J=2.4 Hz, H-3), 6.3 (dd, 1H, J=5.9 and 2.4 Hz, H-5); ^{13}C-NMR (75 MHz, $CDCl_3$) 179.2 (C-4), 163.9 (C-2), 154.8 (C-6), 117.1 (C-5), 112.3 (C-3), 131.1 (C'-1), 125.7 (C'-2), 129.4 (C'-3), 131.4 (C'-4); MS *m/e* (relative intensity) 172 (M^+, 98), 144(78), 115(36), 102(100), 77(38). Anal. Calcd for $C_{11}H_8O_2$: C, 76.73; H, 4.68. Found; C, 76.62; H, 4.89.

In conclusion, the copper sulfate-catalyzed reaction of diazomalonaldehyde with enol silyl ether provides a convenient method for construction of 2-substituted γ-pyrones.

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8. Trimethylsilanol was detected by GC-Mass from crude reaction mixture.

Photochemical Interaction between Harmaline and DNA

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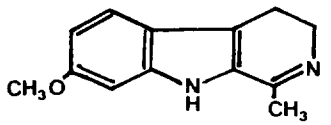
A few β-carboline alkaloids have been known to be phototoxic to bacteria and yeast.[1-4] Several observations strengthen the view that DNA and probably other macromolecules can serve as targets for the phototoxicity of β-carbolines but its action mechanism is not fully understood.[5-8] In the course of studies on the elucidation of phototoxicity mechanism of β-carboline alkaloids, we[9,10] previously reported that these compounds photochemically produce singlet oxygen and superoxide anion radicals in the presence of oxygen, while produce carbolinyl radicals under anaerobic conditions. One of β-carboline derivatives, harmaline photochemically produces a cycloaddition product with fumaronitrile.[11]



Harmaline

Scheme 1.

Photochemical reaction of harmaline with DNA was studied in order to make insight into the phototoxicity mechanism of β-carbolines. UV absorption spectrum of harmaline was not so strongly but somewhat affected by increasing DNA (Sigma, type I) concentration indicating weak ground state complexation between harmaline and DNA. UV absorption spectral changes of harmaline (1×10^{-4} M in H_2O) in the presence of calf thymus DNA (0.05 mg/cc) with time of irradiation show that the absorption maximum of harmaline at 375 nm gradually decreased and an isosbestic point was observed at 320 nm. Similar results were obtained when the reactions were run without DNA but far more slower rate. The relative absorbance decrease at 375 nm was in the order of the reaction runed with DNA under N_2> with DNA under O_2> without DNA under N_2 or O_2. The absorbance at 375 nm of harmaline in the presence of DNA under N_2 decreased more effectively than under O_2 strongly suggesting that the double bond of harmaline disappeared *via* photochemical reaction with DNA and this photoreaction is likely to proceed through its triplet excited state.

The photochemical modification in the helical structure of DNA in the presence of harmaline was investigated by monitoring the changes in circular dichroism spectrum. A 0.01 M NaCl solution of harmaline (1×10^{-4} M) and calf thymus DNA (A=1.0 at 260 nm) was irradiated with 350 nm UV light under N_2. The CD spectral changes with time of irradiation are shown in Figure 1. Both positive and negative band of the CD spectrum decreased significantly on irradiation. It has been known that when DNA is irradiated in the presence of the bifunctional psoralen, 8-MOP, both the negative and positive bands in the CD spectrum were affected to a similar extent.[12] However, in the presence of monofunctional 1,4-diphenylbutadiyne[13] or 5,7-dimethoxycoumarin, the negative band of the CD spectrum remained unperturbed but positive band increased with time of irradiation. The results suggest that the photochemical interaction of harmaline and DNA involves cross-linking of DNA double strands.

