

## Photodegradation Products of a New Antibacterial Fluoroquinolone Derivative, Orbifloxacin, in Aqueous Solution

Tetsu MORIMURA,\* Yoshifumi NOBUHARA, and Hayashi MATSUKURA

Department of Chemical Analysis, Pharmaceutical Research Laboratories, Dainippon Pharmaceutical Co., Ltd., 1-5-51, Ebie, Fukushima-ku, Osaka 553, Japan. Received August 2, 1996; accepted October 7, 1996

A new antibacterial fluoroquinolone derivative, orbifloxacin (ORFX), is decomposed photochemically in aqueous solution. When ORFX solution was irradiated with a chemical lamp or sunlight, three major photodegradation products were isolated by preparative HPLC. These degradation products were identified by electron-impact mass spectrometry, liquid-secondary-ion mass spectrometry and <sup>1</sup>H-NMR spectroscopy.

Moreover, the photodegradation pathway was investigated by a similar study using several fluoroquinolone derivatives which were presumed to be the intermediates of the photoreaction of ORFX. Consequently, it was found that two main photochemical reactions, the decomposition of the dimethylpiperazinyl moiety and the elimination of the cyclopropyl group, take place in ORFX. The detected structures of photodegradation products and the photodegradation studies of the postulated intermediates suggested that the photodecomposition of the dimethylpiperazinyl ring at the 7-position and the elimination of the cyclopropyl group at the 1-position occurred concurrently with the release of fluorine at the 8-position.

**Key words** photodegradation product; photodegradation pathway; fluoroquinolone derivative; orbifloxacin

Fluoroquinolone derivatives have been widely used for the treatment of infections, and their mechanism of the action has been considered to be the inhibition of DNA gyrase. The photochemistry of fluoroquinolone derivatives has been considered important in the dermatological field.<sup>1-7)</sup>

Some fluoroquinolone derivatives are easily decomposed in aqueous solutions photochemically, and there have been some reports on the photochemistry of fluoroquinolones. Ferguson *et al.*<sup>8)</sup> determined that UV irradiation of ciprofloxacin reduced antibacterial activity due to photodegradation of the drug. Tiefenbacher *et al.*<sup>9)</sup> reported photodegradation products of some fluoroquinolones, and concluded that quinolone drugs should be strictly protected from any light during storage and administration.

A new fluoroquinolone, orbifloxacin (ORFX), is a synthetic antimicrobial agent with broad-range activity against gram-negative and gram-positive organisms, and it has been prepared in injections for the treatment of animal infections. Although ORFX is extremely thermostable,<sup>10)</sup> its photodegradation takes place rapidly in aqueous solution.<sup>11)</sup>

In a previous report<sup>11)</sup> on the photodegradation kinetics of ORFX, we discussed some factors affecting its photostability such as light source, initial concentration of the drug and pH of the solution. Furthermore, it was reported<sup>11)</sup> that there were large differences in photostability among cationic, anionic and neutral forms (or zwitterion) of the compound.

This paper describes the photodegradation products of ORFX after irradiation with sunlight or a chemical lamp in aqueous solution.

### Experimental

**Materials** ORFX and authentic compounds were prepared in our laboratories (Fig. 1). These compounds are as follows: ORFX (1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid); AT-4929 (1-cyclopropyl-5,6-difluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-

3-carboxylic acid); AT-6445 (7-(2-aminopropylamino)-1-cyclopropyl-5,6-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid); AT-6855 (7-(2-aminopropylamino)-1-cyclopropyl-5,6,8-trifluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid); AT-7592 (5,6,8-trifluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid). All other chemicals were of reagent grade.

**Photodegradation Study** Each of ORFX, AT-4929, AT-6855 and AT-7592 was dissolved in water (0.01 mg/ml), and these solutions were used as the sample solutions. Twenty-five milliliters of each of these sample solutions was put into a test tube of Pyrex glass with a stopper, and the exposure test was performed at room temperature by irradiating these sample solutions in a distance of approximately 200 mm with a chemical lamp (wavelength; about 300–400 nm, FL20S BL, 20W, Toshiba Electric Co., Ltd., Tokyo). At appropriate time intervals, a portion (0.4 ml) of the sample solution was withdrawn from the test tube and assayed by quantitative HPLC.

