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Photochemistry of Antibiotics. I. Oxidative Coupling of *o*-Aminophenol by Photoirradiation

Tetsuro Ikekawa, Nobuaki Uehara, and Tomoko Okuda

National Cancer Center Research Institute[1]

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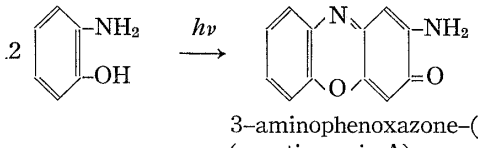
It was demonstrated that 3-aminophenoxazone-(2) (questiomycin A) was obtained by photooxidative coupling of *o*-aminophenol. The photoreaction proceeded in the UV region of *o*-aminophenol and did not proceed without oxygen. Some photosensitizers could induce the photoreaction by irradiation of wave length longer than 300 mμ. The reaction was suggested to be a first order reaction with respect to the concentration of *o*-aminophenol. By means of electron spin resonance, the reaction mechanism was discussed.

The antitumor antibiotics, actinomycins, have 3-aminophenoxazone derivatives as a chromophore[2] and it is suggested that they are derived from dimerization of *o*-aminophenol derivatives.[3]

Didemethylactinoin (3-aminophenoxazone 1,8-dicarboxylic acid), 2-amino-1-naphthol and 1-amino-2-naphthol have carcinogenic activities for bladder.[4] Considering that some antitumor agents have carcinogenisity, *o*-aminophenol derivatives are interesting in cancer study.

Questiomycin A,[5] one of the phenoxazine antibiotics, is found with questiomycin B, *o*-aminophenol, which is suggested to be a precursor of questiomycin A.

In the present work, questiomycin A is obtained by photoirradiation of *o*-aminophenol and some evidence is discussed concerning the reaction mechanism. *o*-Aminophenol is dissolved in the organic solvent and irradiated with high pressure mercury lamp for several hours. After purification of the photoproducts by alumina chromatography and sublimation, questiomycin A (3-aminophenoxazone-(2)) is obtained as one of the photoproducts. It is confirmed by mixed melting points (mp 250—251°), infrared spectra and thin-layer chromatography that the photoproduct is identical with the authentic sample synthesized by yellow mercury oxide.[6]



3-aminophenoxazone-(2)
(questiomycin A)

Chart 1

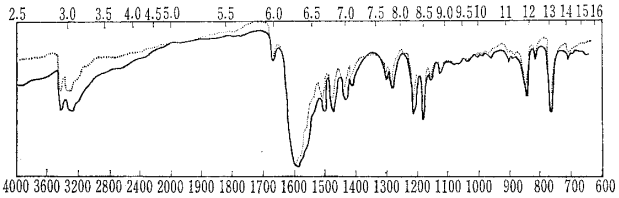


Fig. 1. Infrared Spectra of 3-Aminophenoxazone-(2) and the Photoproduct (KBr Tablet)

------ 3-Aminophenoxazone-(2) —— Photoproduct

1) Location: 5-1, *Tsukiji, Chuo-ku, Tokyo.*
2) H. Umezawa, "Recent Advances in Chemistry and Biochemistry of Antibiotics," Microbial Chemical Research Foundation, 1964, p. 120.
3) E. Katz and H. Weissbach, *J. Biol. Chem.*, **237**, 882 (1962); L.R. Morgam, Jr. and D.M. Weibmorb, *Biochem. Biophys. Acta*, **82**, 645 (1964).
4) C.M. King, S.F. Chang, and H.R. Gutmann, *J. Biol. Chem.*, **238**, 2206 (1963); E. Boyland, *Acta Unio Intern. Contra. Cancrum.*, **16**, 273 (1960).
5) K. Anzai, K. Isono, K. Okuma, and S. Suzuki, *J. Antibiotics*, **13A**, 125 (1960).
6) O. Fisher and O. Jonas, *Chem. Ber.*, **27**, 2782 (1894).

In addition, these two compounds show identical spectra by high resolution mass spectrometry, and the parent peak observed is m/e 212.055 for the calculated value 212.059 of $C_{12}H_8O_2N_2$. The fragmentation also verifies the chemical structure of questiomycin A. Thus it is demonstrated that questiomycin A, 3–aminophenoxazone–(2) is obtained by photooxidative coupling of *o*–aminophenol.

