containing phosphate buffer (pH 7.4) from the in situ decomposition (25 °C) of 1-methyl-4-(2-carboxyethyl)naphthalene endoperoxide[16] also effected the conversion (by ESR) of **3** into **4**. Collectively, these observations indicate the intermediacy of singlet oxygen in the formation of **4**.

Nitroxide **3** also responds to singlet oxygen under biologically relevant conditions. Unilamellar and multilamellar dimyristoylphosphatidyl choline vesicles doped with 2 mol % of **3** were separately suspended in phosphate buffer (pH 7.4) at 32 °C (T_m = 24 °C) containing methylene blue and irradiated open to air for several minutes. Aliquots were removed periodically and diluted with 2 volumes of MeOH in order to destroy the vesicles and give isotropic ESR spectra. The spectra indicated that the conversion of **3** to **4** in the vesicles had taken place.[17,18]

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(17) Oxygen (and presumably singlet oxygen as well) is known to partition effectively into lipid bilayers from aqueous solution: Subczynski, W. K.; Hyde, J. S. *Biophys. J.* **1983**, *41*, 283.

(18) Qualitatively, the conversion of **3** into **4** in the vesicles may be monitored directly by ESR, although the spectra have the broadened appearance expected for probe incorporated in lipid bilayer. Spectral titration (Jost, P.; Griffith, O. H., In "Spin Labeling. Theory and Application"; Berliner, L. J., Ed.; Academic Press: New York, 1976; pp 268-271) of the isotropic digitized spectra of **3** and **4** indicated that as little as a 15% conversion of **3** into **4** could be detected.

(19) The spectrum was analyzed by a least-squares fit of the line shapes: J. Heinzer, "Least Squares Fitting of Isotropic Multiline ESR Spectra"; Computer Program ESRCON, QCPE, 1971; No. 197. With this method it is possible to extract the accurate coupling values even though all of the expected spectral lines are not experimentally resolved. The spectral parameters are consistent with those obtained by the approach of: Duncan, J. L.; Forrester, A. R.; McConnachie, G.; Mallinson, P. D. *J. Chem. Soc., Perkin Trans. 2* **1973**, 718.

(20) Best fit parameters were calculated by using several spin models, including those in which the two methoxy groups were either identical, nonidentical, or neglected altogether. The a_{OCH_3} values were strongly correlated only with the line-width parameter (correlation constant, ~0.49: Castellano, S.; Bothner-By, A. A. *J. Chem. Phys.* **1964**, *41*, 3863), suggesting that methoxy couplings are present but are of much smaller magnitude than the modulation amplitude employed.

Direct Observation of Benzoyloxyl Radicals in Photodecomposition of Dibenzoyl Peroxides with a Time-Resolved EPR Technique

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Diaroyl peroxides are well-known as radical initiators; they decompose thermally and photochemically to give radicals which in turn induce a variety of reactions in solution.[1,2] Many studies have been made to clarify the reaction mechanism.[3] On the basis of product and other analyses it is considered that peroxide bond scission occurs in the first stage of the reaction and decarboxylation

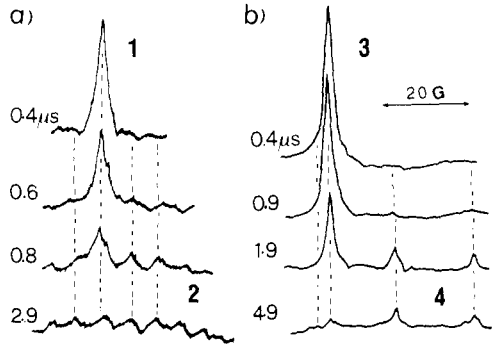

Figure 1. Time-resolved EPR spectra of the intermediate radicals produced by laser irradiation of (a) BPO and (b) MeO-BPO in carbon tetrachloride at room temperature. All signals show absorptions of microwave. The radicals **1-4** observed were assigned as $C_6H_4COO\cdot$ (**1**), $\cdot CCl_3$ (**2**), $CH_3OC_6H_4COO\cdot$ (**3**), and $\cdot CH_2OC_6H_4X$ (**4**).

