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Photodecomposition of Procaine Hydrochloride in Aqueous Solution after Exposure to a Fluorescent Lamp

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Introduction

Early reports by Briggs and Callow[1)] and Bullock and Cannell[2)] indicated that procaine hydrochloride, an anesthetic, is hydrolyzed to yield paraaminobenzoic acid and diethylethanolamine in aqueous solution, thus reducing the drug's efficacy. In addition to being hydrolyzed, color of an aqueous solution of the compound gradually changes to dark yellow upon prolonged autoclaving and storage. These observations were expanded in later studies including environmental factors such as pH- and temperature elevation and the presence of oxygen, which affect stability of the solution.[3-5)]

Procaine hydrochloride, on the other hand, is stable to visible light in aqueous solution (Japanese pharmacopoeia XI, JP XI). Very little is known, however, about the effects of ultraviolet (UV) light, which may influence the drug.

The purpose of this experiment is to examine the photodecomposition of a procaine solution after exposure to a commonly available fluorescent lamp which including a UV region (Fig. 1).

Methods

1. Test solution

Procaine hydrochloride (PHC, JP) was dissolved in distilled water and diluted to give solutions of 0.5, 1 and 2%. The sample solutions were adjusted with 0.1 N hydrochloric acid to pH 4, 5 and 6. They were used in tests immediately after preparation.

2. Exposure test procedure

Fifty milliliter vessels filled with 10 ml of each solution (aqueous layer, 10 mm) were exposed di-

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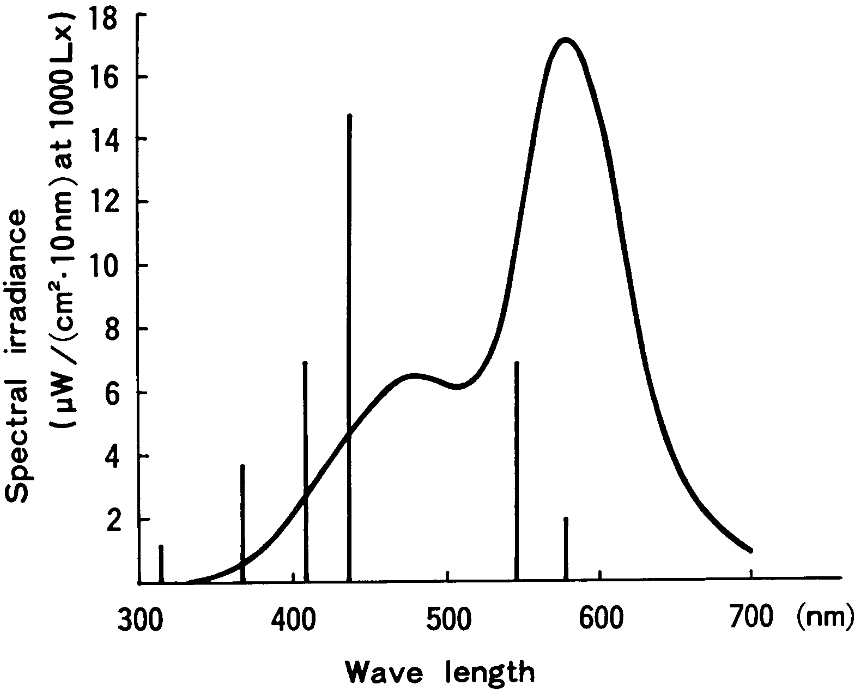

Fig. 1. Spectral Irradiance of Common Aveilable Fluorescent Lamp Used for Exposure Test

rectly to a 20 W fluorescent lamp (FL, Type FL-20sw, Mitsubishi Electric, Co., LTD., Tokyo) for 100, 300 and 600 hours, respectively. The distance from the test vessel to the light source was 40 cm, and the illuminance on the solution surface was 1,030 Lx, as measured with an illumination meter (Model UVR-365, Tokyo Optical Instruments Co., LTD., Tokyo). Evaporation was compensated for intermittently during exposure by adding distilled water (0.05–0.1 ml) up to the correct level. All experiments were carried out at room temperature.

3. Measurements of discoloration

The degree of discoloration of each solution after FL exposure was measured with a Leitz photoelectric colorimeter at a wave length of 415 nm.

4. Identification of photodecomposed products by TLC

Two microliter samples of the 2% solutions at pH 6 after 100, 300 and 600 hours of FL exposure were subjected to thin-layer chromatography (TLC, Kiesel gel 60, Thickness 4 mm, 20 × 20 cm, Merck) until the solvent (methanol-benzene-ammonia, 20:79:1) front reached to 10 cm from origin, which took approximately 30 min. After the plate dried in air, it was lightly sprayed with Ehrlich's reagent until the background was an even yellow.

