Facile Generation of Hydroxyl Radical by Photolysis of Pyrimido[5,4-*g*]pteridinetetrone *N*-Oxides in Aqueous Solution. A New Efficient DNA-photocleaving Agent

Magoichi Sako, Katsuyuki Nagai and Yoshifumi Maki*

Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan

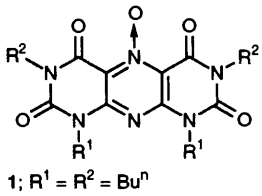
Photolysis of pyrimido[5,4-*g*]pteridinetetrone *N*-oxide **2** in water with UV–VIS light (>355 nm) provides a convenient and efficient method for the clean generation of hydroxyl radicals, which are useful as DNA-cleaving agents.

There have been a variety of methods for generating hydroxyl (OH) radicals involving radiolysis,[1] photolysis of peroxide species,[2,3] Fenton's type reactions,[4] and dissolution of potassium peroxonitrite;[5] a clean and facile method, however, is still desirable in OH radical chemistry and for constructing a new class of agents such as DNA cleaving species.

In this context, we report herein such a method for generating OH radicals by irradiation (>355 nm) of compound **2** in aqueous solution without additives. We also describe its use as a photochemical DNA-cleaving agent[6] and the preparation of ^{18}O-labelled *N*-oxide **2*** resulting in the generation of ^{18}O-labelled OH radical.

The practical use of photoexcited heterocyclic *N*-oxides as OH radical generators has not been fully documented.[7]† This can be ascribed to the fact that most heterocyclic *N*-oxides undergo preferentially, intramolecular rearrangements rather than oxidation of cosubstrates under photochemical conditions.[7]

Our previous work[8,9] has demonstrated that compound **1**[10] exhibits a number of remarkable photochemical properties unique to heterocyclic *N*-oxides: **1** functions efficiently under irradiation (>355 nm) in aprotic solvents (*e.g.* dry acetonitrile) as an electron acceptor and as an agent for oxygen-atom transfer or dehydrogenation, depending upon the nature of the cosubstrates, without any accompanying appreciable intramolecular rearrangements.

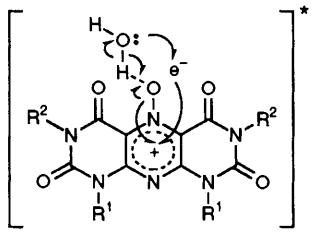


1; $R^1 = R^2 = Bu^n$
2; R^1 = MOM, R^2 = Me
2*; **2** with ^{18}O in the *N*-oxide position

Our strategies for generating OH radicals from pyrimido[5,4-*g*]pteridinetetrone *N*-oxides are based on the novel photochemical nature of the *N*-oxide system and on the assumption that water-soluble derivatives of **1** may efficiently generate OH radicals in a bimolecular fashion from the water-solvated excited form, see Scheme 1.

In a manner similar to the case of **1**,[10] the *N*-oxide **2** [m.p. 264 °C; λ_{max}: 360 (ε 1.5 × 10^4) nm in water] was easily prepared in *ca.* 50% yield by the oxidation of 6-amino-1-methoxymethyl-3-methyl-5-nitrosouracil with lead tetraacetate in acetic acid, together with furazano[3,4-*d*]pyrimidinedione derivative. The *N*-oxide **2** was very stable and, in contrast with **1**, highly soluble in water. Synthesis of ^{18}O-labelled *N*-oxide **2*** (^{18}O-content = 56%) was achieved using a 6-amino-5-^{18}O-labelled nitrosouracil derivative as starting material, prepared by the nitrosation of the corresponding 6-aminouracil derivative with nitrosonium tetrafluoroborate pretreated with ^{18}O-labelled water (^{18}O-content = 97%) in dry acetonitrile at 0 °C for 3 min.‡

The *N*-oxide **2** is stable in dry acetonitrile under irradiation with UV–VIS light (>355 nm) but is gradually deoxygenated in water, *e.g.* the photolysis of **2** (500 μmol dm^{-3}) in water was complete after 1 h giving the corresponding deoxygenated pyrimidopteridinetetrone almost quantitatively. The genera-



Scheme 1 The proposed mechanism for the bimolecular generation of hydroxyl radicals by photolysis of pyrimido[5,4-*g*]pteridinetetrone *N*-oxides in water

