Novel Photodegradation of the Antifungal Antibiotic Pyrrolnitrin in Anhydrous and Aqueous Aprotic Solvents

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The UV irradiation of pyrrolnitrin (**1a**), which is an antibiotic clinically useful against dermatophytosis and possesses a unique 2-(pyrrol-3-yl)nitrobenzene moiety in the molecule, in an anhydrous aprotic solvent resulted in the exclusive formation of transient 7,4′-dichlorospiro[1,3-dihydrobenzo(*c*)isoxazole-3,3′-pyrrolin-2′-one] (**2a**) via the intramolecular oxidation of the juxtaposed pyrrole ring by the triplet-excited nitro group. The irradiation in an aqueous aprotic solvent, however, allowed the concurrent occurrence of intramolecular cyclization by the singlet-excited nitro group in **1a** and the hydroxylation at the 2-position of the pyrrole ring by water to afford 3,7-dichloro-8-hydroxy-8,8a-dihydropyrrolo[2,3-*b*]indol-2-one (**3a**), accompanied by the formation of **2a**. Elongation of the irradiation time in these photoreactions caused a rapid consumption of the products, **2a** and **3a**, to give undetermined polar polymeric products. The present results indicate that the photodegradation of **1a** is significantly influenced by the presence of water in the reaction media and by the nature of its excited state. Thus, the loss of the antifungal activities by the photosensitive antibiotic **1a** was chemically proved.

I. Introduction

Pyrrolnitrin (**1a**), 3-chloro-4-(3-chloro-2-nitrophenyl)-pyrrole,[1] is an antibiotic produced from L-tryptophan [2] by *Pseudomonas* species,[3] *Burkholderia cepacia*,[4] *Myxococcus fulvus*,[5] *Serratia* species,[6] and *Enterobacter agglomerans*.[7] The antibiotic **1a** is on the market today as an externally used chemotherapeutic agent for tinea pedis and other types of dermatophytosis[8] on the basis of its inhibitory activities toward NADH- and succinate-supported electron transport in mitochondria[4,9] and on the destructive influence to the cell membrane by combination with phospholipids.[10] Furthermore, the antibiotic **1a** has been recently documented to play a very important role as an effective biocontrol agent for the soilborne fungal pathogens in a variety of plants[11] and to exhibit an appreciable inhibiting activity against *Mycobacterium tuberculosis* and *M. avium*.[12]

This antibiotic has a unique structure; i.e., the dihedral angle of the pyrrole ring to the benzene ring was shown to be 52° and the plane through the nitro group is almost at a right angle (88°) to the plane of the benzene ring on the bais of its X-ray crystal analysis,[13] suggesting the absence of a long conjugation system in the molecule. An extreme lability of **1a** [λ_{max} 252 (ϵ 7.5 × 10^3) nm, accompanied by the tailing of the peak to 400 nm] upon exposure to sunlight, however, was observed in early investigations, resulting in significant destruction of its antifungal activity.[14] In view of these observations and from our (M.S. and Y.M.) continuous interests in the

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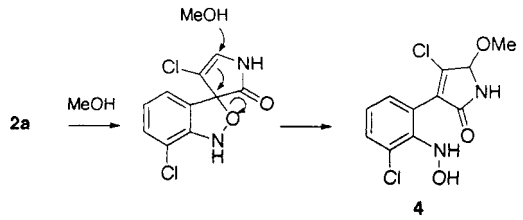
photochemical reactivity of antibiotics and medicines,[15] we attempted to clarify the photodegradation modes of **1a**.

II. Results and Discussion

Photoreactions of Pyrrolnitrin (1a). The antibiotic **1a** was stable in dry acetonitrile even under reflux for 1 day. In sharp contrast to the high stability under thermal conditions, **1a** was extremely labile upon exposure to UV (>280 nm) light resulting in a smooth consumption ($\Phi = 0.1$) accompanied by color change in the solution from pale yellow to brown. The TLC analysis of the reaction mixture showed that a polar compound (**2a**) was formed as the major product during the initial stage, together with undetermined polar polymeric products, e.g., the yield of **2a** was 28% after the irradiation for 10 min, though the 35% recovery of the starting **1a** was observed under the conditions employed. Elongation of the irradiation time caused degradation of the photoproduct **2a** [λ_{max} 286 (ϵ 3.0 × 10^3) nm] leading to polymeric compounds of which the ^{1}H NMR spectrum showed the appearance of unassignable multiplet signals in the aromatic region. The use of dry THF, chloroform, or diethyl ether as a solvent for the photoreaction of **1a** was more effective (47−72% yields after a 10-min irradiation) for the formation of **2a**. Although **1a** is thermally stable in 5% aqueous acetonitrile, the UV irradiation of **1a** in the same aqueous solvent allowed the formation of another polar compound (**3a**) in 20% yield together with **2a** (24%). The product distribution of **2a** and **3a** in this photoreaction was significantly dependent on the water content in the reaction media; an increase in the water content of the solvent caused the preferential formation of **3a** than that of **2a**, e.g., the yields of **2a** and **3a** in 20% aqueous acetonitrile were 11% and 40%, respectively, after a 10 min irradiation.

