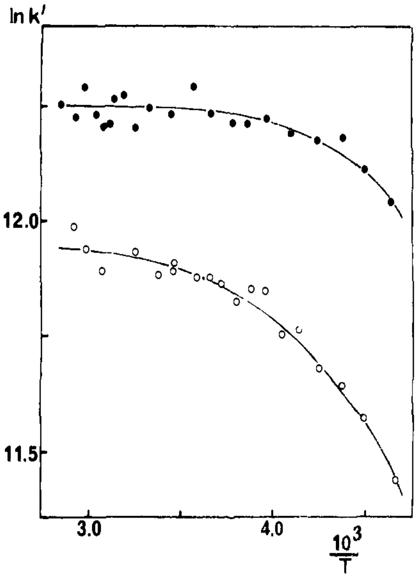


Figure 1. Arrhenius plot of ln k' vs. $10^3/T$ for oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}) and DPBF [1.25×10^{-4} (●) and 5.0×10^{-5} mol L^{-1} (○)].

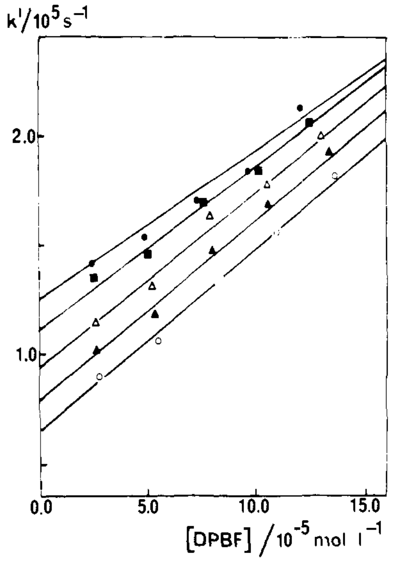


Figure 2. Dependence of first-order constant for DPBF bleaching, k', on DPBF concentration for oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}) at 60 (●), 13 (■), −23 (△), −38 (▲), and −51 °C (○).

10-MeV linear accelerator. Details of the procedure have been described.[11] In all DPBF bleaching experiments the optical density change at 415 nm during the bleaching event correspond to less than 10% removal of this O_2^* ($^1\Delta_g$) monitor. In addition the substrate concentration was considerably higher than that of DPBF and experiments were arranged so that monitor and substrate removed comparable amounts of O_2^* ($^1\Delta_g$). The assumptions upon which eq 9 is based were thus valid for our experiments.

2. Variable-Temperature System. The variable-temperature system was as designed by Wardman.[13] A variable-temperature run (−50 to 80 °C) was carried out on a single stock solution, the cell sample being automatically replenished after each shot at a given equilibrated temperature. Thus concentration changes due to solvent density variation had to be corrected for. These concentration changes resulted in smaller, but perfectly adequate, DPBF bleaching signals at higher temperatures.

3. Materials. Toluene was refluxed over lithium aluminum hydride for 48 h and fractionated under nitrogen.[14] Naphthalene (BDH, OAS) was used as supplied. Diphenylisobenzofuran (Aldrich) was twice recrystallized from methanol/water and stored in the dark. Furan (Hopkin and Williams) was washed with 5% aqueous potassium hydroxide (5 × 20 mL per 100 mL), dried over anhydrous sodium sulfate, and distilled from sodium. Cyclopentadiene was prepared immediately before use by thermal decomposition of the dimer. 2,5-Dimethylfuran was distilled. 2,3-Diphenylfuran,[15] 2,5-diphenylfuran,[16] 2,3,4,5-tetraphenylfuran,[17] and 2,5-di-*tert*-butylfuran[18] were prepared as described. 2,5-Di(*p*-chlorophenyl)furan was prepared according to the method described for 2,5-diphenylfuran,[16] benzene being replaced by chlorobenzene. This gave an intermediate *trans*-di(*p*-chlorobenzoyl)ethylene (65%, mp 173–175 °C, 1:1 EtOH/$CHCl_3$) and the desired product (70%, mp 169–170 °C, EtOH and 3:1 EtOH/$CHCl_3$). Indole (BDH) was used as received. 3-Methylindole was recrystallized from EtOH/H_2O. 2,3-Dimethylindole and 3-methyl-2-phenylindole were prepared by Fischer condensation of phenylhydrazine with the appropriate ketone in refluxing acetic acid and recrystallized from EtOH/H_2O, mp 106–108 and 91–92 °C, respectively.