5. Isolation and identification of PHC and its photodecomposed products

Two hundred microliter samples of the 2% solutions at pH 6 after 100, 300 and 600 hours of FL exposure were subjected to TLC and spots (located by UV light detection) with Rf values similar to the references were teared off and extracted with chloroform, respectively. The solvent was evaporated and each residue sample was dissolved in 200 ml or 4 ml of sodium acetate-acetic acid buffer (pH 5.6).⁶⁾

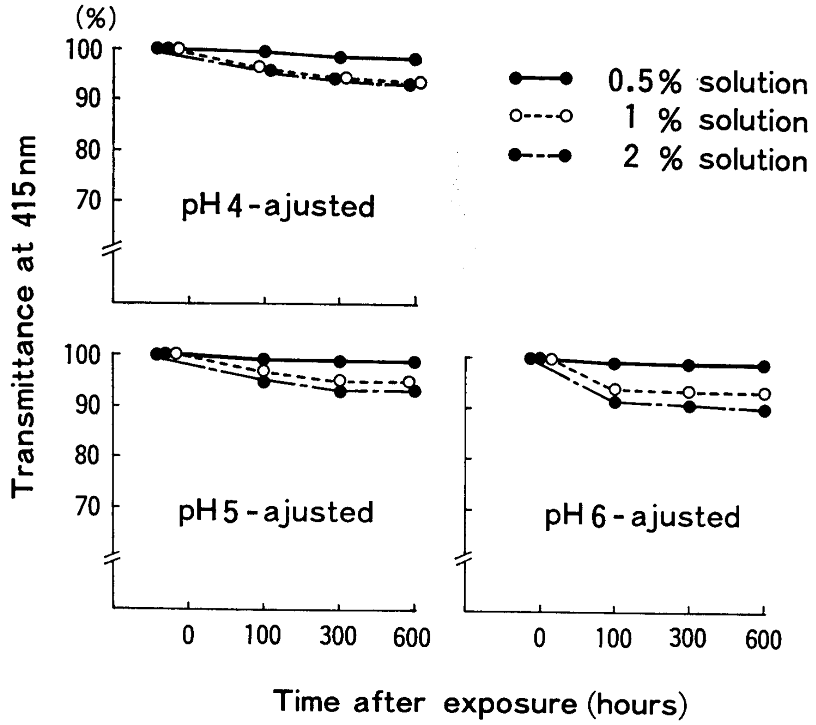

Fig. 2. Degree of Discoloration of Each Procaine Solutions Measured with Leitz Photoelectric Colorimeter at a Wave Length of 415 nm

Each sample solutions were identified respectively by spectrophotometry (Hitachi Spectrophotometer Type 124, Hitachi Co., LTD., Tokyo).

6. Statistical evaluation

Significant differences of mean values (5 determinations) were evaluated by usinfg the X^2-text.

Results

1. Degree of discoloration

In the early stage of exposure, color of the solution was not changed. As the exposure period proceeded, a slight yellowish colorchange was observed in samples at higher pHs and higher concentrations. As can be seen in Fig. 2, the 0.5% solution showed almost no discoloration even after 600 hours of exposure regardless of the pH level. The 1 and 2% solutions at pH 4 and 5 showed slight decrease of transmittance after exposure. The 2% solutions at pH 6 showed an apparent decrease of transmittance. The rates after each exposure were 94.1, 92.3 and 90.5%, respectively. There was no statistically significant difference between them (Table 1). In addition, control solutions stored in the dark did not undergo any decomposition or discoloration.

2. Identification of photodecomposition components by TLC

The TLC plate was sprayed with Ehrlich's reagent to distinguish PHC, para-aminobenzoic acid (PABA) and aniline (PABA's decarboxylation product at elevated temperatures)[7,8] from other compounds. Two spots appeared with Rf values close to those of the PHC and PABA references,

Table 1. Degree of Discoloration and Photodecomposition of PHC

Exposure time (hours)	Remaining procaine (%)		Transmittance at 415 nm (%)
0	100.0		100.0
100	92.5	*	94.1
300	83.6		92.3
600	82.1		90.5

* : $P<0.05$ (X^2-test, 3.98)

but aniline was not detected (detection limit 0.1 μg) (Fig. 3). The spot equivalent to the PABA reference tended to increase in size and show slightly yellowish discoloration as the exposure time increased.

