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Photodegradation of Antifungal 3,3'-Diindolylmethane by Xenon Lamp

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Abstract: The photodegradation of 3,3'-diindolylmethane (DIM), which has the antifungal activity against *Xanthomonas campestris* pv. *oryzae*, *Cochliobolus miyabeanus* and *Pyricularia oryzae*, was investigated under xenon lamp irradiation. The maximal degradation of DIM in 12.5 ppm methanol solution was observed in the UV range of 280 nm to 290 nm. The range of wavelengths for the maximal degradation shifted to longer wavelengths as DIM concentration in methanol solution increased. For example, the range shifted from 280-290 nm in 12.5 ppm solution to 320-330 nm in 200 ppm solution. Nine compounds were detected as the photodegradation products of DIM and their structures were tentatively determined by gas chromatography-mass spectrometry as 3-formylindole, indole, 2-aminophenol, indole-2,3-dione, methyl 2-aminobenzoate, indole-2-one, 3-methoxymethylindole, methyl 2-ethylaminobenzoate, and 1-(2-dimethylaminophenyl)ethanone, respectively.

INTRODUCTION

3,3'-Diindolylmethane (DIM, **I**) is a product of enzymatic oxidation of 3-indolylacetic acid (IAA),^{1,2)} and also a main degradation product formed by autoclaving gramine.³⁾ Recently, DIM was found to exhibit the biological activity toward bacterial leaf blight of rice,⁴⁾ and *Helminthosporium* leaf spot (Katagiri, unpublished data) by inhibiting the growth of *Xanthomonas campestris* pv. *oryzae*, and *Cochliobolus miyabeanus*, respectively. DIM was also reported to suppress the incidence of rice blast disease caused by *Pyricularia oryzae*.⁵⁾ For the environmental degradation of DIM, decomposition by sunlight

is one of the possible routes. It is, therefore, important to find out to what extent the photodegradation occurs and to identify its products from the view point of environmental safety.

In this paper, the photodegradation of DIM in methanol solution under xenon lamp irradiation is investigated to find out the photodegradation characteristics of DIM and characterization of the photodegradation products.

MATERIALS AND METHODS

1. Procedures

The test compound DIM (**I**) was synthesized from indole and formaldehyde according to the method of Grambow *et al.*⁵⁾ A small amount of DIM (**I**) was dissolved in 100 ml of methanol to make 12.5 ppm, 50 ppm, 100 ppm and 200 ppm solutions. Two ml of each DIM (**I**) solution was taken into a quartz cubic cell (1 × 1 × 5 cm). The cells were irradiated at 13 points between 199 nm and 358 nm wavelengths by using a JASCO CRM-FA xenon lamp irradiator. After 30 min to 120 min irradiation, 2 μ l of each solution was subjected to gas chromatography (GC) for the quantitative analysis of DIM (**I**). The irradiated solution was also applied to GC-mass spectrometry (GC-MS) for the identification of photodegradation products.

2. Gas Chromatography

A Shimadzu 7A, equipped with a flame thermionic detector, was used with a 3% OV-101 glass column, 0.3 cm × 0.9 m column length. Flow rate: N₂ 50 ml/min, column temp.: 230°C, injector temp.: 250°C, detector temp.: 250°C.

3. Gas Chromatography-Mass Spectrometry

A Hewlett Packard 5890 GC and a 5970 B Series Mass Selective Detector equipped with a splitless injector, were used with a cross-linked methyl silicone capillary column, 12 m length, 0.33 μ m film thickness, 0.2 mm i.d. The head pressure was 9 psi. E.M. volts: 1600, initial time: 1 min, initial temp.: 100°C, final temp.: 240°C, program rate: 40°C/min, interface temp.: 250°C.

RESULTS AND DISCUSSION

The ultraviolet (UV) absorption spectrum of DIM (**I**) suggests that UV light in the range of 190 nm to 320 nm was significantly absorbed and participated in the photochemical transformation

Fig. 1 UV absorption spectrum of 3,3'-diindolylmethane (DIM) in methanol solution.

× 1: 2.5 ppm solution, × 10: 25 ppm solution.

Fig. 2 Recovery rates of 3,3'-diindolylmethane (DIM) in 12.5 ppm methanol solution after xenon lamp irradiation.

■: 30 min irradiation, ▲: 60 min irradiation, ●: 120 min irradiation.

(Fig. 1). As shown in Fig. 2, DIM (I) in 12.5 ppm methanol solution was easily degraded by 30 min to 120 min irradiation of UV light from a xenon lamp. An exponential decrease in the concentration of DIM (I) accompanied an arithmetic increase in irradiation time until the recovery rates of DIM (I) became lower than 1%.

