by crystallography, the current method leads to the derivation of molecular structures and interactions between molecules in systems where a single-crystal study is not available. To the best of our knowledge, the example presented here is the first ^1H CRAMPS NMR study concerning the thermal phase alteration of amino acids in the solid state.

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Phthalimide Hydroperoxides as Efficient Photochemical Hydroxyl Radical Generators. A Novel DNA-Cleaving Agent

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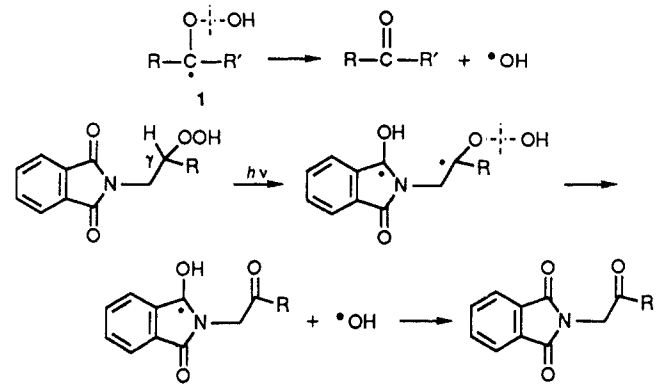
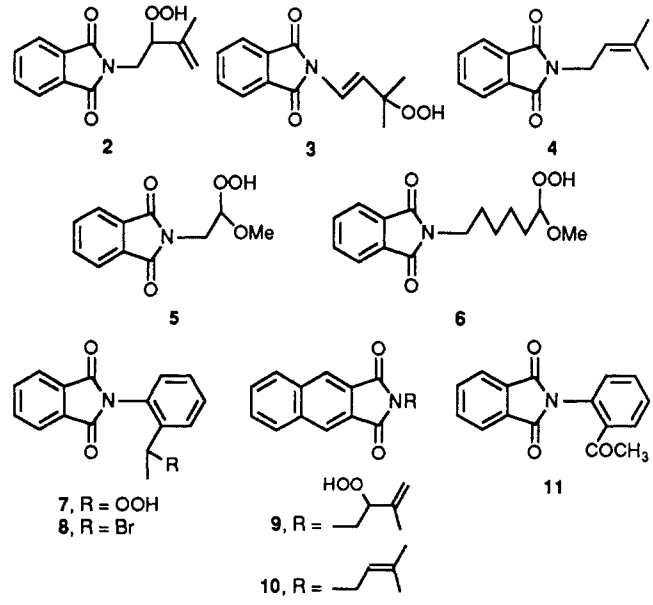
While a variety of methods for the generation of hydroxyl radical are known,[1] the use of organic hydroperoxides as a hydroxyl radical source has not been fully realized.[2,3] Hydroperoxides that can efficiently generate hydroxyl radical by photoirradiation with long-wavelength light (>300 nm) without using metal ions would be an extremely useful hydroxyl radical source, particularly for the design of DNA-cleaving molecules.[3] We report herein an efficient method for generating hydroxyl radical by irradiation (>300 nm) of hydroperoxides derived from phthalimides, which may be widely used as a convenient and clean hydroxyl radical source. We also disclose their use as an effective photochemical DNA cleaver.

Our strategy for generating hydroxyl radical is based on the photochemical γ-hydrogen abstraction of phthalimide[6] and the earlier finding[7] that the hydroperoxyalkyl radicals such as **1** undergo extremely facile β-cleavage of the labile O–O bond giving rise to hydroxyl radical as illustrated in Scheme I. Since γ-hydroperoxy ketones normally exist in a cyclic peroxide form, we have chosen hydroperoxides derived from phthalimides as the precursor. Hydroperoxides **2** and **3** are readily available by singlet oxygenation of **4** in a 1:1 ratio, whereas **5** and **6** were prepared from the corresponding dimethyl ketals (ethereal H_2O_2/cat. CF_3SO_3H/0 °C, 80–85%).[8] Hydroperoxide **7** was obtained from

Scheme I



8 (H_2O_2/CF_3SO_3Ag/ether/0 °C, 70%), whereas **9** was prepared by singlet oxygenation of **10** together with an isomeric hydroperoxide. These hydroperoxides purified by silica gel column chromatography were stable in aqueous organic solvents for at least 10 h at ambient temperature.