Results and Discussion

The experimentally determined rate parameter in this work is k', the rate constant for exponential decay of DPBF, which is defined by eq 10. We have previously shown that the O_2^* ($^1\Delta_g$) lifetime is linearly dependent on the radiation intensity[11] and have attributed the phenomenon to radical quenching of this species. The observed first-order dependence is best accommodated by a paramagnetic radical quenching process according to eq 11. If this is the case a rate constant of 4.3×10^9 L mol^{-1} s^{-1} at ambient temperature may be readily calculated[19] for this quenching process.[20]

$$O_2^* (^1\Delta_g) + R^\cdot \rightarrow O_2 (^3\Sigma_g^-) + R^\cdot \qquad (11)$$

In order to assess the origin(s) of temperature effects on k' we have redefined the latter according to the equation

$$k' = k_d + k_{dq}[R^\cdot] + k_r[DPBF] + k_q[Q] \qquad (12)$$

where k_{dq} is the rate constant for radical quenching of O_2^* ($^1\Delta_g$). We have examined the temperature dependence of k_d, k_{dq}, and k_r by determining k' over the range −50 to 80 °C for four oxygenated toluene solutions of naphthalene (10^{-2} mol L^{-1}) containing different DPBF concentrations and at four different radiation doses for a single DPBF concentration. Arrhenius plots of ln k' vs. $10^3/T$ are shown for two different DPBF concentrations in Figure 1. Not surprisingly the plots show curvature since k' is a composite rate constant. The data show more scatter at higher temperatures and this is attributed to (a) the lower DPBF concentration at higher temperatures, due to solvent density variation, leading to lower bleaching yields and therefore lower signal to noise ratios, and (b) the intrusion of naphthalene triplet absorption at the monitoring wavelength (415 nm) for the faster bleaching signals.

Temperature Dependence of k_r. In Figure 2 are shown plots of k' vs. [DPBF] for various temperatures, the plots having been constructed from four experiments of the type depicted in Figure 1 and the data corrected for concentration changes with temperature. Clearly the slope, k_r, is essentially independent of temperature, indicating an activation energy of zero for reaction of O_2^* ($^1\Delta_g$) with DPBF. Only at the highest temperature taken, 60 °C, as mentioned the least precise region, is any deviation apparent. Thus we conclude that the changes in k' in Figure 1 reflect changes in the intercepts in Figure 2, i.e., in $k_d + k_{dq}[R^\cdot]$ [cf. eq 12 where [Q] = 0].

Temperature Dependence of k_d and k_{dq}. In Figure 3 are shown Arrhenius plots of ln k' vs. $10^3/T$ at two different radiation doses for an oxygenated toluene solution containing naphthalene (10^{-2} mol L^{-1}) and DPBF (2.5×10^{-5} mol L^{-1}). Curvature is again observed and, as before, the greatest scatter occurs at the higher temperatures. In Figure 4 are shown linear plots of k' vs. absorbed radiation dose constructed from four experiments of the type depicted in Figure 3. The slopes reflect

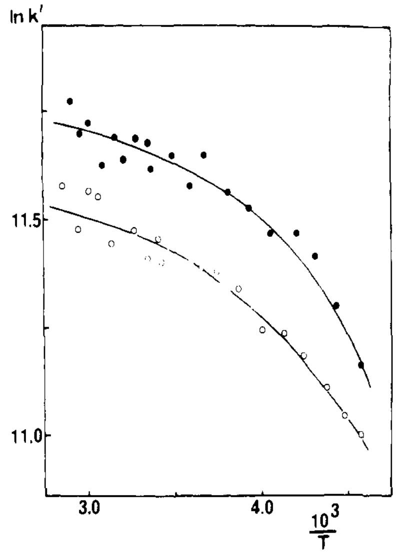


Figure 3. Arrhenius plots of ln k' vs. $10^3/T$ for oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}) and DPBF (2.5×10^{-5} mol L^{-1}) at radiation doses of 7.2 (●) and 4.4 (○) krad/pulse.