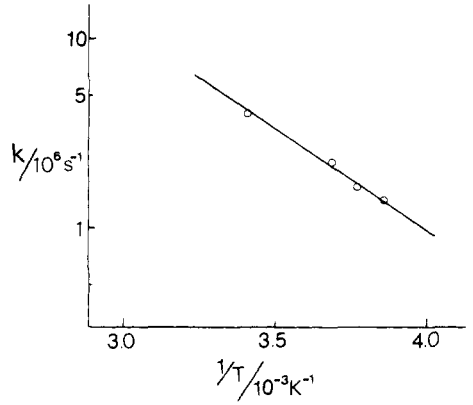

Figure 2. Temperature dependence of the decay rate constant of the benzoyloxyl radical. See the text for the straight line.

of the resulting aroyloxyl radicals follows as the second step.[3] However, no intermediate radicals such as aroyloxyl and aryl radicals have ever been observed yet in the solution systems.[4] Here we report the first observation of EPR spectra of these intermediate species in photodecomposition of some dibenzoyl peroxides by a time-resolved EPR (TREPR) technique with laser irradiation.[5]

We have examined three kinds of dibenzoyl peroxides, dibenzoyl (BPO), bis(4-chlorobenzoyl) (Cl-BPO), and bis(4-methoxybenzoyl) peroxides (MeO-BPO) in carbon tetrachloride at temperatures from -14 to ~20 °C. The sample solutions were irradiated at 308 nm with a Lumonics 861M excimer laser (XeCl, 30 mJ/pulse). The EPR spectra and decays of EPR signals were obtained by a PAR 160 boxcar integrator and a Kawasaki Electronica MR-50E transient memory, respectively. The time resolution of the TREPR system is ca. 0.1 μs.[6] The sample solutions were deaerated by bubbling helium gas.

The TREPR signals were observed for BPO, Cl-BPO, and MeO-BPO. The spectra of BPO and MeO-BPO at 20 °C are shown in Figure 1, parts a and b, respectively. The results for Cl-BPO are qualitatively the same as those for BPO. All the

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signals indicate absorptions of microwave at any times and at any temperatures. Thus we observe the radicals produced from the triplet states of the peroxides. Two kinds of signals, **1-4**, are observed for each system; radicals **1** and **3** appear immediately after the laser irradiation and radicals **2** and **4** are produced at later stages. These four radicals were assigned in reference to the g values and hyperfine coupling constants (hfcc) of the known species. Radical **1** (g = 2.0123, $\Delta H_{1/2} \sim 2.8$ G) and radical **3** (g = 2.0121, $\Delta H_{1/2} \sim 2.2$ G) were assigned as the benzoyloxyl and the 4-methoxybenzoyloxyl radicals by referring to the EPR spectra of $C_6H_5COO\cdot$ (g = 2.0117) and $DOOCCH{=}CHCOO\cdot$ (g = 2.0119) produced by UV and X-ray irradiation of crystals of acetylbenzoyl peroxide[4b,c] and maleic acid.[7] Both radicals **1** and **3** provide relatively large g values and small hfcc ($a_H < 1$ G), which clearly indicates that the benzoyloxyl radicals observed are not π radicals but σ radicals.[7-11] Radicals **2** and **4** were assigned as the trichloromethyl (g = 2.0096, $a_{Cl} \sim 6.2$ G)[12] and the phenoxymethyl-type radicals $\cdot CH_2OC_6H_4X$ (g = 2.0033, $a_H \sim 17.5$ G),[13,14] respectively. The radicals observed for Cl-BPO were assigned as the 4-chlorobenzoyloxyl and the trichlomethyl radicals as in the case of BPO.