3. Identification and estimation of photodecomposition of PHC

The absorption curve for initial PHC samples revealed a characteristic absorption bands with a maximum at 291.5 nm and minimum at 240 nm. The absorption value was 0.67 ($E^{1\%}_{1\,\mathrm{cm}} \times 10^3$) at the maximum (Fig. 4). These results were in good agreement with others reported in the literature.[4,9] The value of maximum absorption after FL decreased gradually with exposure time. The absorption values after 100-, 300- and 600-hour exposures were 0.62, 0.56 and 0.55, respectively. The remaining rates of PHC values estimated from these data at each exposure were 92.5, 83.6 and 82.1%, respectively (Table 1). There was a significant difference between after 100 and 600 hours ($p<0.05$).

4. Identification and determination of the photodecomposition product equivatent to PABA

The spectrum of the original state of PABA (reagent grade) had an absorption maximum and minimum at 267.5 nm and 232 nm, respectively. The absorption value was 1.07 ($E^{1\%}_{1\,\mathrm{cm}} \times 10^{-3}$) at the maximum (Fig. 5). The product absorption curve after 100-hour exposure was almost identical to that of the original PABA. However, as the exposure time increased, the maximum absorption tended to shift to longer wave lengths and the curve was flattened. The maximum and minimum absorption values also increased.

Discussion

We found that a PHC solution was hydrolyzed to yield mainly PABA. We also noted a colorchange after FL exposure. The degree of the photodecomposition could not be recognized by the amount of discoloration, and there appeared to be almost no relationship between the two. Furthermore, as the exposure time increased, the photohydrolyzed PABA was degraded more and lost its characteristic absorption maximum in the UV region. The maximum shifted toward longer wave lengths. These findings indicate that PABA degrades after

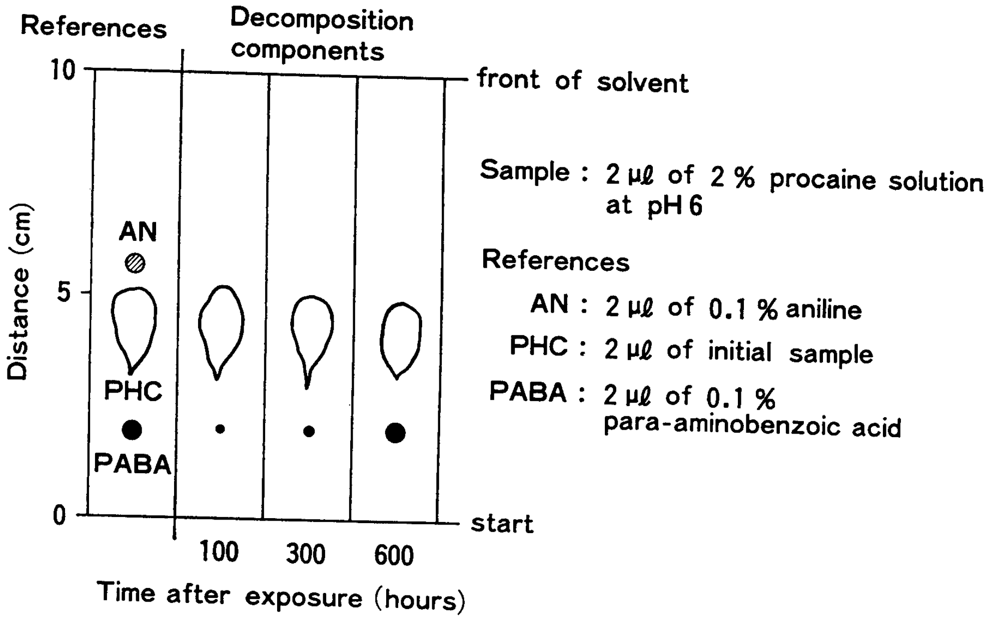

Fig. 3. Two Microliter Samples of the 2% Procaine Solutions at pH 6 after 100, 300 and 600 Hours of FL Exposure were Subjected to TLC until the Solvent (Methanol-benzene-ammonia, 20:79:1) front Reached to 10 cm from Origin