Scheme 1. Formation of Methanol Adduct 4 from 2a



Structures of the Photoproducts 2a and 3a. The product **2a** was very unstable even in an aprotic solvent and at ambient temperature and was converted into undetermined polar polymeric products.[16] However, purification of the reaction mixture of **1a** by flash chromatography using a very short column and toluene−ethyl acetate as an eluent followed by recrystallization from toluene allowed the isolation of **2a**, mp 225 °C dec, as a colorless amorphous powder in the pure state. Contrary to the chemical property of **2a**, the product **3a** was a fairly stable even in a warmed protic solvent and recrystallized from ethanol to afford colorless prism crystals, mp 175 °C dec. Microanalytical results showed that the photoproducts **2a** and **3a** are formulated as $C_{10}H_6N_2O_2Cl_2$, which is the same as that of the starting **1a**.

The remarkable spectral changes of **2a** from **1a** are as follows: (a) a significant shift in the three proton signals assignable to the benzene-ring protons to the higher fields [δ_H 7.57 (dd, J = 2, 8 Hz), 7.69 (t, J = 8 Hz), and 7.75 (dd, J = 2, 8 Hz) ppm for **1a**; δ_H 7.03 (dd, J = 2, 7 Hz), 7.08 (t, J = 7 Hz), and 7.41 (dd, J = 2, 7 Hz) ppm for **2a**] in the ^{1}H NMR spectrum was observed, implying a drastic structural change in the nitro group of **1a** during the reaction. (b) the disappearance of one proton signal for the pyrrole-ring from the aromatic region [δ_H 6.9 and δ_H 7.15 ppm for **1a**: δ_H 7.28 (d, J = 2 Hz) ppm for **2a**] and appearance of a γ-lactam moiety [υ 1733 cm^{-1} (δ_C 174.8 ppm) for the amide carbonyl group; υ 3190 cm^{-1} (δ_H 10.5 ppm) for the amino group] in the ^{1}H NMR and IR spectra were observed, indicating the occurrence of the oxidation at the α-position of the pyrrole ring in **1a** during the photoreaction. On the basis of these spectral data, the structure of **2a** was reasonably assigned as 7,4′-dichlorospiro[1,3-dihydrobenz(*c*)isoxazole-3,3′-pyrrolin-2′-one], which has a novel type of spiro heterocyclic ring-system in the molecule. The structure having a spiro-asymmetric carbon was well supported by its HMBC spectrum showing the presence of cross-peaks of the spiro-centered carbon [δ_C 89.9 (C-3) ppm] to the benzene-ring proton [δ_H 7.03 (H-4) ppm], the pyrrole NH proton [δ_H 9.96 (br d, J = 2 Hz) ppm], and the pyrrole-ring proton [δ_H 7.28 (H-5′; d, J = 2 Hz) ppm]. The isolated product **2a** was optically inactive. Further structural proof was obtained upon treatment of **2a** with methanol at room temperature leading to a ring-opening adduct

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(16) For this reason, all attempts to prepare a single crystal of **2a** suitable for X-ray crystal analysis were unsuccessful.

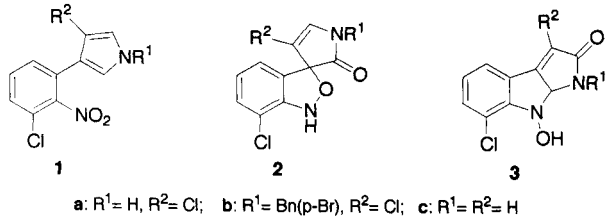

Figure 1.

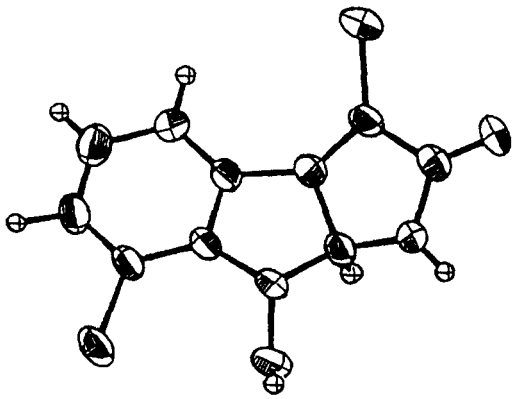

Figure 2. ORTEP drawing of the X-ray crystal structure of **3a**.

(**4**), mp 140 °C dec, of which the formation can be reasonably explained by considering the addition of methanol to the 5′-position and the cleavage of the C_3-O_2 bond in **2a** as shown in Scheme 1.

