ANDP-2, A Novel Acrinol Degradation Product by Light

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To clarify the degradation pathway of acrinol by light, isolation and identification of acrinol degradation products (ANDP) were attempted. ANDP-2, one of the ANDPs, was isolated by extraction with methanol from cloths dampened with acrinol solution, and purified by column chromatography on Diaion HP-10, Sephadex LH-20 and G-25. The structural elucidation of ANDP-2 was examined by infrared, ^{1}H-NMR, ^{13}C-NMR and Fast atom bombardment mass (FAB-MS) spectra studies. From the spectroscopic data, the structure of ANDP-2 was determined to be 4-amino-6-ethoxyl-2,3-dicarboxyquinoline, that was found to be a novel degradation product of acrinol by light. The solution of ANDP-2 has fluorescence with a blue color, though that of acrinol has fluorescence with green. ANDP-2 did not show growth inhibition at a concentration of 100 μg/ml against Gram-positive, -negative bacteria, yeast and fungi.

Key words —— acrinol, acrinol degradation product-2, antimicrobial activity, disinfectant, florescence quantum yield

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INTRODUCTION

Acrinol (2-ethoxyl-6,9-acridinediamine lactate monohydrate) is widely used as a disinfectant in hospitals. Synergistic bacterial effects of acrinol with other antibiotics and the influence of light on the antimicrobial activity of acrinol and of acrinol with other antibiotics have been reported.[1–4] The degradation product of acrinol by light, however, has not been clarified, although color change of the acrinol liniment or ointment occurs by light irradiation. The degradation products of acrinol by light were produced after long time irradiation (more than three month). Therefore, the use of acrinol may yield few clinical problems. To clarify the degradation pathway of acrinol by light, isolation and structural elucidation of acrinol degradation products were attempted.

In previous papers,[5, 6] we reported two novel acrinol degradation products, acrinol degradation products (ANDP)-7 and -8. In this paper, the isolation and structural elucidation of ANDP-2, another novel ANDP, together with the antimicrobial activity of this compound, are described.

MATERIALS AND METHODS

Materials —— Acrinol was purchased from Daiichi Fine Chemical, Co., Ltd. (Toyama, Japan). Nonwovens was purchased from Kuraray Co., Ltd. (Okayama, Japan). All other chemicals were of reagent grade or better.

Apparatus, Analysis, and Detection of ANDP —— HPLC apparatus used was described in the previous paper.[5] The detection of ANDP was routinely measured by HPLC on an apparatus equipped with a UV detector set at 254 nm. Elementary analysis was performed with a CHN CORDER MT-5 (Yanagimoto Co. Ltd., Kyoto, Japan). The IR spectrum was taken in KBr tablets on a JEOL (Tokyo, Japan) JIR-6500W infrared spectrophotometer. Mass spectrum Fast atom bombardment mass (FAB-MS) was measured with a JEOL JMS-700 M station spectrometer. ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H COSY, ^{1}H-^{13}C COSY and HMBC spectra with tetramethylilane (TMS) as internal standard were taken in dimethylsulfoxide-d_6 at 500 MHz on a JEOL JMN-A500 spectrometer.

Light irradiation was done in a germ-free box (15 W, 40 cm, Ikemoto Scientific Technology Co. Ltd., Tokyo, Japan). UV light irradiation was done

in a clean bench (15 W, 40 cm, Air Tech Co. Ltd., Tokyo, Japan). The excitation and fluorescence spectrum were taken on a Hitachi fluorescence spectrophotometer F-4500. The fluorescence quantum yield was measured in various solvents (Table 1).

Degradation of Acrinol —— Degradation of acrinol by light was done as described in the previous paper.[5)]

Degradation of ANDP-7 and Detection of ANDP-2 —— 4 sheets of nonwovens (300 × 300 × 1.3 mm) were dampened with methanol solution of ANDP-7 (100 mg/100 ml). Air drying nonwovens dampened with methanol solution of ANDP-7 are irradiated with light for more than three months. The detection of degradation products of ANDP-7 was measured by HPLC as described in the previous paper.[5)]

Antimicrobial Activity —— The conventional serial agar dilution method was applied in this study using a bouillon agar or glucose bouillon agar as an assay medium. Suspensions of test organisms were prepared using precultured organisms (optical density (OD) = 1.0). One loopful of each suspension was streaked on an assay medium. After incubation at 37°C (24 hr for bacteria and 2–3 days for yeast and fungi), antimicrobial activity (minimum growth inhibitory concertrations (MICs)) of ANDP-2 was measured. The test organisms used for the study are given in Table 2.

