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1,8-Naphthalimide Hydroperoxides as Novel Intercalating DNA Cleavers

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ABSTRACT

Two new naphthalimide hydroperoxides **3** *and* **4** *have been synthesized and their interactions with DNA investigated. They efficiently intercalate into (in the dark) and cleave (under irradiation, 365 nm) pBR322 and pUC19 DNA. Their DNA-cleaving activities depend on incubation time and temperature in the dark, concentration of drugs, and types of buffers. In contrast, DNA cleavage by* **1**, *a phthalimide peroxide that has been reported to act as a DNA cleaver, was not visible under the same condition. Copyright © 1996 Elsevier Science Ltd*

INTRODUCTION

DNA cleavage by active species has received much current interest.[1] Of all the different oxygen species involved in DNA damage, the hydroxyl radical is the most active species. There have been a variety of methods for generating hydroxyl radicals involving radiolysis,[2] Feton's type reactions,[3] dissolution of potassium peroxonitrite,[4] and photolysis of peroxide species[5,6] and heterocyclic N-oxides[7] that generate ·OH either by low-energy irradiation such as long-wavelength (>350 nm), or, more preferably, by visible light irradiation. Such molecules, referred to as 'photo-Feton reagents', are particularly attractive as a controllable and mechanistically less complicated ·OH

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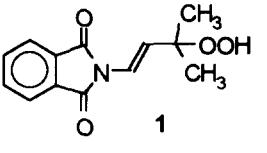
source for applications in a number of biologically important reactions such as cross-linkage of biopolymers,[8,9] cleavage of DNA[10,11] or proteins[12–14] and lipid peroxidation.[15] 1,8-Naphthalimide type dyes usually have strong fluorescence,[16] and we have previously reported their uses as electro-optically sensitive materials,[17,18] singlet oxygen probes[19] and DNA intercalaters.[20,21,22] We report herein DNA-cleaving peroxide derivatives possessing a 1,8-naphthalimide chromophore as intercalating photochemical DNA-cleaving agents.

RESULTS AND DISCUSSION

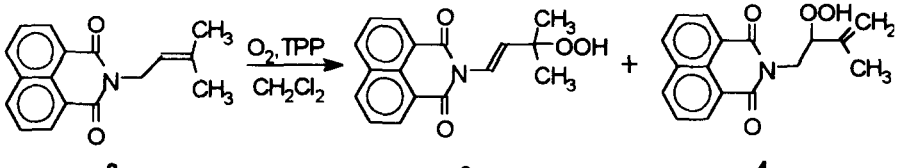
The methodology for the preparation of 1,8-naphthalimide peroxides is displayed in Scheme 1. Naphthalimide **2** was oxygenated by singlet oxygen at −30 to −10°C to give a mixture of **3** and **4**, which was separated by TLC to afford the naphthalimide peroxides **3** and **4** in a *ca* 1:1 ratio; their structures were confirmed by ^{1}H NMR, IR and MS.

A solution of **3**, toluene and PBN (*N-tert*-butyl-α-phenylnitrone) was irradiated (> 300 nm) to give very strong ESR signals (as shown in Fig. 1), which indicates that 1,8-naphthalimide peroxides **3** can efficiently generate hydroxyl radicals. A similar spectrum was obtained for **4**.

The DNA-cleaving activities of **3** and **4** have been tested and their reactivities compared with the phthalimide **1**, which is also believed to cleave DNA.[23] As shown in Fig. 2, at 37°C compounds **3** and **4** clearly converted form I DNA (covalently closed circular) into form II (nicked circular) and form III (linear duplex) DNAs in a typical single-strand-cleavage, but the DNA cleavage by **1** was not obvious. The relative amounts of cleavage in lanes 11, 12 and 13 indicate that the DNA-cleaving activities of the above



1



2 **3** **4**

Scheme 1.