**Isolation of Photodegradation Products** ORFX (500 mg) was dissolved in 1000 ml of water and this solution was exposed to sunlight or a chemical lamp for 4–6 h. The sample solution after irradiation was adjusted to pH 6–7 with 0.1 N NaOH or 0.1 N HCl solution, then loaded

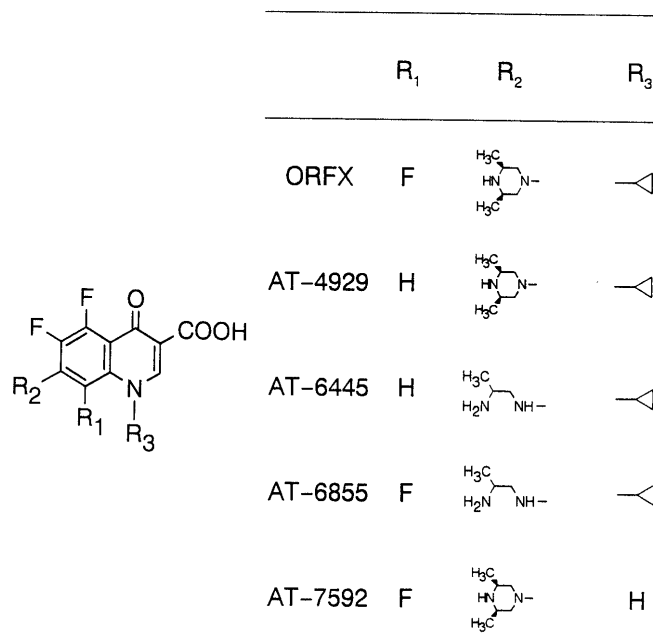


Fig. 1. Structures of Fluoroquinolones

\* To whom correspondence should be addressed.

onto a Sep-Pak cartridge (Waters, 20 ml). The cartridge was washed with distilled water and eluted with methanol. The eluted solution was concentrated and fractionated by preparative HPLC. The collected fractions were evaporated to concentrate under a vacuum at 60°C, neutralized with HCl or NaOH solution and loaded onto a Sep-Pak cartridge. The cartridge was washed with distilled water. Photodegradation products were obtained by elution from the cartridge with methanol and the eluted solution was evaporated to dryness under a vacuum.

**Preparative HPLC** The photochemical degradation products were isolated by means of preparative HPLC consisting of a Waters 600F pump equipped with a Waters 600E system controller, a Waters 170 sample loader, a Waters Fraction Collector and a Shimadzu SPD-6A UV spectrophotometric detector. A Develosil ODS-7 column (7  $\mu$ m, 250  $\times$  8 mm i.d.) was used. A mixture of 0.1 M citrate buffer (pH 3.5)–methanol–dioxane (84:12:5) was used as the mobile phase at a flow rate of 4.0 ml/min. The column eluent was monitored at 290 nm.

**Quantitative HPLC** The HPLC system consisted of a Shimadzu LC-9A pump, an SIL-6A auto-injector, an SPD-2A UV spectrophotometric detector, a C-R5A Chromatopac and a reversed-phase column (Develosil ODS-7, 250  $\times$  4 mm i.d.). HPLC conditions were the same as in preparative HPLC.

**Mass Spectrometry** Liquid-secondary-ion (LSI) mass spectra and electron-impact (EI) mass spectra were obtained with a Hitachi M-80B mass spectrometer using a direct inlet system.

**<sup>1</sup>H-NMR** <sup>1</sup>H-NMR spectra were obtained at 300 MHz in DMSO-*d*<sub>6</sub> using a Varian XL 300 NMR spectrometer.

## Results and Discussion

**Structure Elucidation of Photodegradation Products of ORFX** Although ORFX is extremely thermostable, it decomposes rapidly in aqueous solution under irradiation with a chemical lamp or a fluorescent lamp. Its

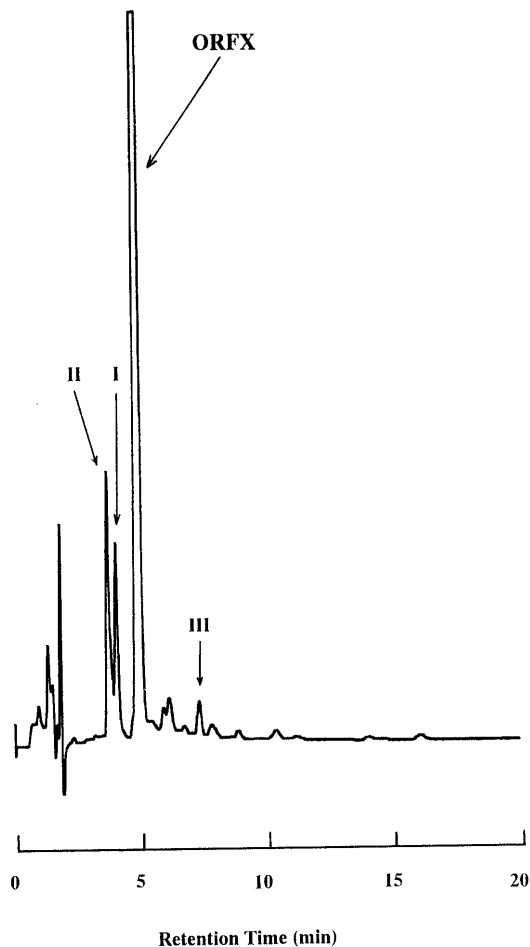


Fig. 2. HPLC Chromatogram of ORFX Solution (pH 6.2) after Irradiation with a Chemical Lamp for 20 min

photodegradation rate is strongly dependent on the pH of the solution.<sup>11)</sup>

A HPLC chromatogram of ORFX solution (0.01 mg/ml, pH 6.2) irradiated with a chemical lamp is shown in Fig. 2. There were at least 10 degradation products in the resulting solution.