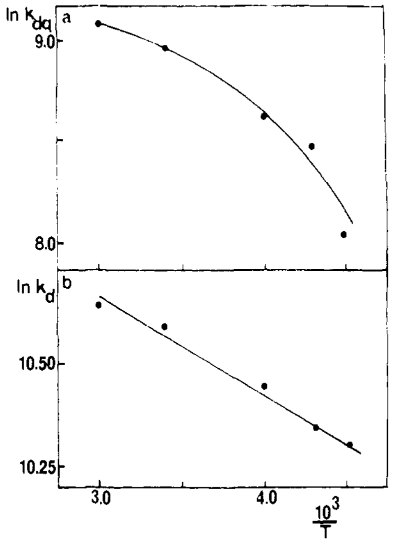


Figure 5. Arrhenius plots of (a) ln k_{dq} and (b) ln k_d vs. $10^3/T$. Values obtained from the slopes (k_{dq}) and the intercepts ($k_d + k_r$[DPBF]), respectively, of Figure 4.

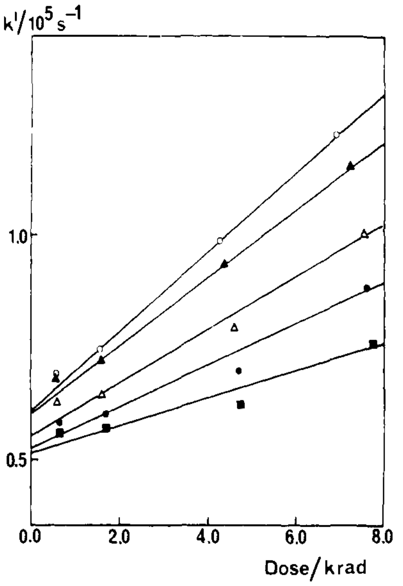


Figure 4. Dependence of first-order constant for DPBF bleaching, k', on absorbed radiation dose for oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}) and DPBF (2.5×10^{-5} mol L^{-1}) at 60 (○), 20 (▲), −23 (△), −40 (●), and −51 °C (■).

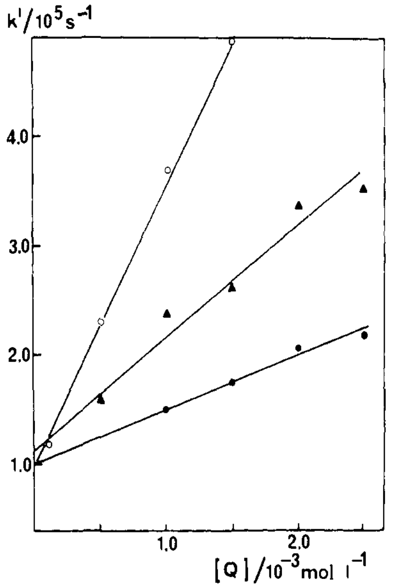


Figure 6. Dependence of first-order constant for DPBF bleaching, k', on the concentrations of 2,3,4,5-tetraphenylfuran (○), 2,5-diphenylfuran (▲), and 2,5-di(p-chlorophenyl)furan (●) for oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}) and DPBF (2.5×10^{-5} mol L^{-1}).

the variation of k_{dq}, in krad s^{-1}, with temperature. The radiation yield for radicals in toluene is only known within the limits $G = 1.0$–2.5.[11] Calculations based on a value of 1.75 and the slopes in Figure 4 lead to k_{dq} values varying from 4.9×10^9 L mol^{-1} s^{-1} at 60 °C to 1.7×10^9 L mol^{-1} s^{-1} at −51 °C. The Arrhenius plot for k_{dq} is not linear (Figure 5), indicating that this rate constant is also composite in nature, possibly owing to contributions from different types of radical.[20] These considerations, although of interest, do not change the overall picture described below.

The intercepts in Figure 4 correspond to $k_d + k_r$[DPBF]. The term k_r[DPBF], corrected for concentration changes with temperature, has been subtracted from these intercepts to allow an Arrhenius plot of ln k_d vs. $10^3/T$. The plot is essentially linear and corresponds to an activation energy in toluene of ~0.5 kcal mol^{-1}. This is to be compared with a value of ~0.7 kcal mol^{-1} which we deduce from data for chloroform solution published by Long and Kearns.[10] However, the Arrhenius plot obtained by these authors showed curvature.