Characteristics of the spectra of the photoproduct **3a** compared with those of **1a** are as follows: (a) A slight signal-shift of the benzene-ring protons in **1a** to the higher fields [δ_H 7.18 (t, J = 8 Hz), 7.48 (dd, J = 3, 8 Hz), and 7.57 (dd, J = 3, 8 Hz) ppm for **3a**] in the ^{1}H NMR spectrum was observed, suggesting a small structural change in the nitro group of **1a** during the reaction. (b) The disappearance of two proton signals for the pyrrole ring from the aromatic region and appearance of a signal attributed to a γ-lactam moiety [υ 1720 cm^{-1} (δ_C 169.8 ppm) for the amide carbonyl group; υ 3255 cm^{-1} (δ_H 9.15 ppm) for the amide N−H group] and a methine proton [δ_H 5.89 (d, J = 3 Hz) ppm; δ_C 87.0 ppm] in the NMR and IR spectra were observed. This is indicative of oxidation at the α-position on the pyrrole ring in **1a** and of the intramolecular cyclization at another α-position of the pyrrole ring. On the basis of these spectral data and its HMBC spectrum, the structure of **3a** was assigned to 3,7-dichloro-8-hydroxy-8,8a-dihydropyrrolo[2,3-*b*]indol-2-one and was finally confirmed by its X-ray crystallographic analysis (see Figure 2). The product **3a**, which has a single chiral center [δ_C 87.0 (C-8a) ppm] in the molecule, is a 1:1 mixture of stereoisomers from X-ray analysis.

Mechanistic Aspects. No interconversion between the photoproducts **2a** and **3a** was observed under analogous irradiation conditions, clearly indicating that these products are formed via different reaction paths during the present photoreaction. Further experiments were carried out to elucidate the reaction mechanisms for the formation of the spiro-benzoisoxazoline **2a** and the pyrroloindoline **3a** during the photoreaction of **1a** by employment of a triplet-sensitizer and intersystem-crossing accelerator. The consumption of **1a** and the formation of

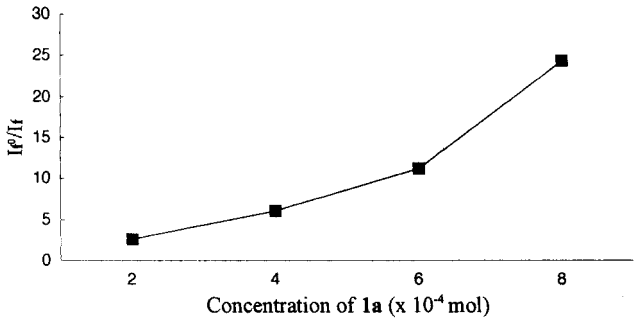

Figure 3. Four samples of acetrophenone (0.2) mmol) in dry acetonitrile containing pyrrolitrin **1a** (0.2, 0.4, 0.6, and 0.8 mmol) were prepared. The fluorescence intensities of these samples obtained by excitation at 282 nm were measured at the emission wavelength of acetophenone (308 nm). If^0: The fluorescence intensity of acetophenone itself. If: The fluorescence intensities of the samples containing **1a**.

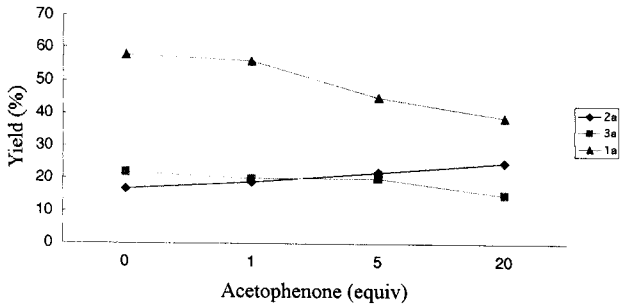

Figure 4. Effects of triplet sensitizer on the Photoreactions of pyrrolnitrin **1a**.

2a during the photoreaction using the aqueous acetonitrile as a solvent were markedly accelerated by the addition of acetophenone, a well-known triplet sensitizer,[17] in a concentration dependent manner as shown in Figure 4 (see also Figure 3). In contrast to these facts, the formation of **3a** was evidently decreased under the triplet-sensitized conditions. The addition of tribromomethane, an accelerator for the intersystem-crossing from a singlet-excited state to a triplet-excited state,[18] to the photoreaction media resulted in a significant increase in the formation of **2a** and the decrease in the formation of **3a** in an accelerator-concentration dependent way (see Figure 5). These facts strongly suggest that the triplet-excited state of **1a** is related to the formation of **2a** and its singlet-excited state plays a role in the formation of **3a**.

Taking the above facts and the photochemical reactivity of the nitro function[19,20] into consideration, we propose a plausible reaction sequence for the formation of **2a** during the photoreaction of **1a** involving the intramolecular addition of the triplet-excited nitro group to the

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(18) For an example, see Nicodem, D. E.; da Cunha, M. F. V. *J. Photochem. Photobiol., A* **1997**, *107*, 165−167.

