# ANDP-7, 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. A novel acrinol degradation product, ANDP-7, 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 and Sephadex LH-20. The structural elucidation of ANDP-7 was examined by $^{1}$H-NMR, $^{13}$C-NMR and EI-mass spectra sudies. From the spectroscopic data, the structure of ANDP-7 was determined to be 9-amino-7-ethoxypyrrolo[3,4-*b*]quinoline-1,3-dione, that was found to be a novel degradation product of acrinol by light. Antimicrobial activities of ANDP-7 against Gram-positive and -negative bacteria were 5 to 100-fold higher than those of acrinol. ANDP-7 was also active against yeast and fungi. Nevertheless, acrinol did not show growth inhibition even at a concentration 100 $\mu$g/ml.

**Key words** —— acrinol, acrinol degradation product, acrinol degradation products-7, disinfectant, fluorescence

## INTRODUCTION

Acrinol (2-ethoxyl-6,9-acridinediamine lactate monohydrate) is widely used as a disinfectant in hospitals. Synergistic bactericidal effects of acrinol with other antibiotics and the influence of light on the antimicrobial activity of acrinol solution 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. In the previous paper,[5] we reported a novel acrinol degradation product, acrinol degradation products-8 (ANDP-8) (2-ethoxyl-6,9-acridinediamine-5,8-dione).

In this paper, the isolation and structural elucidation of ANDP-7, another novel acrinol degradation product, together with the antimicrobial activity of this compound, are described.

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## 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 Acrinol Degradation Products** —— HPLC apparatus used was described in the previous paper.[5] The detection of acrinol degradation products was routinely measured by HPLC on an apparatus equipped with a UV detector set at 254 nm. The IR spectrum was taken in KBr tablets on a JEOL JIR-6500W infrared spectrophotometer. Mass spectrum and high resolution mass spectrum (HR-MS) were measured with a JEOL JMS- GC mate spectrometer. $^{1}$H NMR, $^{13}$C NMR, $^{1}$H-$^{1}$H COSY, $^{1}$H-$^{13}$C COSY and HMBC spectra with TMS as internal standard were taken in methanol-d 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 spectram 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-8 and Detection of ANDP-8 Degradation Products** —— 4 sheets of nonwovens (300 × 300 × 1.3 mm) were dampened with methanol solution of ANDP-8 (200 mg/200 ml). Air drying nonwovens dampened with methanol solution of ANDP-8 are irradiated with light for more than three months. The detection of degradation products of ANDP-8 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. The test organisms used for the study are given in Table 2.

**Table 1.** Fluorescence Spectral Data of Acrinol and ANDP-7 in Various Solvents

| | $F\lambda_{max}$ nm (Quantum yield)[a)] | | |
|---|---|---|---|
| | Methanol | Ethanol | Benzene |
| Acrinol | 490 (0.332) | 489 (0.336) | 511 (< 0.01) |
| ANDP-7 | 470 (0.367) | 466 (0.321) | 430 (0.183) |

*a*) excitation at 370 nm.

## RESULTS AND DISCUSSION

### Isolation and Purification of ANDP-7

The mixture of acrinol degradation products was obtained from Nonwovens as described in the previous paper.[5)]

The mixture of acrinol degradation products was adsorbed on Diaion HP-10 column (2.5 × 40 cm). After washing with water, acrinol degradation products were eluted with 20, 40, 60, 80 and 100% methanol (500 ml, each). ANDP-7 was eluted with 80% methanol. 80% methanol fraction contains ANDP-7, -8 and -9. 80% methanol fraction concentrated *in vacuo* was dissolved in a small volume of methanol and applied to Sephadex LH-20 column (1.5 × 70 cm). ANDP-7 was eluted with 70% methanol. The fractions containing ANDP-7 were pooled and concentrated *in vacuo* to gave 50 mg of the reddish orange powder. The yield of purified ANDP-7 was 5%.