Temperature Dependence of k_q for O_2^* ($^1\Delta_g$) Quenchers. We have extended the above approach to examine the temperature dependence of the rate constants for quenching in toluene of O_2^* ($^1\Delta_g$) by those substrates listed in Table I. The quenching rate constants at room temperature were determined in the standard manner[11] from the slopes of plots of k' vs. [Q] according to eq 10 and 12. In all cases linear plots were obtained as typified by those shown in Figure 6.

In order to determine the effect ot temperature on k_q for individual substrates, it was not necessary to determine separately the effect on k_d, k_{dq}, and k_r but only the composite contribution to k'. Thus O_2^* ($^1\Delta_g$) quenching experiments

Table I. Rate Constants (k_q) and Activation Parameters for Reaction in Toluene of Furans and Indoles with O_2^* ($^1\Delta_g$); Substrate Ionization Potentials[a]

substrate	k_q/L mol^{-1} s^{-1}	$\Delta H^{\ddagger}$/kcal mol^{-1}	$\Delta S^{\ddagger}$/cal mol^{-1} deg^{-1}	IP/eV
1,3-diphenylisobenzofuran	8.1×10^8	0.0 ± 0.4	−19.7	6.81^b
2,5-dimethylfuran	6.3×10^8	0.0 ± 0.4	−20.2	7.96^b
2,3,4,5-tetraphenylfuran	2.8×10^8	0.0 ± 0.4	−21.8	7.59^b
2,5-di-*tert*-butylfuran	2.3×10^8	0.0 ± 0.4	−22.5	
2,5-diphenylfuran	1.0×10^8	0.0 ± 0.4	−23.9	7.34^b
2,3-diphenylfuran	6.2×10^7	0.0 ± 0.4	−24.8	
2,5-di(*p*-chlorophenyl)furan	5.8×10^7	0.0 ± 0.4	−25.0	7.52^b
cyclopentadiene	3.9×10^7	0.0 ± 0.4	−25.8	8.58^c
furan	1.1×10^7	0.0 ± 0.4	−28.3	8.90^d
2,3-dimethylindole	1.0×10^8	0.0 ± 0.4	−23.7	7.30^b
3-methyl-2-phenylindole	2.1×10^7	0.0 ± 0.4	−27.0	
3-methylindole	9.2×10^6	0.0 ± 0.4	−28.7	7.52^b
indole	7.7×10^5	0.0 ± 0.4	−33.8	7.79^d

[a] Adiabatic values from PE spectroscopy unless otherwise stated. [b] This work.[21] [c] Vertical value from PE spectroscopy.[22] [d] Reference 23.

were performed between −50 and 80 °C under experimental conditions, including radiation dose, which were identical with those of a "background" experiment carried out in the absence of quencher. Subtraction of the "background" k' and correction for changes in quencher concentration with temperature allowed evaluation of k_q over the said temperature range. Arrhenius plots for all furans (plus cyclopentadiene) and indoles which were examined gave activation energies of zero within experimental error.[24,26] Typical plots are shown in Figure 7 for 2,5-diphenylfuran and cyclopentadiene. The solid line shown is arbitrarily drawn to represent the slope expected for a diffusion-controlled process in toluene, $E_a \sim 3.5$ kcal mol^{-1}.[9] In Table I activation parameters in the form of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are given for all substrates examined. Entropies of activation were computed assuming that the measured values of k_q were composed solely of the Arrhenius preexponential factor.[28]

Conclusions

It is known that furans react with O_2^* ($^1\Delta_g$), as do 1,3-dienes to give initially endo peroxides,[31] previously accepted as products of a classical Diels-Alder cycloaddition process (Scheme Ia). The Diels-Alder reaction is itself now the subject of considerable mechanistic controversy,[32] said to proceed via a highly unsymmetrical biradicaloid species according to MINDO/3[33] and via a synchronous reaction according to two ab initio calculations.[34,35] Indeed recent MINDO/3 data suggest that the reaction of butadiene with O_2^* ($^1\Delta_g$) yields the endo peroxide which is formed via production of a perepoxide (Scheme Ib), E_a = 10.8 kcal mol^{-1}.[36] No gas-phase experimental data is available for comparison.