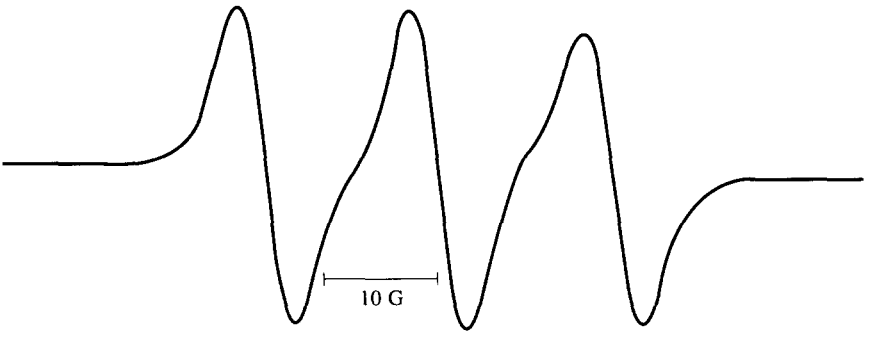


Fig. 1. ESR spectrum of the hydroxyl radical spin adduct of PBN produced during photoirradiation of **3** in toluene containing PBN as trapper.

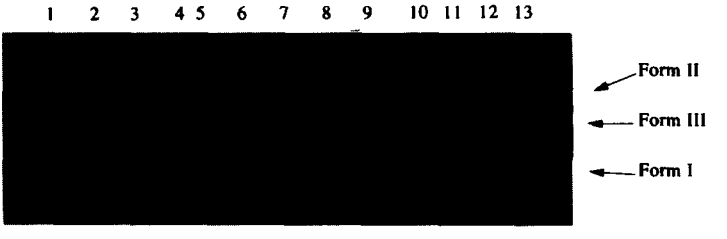


Fig. 2. DNA cleavage by hydroperoxides **3** (lanes 3, 7, 11), **4** (lanes 4, 8, 12) and **1** (lanes 5, 9, 13). pUC19 DNA (1.0 μg) was incubated in 10 μl of 1 × TBE with drugs (400 μM concentration) at 4°C for 18 h (lanes 2–5), at 20°C for 0 h (lanes 6–9), at 37°C for 18 h (lanes 10–13) in the dark, respectively, and irradiated for 30 min at 365 nm. The samples were analysed by gel electrophoresis in 1% agarose and the gel was stained with ethidium bromide. Lane 1: DNA alone without irradiation; lanes 2, 6, 10: DNA control.

three drugs are in the order: **4** > **3** > > **1**. Similar cleavages were also observed using pBR322 DNA.

Other DNA-intercalating naphthalimides and the relative DNA-cleaving activities of **3**, **4** and **1** encouraged us to suspect that the hydroxyl radicals were formed at least partly in the DNA matrix by photolysis of the DNA-intercalated hydroperoxides **3** and **4**. Therefore, we examined whether the naphthalimide peroxides investigated herein undergo intercalation. Indeed, the formation of a molecular complex between pUC19 DNA and the naphthalimide **3** was confirmed by strong quenching of the fluorescence (Fig. 3). From the fluorescence data, the binding parameter of the DNA complex was calculated to be 1.056×10^5 according to the reported methods.[24] The fluorescent intensity changes irregularly for **4** in various concentrations upon mixing with pUC19 DNA (Fig. 3). Such changes in emission intensity may be attributed to the environmental change of compound intercalated into the base pairs of DNA.[25,26] Furthermore, the fact that prolonged incubation at 37°C in the dark strongly increases the DNA-cleaving activities of **3** and **4**