### Physico-Chemical Properties of ANDP-7

ANDP-7 was obtained as reddish orange powder with decomposition point at 245°C. It was soluble in methanol, ethanol, slightly soluble in water and insoluble in other common organic solvents. The solution of ANDP-7 has fluorescence with a violet color, though that of acrinol has fluorescence with green. The fluorescence spectrum and florescence quantum yield of ANDP-7 are shown in Fig. 1 and Table 1, respectively. The quantum yield of ANDP-7 was almost the same as that of acrinol. Absorptions were observed at 3380, 3281, 3200, 1715 and 1650 $cm^{-1}$ in the IR spectrum due to the amino and

**Table 2.** Antimicrobial Activities of ANDP-7

| Test organism | MIC ($\mu$g/ml) | | |
|---|---|---|---|
| | ANDP-7 | ANDP-8[5)] | Acrinol[5)] |
| *Bacillus subtilis*[a)] | 10 | 1 | 100 |
| *Staphylococcus aureus*[a)] | 2 | 1 | 100 |
| *Micrococcus luteus*[a)] | 1 | 10 | 100 |
| *Escherichia coli*[a)] | 20 | 20 | 100 |
| *Proteus vulgaris*[a)] | 20 | > 100 | > 100 |
| *Pseudomonas aeruginosa*[a)] | 20 | 100 | 100 |
| *Candida albicans*[b)] | 20 | 10 | > 100 |
| *Saccharomyces cerevisiae*[b)] | 10 | 10 | > 100 |
| *Aspergillus niger*[b)] | 10 | 10 | > 100 |
| *Aspergillus oryzae*[b)] | 10 | 10 | > 100 |
| *Penicillium chrysogenum*[b)] | 10 | 10 | > 100 |

*a*) bouillon agar, *b*) glucose bouillon agar.

imido group, respectively.

The molecular ion peak of ANDP-7 was obtained at *m/z* 257 ($M^+$) by EI-MS. The elementary analysis of ANDP-7 generated $C_{13}H_{11}N_3O_3$ as the molecular formula, which agreed with the *m/z* 257 ($M^+$) as ion peak on the EI-MS and agreed with the *m/z* 257.08215 as ion peak on HR-MS ($C_{13}H_{11}N_3O_3$ = 257.08002, error –2.1 mili mass). The presence of imido group in ANDP-7 was revealed by the fragment ion corresponding to loss of *m/z* 28, 57(28 + 29) and 71 from molecular ion. The fragment ion peaks at *m/z* 186 indicate A and B rings of acrinol (Fig. 2). Imido group was thought to form the new ring of ANDP-7. The presence of imido group was supported by $^{13}C$ NMR spectral data, which are summarized in Table 3. The $^{13}C$ NMR spectrum of ANDP-7 showed the 13 carbons. The analysis of DEPT spectrum indicated that ANDP-7 consisted of the following functional groups : $CH_3 \times 1$, $CH_2$-O × 1, CH = × 3, C = × 6, C = O × 2.

