

## COMMUNICATIONS TO THE EDITOR

Production of Singlet Oxygen and Superoxide Anion Radicals by  $\beta$ -Carbolines

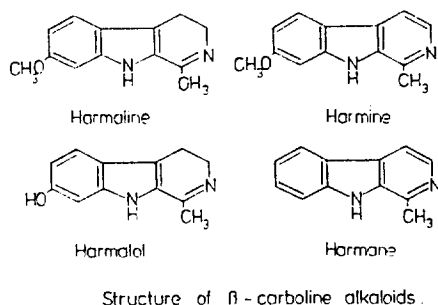
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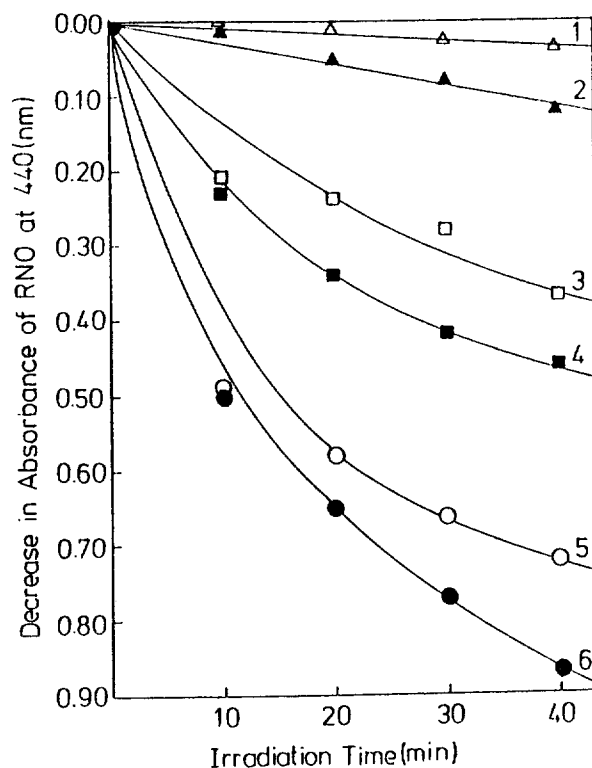
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The  $\beta$ -carboline alkaloids are distributed in a variety of plant families such as *peganum harmala* and have versatile physiological and pharmacological properties. Some of these compounds have been used as a new fluorescence standard<sup>1</sup> and the binding of  $\beta$ -carbolines to DNA was studied in relation to their hallucinogenic activities.<sup>2,3</sup> Recently, Mckenna *et al.* studied UV mediated cytotoxic activity of  $\beta$ -carbolines using yeast and bacteria bioassay systems<sup>4</sup> and it has suggested that DNA is a target for the phototoxicity of these compounds.<sup>5-7</sup>



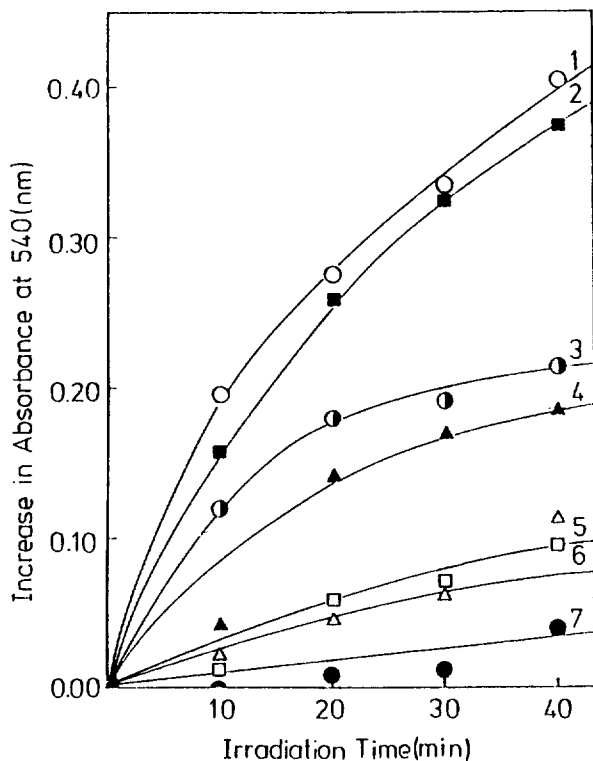
In previous studies at this laboratory,<sup>7</sup> we reported photopolymerization of vinyl monomers initiated by  $\beta$ -carbolines as an evidence for the photochemical radical formation of these compounds. In this paper, we report  $\beta$ -carbolines produce singlet oxygen ( $^1O_2$ ) and superoxide anion radicals ( $O_2^-$ ) through a sensitization mechanism in which the photoexcited  $\beta$ -carboline reacts with molecular oxygen to form reactive  $^1O_2$  or  $O_2^-$ . These reactive forms of oxygen possibly cause cellular damage that eventually contributes to the phototoxicity of  $\beta$ -carbolines.

