Acrinol Degradation Products (ANDP)-8, A Novel Acrinol Degradation Product by Light

Teruo Tanaka,*,[a] Tomonori Iijima,[a] Kiyoka Nakamoto,[a] Yuki Kitaguchi,[a] Katushi Takeda,[a] Toshiharu Tanaka,[b] and Yutaka Kido[a]

[a]Division of Instrumental Analysis, Institute of Resource Development and Analysis, Kumamoto University, 5–1, Oe-honmachi, Kumamoto 862–0973, Japan and [b]Aso Pharmaceutical Co., Ltd., 91–1, Tsukure, Kikuyomachi, Kikuchi-gun, Kumamoto 869–1101, Japan

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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-8, 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-8 was examined by infrared, ^{1}H-NMR, ^{13}C-NMR and electron impact ionization (EI)-mass spectra studies. From the spectroscopic data, the structure of ANDP-8 was determined to be 2-ethoxyl-6, 9-acridinediamine-5, 8-dione, that was found to be a novel degradation product of acrinol by light. Antimicrobial activities of ANDP-8 against Gram-positive bacteria were 10 to 100-fold higher than those of acrinol. ANDP-8 was also active against yeast and fungi. Nevertheless, acrinol did not show growth inhibition even at a concentration of 100 μg/ml.

Key words —— acrinol, acrinol degradation product-8, antimicrobial activity, disinfectant, acrinol degradation product

INTRODUCTION

Acrinol (2-ethoxyl-6,9-acridinediamine lactate monohydrate) is widely used as a disinfectant in hospital. Synergistic effects of acrinol with other antibiotics and the influence of light on the antimicrobial activity of acrinol solution have been reported.[1–4] Degradation of acrinol by light, however, has not been clarified, although color change of the acrinol liniment or ointment occurs by light irradiation.

In this paper, isolation and structural elucidation of acrinol degradation products (ANDP)-8 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 Acrinol Degradation Products —— HPLC was done using jacketed stainless steel analytical columns (25 × 4.0 mm i.d.) packed Mightysil RP-18 (5 μm). The mobile phase was CH_3OH-H_2O (70 : 30, v/v) containing 0.005 M heptanesulfonic acid. The detection of acrinol degradation products was routinely measured by HPLC on an apparatus equipped with a UV detector set at 254 nm. HPLC chromatogram of acrinol degradation products by light is shown in Fig. 1. The IR spectrum was taken in KBr tablets on a JEOL JIR-6500W infrared spectrophotometer. Mass spectrum was 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 heteronuclear multiple bond correlation (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)

Degradation of Acrinol —— 20 sheets of nonwovens (300 × 300 × 1.3 mm) were dampened with methanol solution of acrinol (1 g/l). Air-drying nonwovens dampened with methanol solution of acrinol are irradiated with light or UV light for more than three months. After irradiation, acrinol degradation products were obtained from the methanol extracts of this cloth. Methanol extraction (500 ml) was done 3 times at room temperature.

*To whom correspondence should be addressed: Division of Instrumental Analysis, Institute of Resource Development and Analysis, Kumamoto University, 5–1, Oe-honmachi, Kumamoto 862–0973, Japan. Tel. & Fax: +81-96-371-4809; E-mail: tfujii@gpo.kumamoto-u.ac.jp

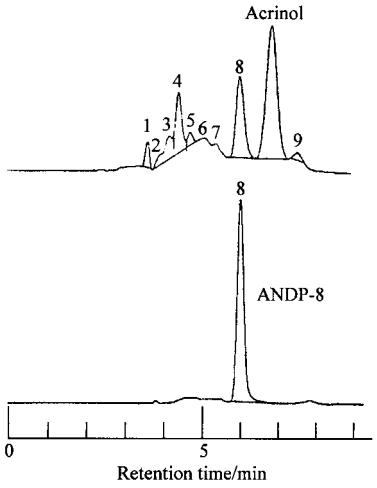

Fig. 1. HPLC Chromatogram of Acrinol Degradation Products by Light

Table 1. Antimicrobial Activities of Acrinol and ANDP-8

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

a) Bouillon agar, *b*) Glucose bouillon agar.