The amount of 3–aminophenoxazone–(2) obtained by photoirradiation of *o*–aminophenol can be determined by measurement of intensity at absorption maximum of the photoproduct (435 mμ), because the starting compound has no absorption in this region. Therefore, after a 10^{-3} mole methanol solution of *o*–aminophenol is irradiated with a 100 watt high pressure mercury lamp without cutting ultraviolet (UV) light, absorption intensity at 435 mμ is measured.

At the initial stage of the photoreaction, the amount of 3–aminophenoxazone–(2) obtained by irradiation of *o*–aminophenol is proportional to the irradiation time. It is suggested from Fig. 2 that this reaction is a first order reaction with respect to the concentration of *o*–aminophenol.

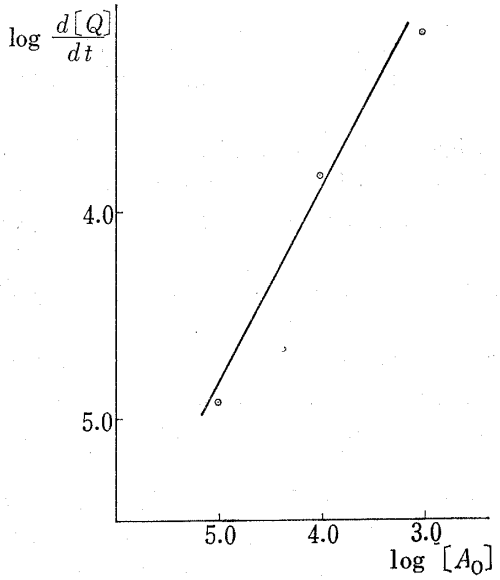


Fig. 2. Time Course of the Photoreaction

$[A_0]$: initial concentration of *o*–aminophenol
$[Q]$: concentration of 3–aminophenoxazone–(2) after t minutes

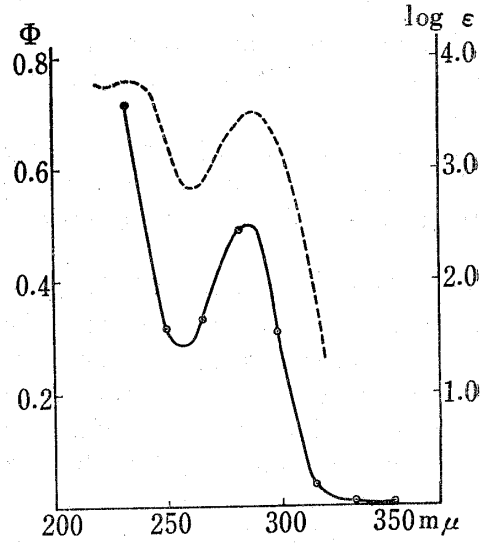


Fig. 3. Effect of Wave Length on the Photoreaction

——: numbers of 3–aminophenoxazone–(2) molecules obtained by one photon (Φ).
------: ultraviolet absorption of *o*–aminophenol
reaction temperature: 16°

Monochromatic photoirradiation is carried out using a Concative Grating Radiation Monochromator at the wave length between 235 and 700 mμ. The concentration of the photoproduct, namely, the numbers of 3–aminophenoxazone–(2) molecules obtained by one photon is calculated by a standard curve given for the monochromator. Fig. 3 shows that the photooxidativereaction occurs in the region below 300 mμ, where *o*–aminophenol has UV absorption. This finding may suggest that *o*–aminophenol molecule absorbs the photoenergy and couples with another molecule. The photoproduct is proved to be only 3–aminophenoxazone–(2) by thin–layer chromatography in monochromatic studies.

This reaction does not proceed at pressures below 10^{-4} mmHg obtained by degassing with an oil diffusion pump. This fact suggests that oxygen is necessary for the reaction.

The effect of irradiated photosensitizers on the reaction is now investigated using the monochromator. As shown in Fig. 4, a small amounts of 4–nitroquinoline N–oxide, riboflavine or methylene blue added to the methanol solution of *o*–aminophenol can induce this

reaction by absorption of photons of wave length longer than 300 mμ. Methylene blue has little effect on the reaction at zero degrees, whereas 4–nitroquinoline N–oxide and riboflavine enhance the reaction rate somewhat at this temperature.

On the other hand, sudan III, methyl red, cresol red, bromphenol blue, indophenol and fluorescein soda have no effect on this reaction. On the basis of the above findings it is suggested that the chemical structure of the photosensitizer is one of the important parameters for this reaction.