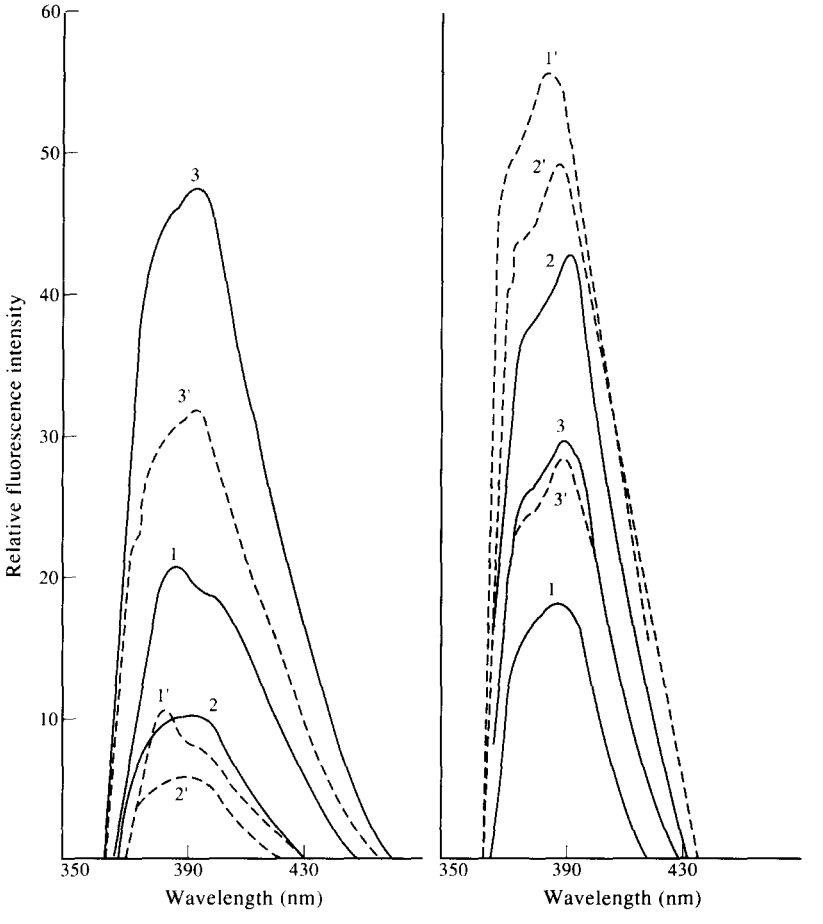


Fig. 3. Fluorescence of **3** [in (a)] and **4** [in (b)] before (-) and after (...) mixing with pUC19 DNA in Tris HCl + CH_3CN (9:1 ratio) system. λ_{ex}: 340 nm for **3**; 343 nm for **4**. The concentration of DNA was held constant (in *ca* 10^{-6} M bp) while that of drugs varied from 10^{-6} to 10^{-4} M.

also proved that naphthalimide hydroperoxides efficiently intercalate into DNA (data not shown). In contrast, the increase of DNA cleavage by **1** was not apparent under the same condition. Unfortunately, because it was not sufficiently fluorescent to give a quantitative estimate of the fluorescence quenching by DNA, the DNA-binding parameter for **1** could not be determined. It is thus unknown whether the higher efficiencies of hydroperoxides **3** and **4** to cleave DNA, in comparison to **1**, are due to their higher DNA-binding constants.

Using **4** as an example, the effects on pUC19 DNA-cleaving efficiency of incubation temperature, concentration of drug and type of buffer were

Scheme 2.

investigated. It was found that the relationship between DNA-cleaving activity and drug concentration was nonlinear, and an appropriate drug concentration can thus be obtained. The DNA-cleaving efficiency of **4** depended on the type of buffer, e.g. **4** cleaved DNA much more efficiently in 1 × TBE (pH 8.0) than in Tris (10 mM, pH 7.4) or in Tris (10 mM, pH 8.0); as previously reported, Tris effectively inhibits DNA cleavage by scavenging HO·.[4] Providing that no decompositions of drugs and DNA take place, the higher the incubation temperature the more efficiently the naphthalimide hydroperoxides **3** and **4** cleave DNA.

According to the above results, it is concluded that 1,8-naphthalimide hydroperoxides interact with DNA in two steps. Initially, they intercalate into DNA in the dark; then, under irradiation, the intercalated 1,8-naphthalimide hydroperoxides generate hydroxyl radicals which cleave DNA (Scheme 2), Thus, naphthalimide hydroperoxides act as efficient intercalating DNA cleavers.