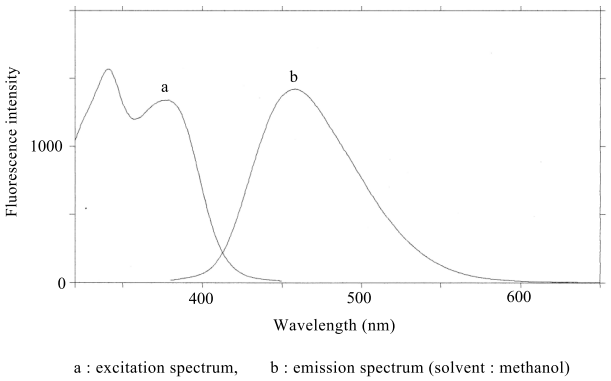


**Fig. 1.** Excitation and Fluorescence Spectrum of ANDP-7

## Structural Elucidation of ANDP-7

In the $^1H$ NMR spectrum of ANDP-7, a series of three coupled aromatic protons were observed at $\delta_H$ 7.84 (dd), 7.52 (d) 7.34 (d). Resonances for a C-methyl ($\delta_H$ 1.38) and an O-methylene proton ($\delta_H$ 4.12) quartet were also observed. The $^1H$ NMR spectrum could account for eight protons. In the $^1H$-$^1H$ COSY spectrum the aromatic proton signal at $\delta_H$ 7.34 (H-6) was correlated with $\delta_H$ 7.84 (H-5). The methyl proton signal at $\delta_H$ 1.38 (H-11) was correlated with $\delta_H$ 4.12 (H-10). In the $^{13}C$ NMR spectrum of ANDP-7 resonances for one methyl carbon ($\delta_C$ 15.02) and an oxygen-bearing methylene carbon ($\delta_C$ 65.09), nine resonances for aromatic-type carbons, one of which was oxygen-bearing carbon ($\delta_C$ 159.69), three of which were substituted by proton ($\delta_C$ 132.77, 124.86, 104.74), and five of which were without proton (169.53, 151.42, 150.97, 146.65, 122.29) indicated the A and B rings of acrinol (Fig. 3), which was also supported by the EI-MS fragmentation ion peak of ANDP-7 (*m/z* 186). In the $^{13}C$ NMR spectrum of ANDP-7 resonances for two carbonyl carbons ($\delta_C$ 171.33, 171.33) formed a new ring of ANDP-7. Two carbonyl carbons were thought to be imido, also supported by EI-MS fragmentation ions peak of ANDP-7 (*m/z* 229, 200, 186).

In the HMBC experiments (Fig. 4) the aromatic proton signals at $\delta_H$ 7.52 (H-8), 7.34 (H-6) and 7.84

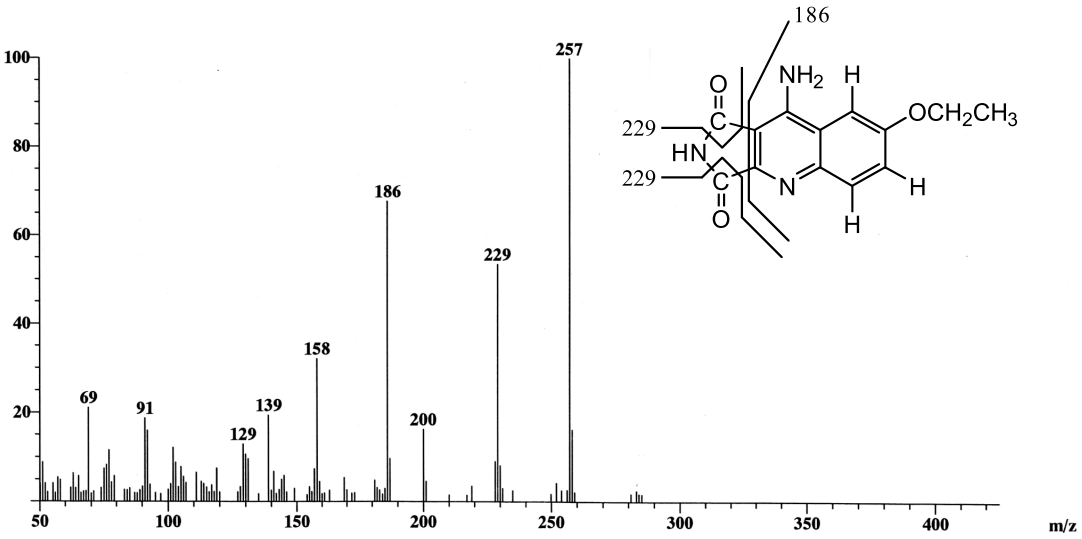


**Fig. 2.** EI-MS Spectrum of ANDP-7

(H-5) were correlated with $\delta_C$ 124.86 (C-6), $\delta_C$ 146.65 (C-4a), $\delta_C$ 159.69(C-7) and $\delta_C$ 122.29 (C-8a), respectively. The oxygen-bearing methylene proton signal at $\delta_H$ 4.12 (H-10) was correlated with $\delta_C$ 159.69 (C-7). From these results the structure of ANDP-7 was deduced to be 9-amino-7-ethoxypyrrolo[3,4-*b*]quinoline-1,3-dion as shown in Fig. 3. ANDP-7 is thought to be a novel compound.