Photo-crosslinking of DNA by harmaline is further demonstrated by melting temperature profiles. As shown on Figure 2, the irradiation of DNA in the presence of harmaline under

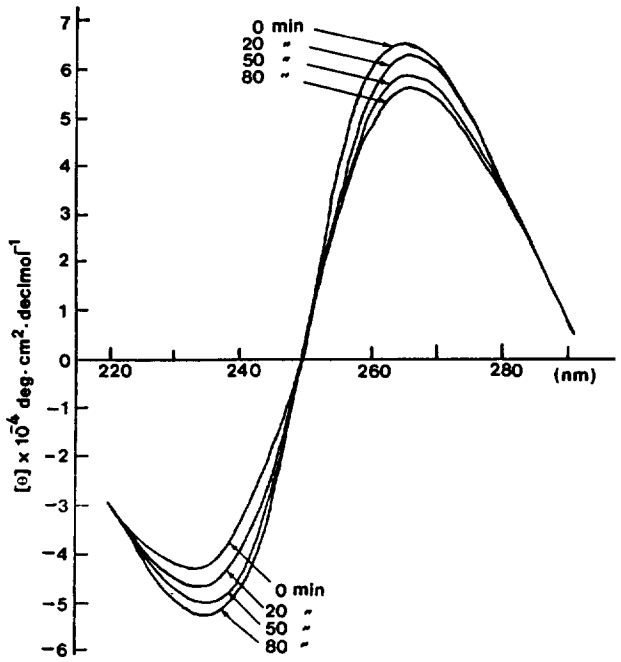


Figure 1. Circular dichroism spectral changes of DNA in the presence of harmaline (1×10^{-4} M) with time of irradiation under N_2.

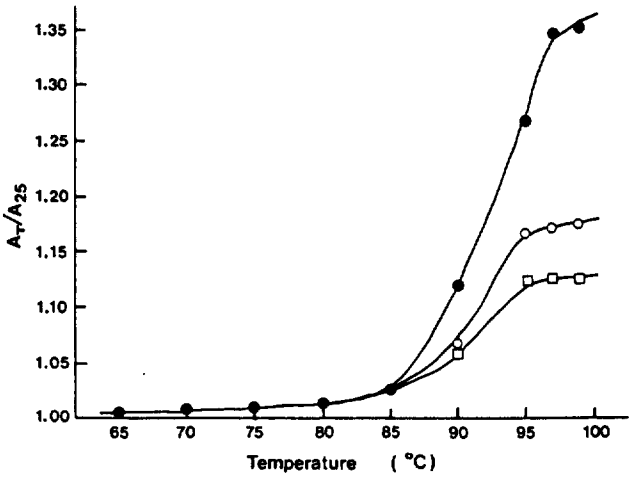


Figure 2. Melting temperature profile of calf thymus DNA (●) and after treatment with harmaline and irradiation at 350 nm for 0 min (○) and 45 min (□) under N_2. The ratio of the absorbance at 260 nm observed at T℃ and at 25℃ (A_T/A_{25}) is plotted as a function of temperature.

N_2 results in a shift of the melting temperature profile toward higher temperatures. It has been known that the photobinding of 8-MOP with DNA, which involves cross-linking of double strands, the melting temperature profile shifts toward higher temperatures.[14] However, in the presence of monofuctional compounds such as angelicine,[12] 1,4-diphenylbutadiyne,[13] and 5,7-dimethoxycoumarin which involve binding single strand only, the melting temperature profile shifts toward lower temperatures.

The photochemical changes of DNA in the presence of harmaline were also studied by agarose gel electrophoresis. An aqueous solution of DNA (800 μg/ml) and harmaline (5×10^{-4} M) was irradiated with 350 nm UV light. The solution was applied to agarose gel electrophoresis (tris buffer, 50 V) after dialysis to remove low molecular weight materials. The mobility of DNA icreased when the reaction was run under O_2 and decreased under N_2 compared with untreated DNA. The results suggest that the molecular weight of DNA decreases by photocleavage of DNA under aerobic conditions but increases by photo-crosslinking of DNA under anaerobic conditions.

It is reminiscent that harmaline efficiently produces singlet oxygen and superoxide anion radicals under O_2[9] and radicals under N_2.[10] As DNA molecules have been known to split by superoxide anion radicals,[15] the results can be explained as follows: DNA molecule is likely to be cleaved by the superoxide anion radicals produced by the photochemical reaction of harmaline with oxygen under aerobic conditions, while photocrosslinked by two steps - that is photochemical reaction of harmaline and photochemically produced harmainyl radical with DNA under anaerobic conditions. The nature of photoproducts formed between harmaline and DNA will be pursued further in this laboratory.

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