The time course of the photodegradation of ORFX in solution (0.01 mg/ml, pH 6.2) is shown in Fig. 3. According to the progress of the reaction, the concentration of compound I increases with time. In contrast, the concentration of compound II first increases, reaches a maximum, and then decreases. After a time lag of about 10 min, the concentration of compound III increases with time.

Moreover, compound II isolated by preparative HPLC was dissolved in water (0.01 mg/ml) and this solution was irradiated with a chemical lamp for 30 min before HPLC assay.

In this experiment, the photodegradation of compound II yielded compound III, but the formation of compound III from compound II in aqueous solution was not observed under a light-resistant condition. These results suggest that the photodegradation of ORFX yielding compound III involves compound II as an intermediate.

For the isolation and identification of the photochemical degradation products of ORFX, the sample solution was irradiated with sunlight or a chemical lamp. The same degradation products were detected by irradiation of both light sources. Three degradation products, compounds I, II and III, were isolated from the solution by preparative HPLC.

Compound I was eluted at 4.0 min on the reversed-phase HPLC. The formation of compound I was observed mainly under acidic and neutral conditions rather than in an alkaline condition. The LSI mass spectrum of compound I showed a protonated molecule ( $M+H$ )<sup>+</sup> at  $m/z$  338. In

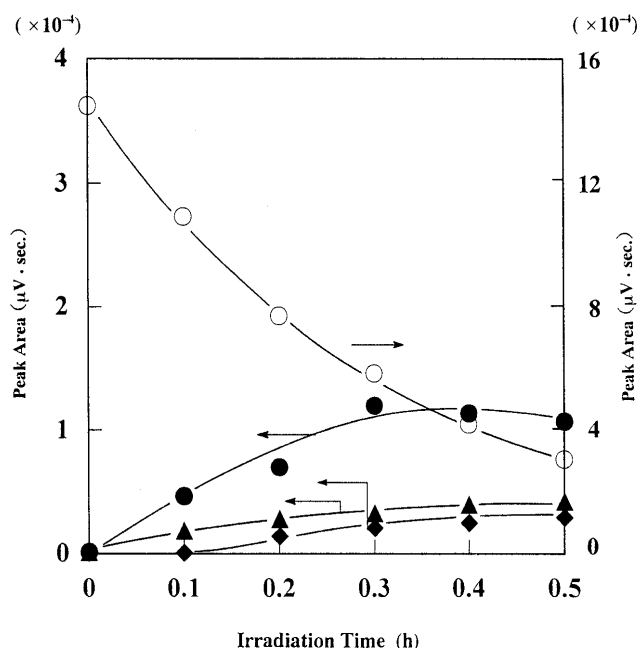


Fig. 3. Time Course of Photodegradation of ORFX in solution (pH 6.2)

○, ORFX; ▲, compound I; ●, compound II; ◆, compound III.

Table 1. <sup>1</sup>H-NMR Chemical Shifts (ppm) for ORFX and Compound I in DMSO-*d*<sub>6</sub>

H position	ORFX		Compound I	
	Chemical shift (ppm)	<i>J</i> (Hz)	Chemical shift (ppm)	<i>J</i> (Hz)
-CH <sub>2</sub> -CH(CH <sub>3</sub> )-NH-CH(CH <sub>3</sub> )-CH <sub>2</sub> -	0.99 (d)	6.2	1.05 (d)	6.4
-CH <sub>2</sub> -CH(CH <sub>3</sub> )-NH-CH(CH <sub>3</sub> )-CH <sub>2</sub> -	2.76 (m) <sup>a)</sup>		2.36 (m) <sup>a)</sup>	
	3.35 (m) <sup>b)</sup>		3.42 (m) <sup>b)</sup>	
-CH <sub>2</sub> -CH(CH <sub>3</sub> )-NH-CH(CH <sub>3</sub> )-CH <sub>2</sub> -	2.89 (m)		3.02 (m)	
CH <sub>2</sub>   >CH- CH <sub>2</sub>	1.11—1.18 (m)		—	
CH <sub>2</sub>   >CH- CH <sub>2</sub>	4.10 (m)		—	
C <sub>2</sub> -H	8.63 (s)		8.64 (s)	
C <sub>8</sub> -H	—		6.93 (dd)	7.8, 2.2
3-COOH	n.d. <sup>c)</sup>		17.76 (brs)	

a) Axial proton. b) Equatorial proton. c) Not detected.

