electron-transfer step. A similar mechanistic proposal has been made regarding the reaction of **1** with dimethyl fumarate and dimethyl maleate.[28] Further work is focusing on the mechanism of the double carbonylation and on developing methods of removing these ligands from the metal centers.

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Supplementary Material Available: Tables of atom positions, temperature factors, and bond distances and angles for **2** and **3** (25 pages). Ordering information is given on any current masthead page.

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DNA Cleavage by Oxygen Radicals Produced in the Absence of Metal Ions or Light

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The availability of molecules that mediate DNA strand scission has facilitated the implementation of strategies for DNA sequencing,[1] as well as studies of DNA conformation[2] and the way in which small molecules bind to DNA.[3] In addition to the exploration of novel mechanistic strategies for DNA cleavage,[4] new DNA cleaving agents are of substantial practical interest as potential antitumor agents[5] and as prosthetic groups for antisense oligonucleotides[6] that can destroy a bound target sequence in a site-selective fashion.[7] The latter two applications, however, require that DNA cleavage obtain efficiently under conditions compatible with physiological function, using only those cofactors normally present within a cell. Relatively few molecules meet this requirement; most of these are natural products[5,8] rather than designed reagents.

There are a number of examples of highly efficient DNA strand scission by reagents that effect the (metal-centered) generation of diffusible oxygen radicals,[2,3,9] but these typically require either a strong oxidant or high concentrations of a reducing agent for oxygen radical generation. Presently, we describe the preparation of a phenazine di-*N*-oxide derivative designed to produce diffusible oxygen radicals, and concomitant DNA strand scission, under physiological conditions.

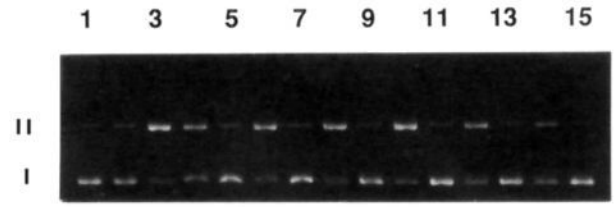

Figure 1. Cleavage of supercoiled ccc DNA by phenazine di-*N*-oxide **1** under aerobic conditions. A reaction mixture (40 μL total volume) containing 200 ng of replicative form *φX174* DNA in 2.5 mM sodium cacodylate, pH 7.5, was treated with compound **1** + 100 μM DTT at 37 °C for 1 h and then analyzed by agarose gel electrophoresis. Lane 1: DNA alone. Lane 2: 100 μM DTT. Lane 3: 10 μM Fe^{2+} + 0.03% H_2O_2. Lanes 4–15: DNA + **1** at 500 μM (lanes 4 and 5), 250 μM (lanes 6 and 7), 100 μM (lanes 8 and 9), 50 μM (lanes 10 and 11), 25 μM (lanes 12 and 13), and 10 μM (lanes 14 and 15) concentrations in the presence (even-numbered lanes) or absence (odd-numbered lanes) of 100 μM DTT.

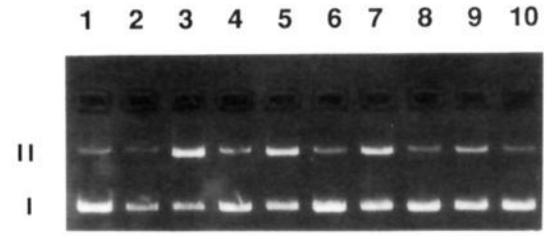

Figure 2. Cleavage of supercoiled DNA by **1** under anaerobic conditions in the presence of DTT and NADPH. Cleavage was carried out at 25 °C for 2 h. Lane 1: DNA alone. Lane 2: 1 mM DTT. Lane 3: 1 mM DTT + 50 μM **1**. Lane 4: 100 μM DTT. Lane 5: 100 μM DTT + 50 μM **1**. Lane 6: 1 mM NADPH. Lane 7: 1 mM NADPH + 50 μM **1**. Lane 8: 100 μM NADPH. Lane 9: 100 μM NADPH + 50 μM **1**. Lane 10: 50 μM **1**.

Scheme I. Proposed Mechanism for the Reductive Activation of Phenazine di-*N*-oxide **1**

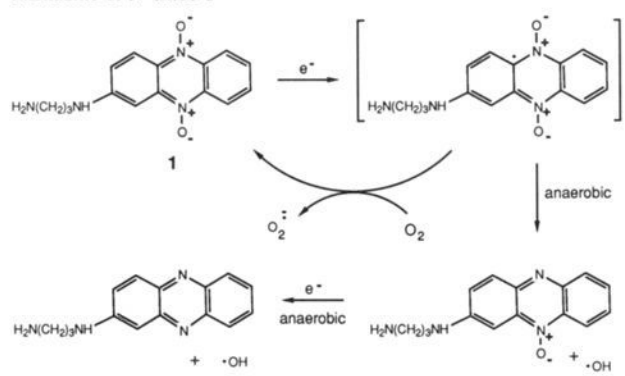

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Table I. Reductive Deoxygenation of Phenazine Di-*N*-oxide 1

conditns[a]	ratio[b,c] phenazine di-*N*-oxide 1/ phenazine mono-*N*-oxide	phenazine di-*N*-oxide 1/ phenazine
aerobic	19.1	-[d]
anaerobic	7.5	9.5