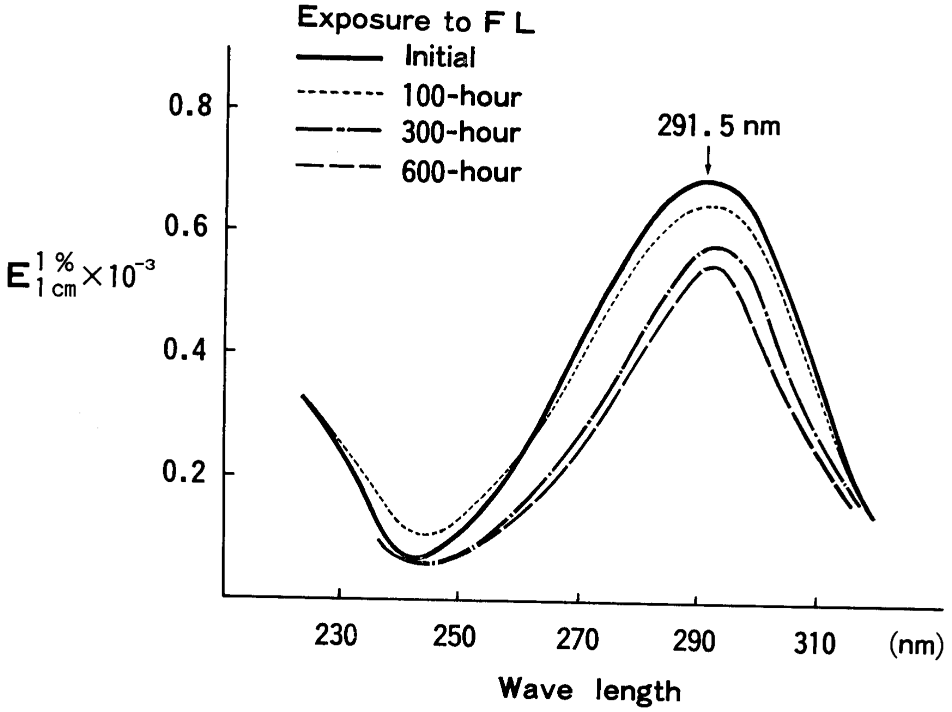

Fig. 4. Partly Decomposed PHC was Determined by the Changes of its Characteristic Absorption Band in Comparison with those of Initial PHC

long UV exposure into substances with different absorption characteristics. It is of interest to note that PABA is used as a sunscreen agent because it absorbs UV irradiation well and is discolored by light and air.[10] In addition, the JP XI note states that oxidation of PABA may result in PHC discoloration. The PHC hydrolysis reaction is known to obey first-order reaction kinetics,[4,11] but our results did not confirm this.

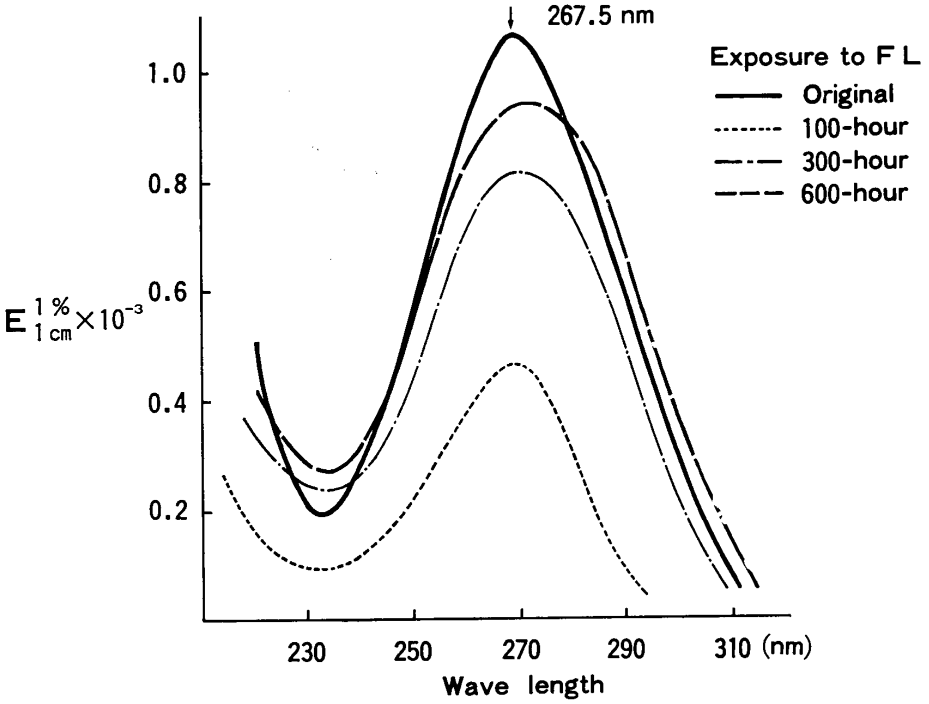

Fig. 5. The Decomposition Product Equivalent to PABA was Identified and Determined by its Absorption Bands in Comporison with those of the Original State of PABA