The maximal degradation of DIM (I) in 12.5 ppm methanol solution was observed in the range of 280 nm to 290 nm, where the energy intensity was 0.40×10^5 to 0.49×10^5 erg/cm²·sec (Fig. 3). In this range of wavelengths, the rates of recovery of DIM (I) in 12.5 ppm methanol solution

Fig. 3 Energy intensity of a xenon lamp used for the irradiation of 3,3'-diindolylmethane (DIM).

Fig. 4 Recovery rates of 3,3'-diindolylmethane (DIM) in methanol solution after 60 min xenon lamp irradiation.

○: 12.5 ppm solution, ●: 50 ppm solution, ▲: 100 ppm solution, ■: 200 ppm solution.

after 30 min, 60 min, and 120 min irradiation were lower than 4.1%, 0.8%, and 0.78%, respectively. The recovery rates of DIM (I) at different concentrations after 60 min xenon lamp irradiation are shown in Fig. 4. The range of wavelengths for the maximal degradation shifted to longer wavelengths with an increase in DIM (I) concentration in methanol as the range shifted from 280–290 nm in 12.5 ppm solution to 320–330 nm in 200 ppm solution. The recovery rate

Fig. 5 Structures of 3,3'-diindolylmethane (DIM, I) and photodegradation products of DIM by xenon lamp irradiation.

Parent ions in mass spectrometry are shown under the structures.

of DIM (I) after 60 min irradiation at 322 nm was lower than 0.6% in even 200 ppm methanol solution. The wavelengths for 50% recovery after constant irradiation for 60 min were 344 nm in 12.5 ppm solution, 351 nm in 50 ppm solution, 356 nm in 100 ppm solution, and 361 nm in 200 ppm solution, respectively (Fig. 4). According to Crosby and Li,⁶⁾ a wavelength of 286.3 nm is the shortest ever recorded on the earth surface, and a solar energy is probably negligible below 295 nm. In the environmental conditions where the wavelengths of sunlight are above 295 nm, in methanol solution is expected to be easily decomposed, since the spectral distribution of the xenon arc is close to sunlight.⁷⁾ Therefore, it is necessary to develop the suitable formulations for the practical use of DIM (I).

In the photodegradation of DIM (I), two processes preceded the cleavage of the molecular linkage. The first process was cleavage between the methylene carbon and the indole skeletons, and the second, cleavage of the indole ring. Nine structures were identified as the photodegradation products of DIM (I) by GC-MS (Fig. 5). The products III (*Rt* 5.044 min on GC-MS), V (*Rt* 3.107 min) and IX (*Rt* 2.583 min) were identified as 3-formylindole, indole and 2-aminophenol, respectively, based on the co-chromatography

of authentic compounds. The products IV (*Rt* 4.706 min), VI (*Rt* 3.403 min) and X (*Rt* 3.998 min) were identified as indole-2,3-dione, methyl 2-aminobenzoate and indole-2-one, respectively, based on the NBS (National Bureau of Standard, U.S.A.) Library Research System. The products II (*Rt* 4.624 min), VII (*Rt* 4.462 min) and VIII (*Rt* 4.325 min) were tentatively identified as 3-methoxymethylindole, methyl 2-ethylaminobenzoate and 1-(2-dimethylaminophenyl)ethanone, respectively, based on the mass fragmentations of the products. Besides the above products, five unknown photodegradation products were observed. Some of them retained at least one indole skeleton and others had only a phenyl ring. The parent ions in mass spectrometry of the unknown products were m/z 262, 179, 163, 161 and 147, respectively.

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要 約

抗菌性 3,3'-diindolylmethane のキセノンランプによる光分解

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イネごま葉枯病原菌, イネ白葉枯病原菌およびイネいもち病原菌に対して抗菌活性を有する 3,3'-diindolylmethane (DIM) のキセノンランプ分光照射による光分解について検討した。12.5 ppm の DIM メタノール溶液では波長域 280 nm から 290 nm において最大分解率が得られた。この最大分解を示す波長域は, DIM 溶液濃度の増加に伴い長波長側へと移動し, 200 ppm 溶液では 320 nm から 330 nm の波長域に達した。DIM の光分解生成物としては, 九つの化合物が検出されガスクロマトグラフィー-マススペクトロメトリーにより, それぞれ 3-formylindole, indole, 2-aminophenol, indole-2,3-dione, methyl 2-aminobenzoate, indole-2-one, 3-methoxymethylindole, methyl 2-ethylaminobenzoate, および 1-(2-dimethylaminophenyl)ethanone と同定された。