Table 1. Fluorescence Spectral Data of Acrinol,[6)] ANDP-7[6)] and ANDP-2 in Various Solvents

	$F\lambda_{max}$ nm (Quantum yield)[a)]		
	Water	Methanol	Ethanol
Acrinol	511 (0.242)	490 (0.332)	489 (0.336)
ANDP-7	—	470 (0.367)	466 (0.321)
ANDP-2	432 (0.642)	436 (0.176)	441 (0.277)

a) excitation at 370 nm.

RESULTS AND DISCUSSION

Isolation and Purification of ANDP-2

The mixture of ANDP was obtained from Nonwovens as described in the previous paper.[5)] HPLC chromatogram of ANDP by light is shown in Fig. 1. The ANDP were gradually increased by light irradiation time (three months 8–10% degradation, six months 35–40% degradation)

The mixture of ANDP was adsorbed on Diaion HP-10 column (2.5 × 40 cm). After washing with water, ANDP were eluted with 20%, 40%, 60%, 80% and 100% methanol (500 ml, each). ANDP-2 was eluted with 20% and 40% methanol. 20% and 40% methanol fractions contain ANDP-1, -2, -3 and -4. 20% and 40% methanol fractions were

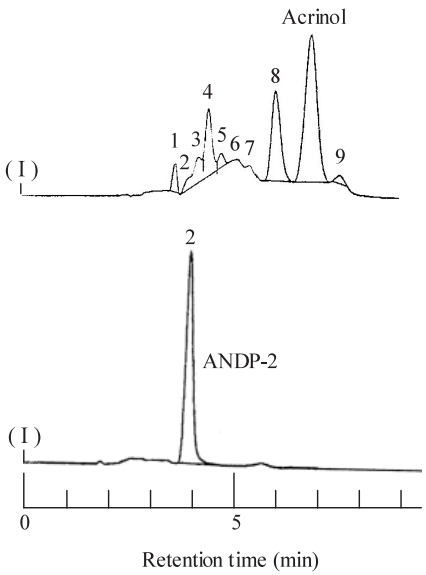

Fig. 1. HPLC Chromatogram of ANDP by Light
Detector, 254 nm; sensitivity, 0.1 a.u.f.s,; I = injection point

Table 2. Antimicrobial Activities of ANDP-2

Test organism	MIC (μg/ml)			
	ANDP-2	ANDP-7[6)]	ANDP-8[5)]	Acrinol[5)]
Bacillus subtilis (a) PCI 219	> 100	10	1	100
Staphylococcus aureus (a) IFO 3060	> 100	2	1	100
Escherichia coli (a) IFO 3301	> 100	20	20	100
Proteus vulgaris (a) IFO 3167	> 100	20	> 100	> 100
Pseudomonas aeruginosa (a) IFO 3448	> 100	20	100	100
Candida albicans (b) IFO 0583	> 100	20	10	> 100
Saccharomyces cerevisiae (b) IFO 0305	> 100	10	10	> 100
Aspergillus niger (b) IFO 4066	> 100	10	10	> 100
Penicillium chrysogenum (b) IFO 4626	> 100	10	10	> 100

(a); bouillon agar, (b); glucose bouillon agar.

combined and concentrated *in vacuo*. This crude powder was dissolved in a small volume of water and applied to Sephadex LH-20 and G-25 columns (1.5×70 cm, each). ANDP-2 was eluted with water. The fractions containing ANDP-2 were pooled and concentrated *in vacuo* to gave 15 mg of white powder. The yield of purified ANDP-2 was 1.5%.

Physico-chemical Properties of ANDP-2

ANDP-2 was obtained as white powder with decomposition point at 251°C. It was soluble in dimethyl sulfoxide, slightly soluble in water and methanol and insoluble in other common organic solvents. The solution of ANDP-2 has fluorescence with a blue color, though that of acrinol has fluorescence with green. The fluorescence spectrum and florescence quantum yield of ANDP-2 are shown in Fig. 2 and Table 1, respectively. The quantum yield of ANDP-2 in water was stronger than that of acrinol. The infrared spectrum of ANDP-2 in KBr disk is shown in Fig. 3. Absorptions were observed at 3365, 3195 and 1612 cm^{-1} in the IR spectrum due to the amino and carboxyl group, respectively. FAB-MS spectrum of ANDP-2 (positive) is shown in Fig. 4.

The molecular ion peak of ANDP-2 was obtained at m/z 276 (positive 277, negative 275) by FAB-MS. The elementary analysis of ANDP-2 (Calcd C: 46.52, H: 4.38, N: 10.14, Found C: 46.48, H: 4.40, N: 10.12) generated $C_{13}H_{12}O_5N_2$ as the molecular formula, which agreed with the m/z 276 (M^+) as ion peak on the FAB-MS. The presence of carboxyl group in ANDP-2 was revealed by the fragment ion corresponding to loss of m/z 45 from molecular ion. The presence of carboxyl group was supported by ^{13}C-NMR spectral data, which are summarized in Table 3. The ^{13}C-NMR spectrum of ANDP-2 showed the 13 carbons. The analysis of DEPT spectrum indicated that ANDP-2 consisted of the following functional groups: $CH_3 \times 1$, CH_2-O $\times 1$, CH = $\times 3$, C = $\times 6$, C = O $\times 2$.

