Cercosporin, a Novel Photodynamic Pigment Isolated from *Cercospora kikuchii*

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In this paper we are going to report the photodynamic action of cercosporin, a deep red pigment which was first isolated in 1957 by Kuyama and Tamura¹⁾ from the cultured mycelia of *Cercospora kikuchii* (Matsumoto *et al.* Tomoyasu) Gardner, a pathogenic fungus of purple speck of Japanese soybean. The chemical structure of the formula of **1** was elucidated

independently by Lousberg *et al.*²⁾ and Yamazaki *et al.*³⁾ Several naturally occurring red pigments, such as erythroaphines,⁴⁾ fagopyrin⁵⁾ and elsinochromes⁶⁾ have been known to show a photodynamic nature. Because of the structural similarity of cercosporin to these compounds, experiments were carried out to clarify the physiological activity of **1**, using mice and microbes as testing organisms.

Mice that were administered cercosporin and were irradiated continuously by the incandescent bulb died within 24 hr, lethal dose being 0.3 mg per 15 g mouse, whereas those fed in the dark revealed no symptoms even though they were dosed ten times as much as the former group. The latter excreted more feces in which considerable amount of **1** was detected by TLC.

Distribution of **1** in the mouse body was studied by means of whole body autoradiography using ¹⁴C-labelled cercosporin, the preparation of which was previously reported.³⁾ Each animal was intraperitoneally injected 0.09 μ Ci of **1**. The accumulation of radioactivity in a particular organ was not observed in any of the autograms obtained and it distributed rather evenly through whole body except central nervous system. In case of mice exposed to light, however, the relatively stronger activity was detected in the liver and eye-ground, whereas in case of those fed in the dark the more rapid transfer of the isotope to small intestine was found and excretion was clearly observed. These facts suggested that in the dark, **1** was detoxicated through a route; intraperitoneum $\rightarrow$ blood $\rightarrow$ liver $\rightarrow$ bile-duct $\rightarrow$ small intestine $\rightarrow$ feces. In the presence of light **1** attacked, being transferred by blood, cells and tissues before the detoxication had been started.

Effect of this pigment on bacteria was determined by plating broth dilution method, using *B. subtilis* and *E. coli* as testing microbes. The gram positive and negative bacteria suspended in 1/50 M phosphate buffer were irradiated. The duplicate results shown in Table I suggested that the number of surviving bacteria obviously reduced only when the suspension containing the pigment were exposed to light. Weiss⁷⁾ reported that gram positive bacteria were more sensitive to the photosensitization process than gram negative organisms and *E. coli* was not affected by the presence of elsinochromes. In case of cercosporin, however, both gram positive and negative bacteria were equally inhibited and the difference between them could not be observed.

The necessity of molecular oxygen in the photodynamic action has been known.⁸⁾ A series of experiment was therefore carried out to confirm it *in vitro* by the oxygenation of 2,5-dimethylfuran. The methanol solution containing the substrate and a small amount of **1** was brought into contact with oxygen gas in a closed glass vessel, irradiating the visible light from outside. The product isolated as

TABLE I. EFFECT OF CERCOSPORIN ON BACTERIA IN THE PRESENCE OF LIGHT

	Surviving number of bacteria (ml ⁻¹)			
	<i>B. subtilis</i>		<i>E. coli B</i>	
Bacterial population at start of test	4.4×10^{10}	7.8×10^7	1.0×10^8	3.5×10^8
Bacteria + cercosporin ^{a)} in the light	1.4×10^6	5.5×10^4	$< 10^5$	3.7×10^4
Bacteria + MeOH in the light	3.6×10^{10}	1.0×10^7	2.7×10^8	2.0×10^8
Bacteria + cercosporin in the dark	1.5×10^{11}	7.6×10^7	1.2×10^9	2.6×10^8
Bacteria + MeOH in the dark	1.3×10^{10}	7.6×10^7	1.1×10^9	3.0×10^8
Concentration of cercosporin (ml ⁻¹)	0.022 mg	0.024 mg	0.024 mg	0.024 mg

^{a)} Cercosporin was dissolved in a small portion of MeOH and added to the buffer.

an oil gradually turned to white crystals (70% yield), which were found to be monohydrated endo-peroxide of 2,5-dimethylfuran, $C_6H_8O_3 \cdot H_2O$. The reaction failed to occur when oxygen was replaced by nitrogen. **1** also catalyzed the degradation reaction of amino acids; tryptophan was decomposed very rapidly to give many products. Cysteine gave cystine and cysteic acid though a half of the starting material was recovered. Methionine yielded exclusively its sulfoxide, and histidine, aspartic acid.

ESR spectrum of **1** shows that the radical formed by irradiation at rather high *g*-value, 2.056, as compared with other organic radicals may be due to the quinoid group in **1**. The intensity of the signal was completely dependent both on the light intensity and the period of irradiation. Detailed analysis of hyperfine structure of the signal is under investigation.

Cercosporin and other pigments above cited have the same poly-aromatic nucleus. The photosensitivity of these compounds may be explained that the excited state of the molecules by the absorbed light is transferred to the molecular oxygen, which then attacks the

substrates. The reason of the death of mice and bacteria can also be ascribed to the photosensitized oxygenation of the cell constituents, such as amino acid residues of proteins and bases of nucleic acids.

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