**Table 3.** NMR Spectral Data for ANDP-7 in $CD_3OD$

| position | $\delta_C$ | $\delta_H$ |
|---|---|---|
| 1 | 171.33 | |
| 3 | 171.33 | |
| 3a | 169.53 | |
| 4a | 146.65 | |
| 5 | 132.77 | 7.84 (dd, *J* = 9.1, 2.4, 1 H) |
| 6 | 124.86 | 7.34 (d, *J* = 9.1, 1 H) |
| 7 | 159.69 | |
| 8 | 104.74 | 7.52 (d, *J* = 2.3, 1 H) |
| 8a | 122.29 | |
| 9 | 150.97 | |
| 9a | 151.42 | |
| 10 | 65.09 | 4.12 (ddq, *J* = 6.7, 7.3, 6.7, 2 H) |
| 11 | 15.02 | 1.38 (t, *J* = 6.7, 3 H) |

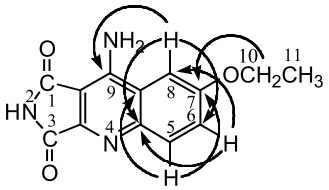

**Fig. 3.** Deduced Structure of ANDP-7

At first, ANDP-7 was observed on the HPLC chromatogram of ANDP-8 degradation product after one month irradiation. The concentration of ANDP-8 decreased inversely with respect to the increase of the concentration of ANDP-7 and another degradation products (ANDP-6, -5, ⋯-1) with the progress of time. It was assumed that ANDP-8 was converted to ANDP-7. This reaction was thought to be rearrangement by light.

## Antimicrobial Activity of ANDP-7

The antimicrobial spectra of ANDP-7, -8[5] and acrinol[5] determined by agar dilution method are shown in Table 2. ANDP-7 was active mainly against Gram-positive bacteria, and in particular the minimum inhibitory concentration against *Micrococcus luteus* or *Stapylococcus aureus* on bouillon agar were 1 or 2 $\mu$g/ml, respectively. This compound was also active against Gram-negative bacteria. Antimicrobial activities of ANDP-7 against Gram-positive and Gram-negative bacteria were 5 to 100-fold higher than those of acrinol and was thought to be due to the imido group in ANDP-7. This compound was also active against yeast and fungi. Nevertheless, the acrinol did not show growth inhibition even at a concentration of 100 $\mu$g/ml.

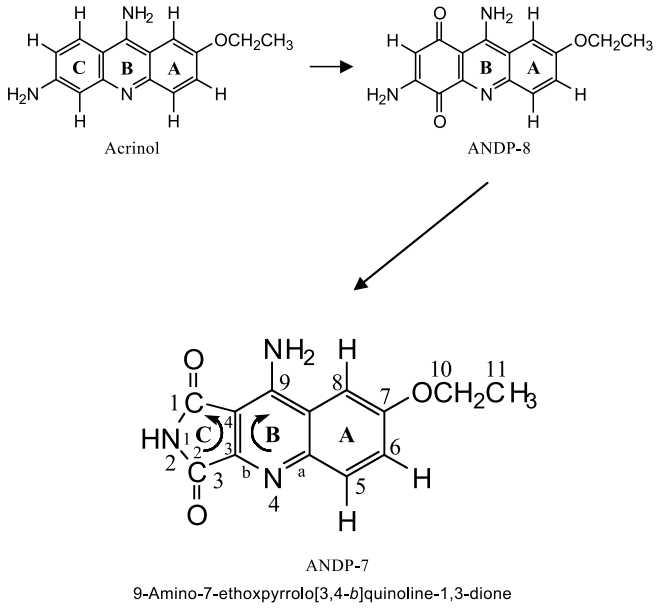


**Fig. 4.** $^1H$-$^{13}C$ Connectivities of ANDP-7 as Revealed by HMBC Experiments

Further examination of the acrinol degradation products by light will be reported in a separate paper.

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