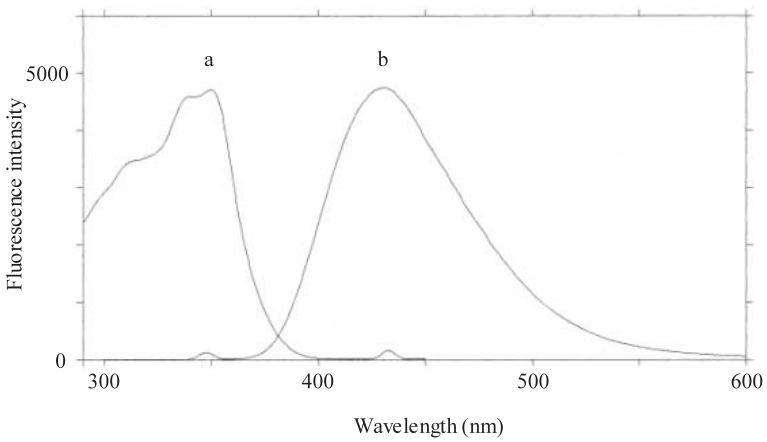

Fig. 2. Excitation and Fluorescence Spectrum of ANDP-2
a; excitation spectrum, b; emission spectrum (solvent: water)

Fig. 3. IR Spectrum of ANDP-2 (KBr)

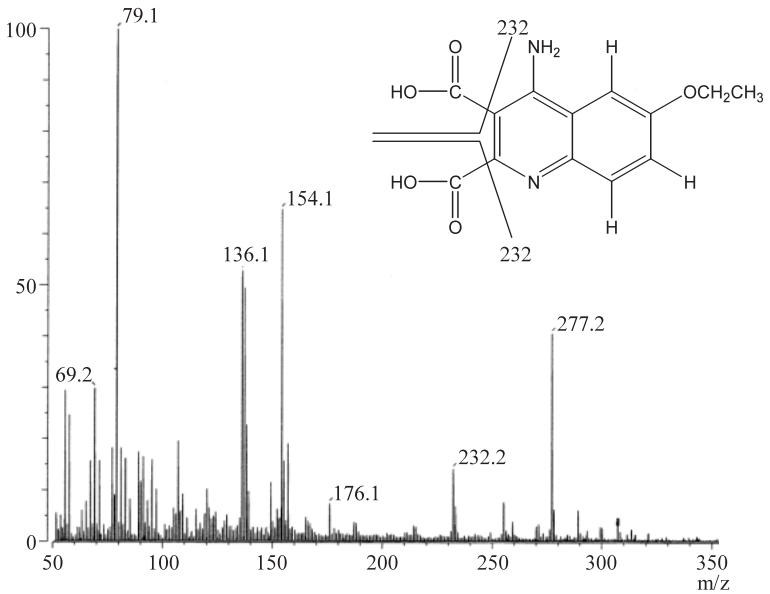

Fig. 4. FAB-MS Spectrum of ANDP-2 (positive)

Table 3. NMR spectral data for ANDP-2 in DMSO-d_6

position	δ_C	δ_H
1a	137.97	
2	166.97	
3	151.99	
4	154.99	
4a	118.61	
5	103.08	7.69 (s, 1 H)
6	156.48	
7	123.45	7.38 (d, J = 8.54, 1 H)
8	127.27	7.85 (d, J = 8.54, 1 H)
9	169.15	
10	169.15	
11	63.80	4.18 (q, J = 7.3, 6.7, 6.7, 2 H)
12	14.40	1.40 (t, J = 7.3, 3 H)
-OH		8.97 (broad, 2 H)