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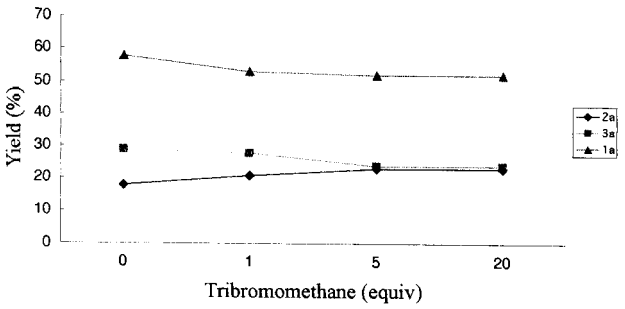
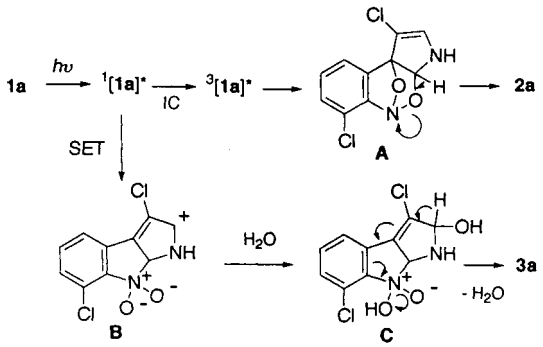


Figure 5. Effects of IC-accelerator on the photoreactions of pyrrolnitrin **1a**.

Scheme 2. Photodegradation Modes of Pyrrolnitrile (1a)



pyrrole ring (cf. **A**) followed by collapse to give **2a** as shown in Scheme 2. An analogous type of reaction sequence in the photochemistry of nitro compounds has been generally accepted.[20]

The formation of **3a**, involving hydroxylation at the 2-position of the pyrrole ring in **1a**, is not reasonably explained by virtue of the mechanism proposed for the formation of **2a**, since the 2-position of the pyrrole ring in **1a** is quite separated from the nitro group,[13] thus inducing the photochemical intramolecular oxygen-transfer. To determine the source of the amide carbonyl oxygen of **3a**, the photoreaction of **1a** was carried out in 30% aqueous acetonitrile containing ^{18}O-labeled water. The Mass and IR spectra of the two photoproducts obtained in this reaction showed no insertion of the labeled oxygen atom into the molecule of **2a** and regioselective insertion into the carbonyl oxygen in **3a** [a molecular ion peak (*m*/*z* 258) for the labeled compound $C_{10}H_6{}^{35}Cl_2N_2{}^{16}O_1{}^{18}O_1$ and a loss of $C^{18}O$ (*m*/*z* 30) from the fragment ion [*m*/*z* 240 ($M^+ - 18$)] under ionizing radiation were observed in the mass spectrum; the stretching vibration band of the carbonyl group (ν 1720 cm^{-1} for **3a**) was significantly shifted to a lower field (ν 1691 cm^{-1} for the ^{18}O-labeled **3a**)]. These facts evidently indicate that the oxygen-atom at the 2-position in **3a** originated from the water molecule in the media. Based on this observation and the results obtained in the triplet-sensitized or intersystem-crossing accelerated experiments described above, we present a reaction sequence

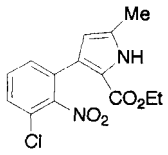


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Figure 6.

for the formation of **3a** in the present photoreaction of **1a** involving a single-electron oxidation of the electron-rich pyrrole ring by singlet-excited nitro group ($E^{red}_{1/2}$ of **1a** = −1.31 V vs SCE in dry acetonitrile) followed by the intramolecular cyclization (cf. **B**) and the concurrent occurrence of the hydroxylation at the 2-position of the pyrrole ring by water to give the tricyclic intermediate (**C**) as shown in Scheme 2.

Analogous results were obtained for the UV irradiation of *N*-(4-bromobenzyl)pyrrolnitrin (**1b**) and the dechlorinated pyrrolnitrin **1c**[21] in aqueous acetonitrile to afford the corresponding spiro-benzoisoxazolines (**2b**,**c**) and pyrroloindolines (**3b**,**c**), respectively. In principle, the present photoreactions seem to be general for the pyrrole antibiotics involving isopyrrolnitrin,[22] oxypyrrolnitrin,[21] dechloropyrrolnitrin,[21] and bromonitrin.[23] 3-(3-Chloro-2-nitrophenyl)-2-ethoxycarbonyl-5-methylpyrrole (**5**),[21b,24] a 2,5-disubstituted analogue of **1a**, was very stable even after a prolonged irradiation time (e.g., for 5 h with a 100-W UV lamp). This observation is of interest in connection with the present photoreactions and the structural design of photostable pyrrolnitrin derivatives. The isolated photoproducts **2a**−**c** and **3a**,**b** exhibited no remarkable antifungal activities against *Aspergillus fumigatus*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes*.

III. Conclusions

The photodegradation mode of pyrrolnitrin (**1a**) significantly depends on the presence of water in the reaction media. In an anhydrous aprotic solvent, **1a** underwent intramolecular oxidation by the nitro group on the α-position of the pyrrole ring in a triplet-excited state leading to the spiro-benzoisoxazoline **2a**. In an aqueous aprotic solvent, the UV irradiation of **1a** allowed the occurrence of a photochemical hydroxylation on the pyrrole ring and intramolecular cyclization with the neighboring nitro group to give the tricyclic pyrroloindoline derivative **3a**. To the best of our knowledge, the photochemical hydroxylation of the pyrrole ring by water is unprecedented. These photoreactions were widely observed in the 2,5-unsubstituted pyrrolnitrins.