The reaction of the indole nucleus with O_2^* ($^1\Delta_g$) has generally been accepted as a typical example of the oxidation of electron-rich olefins,[37] previously thought to proceed via concerted dioxetane formation (e.g., Scheme IIa). However, data from MINDO/3 calculations on the reaction of 2,3-dihydropyran and vinylamine are claimed to indicate the intermediacy of a zwitterionic species (e.g., Scheme IIb) in such reactions;[38] this receives some support from a proposal by Saito[39] that the initial product of reaction of O_2^* ($^1\Delta_g$) with indoles is a species of type A. This suggestion was based on low-temperature product analysis and the results are, in our opinion, open to alternative interpretation.

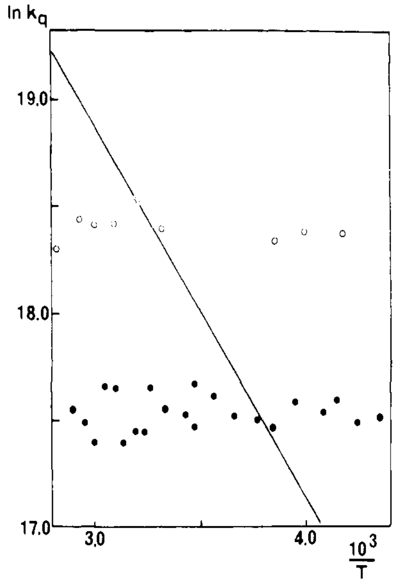
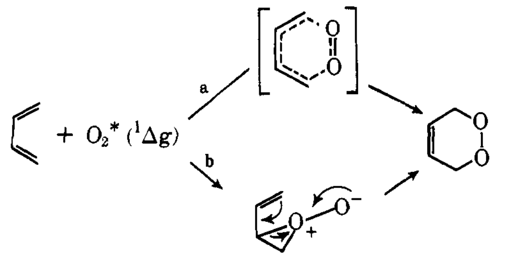
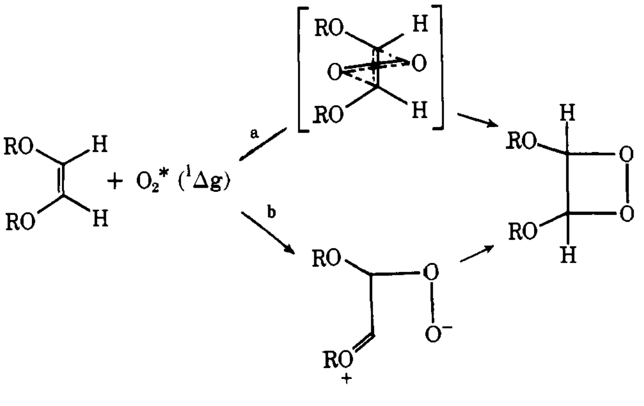


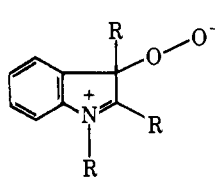
Figure 7. Arrhenius plots of ln k_q vs. $10^3/T$ for 2,5-diphenylfuran (○) and cyclopentadiene (●). Data were obtained from oxygenated toluene solutions containing naphthalene (10^{-2} mol L^{-1}), DPBF (2.5×10^{-5} mol L^{-1}), 2,5-diphenylfuran (○, 10^{-3} mol L^{-1}), and cyclopentadiene (●, 2.4×10^{-3} mol L^{-1}). The solid line shows the slope anticipated for a diffusion-controlled reaction.[9]

Scheme I



Scheme II





A

Activation Parameters. The key finding in the present work is that the experimental enthalpies of activation for reaction of O_2^* ($^1\Delta_g$) with a series of seven furans, cyclopentadiene, and four indoles are zero whereas entropies of activation fall in the range −18 to −34 eu. If the quenching reaction is predominantly chemical in nature,[28] the zero enthalpy of activation may indicate either (a) a single-step reaction, purely entropy controlled or (b) a composite reaction, for instance, one in which the heat of exothermic intermediate formation is canceled out by the enthalpy of activation of the subsequent product-forming step. However, the spread of rate constants observed, 7.0×10^5 to 8.0×10^8 L mol^{-1} s^{-1}, together with the existence of two distinct overall mechanisms, one for furans and one for indoles, makes it extremely unlikely that, for all 13 substrates in Table I, the zero enthalpy of activation is due to a fortuitous matching of individual enthalpy contributions in a complex process. We therefore conclude that the reactions under consideration involve either (a) a single step leading directly to endo peroxide (furans and cyclopentadiene) or dioxetane (indoles) which is purely entropy controlled or (b) production in a *rate-determining* entropy-controlled step of an intermediate (e.g., perepoxide) which rapidly rearranges to products.