The decays of the EPR signals were observed at low microwave powers (≤0.1 mW) to avoid complication due to spin-spin relaxation.[15] The important result obtained is that the decay time of radical **1** is in good agreement with the rise time of radical **2**. A similar relation was also found for radicals **3** and **4**. These results suggest that the stepwise processes occur between radicals **1** and **2** and radicals **3** and **4**. We obtained the decay times (τ) of 0.25, 0.72, and 1.6 μs at 20 °C for the benzoyloxyl radicals produced from BPO, Cl-BPO, and MeO-BPO, respectively. These decay times are considered to be the lifetimes of these radicals for the following reasons. First, the values are much shorter than those (several microseconds) usually obtained for spin-lattice relaxation (SLR) times in solution. Second, the relation of $\tau_{MeO\text{-}BPO} > \tau_{BPO}$ is not expected for the SLR process but is consistent with the lifetimes from the thermal decomposition of BPO and MeO-BPO.[16,17] The decay times of radicals **2** and **4** are much longer (36 and 3-4 μs at 20 °C, respectively) and cannot be easily identified as the lifetimes or the SLR times.

We also examined temperature dependence of the lifetime of benzoyloxyl radical. The result is shown in Figure 2. When we assume that an Arrhenius type equation, $k(T) = k_0 \exp(-E_a/kT)$, holds in this case, we obtain $k_0 \sim 2 \times 10^{10}$ s^{-1} and $E_a \sim 5$ kcal/mol from the straight line of Figure 2. The rate of decarboxylation extrapolated to 130 °C ($\sim 4 \times 10^7$ s^{-1}) is in order of magnitude agreement with the value ($\sim 1 \times 10^8$ s^{-1}) estimated by CIDNP[18a] and spin trapping techniques[18b] for the thermal decomposition of BPO. However, the present values of k and E_a are quite different from the often quoted values ($k \sim 10^4$ s^{-1} and $E_a \sim 14$ kcal/mol).[19]

Finally, we summarize the reaction paths to explain the observations; the phenyl radical has not been observed yet.[20]

$$(C_6H_5COO)_2 \rightarrow C_6H_5COO\cdot \rightarrow [C_6H_5\cdot] + CO_2$$

$$[C_6H_5\cdot] + CCl_4 \rightarrow C_6H_5Cl + \cdot CCl_3 \quad (1)$$

$$(CH_3OC_6H_4COO)_2 \rightarrow CH_3OC_6H_4COO\cdot$$

$$CH_3OC_6H_4COO\cdot + (CH_3OC_6H_4COO)_2 \rightarrow CH_3OC_6H_4COOH + \cdot CH_2OC_6H_4COOOCOC_6H_4OCH_3 \quad (2)$$

(20) The phenyl radical has been reported to react very fast ($k \sim 10^6$ M^{-1} s^{-1}) with carbon tetrachloride.[3c]

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Mechanism-Based Inhibitors of Dopamine β-Hydroxylase Containing Acetylenic or Cyclopropyl Groups

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The hydroxylation of dopamine at the benzylic position to form norepinephrine catalyzed by the copper-containing protein dopamine β-hydroxylase is unusual in that it involves the hydroxylation of an aliphatic carbon-hydrogen bond.[1] Advantage has been taken of the broad substrate specificity of this enzyme[2] to develop mechanism-based inhibitors as probes of the mechanism of C-H bond activation.[2b,e,3] We have recently shown that variously substituted 3-phenylpropenes are mechanism-based inhibitors of dopamine β-hydroxylase and have proposed that the enzyme-bound intermediate which partitions between hydroxylation and inactivation is a benzylic radical, as shown in Scheme I.[3] This was based on a ρ value of −1.2 for the effect of ring substitution upon the rate of inactivation. However, such a result would not be inconsistent with a mechanism in which a second electron is removed from the benzylic radical intermediate to form a benzylic carbonium ion which then partitions between inactivation and hydroxylation. Such a mechanism would involve nucleophilic attack by an enzyme group on a Michael-type acceptor. In order to distinguish between these two possibilities, we have determined the effect upon inactivation of replacing the allylic side chain of the phenylpropenes with a propargyl group. In addition, we have also tested benzylcyclopropanes as inhibitors as a further test of a radical mechanism.

3-Phenylpropynes[4] and benzylcyclopropanes[5] containing either

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