Antimicrobial Activity —— The conventional serial agar dilution method was applied in this study using a bouillon agar (Ehrlich meat extract 0.5%, Polypepton 1.0%, NaCl 0.3%, Agar 2.0%) or glucose bouillon agar (Ehrlich meat extract 0.5%, Polypepton 1.0%, NaCl 0.3%, Agar 2.0%, Glucose 1.0%) as an assay medium. The test organisms used for the study are given in Table 1.

RESULTS AND DISCUSSION

Degradation of Acrinol and Isolation of ANDP-8

HPLC chromatogram of methanol extract from nonwovens, which were dampened with methanol solution of acrinol and allowed to stand for more than three months in the dark room, revealed only acrinol. Even after more than three months of light irradiation, acrinol solution and acrinol powder were only a little degraded. Degradation of acrinol was carried out by the above method. More than nine components were detected by HPLC in the mixture of acrinol degradation products extracted with methanol from nonwovens irradiated with light or UV light. (Fig. 1) Both degradation products contained the same components. Methanol extract from nonwovens 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-8 was eluted with 80% methanol. 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-8 was eluted with 80% methanol. The fraction containing ANDP-8 were pooled and concentrated *in vacuo* to form red powder. The purity of ANDP-8 was measured by HPLC.

Physico-Chemical Properties of ANDP-8

ANDP-8 is a dull red amorphous powder with MP at 292–294°C. It is soluble in mehanol, ethanol, slightly soluble in water and insoluble in other common organic solvents. The solution of ANDP-8 was non-fluorescent. The infrared absorption spectrum of ANDP-8 in KBr disk is shown in Fig. 2.

Absorptions were observed at 3386, 3286, 3201, 1686 and 1618 cm^{-1} in the IR spectrum due to the amino and carbonyl groups, respectively.

The molecular ion peak of ANDP-8 was obtained at *m/z* 283 (M^+) by electron impact ionization (EI)-MS. The elementary analysis of ANDP-8 generated $C_{15}H_{13}N_3O_3$ as the molecular formula, which agreed with the *m/z* 283 (M^+) as ion peak on the EI-MS. The molecular ion peak of acrinol is *m/z* 253, which indicated that ANDP-8 is a derivative of acrinol. The presence of two carbonyl groups in ANDP-8 was revealed by the fragment ion corresponding to a loss of *m/z* 27, 55 (27 + 28) from molecular ion. The fragment ion peaks at *m/z* 228 and 256 (Fig. 3) indicated that two carbonyl moieties were substituted in acrinol. This observation was supported by the ^{13}C NMR spectral data, which is summarized in Table 2. The ^{13}C NMR spectrum of ANDP-8 showed the 15 carbons. The analysis of DEPT spectrum indicated that ANDP-8 consisted of the following functional groups : $CH_3 \times 1$, CH_2-O × 1, CH= × 4, C= ×

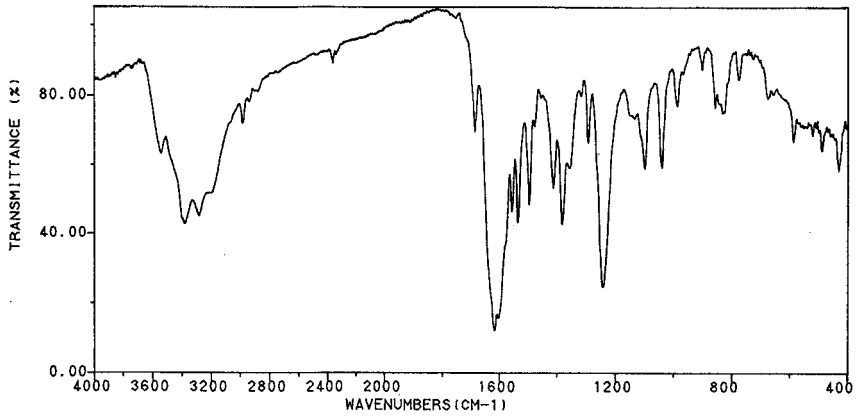

Fig. 2. IR Spectrum of ANDP-8 (KBr)