Upon photoexcitation, phthalimide derivatives **2** and **5** possessing a secondary hydroperoxy group at the γ-position induce hydroxylation of saturated hydrocarbons and benzene. For example, external irradiation of a solution of **2** (32 mM) in benzene in a sealed test tube with a 100-W high-pressure mercury lamp through a Pyrex filter (>280 nm) for 7 h at ambient temperature produced phenol (10%),[9] whereas irradiation of a solution of **2** (2 mM) and adamantane (10 mM) in acetonitrile under aerobic conditions for 2 h followed by extractive workup with *n*-pentane provided 1-adamantanol (25%), 2-adamantanol (7%), and adamantanone (7%).[9,10] Preferential attack on the tertiary

(1) (a) Czapski, G. *Methods Enzymol.* **1984**, *105*, 209. (b) Walling, C. *Acc. Chem. Res.* **1975**, *8*, 125. (c) Bielski, B. H.; Gebicki, J. M. *Free Radicals Biol.* **1977**, *3*, 1. (d) Halliwell, B.; Gutteridge, M. C. *Biochem. J.* **1984**, *219*, 1. (e) Sie, H. *Oxidative Stress*; Academic Press: New York, 1985.

(2) Ogata, Y.; Tomizawa, K.; Furuta, K. *The Chemistry of Peroxides*; Patai, S., Ed.; Wiley: New York, 1983; p 711.

(3) While the generation of hydroxyl radical by 254-nm irradiation of hydrogen peroxide and alkyl hydroperoxides has been well established,[2] only few are known for the unimolecular photochemical generation of hydroxyl radical by irradiation of hydroperoxides with long-wavelength light. These examples include photochemical generation of hydroxyl radical from α-keto hydroperoxides[4] and α-azo hydroperoxides.[5]

(4) Sawaki, Y.; Ogata, Y. *J. Am. Chem. Soc.* **1976**, *98*, 7342.

(5) Tezuka, T.; Narita, N. *J. Am. Chem. Soc.* **1979**, *101*, 7413.

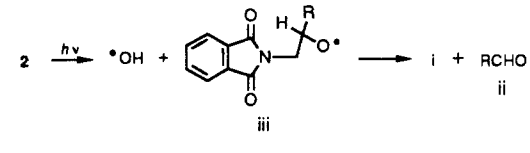
(6) (a) Coyle, J. D. *Synthetic Organic Photochemistry*; Horspool, W. H., Ed.; Plenum: New York, 1984. (b) Mazzocchi, P. H. *Org. Photochem.* **1981**, *5*, 421. (c) Kanaoka, Y. *Acc. Chem. Res.* **1978**, *11*, 407.

(7) (a) Russell, G. A. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973. (b) Howard, J. A.; Chenier, J. H. B. *Can. J. Chem.* **1980**, *58*, 2808.

(8) Myers, A. G.; Fundy, M. M.; Lindstrom, P. A., Jr. *Tetrahedron Lett.* **1988**, *29*, 5609.

(9) Yields determined by GC are based on hydroperoxides initially used.

(10) Products derived from **2** were *N*-(2-methyl-3-oxo-1-buten-4-yl)-phthalimide (10%), *N*-(hydroxymethyl)phthalimide (i) (68%), and methacrolein (ii) (64%) under aerobic conditions. While the former product results from γ-hydrogen abstraction (Scheme I), the latter two products (i and ii) are assumed to arise from β-scission of radical iii formed via homolysis of hydroperoxide **2** initiated by intramolecular energy transfer from photoexcited phthalimide chromophore. Mechanistic details of the photoreaction of these hydroperoxides will be published in a forthcoming paper.



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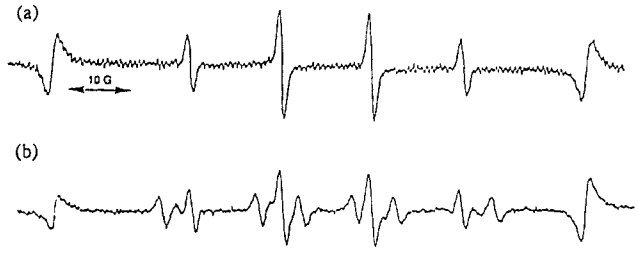