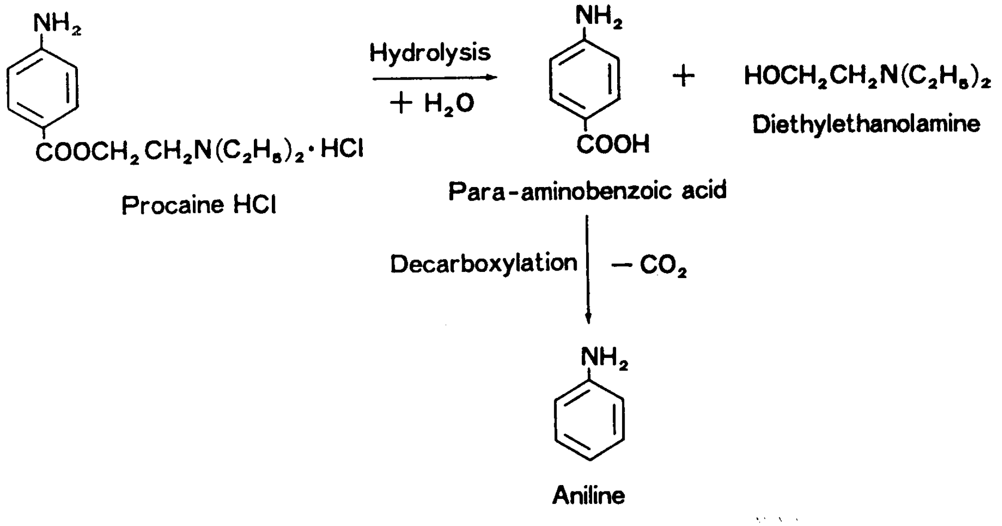

Fig. 6. Scheme of PHC Degradation

The patterns of PHC photodecomposition were very similar to those of thermal decomposition.[4,10] Matsuda and Itoh[12] explained that the decomposition of PHC in light may be due to the heat associated with light absorption. However, some reports have noted that the order of the thermal reaction differs from that of the photochemical reaction in several ways.[13,14]

Saito[15] stated that approximately 650 hours ($6.5\times10^5\times$Lx hour) of exposure to FL or to UV light for 1 hour is equivalent to the practical storage period of 6 months in a hospital pharmacy. This may suggest that the present 600-hour exposure to FL is long enough to observe the photoliable property of PHC.

Kawamura *et al.*[9] reported that autoclaved 1% PHC solution at pH 4 left for 12 months at room temperature showed a 4.5% loss of the componenet. Interestingly, similar observations of the same solution were noted after the 600-hour FL exposure. In addition, a previous report by Biggs[16] indicated that a 0.2% PHC solution in ampules left in a refringerator and in diffused sunlight for 3 months showed losses of 5.2% and 17%, respectively.

Zollner and Vastagh[7] and Grier[8] have suggested that reaction with aniline which was formed by decarboxylation of PABA and diethylethanolamine may produce a colored solution at elevated temperatures (Fig. 6). However, no aniline was found under the present exposure conditions. It might be present after more exposure.

Several reports[17,19] have shown that photodecomposition is more efficient at short wave lengths, moreover drugs decompose most thoroughly at the wave length of their maximum absorption. Kuboki and Saito[20] observed a marked yellowish colorchange of natural-pH PHC solution after exposure to a room bacteriocidal lamp (280 ~ 290 nm) for some time. These data suggest that the UV region of FL may be responsible for the photolytic degradation or discoloration of PHC.

When 0.5% solution was exposed to FL, neither photodecomposition nor discoloration were detected regardless of pH or exposure time. A 2% solution at pH 6 showed an apparent decrease of PHC components and discoloration after longer exposure. In a photochemical reaction, the absorption of light supplies the molecules with adequate energy to perpetuate the reaction.[13] It appears that more photodecomposition and discoloration should occur with rich molecules present in higher concentrations. By extending the UV-exposure time, increases of drug photodecomposition have been reported by several investigators.[13,14] Furthermore, PHC hydrolysis has been shown to be increased with increasing hydroxyl ion activity in the solution.[4,11]

An ester PHC is also subject to oxidative decomposition, but such degradation can be controlled by removing oxygen.[14] This experiment was conducted under an oxygen-free atmosphere. Therefore under the present conditions, it may be difficult to explain whether the cause of this degradation was photolytic or photo-oxidative.

Finally, we found that a PHC solution is susceptible to UV-photochemical degradation and the influence of even a room FL cannot be ignored in terms of quality control of the PHC preparations.

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