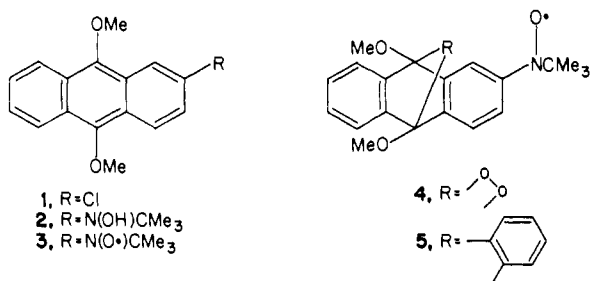## 2-(9,10-Dimethoxyanthracenyl)-*tert*-butylnitroxide. An ESR Spectroscopic Indicator for Singlet Oxygen

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We report the synthesis of the title nitroxide **3**[2] and its reaction

1, R=Cl
2, R=N(OH)CMe₃
3, R=N(O•)CMe₃

4, R=
5, R=

Figure 1. X-band ESR spectra of 2 × 10⁻⁴ M solutions of (A) anthracene nitroxide **3** ($g = 2.0059$), (C) endoperoxide nitroxide **4**, and (D) tryptacene nitroxide **5** in deoxygenated (three freeze–thaw cycles or N_2 bubbling) benzene. Instrument parameters: modulation amplitude, 0.25 G; time constant, 0.3 s; scan rate, 6.25 G/min; microwave power, 5 mW. Computer simulation[19] of spectrum (A) gave the essentially indistinguishable spectrum (B). The hyperfine splitting constants (G) used in the simulation: a_N, 11.300; a_{1H}, 4.150; a_{3H}, 0.696; a_{4H}, 1.043; a_{5H}, 0.528; a_{6H}, 1.116; a_{7H}, 0.305; a_{8H}, 0.396; a_{OCH3}, 0.0853[20]; Lorentzian line width, 0.201. Spectra (C) and (D) have also been simulated (not shown) by using the following parameters. (C) a_N, 12.125; a_{1H}, 2.185; a_{3H}, 1.917; a_{4H}, 0.891; line width, 0.319. (D): a_N, 12.325; a_{1H}, 2.220; a_{3H}, 1.883; a_{4H}, 0.888; line width, 0.357. Double integration of spectra C and D show the expected approximate 1:1:2:2:1:1 relative peak intensities within each of the three multiplets.

with singlet oxygen[3] to give endoperoxide **4**. The pronounced differences between the electron spin resonance (ESR) spectra of **3** and **4** constitute a new, ESR-based method[4] for the detection of this biologically important oxidant.[5] Advantages of ESR methodology include high sensitivity without the requirement of optical transparency of the sample.[6]

2-Chloroanthraquinone was reduced ($Na_2S_2O_4$) to the dihydroxy intermediate which was immediately methylated (Me_2SO_4, aqueous NaOH) to give 2-chloro-9,10-dimethoxyanthracene (**1**), mp 154.5–155.5 °C.[7,8] Reaction[9] of its Grignard derivative (prepared by using activated[10] Mg) with 2-methyl-2-nitrosopropane[11] followed by preparative TLC (silica gel, 1% MeOH in CH_2Cl_2) gave the crude hydroxylamine **2** (26%) which was purified by precipitation from benzene–hexanes to give a yellow powder, mp 154–157 °C.[8] Oxidation with oxygen in MeOH containing $Cu(OAC)_2 \cdot H_2O$ and NH_4OH,[12] evaporation of the solvent, and then trituration of the residue with hexanes gave nitroxide **3** (80%). This was purified by precipitation from benzene–pentane to give a yellow powder, mp 115–118.5 °C.[8] The NMR spectrum ($CDCl_3$), after in situ phenylhydrazine reduction,[13] was identical with that of **2**. Figure 1A shows the ESR spectrum of **3**. The spectrum has been simulated (Figure 1B) using the parameters given in the figure legend.
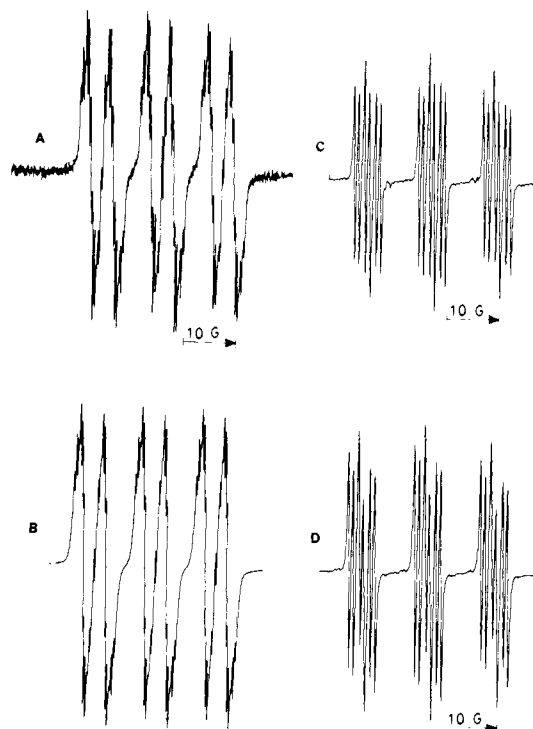
(1) On leave from the Department of Medicinal Chemistry, Hoshi College of Pharmacy, Tokyo, Japan.

