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Photochemical Cleavage of Supercoiled DNA by *N*-Acyloxypyridine-2-thione Acridinyl Derivatives†

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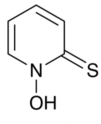
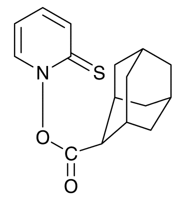
New acyl thiohydroxamates bearing an acridine moiety able to intercalate DNA double strand exhibit the capacity to cleave supercoiled DNA to linear DNA. EPR spin-trapping analysis has been employed to detect the formation of the carbon-centered radicals.

Barton and co-workers[1] have shown that acyl derivatives of *N*-hydroxypyridine-2-thione **1**, the Barton PTOC esters, give, upon photolysis, alkyl radicals which can alkylate physiologically interesting molecules such as purine or pyrimidine bases and nucleosides. Recently we have shown that 1-adamantoyl and 9-acridinylpentanoyl thiohydrosamates **2** and **3** possess cytotoxic activity, probably as a consequence of DNA damage caused by the generation of alkyl radical intermediates.[2] In order to check this possibility, we have synthesized, using methods reported elsewhere,[3] the thiohydroxamates **4** and **5** bearing one or two radical-generating side chains, respectively, linked to a well known DNA-intercalating acridine framework and have examined their behaviour in photodynamic binding experiments on DNA.



1



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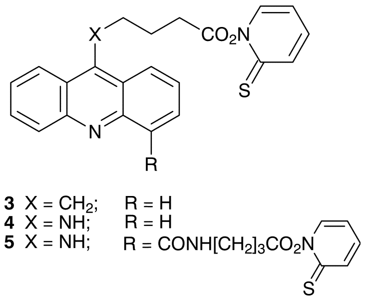

3 X = CH_2; R = H
4 X = NH; R = H
5 X = NH; R = $CONH[CH_2]_3CO_2N$

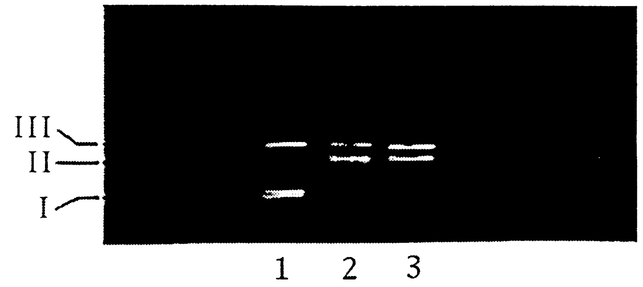

Fig. 1 Photocleavage of pBluescript II KS(+/−) supercoiled DNA induced by compounds **4** and **5**, analysed on 1% agarose gel (tris-acetate buffer) stained with ethidium bromide. Lane 1 pBluescript ii KS(+/−) DNA alone, as unirradiated sample. Lane 2: Lane 1-DNA and **4** (10 mM). Lane 3: Lane 1-DNA and **5** (50 mM). **I**: supercoiled DNA, **II**: linear DNA, **III**: nicked DNA

When a solution of pBluescript II KS (+/−) supercoiled DNA (Stratagene) was irradiated with a 75 W tungsten lamp at 0 °C for 12 h (pH 7) in the presence of compound **4** (10 mM in DMSO–water 20:80), supercoiled DNA was completely transformed into linear DNA. Similarly compound **5** cleaved supercoiled DNA to linear DNA at the concentration of 50 mM under the same experimental conditions (Fig. 1). Since thiohydroxamates such as compounds **2–5** can be cleaved by water under drastic hydrolytic conditions (heating/acids) affording *N*-hydroxypyridine-2-thione **1** and the corresponding carboxylic acid,[4] **1** could photocleave DNA through the formation of hydroxy radicals, as Zard and co-workers[5] have shown. We can exclude that the observed DNA cleavage is attributable to hydroxy radicals as the irradiation of a 1 mM solution of **1** in the presence of supercoiled DNA and under the same conditions used for compounds **4** and **5** did not afford linear DNA. The formation of carbon-centered radicals has been confirmed by EPR experiments. Irradiation with UV light of a DMSO–water (50:50) solution of **4** (10^{-3} M) in the presence of 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) (10^{-2} M) as a spin-trap[6] led to an EPR signal ($a_N = 15.5$, $a_H = 21$ G) that, on the basis of the reported hyperfine coupling constants for the methyl adduct,[6] has been assigned to the 2-adamantyl-DMPO spin adduct. The same experiment carried out on a 50% DMSO solution of *N*-hydroxypyridine-2-thione **1** gave no EPR signal. However, when a DMSO–water (50:50) solution of hydrogen peroxide (10^{-3} M) was irradiated in the presence of DMPO an EPR signal ($a_N = 15.8$, $a_H = 22.4$ G) attributable to the methyl-DMPO spin adduct arising from attack of the hydroxy radical on DMSO[7] was detected. These results show that carbon-centered radicals can be used to cause damage to DNA and likewise the benzoyl analogues studied by Theodorakis and Wilcoxon,[8] Barton's alkylthiohydroxamates could find useful applications in the field of biomedical science.

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†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1998, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

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