We attribute the zero $\Delta H^{\ddagger}$ values to the electronically excited nature of O_2^* ($^1\Delta_g$). Thus, if the reaction of this species with, for instance, cyclopentadiene is of the Diels-Alder type, whatever that might be mechanistically, one would not necessarily expect a $\Delta H^{\ddagger}$ value typical of Diels-Alder reactions of cyclopentadiene in, say benzene, 10-18 kcal mol^{-1},[40] since a route may exist for the utilization of the electronic energy in the progress of the reaction.

An important consequence of the zero $\Delta H^{\ddagger}$ values is that the relative electron-donating abilities of the various substrates should not have a direct bearing on the relative values of rate constants for reaction with O_2^* ($^1\Delta_g$) by affecting the bonding energy requirement at the transition state of the rate-determining step. However, as a referee has rightly pointed out, they may affect $\Delta S^{\ddagger}$ values, e.g., by influencing solvation characteristics, and in the cases of indole and its 2-methyl and 2,3-dimethyl derivatives there does appear to be a correlation between rate constant and substrate ionization potential (Table I). No such correlation is apparent for the larger sample of furans plus cyclopentadiene (Table I) and the significance of the result for the three indoles is impossible to assess.

In contrast to the enthalpies of activation the highly negative entropies of activation in Table I are typical of Diels-Alder processes in inert solvents.[40] Our data are therefore consistent with a simple concerted cycloaddition process for reaction of O_2^* ($^1\Delta_g$) with both furans and indoles. However, if a zwitterionic species such as a perepoxide were produced in a rate-determining step the highly negative $\Delta S^{\ddagger}$ values could reflect a solvent reorganization requirement. Thus no clear mechanistic distinction may be made at this stage.

Substituent Effects. The general trend apparent from Table I is that the rate constant for quenching of O_2^* ($^1\Delta_g$) increases with substitution in the substrate. For reasons discussed in the last section this cannot be a consequence of an increase in the electron-donating ability of the substrate. We can only interpret this phenomenon in terms of steric acceleration due to a lowering of substituent entropy restrictions as the transition state of the rate-determining step is approached. It seems reasonable that restriction of degrees of freedom associated with eclipsed interactions between a substituent and an adjacent hydrogen or substituent on a double bond will become less as rehybridization leading to the transition state of, for example, a concerted Diels-Alder reaction takes place. Any associated effect on $\Delta H^{\ddagger}$ would not manifest itself here since $\Delta H^{\ddagger}$ will in any case be zero for reasons already mentioned. Clearly, an additional and opposite effect on $\Delta S^{\ddagger}$ has to be considered, namely, the restriction of degrees of freedom associated with the proximity of O_2^* ($^1\Delta_g$) and substituents as the transition state is approached. The small size of O_2^* ($^1\Delta_g$) may well cause this term to be relatively unimportant in most cases. However, with bulky substituents at σ-bond forming centers, e.g., at C(2) and C(5) of a furan, a significant negative contribution to $\Delta S^{\ddagger}$ may occur. The fact that 2,5-diphenyl- and 2,5-di-*tert*-butyl- are less reactive than 2,5-dimethylfuran may well be a consequence of this effect.

Further work on substituent effects and the solvent dependence of activation parameters is in progress.

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Lucibufagins. 2. Esters of 12-Oxo-2β,5β,11α-trihydroxybufalin, the Major Defensive Steroids of the Firefly *Photinus pyralis* (Coleoptera: Lampyridae)[1a]

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Abstract: Fireflies of the species *Photinus pyralis* (Coleoptera: Lampyridae) are shown to contain a group of closely related steroidal pyrones, named lucibufagins, which serve a defensive role. The five major steroids from this insect are characterized as esters (**5, 6, 7, 11, 12**) of 12-oxo-2β,5β,11α-trihydroxybufalin (**9**), chiefly on the basis of detailed spectral analyses. Selective proton decoupling ^{13}C NMR experiments played a key role in making these structural assignments. These insect-derived steroids are the first bufadienolides to be isolated and characterized from an invertebrate source.

