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Fungicide Chinomethionate as a New Family of Photoinducible DNA-Cleaving Agents

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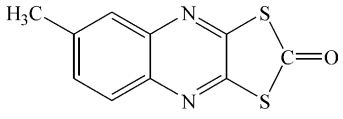
Abstract—It is demonstrated for the first time in this report that chinomethionate is capable of causing efficient DNA cleavage under mild irradiation conditions, a fungicide molecule that processes the simple group of 1,3-dithio-2-one as its reactive functionality.

Chinomethionate (6-methyl-2,3-quinoxalinedithiol cyclic carbonate), a major ingredient of Morestan, is an effective fungicide against mites, aphids, white flies and powdery mildew on many species.[1] One of the structural distinctiveness of this fungicide is the possession of 1.3-dithio-2-one as its reactive functionality. It was reported in the past that field workers using chinomethionate had experienced server skin irradiation when they were exposed to sunlight.[2] In addition, it was found that the half-life of this fungicide on apple leaves in the sun was much shorter than those in the shady areas.[3] Chemical studies, on the other hand, revealed that irradiation of a dilute benzene solution of chinomethionate by UV light gave rise to products arising from the reaction of intermediate radicals with the surrounding oxygen and 1,3-dithio-2-one was the reactive functionality.[4,5] With the aim of identifying new types of photoinducible DNA-cleaving functionality that are readily activated under mild irradiation conditions, the photochemical properties of chinomethionate on target DNA were examined recently in our laboratory. In this communication, we report that fungicide chinomethionate with the functionality of 1,3-dithio-2-one embedded in its molecular structure was capable of causing efficient DNA cleavage under mild irradiation condition, a mode of action that might underlie the previously observed biological behaviors of this fungicide.[1–3] In addition, our studies showed that both carbon-centered and hydroxyl radicals were most likely accountable for the DNA-cleaving process by chinomethionate, a pathway that was consistent with the chemical properties of this fungicide.



Chinomethionate (1)

The DNA-cleaving ability of chinomethionate under irradiation was examined in our studies by monitoring the conversion of circular supercoiled (form I) to circular relaxed (form II) DNA. For this purpose, this fungicide was irradiated at 365 nm by using a Blak-Ray Bench Lamp (15 W) at various concentrations of chinomethionate for 10 min in the presence of $\phi\chi$174RF NDA (30 μM/bp) in acetonitrile: Na_2HPO_4 buffer (25 mM, pH 7.0): triton [10:20:2.5 (v/v/v)]. As shown in Figure 1, single-stranded DNA cleavage was observed at concentration as low as 10 μM and in a concentration-dependent fashion while no DNA cleavage occurred in the absence of chinomethionate (lane 1, Fig. 1). In addition, the yield of this DNA-cleaving reaction increased with the increase of irradiation time (Fig. 2). Under the conditions designed for our investigation, the yield of this DNA-cleaving reaction could reach up to nearly completion in 12 min, illustrating the relatively high efficiency of this DNA-cleaving process by chinomethionate. As a positive control, the reaction of DNA with methyl quinoxalinecarboxylate, a known highly efficient photoinducible DNA-cleaving agent,[6] was

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carried out under the similar reaction conditions to the one designed for chinomethionate. As can be seen from Figure 3, the DNA-cleaving activity of methyl quinoxalinecarboxylate was nevertheless more efficient than the sulfur-containing agent, chinomethionate.

As a reference point for this study, the photolyse of 30 mg of chinomethionate in tetrahydrofuran as solvent was carried in our laboratory by using the same irradiation source set up for our DNA cleavage studies.[7] After 15 h irradiation at room temperature, for example, 6-methyl-1,2,3,4-tetrahydroquinoxaline-2,3-dione (**5**, Scheme 1) was obtained[8] in 65% yield from the corresponding reaction. This experimental result of ours was compatible with the outcome of photodecomposition studies on chinomethionate conducted by Clark and Loeffler in 1980[4] in which carbon-centered and peroxide radicals were generated in the reactions.