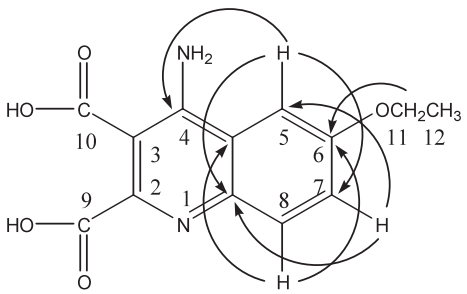

Fig. 5. ^{1}H-^{13}C Connectivities of ANDP-2 as Revealed by HMBC Experiments

Structural Elucidation of ANDP-2

In the ^{1}H-NMR spectrum of ANDP-2, a series of three coupled aromatic protons were observed at δ_H 7.85 (d), 7.38 (d) 6.69 (s). Resonances for a C-methyl (δ_H 1.40) triplet and an *O*-methylene proton (δ_H 4.18) quartet were observed. Hydroxy protons were also observed at δ_H 8.97 (broad). The ^{1}H-NMR spectrum could account for ten protons. In the ^{1}H-^{1}H COSY spectrum, the aromatic proton signal at δ_H 7.38 (H-7) was correlated with δ_H 7.85 (H-8). The methyl proton signal at δ_H 1.40 (H-12) was correlated with δ_H 4.18 (H-11). In the ^{13}C-NMR spectrum of ANDP-2 resonances for one methyl carbon (δ_C 14.40) and an oxygen-bearing methylene carbon (δ_C 63.80), nine resonances for aromatic-type carbons, one of which was oxygen-bearing carbon (δ_C 156.48), three of which were substituted by proton (δ_C 127.27, 123.45, 103.08), and five of which were without proton (166.97, 154.99, 151.99, 137.97, 118.61) indicated the A and B rings of acrinol (Fig. 3). In the ^{13}C-NMR spectrum of ANDP-2 resonances for two carbonyl carbons (δ_C 169.15, 169.15) bounded at B ring. Two carbonyl carbons were thought to be carboxyl acid, supported by IR spectrum (1612 cm^{-1}) and FAB-MS fragmentation ions peak of ANDP-2 (m/z 277–232).

In the heteronuclear multiple bond correlation (HMBC) experiments (Fig. 5) the aromatic proton signal at δ_H 7.69 (H-5) was correlated with δ_C 123.45 (C-7), δ_C 137.97 (C-1a) and δ_C 154.99 (C-4), the aromatic proton signal at δ_H 7.38 (H-7) was correlated with δ_C 103.08 (C-5) and δ_C 137.97 (C-1a), the aromatic proton signal at δ_H 7.85 (H-

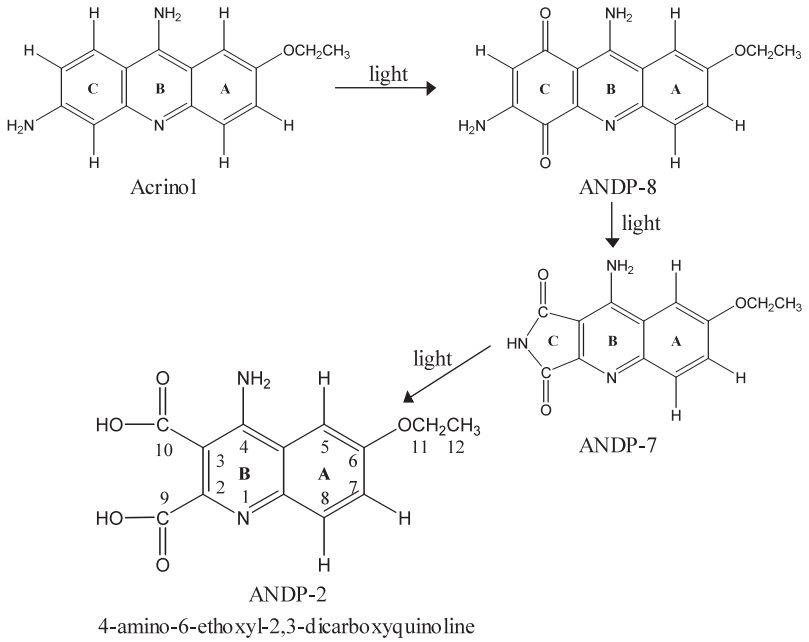

Fig. 6. Degradation Pathway of Acrinol by Light

8) was correlated with δ_C 156.48 (C-6) and δ_C 118.61 (C-4a). The oxygen-bearing methylene proton signal at δ_H 4.18 (H-11) was correlated with δ_C 156.48 (C-6). From these results the structure of ANDP-2 was deduced to be 4-amino-6-ethoxyl-2,3-dicarboxyquinoline as shown in Fig. 4. ANDP-2 is thought to be a novel compound.

At first, ANDP-2 was observed on the HPLC chromatogram of ANDP-7 degradation product after one month irradiation. The concentration of ANDP-7 decreased inversely with respect to the increase of the concentration of ANDP-2 and another degradation products (ANDP-6, -5, -4, -3, -1) with the progress of time. It was assumed that ANDP-7 was converted to ANDP-2 (Fig. 6).

Antimicrobial Activity of ANDP-2

The antimicrobial spectra of ANDP-2, -7,[6] -8[6] and acrinol[5] (no degradation products) determined by agar dilution method are shown in Table 2. ANDP-2 did not show growth inhibition even at a concentration of 100 μg/ml against Gram-positive, -negative bacteria, yeast and fungi. The existence of C-ring is thought to be necessary for antimicrobial activity. The degradation product of acrinol by light were produced after long time irradiation (more than three months). Therefore, the use of acrinol may yield few clinical problems. Further examination of the acrinol degradation products by light will be reported in a separate paper.

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