Experimental Section

General Procedures. All melting points were measured on Yanagimoto micro-melting point apparatus and are uncorrected. Elemental analyses were carried out in the Microana-

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(24) Gosteli, J. *Helv. Chim. Acta* **1972**, *55*, 451−460. Nakano, H.; Umio, S.; Kariyone, K.; Tanaka, K.; Ueda, I.; Nakamura, H. *Chem. Pharm. Bull.* **1969**, *17*, 567−575.

lytical Center of Gifu Pharmaceutical University. Mass spectra were measured on a JEOL JMS-SX 102A instrument with a direct inlet system operating at 70 eV. Infrared (IR) spectra were recorded on a Perkin-Elmer 1640 series FTIR spectrometer from samples as KBr pellets and ultraviolet (UV) spectra with a Shimadzu-260 spectrophotometer from samples in a dilute solution. Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were obtained on a JEOL JNM-EX 400 spectrometer using deuteriochloroform or dimethyl sulfoxide as a solvent and tetramethylsilane as the internal standard. The chemical shifts are expressed in δ value (parts per million). UV Irradiation was carried out under an argon atmosphere with a Riko Rotary Photochemical Reactor (400 or 100 W high-pressure mercury arc lamp; Riko Kagaku Sangyo) through a Pyrex filter at ambient temperature. A JASCO CRM-FA spectroirradiator (2 KW Xe lamp) was used for the measurement of quantum yield. Dry solvents were obtained using standard procedures. Thin-layer chromatography (TLC) analyses were performed on silica gel 60 F-254 plates (Merck Art. 5715, 0.25 mm thick) and TLC-scanning was carried out with a Shimadzu CS-9000 dual-wavelength flying-spot scanner. Rotary evaporation was carried out under reduced pressure with the bath temperature below 35 °C unless otherwise specified. Column chromatographic separation was performed with Merck silica gel 60 (70−230 mesh).

Materials. A dechlorinated pyrrolnitrin (**1c**)[21] and 3-(3-chloro-2-nitrophenyl)-2-ethoxycarbonyl-5-methylpyrrole (**5**)[21b,24] were prepared according to the reported procedures.

Photoreaction of Pyrrolnitrin (1a) in Anhydrous Aprotic Solvent. A solution of **1a** (128 mg, 0.5 mmol) in dry acetonitrile (100 mL) was externally irradiated using a 400 W UV lamp for 10 min with stirring at ambient temperature. TLC analysis (toluene−ethyl acetate: 4/1) of the brown reaction mixture showed 35% of the starting material **1a** remaining and the formation of a more polar product, together with polymeric products observed at the spotted position on the TLC plate. After removal of the solvent under reduced pressure, the resulting residual oil was subjected to a short column elution with toluene-ethyl acetate (4/1) to isolate 7,4′-dichlorospiro[1,3-dihydrobenzo(*c*)isoxazole-3,3′-pyrrolin-2′-one] (**2a**) (32 mg, 25%): mp 225 °C dec (a colorless amorphous powder, recrystallized from toluene); IR 3300, 3190, 1733, 1625 cm^{-1}; UV (MeCN) 286 (ϵ 3.0 × 10^3), 235 (sh, 5.0 × 10^3), 212; ^{1}H NMR (DMSO-d_6) 7.03 (1H, dd, J = 2, 7 Hz, H-4), 7.08 (1H, t, J = 7 Hz, H-5), 7.28 (1H, d, J = 2 Hz, H-5′), 7.41 (1H, dd, J = 2, 7 Hz, H-6), 9.96 (1H, br d, J = 2 Hz, N′H), 10.5 (1H, br, NH); ^{13}C NMR (DMSO-d_6) 89.9, 109.7, 115.3, 120.3, 124.6, 127.5, 129.7, 130.8, 146.6, 174.8; EIMS *m*/*z* (relative intensity) 256 (M$^+$ for $C_{10}H_6{}^{35}Cl_2N_2O_2$, 71), 228 (M$^+$ − CO, 40), 221 (12), 201 (25), 193 (64). Anal. Calcd for $C_{10}H_6Cl_2N_2O_2$: C, 46.72; H, 2.35; N, 10.90. Found: C, 46.94; H, 2.50; N, 10.77. In the HMBC spectrum of **2a**, peak correlations of H-4, N′H, and H-5′ to δ_C 89.9 (C-3) ppm, H-5 to δ_C 115.3 (C-3a) ppm, H-5 to δ_C 127.5 (C-7) ppm, H-4 to δ_C 129.7 (C-6) ppm, H-4 and H-6 to δ_C 146.6 (C-7a) ppm, and H-5′ to δ_C 174.8 (C-2′) ppm were observed.