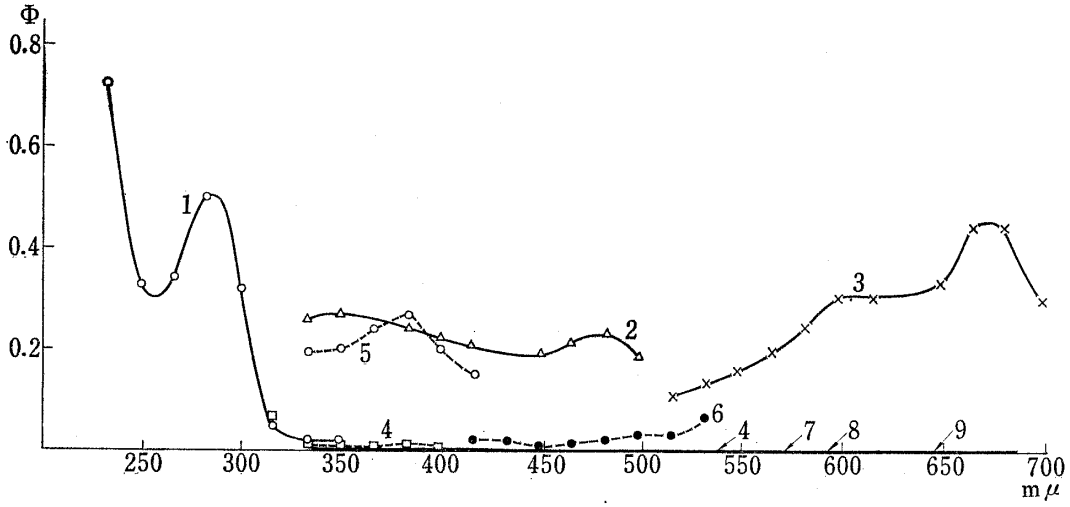


Fig. 4. Photoirradiation with Monochromatic Light

1 : *o*–aminophenol
2 : *o*–aminophenol+riboflavin
3 : *o*–aminophenol+methylene blue
4 : *o*–aminophenol+sudan III
5 : *o*–aminophenol+4–nitroquinoline N–oxide
6 : *o*–aminophenol+methyl red
7 : *o*–aminophenol+cresol red
8 : *o*–aminophenol+fluorescein soda
9 : *o*–aminophenol+bromphenol blue

Electron Spin Resonance (ESR) signal is obtained by photoirradiation of a dioxane or benzene solution of *o*–aminophenol in air. As shown in Fig. 5 the signal disappears when photoirradiation is stopped. However, after prolonged irradiation the sample becomes orange–red, and a signal persists even without photoirradiation. Therefore, this signal, which is different from the signal shown in Fig. 6, is suggested to be that of the reaction product.

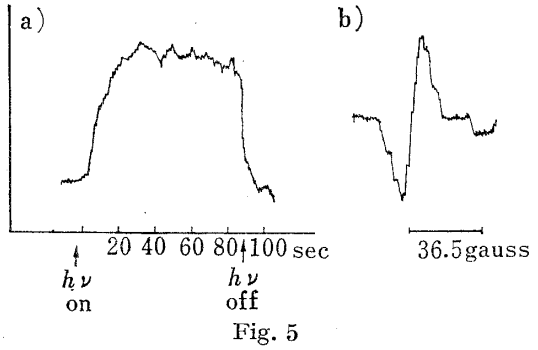


Fig. 5

(a) the change of the signal intensity by photoirradiation to the dioxane solution of *o*–aminophenol
(b) the ESR spectrum of the radical produced from *o*–aminophenol in dioxane

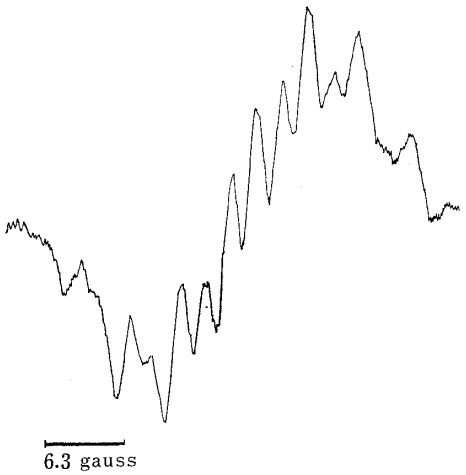


Fig. 6. The Electron Spin Resonance Spectrum of the Radical produced from *o*–Aminophenol in Dioxane

If the sample is degassed with a vacuum pump to a pressure of less than 10^{-4} mmHg, the signal is no longer observed. Signal intensity is larger for a dioxane solution than for a benzene solution. In the dioxane solution hyperfine structure is obtained, as shown in Fig. 6.