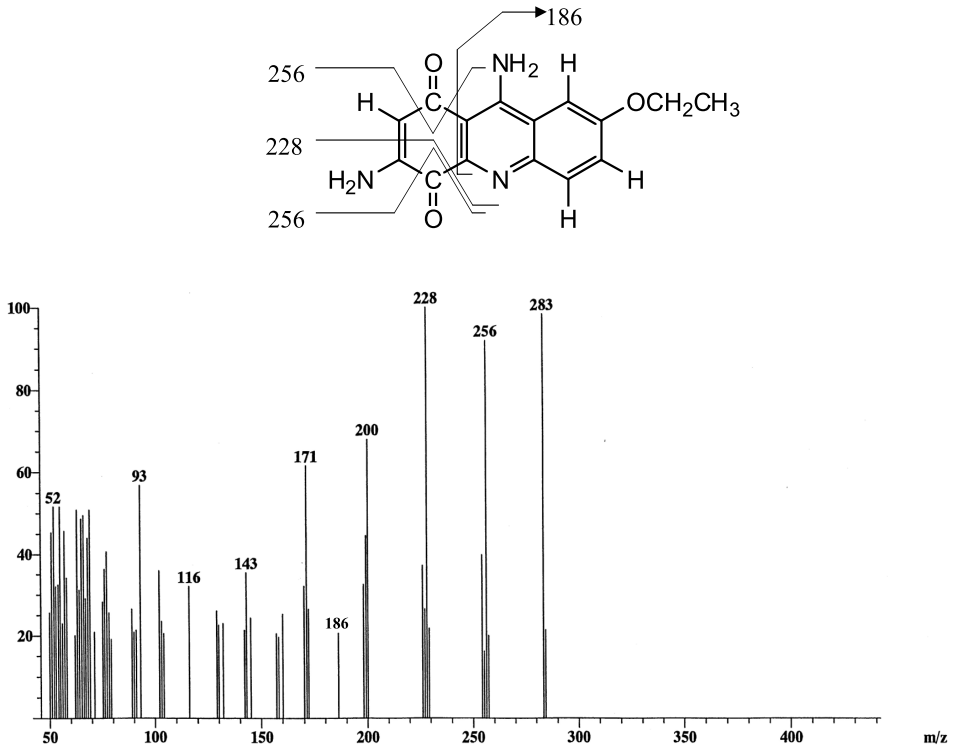

Fig. 3. EI-MS Spectrum of ANDP-8

7, C=O × 2.

Structural Elucidation of ANDP-8

In the ^{1}H NMR spectrum of ANDP-8, two coupled aromatic protons were observed at δ_H 7.93 (d) and δ_H 7.36 (dd), and two singlet signals were observed at δ_H 7.52 and δ_H 5.86. Resonance for a methyl (δ_H 1.47) triplet and an O-methylene (δ_H 4.19) double doublet were also observed. The ^{1}H NMR spectrum could account for nine protons. In the ^{1}H-^{1}H COSY spectrum of ANDP-8 the aromatic proton signal at δ_H 7.36 (H-3) was correlated with δ_H 7.93 (H-4). The methyl proton signal at δ_H 1.47 (H-11) was correlated with δ_H 4.19 (H-10). In the ^{13}C NMR spectrum of ANDP-8 resonances for one methyl carbon (δ_C 15.04) and an oxygen-bearing methylene carbon (δ_C 65.42), nine resonances for aromatic-type carbons, one of which was oxygen-bearing carbon (δ_C 160.23), three of which were substituted by proton (δ_C 132.85, 124.99, 103.71), and five of which