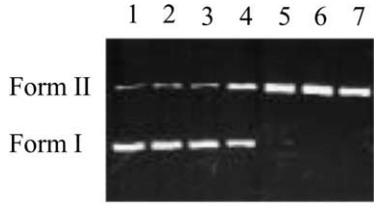


Figure 1. DNA cleavage by chinomethionate activated by UV irradiation.[9] Reactions were incubated in 25 mM Na_2HPO_4 buffer solution (pH 7.0) containing DNA (30 μM/bp), quinomethionate, 10% (v/v) CH_3CN and 2.5% (v/v) triton-X and were irradiated at room temperature for 10 min. Lane 1, DNA alone; lane 2, DNA + 10 μM of **1**; lane 3, DNA + 50 μM of **1**; lane 4, DNA + 100 μM of **1**; lane 5, DNA + 250 μM of **1**; lane 6, DNA + 500 μM of **1**; lane 7, DNA + 750 μM of **1**.

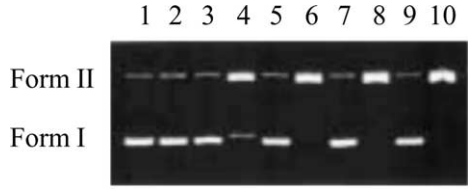


Figure 2. Irradiation-time dependence of DNA-cleavage by chinomethionate. Reactions were incubated in 25 mM Na_2HPO_4 buffer solution (pH 7.0) containing DNA (30 μM/bp), 250 μM of **1**, 10% (v/v) CH_3CN and 2.5% (v/v) triton-X and were irradiated at room temperature. Lane 1, DNA alone without irradiation; lane 2, DNA + **1** without irradiation; lane 3, DNA alone irradiated for 8 min; lane 4, DNA + **1** irradiated for 8 min; lane 5, DNA alone irradiated for 10 min; lane 6, DNA + **1** irradiated for 10 min; lane 7, DNA alone irradiated for 12 min; lane 8, DNA + **1** irradiated for 12 min; lane 9, DNA alone irradiated for 14 min; lane 10, DNA + **1** irradiated for 14 min.

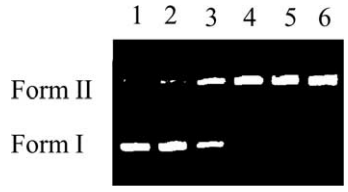
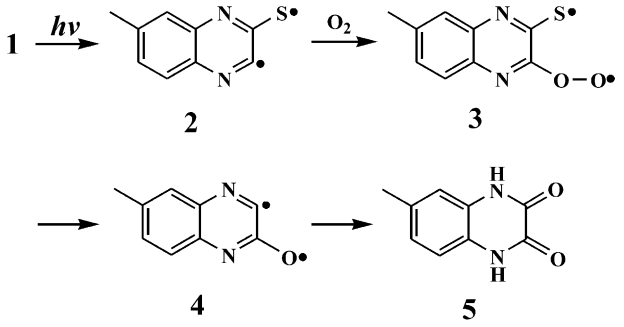


Figure 3. DNA cleavage by methyl 2-quinoxalinecarboxylate (MQ) activated by UV irradiation. Reactions were incubated in 25 mM Na_2HPO_4 buffer solution (pH 7.0) containing DNA (30 μM/bp), MQ, 10% (v/v) CH_3CN and 2.5% (v/v) triton-X and were irradiated at room temperature for 10 min. Lane 1, DNA alone; lane 2, DNA + 1 μM of MQ; lane 3, DNA + 2 μM of MQ; lane 4, DNA + 4 μM of MQ; lane 5, DNA + 8 μM of MQ; lane 6, DNA + 12 μM of MQ.