Figure 1. ESR spectrum of the hydroxyl radical spin adduct of DMPO produced during photoirradiation of hydroperoxide **7**. (a) The sample solution containing 1 μL of hydroperoxide **7** (10 mM) in acetonitrile, 5 μL of DMPO (29.2 mM), 10 μL of sodium cacodylate (20 mM), and 84 μL of water was irradiated with transilluminator (302 nm) at ambient temperature for 10 min. An aliquot (5 μL) of the solution was taken up in a capillary, and the ESR was measured at room temperature. The magnetic fields were calculated by the splitting of Mn^{2+} in MnO (ΔH_{3-4} = 86.9 G) (signals at both ends). The 1:2:2:1 pattern of four lines can be interpreted in terms of equivalent hyperfine splitting constants (a_N = a_H = 14.8 G, g = 2.0058) due to both the nitroxide nitrogen and the β-hydrogen.[14] (b) Addition of ethanol (2.5 mM), a hydroxyl radical scavenger, to the reaction system described above collapsed the signals of the DMPO–OH adduct, resulting in the production of signals due to $CH_3\dot{C}HOH$. Similar ESR spectra were obtained in the photoirradiation of **2** and **9**.

position is consistent with the free-radical oxidation initiated by hydroxyl radical.[11] The photooxidation of cyclododecane with **2** was almost completely inhibited by the addition of typical hydroxyl radical scavengers such as 2-propanol or dimethyl sulfoxide.[12] A similar photooxidation was also observed with **5**, **7**, and **9**. Irradiation of **7** (2 mM) and adamantane (10 mM) in acetonitrile for 7 h under similar conditions provided 1-adamantanol (28%), 2-adamantanol (9%), and adamantanone (5%) together with formation of **11** (85%).[13] In contrast, irradiation of **3** or **6** in the presence of adamantane produced less than 10% of the total oxidation products obtained in the photooxidation using **2** under identical conditions. The generation of hydroxyl radical from **2** and **7** was further confirmed by an ESR spin trapping method using 5,5-dimethylpyrroline *N*-oxide (DMPO). As shown in Figure 1, the only detectable ESR signals were those assignable to the hydroxyl radical–DMPO spin adduct. These results clearly indicate that phthalimides possessing a secondary hydroperoxy group at the γ-position are excellent photochemical hydroxyl radical generators.

We then examined the DNA cleaving activity of these hydroperoxides by using supercoiled circular φX 174 RFI DNA (form I). When a solution of form I DNA and hydroperoxide **2** or **5** was irradiated with transilluminator (302 nm) at 0 °C, single-strand and, to a lesser extent, double-strand breaks were observed, as evidenced by the production of form II (relaxed circular) and form III (linear) DNA, respectively (Table I). Hydroperoxides **7** and **9** also cleaved DNA efficiently. Particularly, **9** cleaved DNA at only 1 mM concentration upon irradiation with 366-nm light at 0 °C, whereas **3** and **6** induced DNA cleavage at more than 200 mM concentrations by 302-nm irradiation.

The present work has demonstrated a unimolecular generation of hydroxyl radical from readily available phthalimide hydroperoxides with long-wavelength light (>300 nm) and their use as a photochemical DNA cleaver. Design of a new class of bifunctional molecules comprising a DNA binding component linked to phthalimide hydroperoxide, as well as an examination of the mechanistic aspects of the photochemistry of these hydroperoxides, is underway in our laboratory.

Table I. Cleavage of Supercoiled Circular φX 174 RFI DNA (Form I) into Nicked Circular DNA (Form II) and Linear DNA (Form III) by Photoirradiation of Hydroperoxides **2** and **5**[a]

hydroperoxide	concn, μM	% form I[a]	% form II[b]	% form III[b]
2	200	44.9	55.1	
5	200	5.3	82.9	11.8
2	20	75.3	24.7	
5	20	48.2	46.8	5.0
DNA alone[c]		87.0	13.0	

[a] The reaction mixtures containing 25 μM DNA (form I) and hydroperoxide **2** or **5** at varying concentrations in 50 mM sodium cacodylate buffer (pH 7.0) were irradiated at a distance of 10 cm from transilluminator (302 nm) at 0 °C for 1 h and analyzed by agarose gel electrophoresis. [b] Forms were obtained from densitometry reading after ethidium bromide staining and photography. [c] The DNA used contained a small amount of form II DNA.