Table 2. NMR Spectral Data for ANDP-8 in CD_3OD

	ANDP-8		Acrinol
position	δ_C	δ_H	δ_C
1	103.71	7.52 (s, 1H)	103.50
2	160.23		156.10
3	124.99	7.36 (dd, J = 9.13, 2.44, 1H)	127.21
4	132.85	7.93 (d, J = 9.13, 1H)	120.09
4a	143.66		135.20
4b	146.32		143.09
5	181.89		96.0
6	150.77		156.02
7	105.75	5.86 (s, 1H)	116.96
8	189.58		126.02
8a	105.62		104.10
9	154.17		154.96
9a	122.97		113.02
10	65.42	4.19 (ddq, J = 6.72, 7.32, 6.72, 2H)	65.40
11	15.04	1.47 (t, J = 6.72, 3H)	15.02

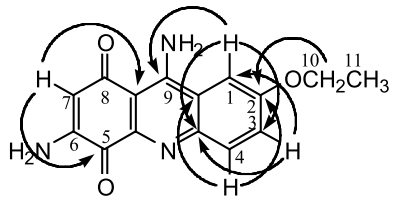

Fig. 4. ^1H-^{13}C Connectivities of ANDP-8 as Revealed by HMBC Experiments

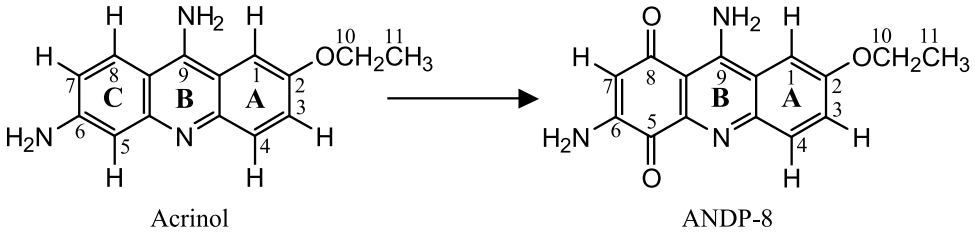

Fig. 5. Deduced Structure of ANDP-8

were without proton (154.17, 146.32, 143.66, 122.97, 105.62) indicated the A and B rings of acrinol (Fig. 3) which were also supported by the EI-MS spectral data (*m/z* 186). In the ^{13}C NMR spectrum of ANDP-8 resonances for two carbonyl carbons (δ_C 189.58, 181.89), two aromatic-type carbons, one of which was substituted by proton (δ_C 105.75), and the other was without proton (δ_C 150.77) formed the new ring of ANDP-8. In the HMBC experiments (Fig. 4) four aromatic proton signals at δ_H 7.52 (H-1), 7.36 (H-2), 7.93 (H-3) and 5.86 (H-7) were correlated with δ_C 124.99 (C-3), δ_C 143.66 (C-4a), δ_C 103.71(C-1), δ_C 143.66 (C-4a), δ_C 160.23 (C-2), δ_C 122.97 (C-9a), δ_C 181.89 (C-5) and δ_C 105.62 (C-8a), respectively. The oxygen-bearing methylene proton signal at δ_H 4.19 (H-10) was correlated with δ_C 160.23 (C-2). From these results the structure of ANDP-8 was deduced to be 2-ethoxyl-6, 9-acridiamine-5, 8-dione as shown in Fig. 5.

ANDP-8 is thought to be a novel compound. ANDP-8 is not a fluorescent substance, while acrinol is fluorescent. This difference may be explained by

the presence of two carbonyl groups.

Antimicrobial Activity of ANDP-8

The antimicrobial spectra of ANDP-8 and acrinol as determined by the agar dilution method are shown in Table 1. ANDP-8 was active mainly against Gram-positive bacteria, and in particular the minimum inhibitory concentration against *Bacillus subtilis* or *Stapylococcus aureus* on bouillon agar was 1 μm/ml. This compound was also active against yeast and fungi. Nevertheless, the acrinol did not show growth inhibition even at a concentration of 100 μg/ml using agar dilution method on the glucose bouillon agar against the following microorganisms: *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium chrysogenum*.

Antimicrobial activities of ANDP-8 against bacteria, yeast and fungi were 10 to 100-fold higher than those of acrinol and was thought to be due to the quinone skeleton (C ring) in ANDP-8.

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

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