It has been recognized previously that simple phenyl radicals are capable of causing efficient single-stranded scissions to DNA.[9] In addition, it was well established in the past that hydroxyl radical generated from oxygen, superoxide radical as well as from hydrogen peroxide could initiate a DNA cleaving process effectively.[10] In order to examine whether the carbon-centered and hydroxyl radicals were indeed the active species accountable for the DNA-cleaving process by chinomethionate, the inhibitory effects of 2,2,6,6-tetramethylpiperidinooxy (TEMPO, carbon-centered radical scavenger) and sodium benzoate (hydroxyl radical scavenger)[9] on this DNA-cleaving process were examined accordingly. As shown in Figure 4, the DNA-cleaving process by chinomethionate was efficiently inhibited by the addition of TEMPO and this inhibitory effect increased with the increase of the concentration of TEMPO (lanes 3–5). These observations could accordingly be considered as the experimental evidence of involvement of carbon-centered radical in the DNA-cleaving reactions. Similarly, addition of sodium benzoate to the corresponding reaction mixture slowed down the rate of the DNA-cleaving reactions (lanes 6–10), indicating that hydroxyl radicals were probably the additional active species involved in the DNA-cleaving process by chinomethionate.

In summary, our studies demonstrate that the simple functionality of 1,3-dithio-2-one embedded in fungicide



Scheme 1. Proposed decomposition mechanism of chinomethionate under UV irradiation.[4]

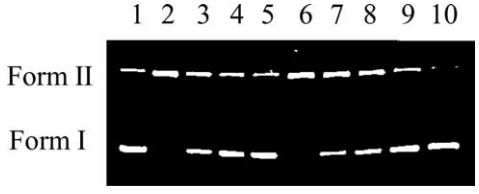


Figure 4. DNA cleavage by chinomethionate inhibited by radical scavengers. Reactions were incubated in 25 mM Na_2HPO_4 buffer solution (pH 7.0) containing DNA (30 μM/bp), 250 μM of **1**, 10% (v/v) CH_3CN and 2.5% (v/v) triton-X and were irradiated at room temperature for 8 min. Lane 1, DNA alone; lane 2, DNA + **1**; lane 3, DNA + **1** + 1 μM of TEMPO; lane 4, DNA + **1** + 10 μM of TEMPO; lane 5, DNA + **1** + 100 μM of TEMPO; lane 6, DNA + **1** + 0.1 μM of sodium benzoate; lane 7, DNA + **1** + 1 μM of sodium benzoate; lane 8, DNA + **1** + 10 μM of sodium benzoate; lane 9, DNA + **1** + 100 μM of sodium benzoate; lane 10, DNA + **1** + 200 μM of sodium benzoate.

chinomethionate was capable of causing efficient DNA cleavage, a process that can readily be activated via mild UV irradiation. In addition, our results indicate that the DNA-cleaving process by chinomethionate was seemingly through the generation and reaction of carbon-centered and hydroxyl radicals. In consideration of the structural simplicity of 1,3-dithio-2-one and the mild irradiation condition as the activating force, this newly discovered photoinducible DNA-cleaving functionality could have certain implications in the design of new diagnostic probes and therapeutic agents.[11]

Acknowledgements

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7. A Blak-Ray Bench Lamp (Model: XX-15L, 365 nm, 15 W, UVP, Inc., USA) was used as the irradiation source of our investigations. All DNA-cleaving reactions by chinomethionate were analyzed by electrophoresis on 1% agarose gels in 1 M of Tris–acetate buffer (100 V, 1 h). The resulting gels were subsequently photographed on a UV transilluminator after attaining with ethidium bromide. The amount of DNA in each band of ethidium-stained gels was quantified using Lumi-Imager (LumiAnalyst 3.1).
8. For **5**, δ_H (DMSO-d_6): 11.873 (s, 1H, NH); 11.848 (s, 1H, NH); 6.910 (s, 1H, ArH); 7.008 (d, $J=8$ Hz, 1H, ArH); 6.901 (d, $J=8$ Hz, 1H, ArH); 2.265 (s, 3H, CH_3).
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