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Constraint of the Spontaneous Intermembrane Movement of Sitosterol by Its 24α-Ethyl Group

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The predominant plant sterol β-sitosterol (referred to as sitosterol below) differs from cholesterol only by the presence of a 24α-ethyl group. This structural difference results in a marked decrease in the amount of dietary sitosterol absorbed compared with dietary cholesterol and a preferential excretion of sitosterol in bile. As a result of these processes, the level of sitosterol and other plant sterols normally found in mammalian tissues and plasma is very low (<1% of total plasma sterols).[1] A loss of sterol recognition capacity occurs in the inherited lipid-storage disease called sitosterolemia with xanthomatosis. This disorder is characterized by the abnormal hyperabsorption of ingested dietary sitosterol[2] and shellfish sterols,[3] with a concomitant increase in the concentrations of plant and shellfish sterols[2,3] and 5α-saturated stanols[4] in the plasma.[5]

Cholesterol is thought to be transported into the lymphatic circulation by a passive diffusion process that depends on bile salts for absorption.[6] A number of processes have been considered

(11) The C2/C3 ratio for the selectivity at secondary vs tertiary positions is 0.56. For related examples, see: (a) Fossey, J.; Lefort, D.; Massoudi, M.; Nedelec, J.-Y.; Sorba, J. *Can. J. Chem.* **1985**, *63*, 678. (b) Lau, T.-C.; Che, C.-M.; Lee, W.-O.; Poon, C.-K. *J. Chem. Soc., Chem. Commun.* **1988**, 1406. (c) Broun, R. B.; Hill, C. L. *J. Org. Chem.* **1988**, *53*, 5762. (d) Saito, I.; Takayama, M.; Matsuura, T. *Tetrahedron Lett.* **1989**, *30*, 2297.

(12) Irradiation of **2** (4.6 mM) and cyclododecane (23 mM) in acetonitrile for 2.5 h gave cyclododecanol (4%) and cyclododecanone (11%), whereas in the presence of 2.3 M of 2-propanol or dimethyl sulfoxide, formation of only small amounts of cyclododecanone (less than 3%) was observed under the conditions.

(13) For analogous photochemical δ-hydrogen abstraction of phthalimide,[6] see also: Kanaoka, Y.; Nagasawa, C.; Nakai, H.; Sato, Y.; Ogiwara, H.; Mizoguchi, T. *Heterocycles* **1975**, *3*, 55.

(14) Finkelstein, E.; Rosen, G. M.; Rauckman, E. J. *Arch. Biochem. Biophys.* **1980**, *20*, 1.

(1) (a) Gould, R. G.; Jones, R. J.; LeRoy, G. V.; Wissler, R. W.; Taylor, C. B. *Metab., Clin. Exp.* **1969**, *18*, 652–662. (b) Salen, G.; Ahrens, E. H., Jr.; Grundy, S. M. *J. Clin. Invest.* **1970**, *49*, 952–967. (c) Bhattacharyya, A. K. *Am. J. Physiol.* **1981**, *240*, G50–G55.

(2) Bhattacharyya, A. K.; Connor, W. E. *J. Clin. Invest.* **1974**, *53*, 1033–1043.

(3) Gregg, R. E.; Connor, W. E.; Lin, D. S.; Brewer, H. B., Jr. *J. Clin. Invest.* **1986**, *77*, 1864–1872.

(4) Salen, G.; Kwiterovich, P. O., Jr.; Shefer, S.; Tint, G. S.; Horak, I.; Shore, V.; Dayal, B.; Horak, E. *J. Lipid Res.* **1985**, *26*, 203–209.

(5) There is also a defect in the ability of the liver to concentrate these sterols preferentially into bile; since sterol esters are not secreted into bile, esterification of these sterols with fatty acids (which is retarded in normal cells) may be responsible for this defect.[3] Loss of the normal sterol substrate specificity of enzymes may result in other modifications of plant and shellfish sterols, contributing to their increased absorption.

(6) (a) Treadwell, C. R.; Vahouny, G. V. In *Handbook of Physiology*; Code, C. F., Heidel, W., Eds.; American Physiological Society: Washington, DC, 1968; Vol. 3, pp 1407–1438. (b) Westergaard, H.; Dietschy, J. M. *J. Clin. Invest.* **1976**, *58*, 97–108.

0002-7863/90/1512-884$02.50/0