† Photolysis of *N*-hydroxy-2-thiopyridone in CH_2Cl_2 to generate OH radicals has been reported previously but without detail description: *cf.* D. H. R. Barton, J. Cs. Jaszberenyi and A. I. Morrell, *Tetrahedron Lett.*, 1991, **32**, 311. The non-photochemcial DNA cleavage by oxidative species including OH radicals produced from a phenazine di-*N*-oxide derivative in the presence of dithiothreitol has been reported. The efficacy of DNA cleavage, however, is not so high: essential complete conversion of form I DNA to form II DNA was achieved at a 50 μmol dm^{-3} concentration of the di-*N*-oxide, *cf.* K. Nagai, B. J. Carter, J. Xu and S. M. Hecht, *J. Am. Chem. Soc.*, 1991, **113**, 5099. The *N*-oxide **2** also mediated the DNA strand scission (only 43% conversion yield at a 50 μmol dm^{-3} concentration of **2**) under the conditions described by Hecht *et al.*

‡ The ^{18}O-content of **2*** was delicately governed by the reaction time for the pretreatment of the nitrosonium salt with $H_2{}^{18}O$ and the conditions were not optimized.

Table 1 Cleavage of supercoiled circular ΦX 174 RF I (Form I) DNA into nicked circular (form II) DNA by photoirradiation of pyrimido[5,4-*g*]pteridinetetrone *N*-Oxide **2** and its inhibition with dimethyl sulfoxide (DMSO)[a]

2	Content of DMSO	% Form I DNA[b]	% Form II DNA[b]
0.5 μmol dm^{-3}	—	43	54
1.0 μmol dm^{-3}	—	5	92
	0.1%	86	13
	1.0%	92	7
2.0 μmol dm^{-3}	—	N.D.	98
	0.1%	47	52
	1.0%	70	29

[a] The reaction mixtures (30 μl total volume) containing 200 ng form I DNA and the *N*-oxide **2** at varying concentrations in 50 mmol dm^{-3} sodium cacodylate buffer (pH 7.5) were irradiated in the absence or presence of DMSO at a distance of 5 cm from a 400 W high-pressure mercury-arc lamp through a $BiCl_3$ solution filter (>355 nm) at ambient temp. for 10 min and then analysed by agarose gel electrophoresis in the presence of ethidium bromide. The DNA used contains a small amount of form II DNA (≈10%) and a trace amount of linear DNA. [b] Yields were estimated by densitometric analysis of a photographic negative of the agarose gel after ethidium bromide staining.

tion of OH radicals in this reaction was confirmed by an EPR spin-trapping method using 5,5-dimethylpyrroline *N*-oxide (DMPO): the OH radical–DMPO spin adduct showed a clear 1 : 2 : 2 : 1 pattern of four lines with $a_N = a_H = 15.0$ G (1 Gauss = 10^{-4} T). The colorimetric quantitative assay of OH radicals[11] showed the generation of *ca.* 2 equiv. of OH radical during the reaction, strongly supporting the assumption described above.§

The ability of **2** to induce photocleavage of DNA was estimated by using supercoiled circular ΦX 174 RF I (form I) DNA. When a buffer solution of form I DNA and **2** was irradiated externally with UV–VIS light (>355 nm) at ambient temperature, an efficient single strand break was observed as evidenced by the production of relaxed circular (form II) DNA with concentration dependence of **2** (see Table 1). Essentially complete conversion of form I DNA to form II DNA was achieved at a 2.0 μmol dm^{-3} concentration of *N*-oxide **2** after irradiation for 10 min. The photochemical DNA cleavage with **2** was effectively inhibited by the addition of an OH radical scavenger, dimethyl sulfoxide, with concentration dependence, supporting that the strand break was induced by OH radicals generated during the reaction. Comparative experiments of photochemical DNA cleavage using tricyclic heterocyclic *N*-oxides such as phenazine *N*-oxide and 1-methoxymethyl-3-methylalloxazine 5-oxide under analogous conditions showed that **2** was much more effective than the *N*-oxides used. No nicking of form I DNA with **2** was observed under the reaction conditions without irradiation.†

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§ The peak irreversibility of **2** [E^{ox}_p = −0.70 V *vs.* SCE in 0.1 mol dm^{-3} phosphate buffer (pH 7.5)] in cyclic voltammetry clearly indicates that the protonated radical of **2** (*cf.* Scheme 1) is very unstable at ambient temperature.

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