A solution of **1a** (2.0 mg, 7.8 μmol) in dry THF, chloroform, or diethyl ether (2.0 mL) was irradiated in a manner similar to that described above. TLC densitometric analyses (toluene-ethyl acetate: 2/1; detector: 254 nm) of the reaction mixtures obtained after the irradiation for 10 min showed the formation of **2a** (the yields of the remaining **1a**) in 47% (15%), 60% (5%), and 72% (12%) yields, respectively. The formation of **2a** after the 20 min irradiation were 50% (in THF), 56% (in chloroform), and 76% (in diethyl ether) yields. In these reactions, the complete consumption of **1a** and the formation of polymeric polar products were observed.

Photoreactions of 1a in Aqueous Acetonitrile. A solution of **1a** (256 mg, 1.0 mmol) in 10% aqueous acetonitrile (250 mL) was externally irradiated with the 400-W UV lamp for 10 min. TLC analysis of the reaction mixture showed the formation of **2a** (17%) and a more polar product, together with the 39% recovery of **1a**. After removal of the solvent under reduced pressure, the resulting residue was subjected to a short column elution with toluene−ethyl acetate (4/1 to 2/1) to isolate **1a** (82 mg, 32%), **2a** (38 mg, 15%), and 3,7-dichloro-8-hydroxy-8,8a-dihydropyrrolo[2,3-*b*]indol-2-one (**3a**) (85 mg, 33%): mp 175 °C dec (colorless prisms, from ethanol); IR 3255, 1720, 1665 cm^{-1}; UV (MeCN) 342 (ϵ 4.1 × 10^3), 275 (sh, 4.9 × 10^3), 245 (1.7 × 10^4); ^{1}H NMR (DMSO-d_6) 5.89 (1H, d, J = 3 Hz, H-8a), 7.18 (1H, t, J = 8 Hz, H-5), 7.48 (1H, dd, J = 3, 8 Hz, H-6), 7.57 (1H, dd, J = 3, 8 Hz, H-4), 9.15 (1H, br d, J = 2 Hz, NH), 10.2 (1H, s, OH); ^{13}C NMR (DMSO-d_6) 87.0, 119.5, 121.3, 122.4, 122.5, 125.4, 133.6, 146.7, 152.2, 169.8; EIMS *m*/*z* (relative intensity) 256 (M$^+$ for $C_{10}H_6{}^{35}Cl_2N_2O_2$, 3), 238 (M$^+$ − H_2O, 28), 210 (8), 66 (100). Anal. Calcd for $C_{10}H_6Cl_2N_2O_2$: C, 46.72; H, 2.35; N, 10.90. Found: C, 46.79; H, 2.43; N, 10.87. In the HMBC spectrum of **3a**, peak correlations of H-8a and NH to δ_C 119.5 (C-3), H-5 to δ_C 121.3 (C-3b), NH and H-8a to δ_C 146.7 (C-3a), and H-4 to δ_C 152.2 (C-7a) were observed.

After the irradiation of **1a** in aqueous acetonitrile containing a variety of water-contents under analogous conditions (for 10 and 20 min), TLC densitometric analyses (toluene-ethyl acetate: 2/1; detector: 254 nm) of the reaction mixtures were carried out and showed the formation of **2a** and **3a** as follows: (a) after the 10 min irradiation: **2a** (28%) with the recovery of **1a** (35%) and no formation of **3a** in the case of anhydrous acetonitrile; **2a** (24%) and **3a** (20%) with a 35% recovery of **1a** in the case of 5% aqueous acetonitrile; **2a** (17%) and **3a** (36%) with a 39% recovery of **1a** in the case of 10% aqueous acetonitrile; **2a** (11%) and **3a** (40%) with a 44% recovery of **1a** in the case of 20% aqueous acetonitrile; **2a** (8%) and **3a** (39%) with a 49% recovery of **1a** in the case of 50% aqueous acetonitrile. (b) after the 20 min irradiation: **2a** (44%) with the recovery of **1a** (14%) and no formation of **3a** in the case of anhydrous acetonitrile; **2a** (44%) and **3a** (30%) with an 18% recovery of **1a** in the case of 5% aqueous acetonitrile; **2a** (23%) and **3a** (47%) with a 22% recovery of **1a** in the case of 10% aqueous acetonitrile; **2a** (17%) and **3a** (53%) with a 26% recovery of **1a** in the case of 20% aqueous acetonitrile; **2a** (13%) and **3a** (48%) with a 35% recovery of **1a** in the case of 50% aqueous acetonitrile.

Quantum Yield for the Consumption of 1a in the Photolysis. The quantum yield was measured at 21 °C using potassium ferrioxalate actinometry at 280 nm. A solution of **1a** (40 mmol) in dry acetonitrile was purged well with argon. After irradiation for 6 min, the consumption of the starting **1a** was assayed by TLC densitometry eluted with toluene−ethyl acetate (2/1).