EXPERIMENTAL

General

Melting points were taken on a digital melting point apparatus made in Shanghai. Infrared spectra were recorded on a Nicolet FT IR-20sx, mass spectra on a Hitachi M 80 and ^{1}H NMR on a Bruker AM-300, using TMS as the internal standard. Fluorescence spectra were measured on a Perkin Elmer LS 50 and ESR on a Bruker ER200D-SRC.

Synthesis of 1,8-naphthalimide hydroperoxides (3), (4)

A solution of 0.252 g (0.95 mmol) of **2** in 40 ml of CH_2Cl_2, which contained 5 mg of tetraphenylporphine (TPP) as a sensitizer, was irradiated externally with a 150 W sodium lamp at −30 to −10°C for 3 h, while a gentle stream of dry oxygen gas was continuously passed through the reaction mixture. After removal of the solvent, the residue was subjected to TLC on silica gel using a 3:1 mixture of petroleum ether (60–90°C)/ethyl acetate; two bands were collected to give **3** (35%) and **4** (34%), respectively:

3: mp, 110.6–111.3°C; ^{1}H NMR (CD_3COCD_3): δ1.47 (s, 3H, 3′-CH_3), 1.48 (s, 3H, 3′-CH_3), 6.72 (*d*, J = 15.2 Hz, 1H, 2′-H), 7.05 (*d*, J = 15.2 Hz, 1H, 1′-H), 7.90 (*dd*, J_{AX} = 8.0, J_{XB} = 7.4 Hz, 2H, 3-H, 6-H), 8.45 (*dd*, J_{XB} = 7.4, J_{AB} = 1.0 Hz, 2H, 4-H, 5-H), 8.60 (*dd*, J_{AX} = 8.0 Hz, J_{AB} = 1.0 Hz, 2H, 2-H, 7-H); IR (Nujol): 3380 (OOH), 1700, 1660, 1620, 1584, 1520, 790 cm^{-1}; MS (EI, 70ev) *m/e* (%): 280 (1.04)[M^+-OH], 264 (22.08)[M^+-OOH], 222 (100)[M^+-C(OOH)Me_2].

4: mp, 158.5–159.0°C; ^{1}H NMR (CD_3COCD_3): δ1.89 (s, 3H, 3′-CH_3), 4.28 (q, J = 5.4 Hz, 1H = CH), 4.52 (q, J = 8.1 Hz, 1H = CH), 4.75 (m, 1H, 2′-H), 4.94 (*d*, J = 1.5 Hz, 1H, N-CH), 4.95 (*d*, J = 3.9 Hz, 1H, n-CH), 7.91 (*dd*, J_{AX} = 8.1 Hz, J_{BX} = 7.5 Hz, 2H, 3nH, 6-H), 8.47 (*dd*, J_{AX} = 8.1 Hz, J_{AB} = 0.9 Hz, 2H, 4-H, 5-H), 8.60 (*dd*, J_{BX} = 7.5 Hz, J_{AB} = 0.9 Hz, 2H, 2-H, 7-H); IR (KBr): 3400(OOH), 1700, 1665, 1630, 1595, 1445, 1250, 785 cm^{-1}; MS (EI 70ev) *m/e* (%): 297 (3.2)[M^+], 280 (34.1)[M^+-OH], 264 (15.7)[M^+-OOH], 210 (100)[M^+-CH(OOH)C(CH_3) = CH_2].