(2) For reviews on nitroxides, see: Keana, J. F. W. *Chem. Rev.* **1978**, *78*, 37. Keana, J. F. W. In "Spin Labeling in Pharmacology"; Holtzmann, J. L., Ed.; Academic Press: New York, 1984; Chapter 1.

(3) "Singlet O₂"; Frimer, A. A., Ed.; CRC Press: Boca Raton, FL, 1984; Vol. 1–4. "Singlet Oxygen"; Wasserman, H. H., Murray, R. W., Eds.; Academic Press: New York, 1979. "Singlet Oxygen"; Ranby, B., Rabek, J. F., Eds.; Wiley: New York, 1978.

(4) The generation of a stable nitroxide radical by singlet oxygen oxidation of 2,2,6,6-tetramethylpiperidine has been described by: Lion, Y.; Gandin, E.; Van de Vorst, A. *Photochem. Photobiol.* **1980**, *31*, 305.

(5) For leading references to other methods of detection, see: Midden, W. R.; Wang, S. Y. *J. Am. Chem. Soc.* **1983**, *105*, 4129.

(6) ESR spectroscopy has been used extensively for the detection of other reactive intermediates, especially through spin-trapping methodology. See: Perkins, M. J. *Adv. Phys. Org. Chem.* **1980**, *17*, 1.

(7) This substance has been reported without physical constants by: Obyknovennaya, I. E.; Vember, T. M.; Veselova, T. V.; Cherkosov, A. S. *Opt. Spectrosk.* **1975**, *38*, 1127.

(8) A satisfactory elemental analysis was obtained.

(9) See, for example: Calder, A.; Forrester, A. R.; McConnachie, G. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2198.

(10) Lai, Y.-H. *Synthesis* **1981**, 585.

(11) Calder, A.; Forrester, A. R.; Hepburn, S. P. *Org. Synth.* **1972**, *52*, 77.

(12) Lee, T. D.; Keana, J. F. W. *J. Org. Chem.* **1976**, *41*, 3237.

(13) Lee, T. D.; Keana, J. F. W. *J. Org. Chem.* **1975**, *40*, 3145.

The endoperoxide **4** was generated by irradiation (150-W sunlamp) of a CH_2Cl_2 (90 mL) solution of **3** (9 mg) containing methylene blue (3 mg) with gentle stirring under air. The ESR spectra of diluted aliquots smoothly changed from that in part A to that in part C of Figure 1 over 5 min. Workup gave 7.2 mg (71%) of **4**[8] as a pale brown solid. Its ESR spectrum (Figure 1C) is seen to be similar to that (Figure 1D) of tryptacene nitroxide **5**, mp 103–105 °C,[8] synthesized for purposes of comparison.[14]

Formation of **4** proceeded well when either *meso*-tetraphenylporphine or immobilized rose bengal was used as the sensitizer or when benzene was the solvent. The ESR spectrum of **3** (2 × 10⁻⁴ M) remained essentially unchanged over 5 min under the photolysis conditions either when the sensitizer was omitted, when oxygen was excluded from the reaction, or when the singlet oxygen quencher β-carotene[3,15] (2 × 10⁻⁴ M) was present (benzene solvent). Singlet oxygen generated chemically in aqueous THF

(14) Details will be forthcoming in the full paper.