[a] Carried out in 80 μL (total volume) of 2.5 mM sodium cacodylate, pH 7.5, containing 16.5 μg of sonicated calf thymus DNA, 50 μM 1, and 5 mM DTT. The reaction mixture was incubated at 25 °C for 22 h in the dark. DNA was removed by precipitation (2 volumes of ethanol, -80 °C), and the supernatant was analyzed by HPLC. [b] The product ratios were determined by reverse-phase HPLC on a Rainin Microsorb C_{18} column (4.6 mm × 10 cm, 3 μm) using a linear gradient of 20% → 60% CH_3OH in 0.1 M sodium phosphate, pH 6.2. [c] Elution volumes; phenazine di-*N*-oxide 1, 8.2 min; phenazine mono-*N*-oxide [2-[(3'-aminopropyl)amino]phenazine 5(10)-mono-*N*-oxide], 12.4 min; phenazine [2-[(3'-aminopropyl)amino]phenazine], 16.3 min. [d] Not detected.

A number of heterocyclic di-*N*-oxides have been reported to exhibit cytotoxicity toward mammalian and bacterial cells.[10] The mechanism of toxicity has been suggested to involve one-electron-reductive activation of the parent *N*-oxides, which could result in the production of $^{\bullet}OH$ and $O_2^{\bullet-}$ (Scheme I).[10c,d,11] While the locus of action of these agents has not been established, it seemed reasonable to anticipate that a heterocyclic di-*N*-oxide capable of binding to DNA and producing diffusible oxygen radicals would effect DNA strand scission.[12] Accordingly, 2-[(3'-aminopropyl)amino]phenazine 5,10-di-*N*-oxide (1) was prepared by treatment of 2-chlorophenazine 5,10-di-*N*-oxide[13] with 1,3-diaminopropane.[14]

Phenazine di-*N*-oxide 1 (10-500 μM concentrations) was incubated aerobically with *φX174* replicative form DNA in the presence of 100 μM dithiothreitol (DTT) (Figure 1). As shown, relaxation of supercoiled DNA was observed at all concentrations (and only where DTT was present) and increased in proportion to the concentration of 1 utilized. Essentially complete conversion of supercoiled (form I) DNA to relaxed (form II) DNA was achieved at 50 μM phenazine di-*N*-oxide, so this concentration was used to study the effects of O_2 and another reducing agent on the facility of DNA cleavage. As shown in Figure 2, compound 1 effected cleavage of the plasmid DNA anaerobically in the presence of both DTT and NADPH. The extent of DNA cleavage was greater with DTT than with NADPH, and also at the higher of the two concentrations employed for each reductant. Repetition of this experiment under aerobic conditions gave similar results.

In order to determine the fate of phenazine di-*N*-oxide 1 under the reductive conditions employed here, compound 1 was incubated with DTT and calf thymus DNA under both aerobic and anaerobic conditions. Reduction products were analyzed by HPLC in comparison with authentic synthetic standards;[14] the results are summarized in Table I. Under anaerobic conditions there was substantial conversion of phenazine di-*N*-oxide 1 to the respective phenazine mono-*N*-oxide(s) and to the parent phenazine derivative. In contrast, little reductive deoxygenation was observed under aerobic conditions. These results are consistent with Scheme I and suggest that reductive activation of phenazine 1 produces $O_2^{\bullet-}$ and $^{\bullet}OH$ under aerobic and anaerobic conditions, respectively. While $^{\bullet}OH$-mediated DNA cleavage might be thought to be substantially more facile, it may be noted that $O_2^{\bullet-}$ can, in principle, be produced catalytically under aerobic conditions.[15]

The present findings establish the utility of phenazine di-*N*-oxide to mediate DNA strand scission under conditions similar to those expected to obtain within an intact cell. The fact that this compound can be activated by the bioreductive agent NADPH argues for the potential therapeutic utility of this species, e.g., as part of an antisense oligonucleotide.[16]

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(15) Although the species responsible for DNA strand scission have not been established definitively, it may be noted that $O_2^{\bullet-}$ can be converted to $^{\bullet}OH$ ($2H^+ + 2O_2^{\bullet-} \rightarrow O_2 + H_2O_2$, and $H_2O_2 + O_2^{\bullet-} \rightarrow {}^{\bullet}OH + {}^{-}OH + O_2$; see: Lesko, S. A.; Lorentzen, R. J.; Ts'o, P. O. P. *Biochemistry* **1980**, *19*, 3023) and that DNA strand scission mediated by 1 was substantially quenched in the presence of DMSO, a known scavenger of $^{\bullet}OH$ (Repine, J.; Pfenninger, O. W.; Talmage, D. W.; Berger, E. M.; Pettijohn, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1001).

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Preparation of Monomeric (η^6-Arene)OsNR Complexes and Their Exchange Reactions with Amines, Alcohols, and Thiols

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Most of the molecules that contain metal-imido groups are derived from the center of the transition series (i.e., group 5-8 metals).[1] Recent advances have extended this class of compounds to zirconium[2] and to iridium,[3] in an effort to generate more reactive M-N linkages. We report here the synthesis of reactive, monomeric, low-valent η^6-arene imido osmium complexes,[4-6] (η^6-

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