Production of  $^1O_2$  was examined by bleaching reaction of p-nitrosodimethylaniline (RNO). A 10ml solution of  $4 \times 10^{-5}$  M RNO, used as a selective scavenger of  $^1O_2$ , was prepared in 0.025 M phosphate buffer (pH 7) and  $10^{-2}$  M histidine was added as a selective acceptor of  $^1O_2$ .  $\beta$ -Carboline was added to give 20  $\mu$ g/ml concentration of the sensitizer. This mixture (7ml) in a Pyrex tube was irradiated with 354 nm UV light



**Figure 1.** The production of  $^1O_2$  determined by the photosensitized bleaching reaction of RNO is plotted as a function of UV irradiation time. The sensitizers used were: (1) without sensitizer, (2) 8-MOP, (3) harmine, (4) harmalol, (5) harmine, (6) harmaline.

for various periods of time. Since the production of  $^1O_2$  by 8-methoxypsoralen (8-MOP) has already been reported,<sup>8</sup> relative  $^1O_2$  producing activity of  $\beta$ -carbolines was compared with that of 8-MOP. Figure 1 shows the generation of  $^1O_2$  by four carboline derivatives and 8-MOP using bleaching reaction of RNO monitored by the absorbance at 440 nm induced by the presence of histidine. The  $^1O_2$  producing activity, based on the use of equivalent concentration for the sensitizer, was found to be in the following order: harmaline > harmine



**Figure 2.** Formation of  $O_2^-$  determined by the photosensitized reduction of NBT in the presence of  $\beta$ -carboline derivatives. The increase in absorption at 540 nm is due to the formation of nitro blue diformazan. The sensitizers used were: (1) harmine, (2) harmalol, (3) riboflavin, (4) harmaline, (5) harmane, (6) 8-MOP, (7) without sensitizer.

> harmalol > harmane > 8-MOP. This order is correspond to the degree of UV absorption of each chemical at 354 nm except harmalol. The relative potency of  $^1O_2$  production of each  $\beta$ -carboline seems to be related to the degree of UV absorption and the triplet quantum yields.

Additional evidence for the production of  $^1O_2$  was obtained by examining the bleaching of RNO and concomitantly carrying out  $^1O_2$  quenching studies with azide ions, DABCO, and under  $N_2$ . When  $NaN_3$  was added, 100% inhibition in  $^1O_2$  production was observed by the  $\beta$ -carboline derivatives tested. About 60% inhibition was observed in the rate of  $^1O_2$  production when DABCO was added or the reaction was carried out under  $N_2$ . Addition of hydroxy radical scavengers<sup>9</sup> such as acetate, benzoate, NaCl and formate resulted in little change in the bleaching reaction of RNO sensitized by  $\beta$ -carbolines indicating that the bleaching of RNO by hydroxy radicals can be excluded from this experiment.

The formation of  $O_2^-$  radicals was studied by monitoring the photosensitized reduction of nitro blue tetrazolium (NBT). A  $1.6 \times 10^{-4}$  M solution of NBT with the sensitizer concentration of 20  $\mu$ g/ml was prepared in 0.01M carbonate buffer (pH 10). The solution in a Pyrex tube was irradiated with 354 nm of UV light and the production of reduced NBT was

monitored by recording the increase in the absorbance of irradiated solutions at 560 nm. Riboflavin was used as a reference compound for selective generation of  $O_2^-$ . The results are shown in Figure 2 which suggest  $O_2^-$  formation by  $\beta$ -carbolines. Harmine and harmalol were found to produce  $O_2^-$  more efficiently than riboflavin. The  $O_2^-$  producing activity was found to be in the order of harmine, harmalol > riboflavin, harmaline > harmane, 8-MOP.

Saito *et al.*<sup>10</sup> reported that an electron transfer reaction giving rise to  $O_2^-$  from  $^1O_2$  is possible in the presence of aromatic amines with oxidation potential less than  $\sim 0.5$  V vs. SCE in highly polar aqueous solvent. To test for generation of  $O_2^-$  from the reaction of  $^1O_2$  with  $\beta$ -carbolines, we studied the effect of  $^1O_2$  quencher on the reduction of NBT in the presence of  $\beta$ -carboline. The formation of nitro blue diformazan sensitized by harmalol was not quenched by DABCO. Thus, it is not possible that the reaction of  $^1O_2$  with  $\beta$ -carboline produces  $O_2^-$ .

The reduction of NBT also takes place in degassed aqueous solution when sensitized by riboflavin and, in this case, triplet riboflavin possibly reduces NBT.<sup>11</sup> To determine whether or not excited  $\beta$ -carboline or  $\beta$ -carbonyl radical participates in the reduction of NBT, the reaction was run under anaerobic ( $N_2$ ) condition. The formation of nitro blue diformazan sensitized by  $\beta$ -carboline derivatives in  $N_2$  was not significant in contrast to riboflavin. Accordingly, it seems unlikely that excited carboline or carbonyl radical reduces NBT.

In summary,  $\beta$ -carboline derivatives photochemically produce singlet oxygen and superoxide anion radicals through energy transfer or electron transfer.

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