(15) The question of singlet oxygen quenching by **3** has been raised by a referee. β-Carotene is a (2 × 10³)-fold more efficient quencher of singlet oxygen than is DABCO (Denny, R. W.; Nickon, A. *Org. React.* **1973**, *20*, 167). The nitroxide 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl (i) is about as efficient a quencher as DABCO in the photooxygenation of 9,10-dimethoxy-1-methylanthracene (Bellus, D.; Lind, H.; Wyatt, J. F. *J. Chem. Soc. D* **1972**, 1199). A 5 × 10⁻² M concentration of nitroxide i was required in order to reduce the relative rate of O_2 uptake to 0.58 of the quencher-free rate. Since the concentration of nitroxide **3** in our experiments is some 500-fold lower than that employed with i as a quencher, it is unlikely that appreciable quenching of singlet oxygen by **3** is occuring here.

(16) Saito, I.; Matsuura, T.; Inoue, K. *J. Am. Chem. Soc.* **1983**, *105*, 3200.

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]

(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

(1) Swern, D., Ed. "Organic Peroxides"; Wiley-Interscience: New York, 1970; Vol. 1; 1971; Vol. 2, 3.

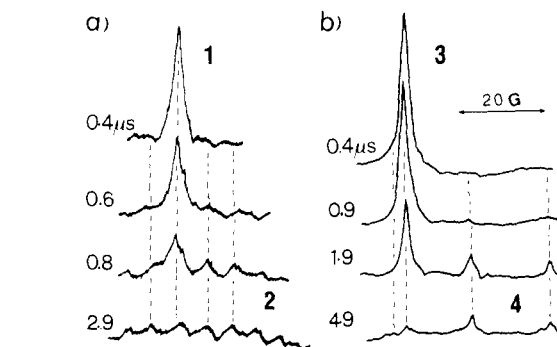(2) Patai, S., Ed. "The Chemistry of Peroxides"; Wiley-Interscience: New York, 1983.

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**).
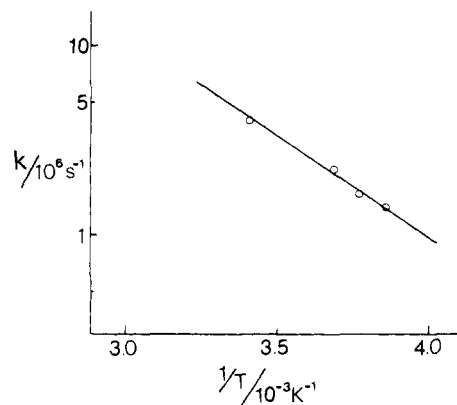

Figure 2. Temperature dependence of the decay rate constant of the benzoyloxyl radical. See the text for the straight line.

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

(3) For photochemical reactions: (a) Nakata, T.; Tokumaru, K. *Bull. Chem. Soc. Jpn* **1970**, *43*, 3315. (b) Kitamura, A.; Sakuragi, H.; Yoshida, M.; Tokumaru, K. *Ibid.* **1980**, *53*, 1393. (c) Scaiano, J. C.; Stewart, L. C. *J. Am. Chem. Soc.* **1983**, *105*, 3609. (d) Walling, C.; Gibian, M. J. *Ibid.* **1965**, *87*, 3413. (e) Poranski, C. F., Jr.; Moniz, W. B.; Sojka, S. A. *Ibid.* **1975**, *97*, 4275. (f) Kaptain, R.; den Hollander, J. A.; Antheunis, D.; Oosterhoff, L. J. *J. Chem. Soc. D* **1970**, 1687.

(4) For reactions in crystalline systems: (a) Box, H. C.; Budzinski, E. E.; Freund, H. G. *J. Am. Chem. Soc.* **1970**, *92*, 5303. (b) Karch, N. J.; Koh, E. T.; Witzel, B. L.; McBride, J. M. *Ibid.* **1975**, *97*, 6729. (c) McBride, J. M.; Merrill, R. A. *Ibid.* **1980**, *102*, 1723.

(5) Muus, L. T.; Atkins, P. W.; McLauchlan, K. A.; Pederson, J. B., Eds. "Chemically Induced Magnetic Polarization"; D. Reidel Publishing: Dordrecht, The Netherlands, 1977.

(6) Yamauchi, S.; Hirota, N. *J. Phys. Chem.* **1984**, *88*, 4631.