Reaction of the Photoproduct 2a with Methanol. Compound **2a** (65 mg, 0.25 mmol) was dissolved in methanol (5 mL) and the solution was stirred overnight at ambient temperature. The resulting crystalline product was collected by suction to isolate 4-chloro-3-(3-chloro-2-hydroxyaminophenyl)-5-methoxy-1,5-dihydropyrrol-2-one (**4**) (45 mg, 62%): mp 140 °C dec; IR 3358, 3297, 1689 cm^{-1}; UV (MeCN) 295, 230 (sh), 213; ^{1}H NMR (DMSO-d_6) 3.20 (3H, s, OMe), 5.45 (1H, d, J = 1 Hz, H-5), 6.92 (1H, t, J = 7 Hz, H-5′), 6.95 (1H, dd, J = 2, 7 Hz, H-4′), 7.37 (1H, dd, J = 2, 7 Hz, H-6′), 7.80 (1H, br, NHOH), 8.45 (1H, br, NHOH), 8.78 (1H, br s, NH); ^{13}C NMR (DMSO-d_6) 51.0, 84.9, 119, 121.2, 121.4, 129.8, 130.0, 136.4, 141.5, 145.4, 168.6; EIMS *m*/*z* (relative intensity) 288 (M$^+$ for $C_{11}H_{10}{}^{35}Cl_2N_2O_3$, 17), 272 (M$^+$ − O, 38), 254 (M$^+$ − ^{35}Cl, 23), 236 (22), 205 (88), 193 (100). Anal. Calcd for $C_{11}H_{10}Cl_2N_2O_3$: C, 45.70; H, 3.49; N, 9.69. Found: C, 45.86; H, 3.52; N, 9.66.

Effects of Triplet Sensitizer or Intersystem-crossing Accelerator on the Photoreactions of 1a in Aqueous Acetonitrile. The solution of **1a** (2 mg, 7.8 μmol) in 10% aqueous acetonitrile (2 mL) was irradiated for 5 min in the presence of acetophenone (its fluorescence was quenched by **1a** in a concentration dependent manner as shown in Figure 3) or tribromomethane with a variety of concentrations and then the product distribution of **2a** and **3a** in these reactions were estimated by TLC densitometric analyses (toluene-ethyl acetate: 2/1; detector: 254 nm) of the reaction mixtures. The results of these reactions are shown in Figures 4 and 5.

Photoreaction of 1a in Aqueous Acetonitrile containing ^{18}O-Labeled Water. A solution of **1a** (10 mg, 0.04 mmol) in a 30% aqueous acetonitrile containing ^{18}O-labeled water (Euriso-top, 99.8 atm% ^{18}O) was irradiated under the same

conditions described above (for 5 min). Column chromatographic separation eluting with toluene−ethyl acetate (4/1) allowed isolation of the expected spiro-benzoisoxazoline (trace) [IR and mass spectral diversity with the unlabeled **2a** was not observed] and the ^{18}O-labeled **3a** (2 mg, 20%) [IR 3250, 1691 cm^{-1}; EIMS *m*/*z* (relative intensity) 258 (M^+ for $C_{10}H_6{}^{35}Cl_2N_2{}^{16}O_1{}^{18}O_1$, 22), 240 ($M^+ - H_2O$, 100), 223 (38), 210 ($M^+ - H_2O - C^{18}O$, 22), 206 (19), 175 (24), 148 (27)].

Preparation of 1-(4-Bromobenzyl)-3-chloro-4-(3-chloro-2-nitrophenyl)pyrrole (1b). To a solution of **1a** (150 mg, 0.58 mmol) in dry *N*,*N*-dimethylformamide (50 mL) containing lithium hydride (9.3 mg, 1.17 mmol) was added 4-bromobenzyl bromide (175 mg, 0.7 mmol), and the mixture was stirred overnight at ambient temperature. After removal of the solvent under reduced pressure, the resulting residue was purified by silica gel column chromatography eluting with *n*-hexanes−ethyl acetate (15/1) to isolate the desired **1b** (240 mg, 96%) as an oily product: ^1H NMR ($CDCl_3$) 4.87 (2H, s, benzylmethylene protons), 6.64 (1H, d, $J = 3$ Hz, H-5), 6.69 (1H, d, $J = 3$ Hz, H-2), 6.96 (2H, d, $J = 8$ Hz, H-2″ and H-6″), 7.37 (2H, m, H-5′ and H-6′), 7.42 (2H, d, $J = 8$ Hz, H-3″ and H-5″), 7.49 (1H, dd, $J = 3$, 6 Hz, H-4′); EIMS *m*/*z* (relative intensity) 424 (M^+ for $C_{17}H_{11}{}^{79}Br^{35}Cl_2N_2O_2$, 4), 169 (100). Anal. Calcd for $C_{17}H_{11}BrCl_2N_2O_2$: *m*/*z* 423.9381. Found: *m*/*z* 423.9396.