Synthesis of phthalimide peroxide (1)

The same procedure described above was used, but the reagents used were changed as follows: 0.292 g of *N*(-3,3-dimethylallyl)phthalimide, 5 mg of TPP, 30 ml of CH_2Cl_2; purification of **1** was effected by recrystallization from a mixture of petroleum ether and $CHCl_3$. mp 111.2°C. ^{1}H NMR (CD_3COCD_3): δ1.39 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 6.81 (t, 2H, -CH = CH-), 7.93 (s, 4H, aromatic ring H); IR(Nujol) v = 3300 (OOH), 2900, 2850, 1765, 1700, 1464, 1380, 794, 720 cm^{-1}; MS (EI 70ev) *m/e* (%): 230 (5.1)[M^+-OH], 214 (82.0)[M^+-OOH], 172 (26.3)[M^+-C(OOH)Me_2], 104 (100).

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REFERENCES

1. Meunier, B., Pratviel, G. & Bernadou, J., *Bull Soc. Chim. Fr.*, **131** (1994) 933.
2. Bielski, B. H. & Gebicki, J. M., *Free Radicals Biol.*, **3** (1977) 18.
3. Imlay, J. A., Chin, S. M. & Linn, S., *Science*, **240** (1988) 640.
4. King, P. A., Anderson, V. E., Edwards, J. O., Gustafson, G., Plumb, R. C. & Suggs, J. W., *J. Amer. Chem. Soc.*, **114** (1992) 5430.
5. Ogata, Y., Tomizawa, K. & Furuta, K., *The Chemistry of Peroxides*, ed. S. Patai. Wiley, New York, 1983.

6. Adam, W., Cadet, J., Dall'Acqua, F., Epe, B., Ramaiah, D. & Saha-Moller, C. R., *Angew., Chem. Int. Ed. Engl.*, **34** (1995) 107.
7. Sako, M., Nagai, K. & Maki, Y., *J. Chem. Soc. Chem. Commun.* (1993) 750.
8. Rana, T. M. & Meares, C. F., *Bioconjugate Chem.*, **1** (1990) 357.
9. Davis, K. J. A., *J. Biol. Chem.*, **262** (1987) 9895.
10. Mack, D. P., Sluka, J. P., Shin, J. A., Griffin, J. H., Simon, M. I. & Dervan, P. B., *Biochemistry*, **29** (1990) 6561.
11. Prigodich, R. V. & Martin, C. T., *Biochemistry*, **29** (1990) 8017.
12. Rana, T. M. & Meares, C. F., *J. Amer. Chem. Soc.*, **112** (1990) 2457.
13. Schepartz, A. & Cuenoud, B., *J. Amer. Chem. Soc.*, **112** (1990) 3247.
14. Hoyer, D., Cho, H. & Schultz, P. G., *J. Amer. Chem. Soc.*, **112** (1990) 3249.
15. Dix, T. A. & Alkens, J., *Chem. Res. Toxicol.*, **6** (1993) 218.
16. Peters, A. T. & Bide, M. J., *Dyes and Pigments*, **6** (1985) 349.
17. Qian, X., Zhu, Z. & Chen, K., *Dyes and Pigments*, **11** (1989) 13.
18. Qian, X., Zhu, Z., Chen, K., Yin, Q. & Zhu, G., *Mater. Chem. and Phys.*, **23** (1989) 335.
19. Adam, W., Qian, X. & Saha-Moeller, C. R., *Tetrahedron*, **49** (1993) 417.
20. Qian, X., *Proc. 1st Young Scientists of China Association of Science and Technology, China Press of Science and Technology*, Beijing, China, 1992, 252.
21. Qian, X., Tang, J., Zhang, J. & Zhang, Y., *Dyes and Pigments*, **25** (1994) 109.
22. Tao, Z., Qian, X. & Tang, J., *Dyes and Pigments*, in press.
23. Saito, I., Takayama, M. & Matsuura, T., *J. Amer. Chem. Soc.*, **112** (1990) 883.
24. Gupta, M. & Ali, R., *J. Biochem.*, **95** (1984) 1253.
25. Barton, J. K., Dannishefsky, A. T. & Goldberg, J. M., *J. Amer. Chem. Soc.*, **106** (1984) 2172.
26. Jenkins, Y. & Barton, J. K., *J. Amer. Chem. Soc.*, **114** (1992) 8736.