Introduction

The remarkable ability of fireflies (sometimes also called lightning bugs or glowworms) to produce light signals has been celebrated by poets[2] and musicians.[3] Biologists,[4] biochemists,[5] and organic chemists[6] have studied the origin and meaning of these beetles' signals in depth. We have become interested in the chemical defenses of fireflies, and have recently reported that two locally available species, *Photinus ignitus* and *P. marginellus*, contain mixtures of at least three bufadienolides, for which we proposed the generic name lucibufagins.[7] These steroids were shown to render the fireflies distasteful to birds.[7] We now report the complete structures of five lucibufagins isolated from *P. pyralis.*[8] These insect steroids are the first insect-derived bufadienolides to be fully characterized. Data on their deterrent effect on birds and ants will be described elsewhere.

Results and Discussion

Employing the extraction procedure previously described,[7] ~2.8 g of crude steroid was isolated from ~28 000 freeze-dried, lanternectomized animals (*P. pyralis*).[8] This extract was further fractionated by column chromatography, which afforded five compounds (initially designated as A, B, C, D, and E in order of increasing retention volume) in the ratio of 2:1:20:8:3. The major component, compound C, crystallized spontaneously, providing over 1 g of pure material. We focused our first efforts on determining the structure of this compound.

The infrared spectrum of C contains significant bands at 3550 and 3430 (OH), 1750–1700 (overlapping carbonyls), and 1635 and 1535 cm^{-} (C=C), while its ultraviolet spectrum has a single maximum at 298 nm (ϵ 5500). The ultraviolet absorption, together with the observation of a set of three interacting protons in the low-field region of the ^{1}H NMR spectrum of C (Figure 1), indicates the presence of a conjugated system. The chemical shifts and coupling constants of the three low-field protons agree closely with those reported for 5-substituted 2-pyrones[9] and, upon consideration of all of the spectral data, the presence of this pyrone moiety in C is clear.[10,11] The chemical ionization mass spectrum (CIMS) of C establishes a molecular weight of 532, leaving 437 amu to be accounted for by the single substituent on the pyrone ring.

Our initial interpretation of the ^{1}H NMR spectrum led to the hypothesis that C contains two quaternary methyl groups, two secondary acetoxy groups, and a tertiary and two secondary hydroxyl groups. The ^{13}C NMR spectrum shows 28 resonances and confirms the presence of two acetates (δ 20.5, 20.7, 168.8, and 169.2). This accounts for two of a total of five sp^{3} carbons bearing an oxygen substituent. In addition, a set of five resonances (δ 114.9, 120.9, 147.5, 149.8, and 162.5) agree well with expectations for the α-pyrone unit.[12] Finally, a signal observed at δ 212.8 revealed the presence of a ketonic carbonyl group. With 28 carbons directly observed and 10 oxygens implied from the ^{13}C NMR spectrum of C, the molecular formula $C_{28}H_{36}O_{10}$ (mol wt 532) is required by its mass spectrum. Aside from the nine carbon resonances of the pyrone and acetate units, the remaining 19 signals present a pattern which suggests that C contains a saturated steroid nucleus, a conclusion consistent with the molecular formula and with the presence of two upfield methyl singlets in its ^{1}H NMR spectrum.

Steroidal pyrones, previously isolated only from certain toads and a small group of plants,[13] often possess desirable cardiotonic properties. This class of steroids, the bufadienolides, is characterized by a cis-anti-trans-syn-cis steroid skeleton bearing 3β- and 14β-oxygen substituents and a 17β-pyrone substituent. We assumed (and later confirmed) the presence of these same features in compound C, placing an acetoxy group at the C-3 position (vide infra) to derive the part structure **1**.

The electron impact mass spectrum (EIMS) of C, although dominated by cleavage of the acetyl groups (*m/e* 43), does contain useful fragments of higher molecular weight. In addition to the weak molecular ion (*m/e* 532), diagnostic peaks were observed at *m/e* 123, 135, 136, 191, 201, and 204. The three lower molecular weight fragments in this group are characteristic of bufadienolides which are devoid of oxygen substituents at C-15, -16 and -17.[14] The three remaining ions, also prominent in the mass spectrum of arenobufagin (**2**), have

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