Photoreaction of Pyrrolnitrin Derivatives (1b,c). A solution of **1b** (240 mg, 0.56 mmol) in 50% aqueous acetonitrile (240 mL) was irradiated with the 100 W UV lamp for 2 h and the reaction mixture was evaporated to dryness. The silica gel column chromatographic separation of the resulting residue afforded the expected 1-(4-bromobenzyl)-7,4′-dichlorospiro[1,3-dihydrobenzo(*c*)isoxazole-3,3′-pyrrolin-2′-one] (**2b**) (32 mg, 13%) [IR 3228, 1710 cm^{-1}; UV (MeCN) 291; ^1H NMR (DMSO-d_6) 4.54 and 4.60 (each 1H, each d, $J = 16$ Hz, benzylmethylene protons), 6.97 (1H, br d, $J = 8$ Hz, H-4), 7.05 (1H, t, $J = 8$ Hz, H-5), 7.24 (2H, d, $J = 8$ Hz, H-2″ and H-6″), 7.38 (1H, s, H-5′), 7.39 (1H, br d, $J = 8$ Hz, H-6), 7.59 (2H, d, $J = 8$ Hz, H-3″ and H-5″), 10.6 (1H, br, NH); ^{13}C NMR (DMSO-d_6) 45.0, 90.3, 110.7, 115.4, 120.4, 120.9, 124.7, 127.0, 129.8, 130.0, 131.7, 133.3, 135.8, 146.5, 172.9; EIMS *m*/*z* (relative intensity) 424 (M^+ for $C_{17}H_{11}{}^{79}Br^{35}Cl_2N_2O_2$, 7), 346 (8), 255 (8), 220 (8), 193 (92), 169 (100). Anal. Calcd for $C_{17}H_{11}BrCl_2N_2O_2$: C, 47.92; H, 2.60; N, 6,57. Found: C, 48.17; H, 2.84; N, 6.49] and 1-(4-bromobenzyl)-3,7-dichloro-8-hydroxy-8,8a-dihydropyrrolo[2,3-*b*]indol-2-one (**3b**) (97 mg, 40%) [UV (MeCN) 350, 288, 245; ^1H NMR (DMSO-d_6) 4.54 and 4.84 (each 1H, each d, $J = 15$ Hz, benzylmethylene protons), 5.75 (1H, s, H-8a), 7.21 (1H, t, $J = 8$ Hz, H-5), 7.28 (2H, d, $J = 8$ Hz, H-2″ and H-6″), 7.49 (1H, br d, $J = 8$ Hz, H-6), 7.54 (2H, d, $J = 8$ Hz, H-3″ and H-5″), 7.57 (1H, br d, $J = 7$ Hz, H-4), 10.4 (1H, s, NOH); ^{13}C NMR (DMSO-d_6) 45.0, 89.4, 119.0, 120.5, 121.5, 122.5, 125.8, 130.2, 131.3, 131.5, 133.5, 136.1, 145.4, 152.2, 167.0; EIMS *m*/*z* (relative intensity) 424 (M^+ for $C_{17}H_{11}{}^{79}Br^{35}Cl_2N_2O_2$, 10), 371 (8), 169 (100). Anal. Calcd for $C_{17}H_{11}BrCl_2N_2O_2$: *m*/*z* 423.9381. Found: *m*/*z* 423.9401].

Photolysis of **1c** [21] (90 mg, 0.4 mmol) in 50% aqueous acetonitrile (100 mL) under the same conditions followed by column chromatographic separation (toluene−ethyl acetate: 4/1) afforded 7-chloro-spiro[1,3-dihydrobenzo(*c*)isoxazole-3,3′-pyrrolin-2′-one] (**2c**) (20 mg, 22%): IR 3386, 1725 cm^{-1}; UV (MeCN) 286 (ϵ 2.0×10^3), 245 (3.0×10^3), 213; ^1H NMR (DMSO-d_6) 5.35 (1H, d, $J = 5$ Hz, H-4′), 6.88 (1H, br d, $J = 8$ Hz, H-4), 6.96 (1H, d, $J = 5$ Hz, H-5′), 6.98 (1H, t, $J = 8$ Hz, H-5), 7.29 (1H, br d, $J = 8$ Hz, H-6), 9.50 (1H, br, NH), 10.2 (1H, br s, N′H); ^{13}C NMR (DMSO-d_6) 89.6, 108.4, 115.4, 120.0, 124.4, 129.0, 130.5, 134.6, 146.2, 177.4; EIMS *m*/*z* (relative intensity) 222 (M^+ for $C_{10}H_7{}^{35}Cl_1N_2O_2$, 100%), 206 ($M^+ - O$, 28), 204 (31), 194 ($M^+ - CO$, 68), 180 (83), 178 (70). In this reaction, the expected 7-chloro-8-hydroxy-8,8a-dihydropyrrolo-[2,3-*b*]indol-2-one (**3c**) was not isolated in the pure state.

Irradiation of 3-(3-Chloro-2-nitrophenyl)-2-ethoxycarbonyl-5-methylpyrrole (5). A solution of **5** [24] (10 mg, 0.03 mmol) in anhydrous acetonitrile (10 mL) was irradiated under the same conditions described above (with the 100 W UV lamp for 5h). TLC analysis of the irradiated solution showed no consumption of the starting **5**.

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Supporting Information Available: ^1H NMR, ^{13}C NMR, IR, UV, and MS data for **2a**−**c**, **3a**,**b**, and **4**. Tables of crystal data, structure solution and refinement, atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **3a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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