The signals in Fig. 5 and 6 are probably due to the photoexcited *o*–aminophenol molecule, although the radical structure is not known in detail. The free radical thus produced may contribute to initiation and propagation of the photooxidation reaction of *o*–aminophenol.

Experimental

Synthesis of 3-Aminophenoxazone-(2) by Yellow Mercury Oxide——*o*–Aminophenol (3 g) was dissolved in 400 ml benzene, and 1.5 g of yellow mercury oxide was added to this solution. After refluxing in a water bath for 2 hr, the solvent was evaporated *in vacuum*. Purification was made by alumina chromatography eluting with $CHCl_3$– acetone (8:2), and after sublimation, 3–aminophenoxazone–(2) was recrystallized from EtOH. Yield, 0.2 g.

Synthesis of 3-Aminophenoxazone-(2) by Photoirradiation——a) In 500 ml MeOH, *o*–aminophenol (0.5 g) was dissolved and the solution was irradiated with a 100 W high pressure mercury lamp (UM–102, Ushio Denki Co., Ltd.) for *ca.* 10 hr without cutting UV light. Then the solution was evaporated *in vacuum*. The photoproducts were subjected to alumina chromatography and eluted with $CHCl_3$–acetone (8:2). The first band was collected and after purification by sublimation under 15 mmHg at 160°, 3–aminophenoxazone–(2) was obtained, which was recrystallized from EtOH. The identity of this compound with the authentic sample was confirmed with mixed melting point (mp 250—251°C) and IR (KBr Tablet) and thin–layer chromatography of silica gel using $CHCl_3$– acetone (8:2), *Rf* 0.38, benzene–acetone (1:1), *Rf* 0.36. Yield, 0.18 g.

b) *o*–Aminophenol (2 g) was dissolved in MeOH (500 ml) and riboflavine (100 mg) was added to this solution, which was irradiated with a 100 W mercury lamp for 10 hr cutting UV light. After the same purification procedure, 247 mg of 3–aminophenoxazone–(2) was obtained. Yield, 12.4%.

c) *o*–Aminophenol (2 g) was dissolved in MeOH (1500 ml) and irradiated with a 450 W high pressure mercury lamp (Ushio Denki Co., Ltd. UM–452) for 2 hr with water cooling. In this reaction 180 mg of 3–aminophenoxazone–(2) was obtained by the same procedure. Yield, 9.0%.

Time Coures of the Formation of 3-Aminophenoxazone-(2) by Photoirradiation——Methanol solution of *o*–aminophenol (500 ml) was irradiated with a 100 W high pressure mercury lamp (Ushio Denki Co., Ltd. UM–102) without cutting UV light. The optical density at 435 mμ was measured each half hour during irradiation. The value of $d[Q]/dt$ was calculated against the initial concentration of *o*–aminophenol between 10^{-3} and 10^{-5} mole where $[Q]$ was the concentration of 3–aminophenoxazone–(2) obtained by photoirradiation.

Monochromatic Study——One second ml of 10^{-3} mole methanol solution of *o*–aminophenol was put into a quartz glass tube (2 mm diameter) and irradiated with a Concave Grating Radiation Monochromator, model CRM–100 (Japan Spectroscopic Co., Ltd.). The irradiation time was determined by the counter attached to the monochromator. After 10^3 counts of irradiation, 3 ml of methanol was added to each sample and intensity of UV absorption at 435 mμ was measured. The product of each tube by photoirradiation using the monochromator was determined by thin–layer chromatography to be only 3–aminophenoxazone–(2). The total energy given to the sample during irradiation was obtained by the standard curve of the monochromator and the numerical value of 3–aminophenoxazone–(2) molecules per photon was calculated.

The Measurement of the ESR Spectra——The ESR spectra were measured in quartz tube in dioxane solution using a Japan Electron Optics Laboratory's JES–3BX spectrometer with 100 kc/sec field modulation. UV irradiation for the samples was carried out at a distance of 30 cm, using the 100 W high pressure mercury lamp (Ushio Denki Co., Ltd. UM–102) attached to the ESR spectrometer.

The magnetic field was fixed to the resonance field of the radical produced from *o*–aminophenol. When irradiation by the above lamp was made, the signal appeared and showed the maximum intensity within 20 seconds, as shown in Fig. 5. The signal intensity persisted during photoirradiation, but disappeared as soon as the irradiation was stopped.

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