Table 2. <sup>1</sup>H-NMR Chemical Shifts (ppm) for Compound II and III in DMSO-*d*<sub>6</sub>

H position	Compound II		Compound III	
	Chemical shift (ppm)	<i>J</i> (Hz)	Chemical shift (ppm)	<i>J</i> (Hz)
CH <sub>3</sub> -CH(NH <sub>2</sub> )-CH <sub>2</sub> -NH-	1.08 (d)	5.6	1.09 (d)	5.9
CH <sub>3</sub> -CH(NH <sub>2</sub> )-CH <sub>2</sub> -NH-	3.10—3.25 (m)		3.19 (m)	
CH <sub>3</sub> -CH(NH <sub>2</sub> )-CH <sub>2</sub> -NH-	7.32 (s)		7.02 (s)	
CH <sub>2</sub>   >CH- CH <sub>2</sub>	1.03—1.35 (m)		1.18 (m)	
CH <sub>2</sub>   >CH- CH <sub>2</sub>	3.69 (m)		3.66 (m)	
C <sub>2</sub> -H	8.54 (brs)		8.50 (s)	
C <sub>8</sub> -H	7.01 (dd)	7.1, 1.4	6.67 (d)	6.6
3-COOH, 5-OH, -NH <sub>2</sub>			8.30 (brs)	

the EI mass spectrum of compound I, fragment ions were observed at *m/z* 293 (M - CO<sub>2</sub>) and *m/z* 223 (M - CO<sub>2</sub> - C<sub>4</sub>H<sub>8</sub>N).

The <sup>1</sup>H-NMR data of compound I and ORFX are shown in Table 1. In the NMR spectrum of compound I, the signals at δ 1.05 (6H), 2.36 (2H), 3.02 (2H), 3.42 (2H), 8.64 (1H) and 17.76 (1H) suggest that the dimethylpiperazinyl ring and carboxyl group remain unchanged. On the other hand, signals derived from the cyclopropyl ring protons are not observed. Moreover, an aromatic proton appears at δ 6.93, and the coupling constants (7.8, 2.2 Hz) are consistent with the coupling constant of an aromatic proton with *m*- and *p*-fluorine<sup>1,2)</sup> (4—8 Hz and 0—3 Hz, respectively). This result shows that a fluorine atom disappears and a hydrogen attaches to the 8-position.

From the MS and NMR information, it was concluded that compound I is 5,6-difluoro-1,4-dihydro-7-(*cis*-3,5-dimethyl-1-piperazinyl)-4-oxoquinoline-3-carboxylic acid, which is shown in Chart 1.

Compound II, eluted at 3.7 min on the HPLC, represents the major photodegradation product of ORFX in aqueous media. The LSI mass spectrum of compound II showed a protonated molecule (M + H)<sup>+</sup> at *m/z* 338, the same as compound I. In the EI mass spectrum of compound II, fragment ions were observed at *m/z* 337 (M<sup>+</sup>) and *m/z* 293 (M - CO<sub>2</sub>). On the other hand, typical fragments from

the ORFX, *m/z* M - 70 (M - C<sub>4</sub>H<sub>8</sub>N) and M - 114 (M - CO<sub>2</sub> - C<sub>4</sub>H<sub>8</sub>N), were not observed. These facts indicate the absence of a dimethylpiperazinyl group in the molecule.

In the <sup>1</sup>H-NMR data of compound II (Table 2), the signals at δ 1.03—1.35 (4H), δ 3.69 (1H) and δ 8.54 (1H) indicate that the cyclopropyl group remains unchanged. Then, the signal appearing at δ 7.32 as a broad peak, which disappears after the addition of a drop of D<sub>2</sub>O, can be assigned to an exchangeable proton of the -NH group. The signals at δ 1.08 and δ 3.10—3.25, whose integrated area corresponds to the 6 protons, are attributable to the aminopropylamino moiety. The <sup>1</sup>H-NMR spectrum reveals a new aromatic proton at δ 7.01 with coupling constants of 7.1 and 1.4 Hz. This spectral data indicates the replacement of a fluorine atom at the 8-position with a hydrogen.<sup>1,2)</sup>

Based on the MS and NMR information, the authentic compound, AT-6445, was synthesized. The identity of compound II and AT-6445 was confirmed by the identity of the MS and <sup>1</sup>H-NMR data. The structure of compound II is concluded to be AT-6445, which is shown in Chart 1.

Compound III, eluted at 7.2 min on the HPLC, is predominantly represented in an alkaline aqueous media. The LSI mass spectrum showed a molecular ion (M + H)<sup>+</sup>

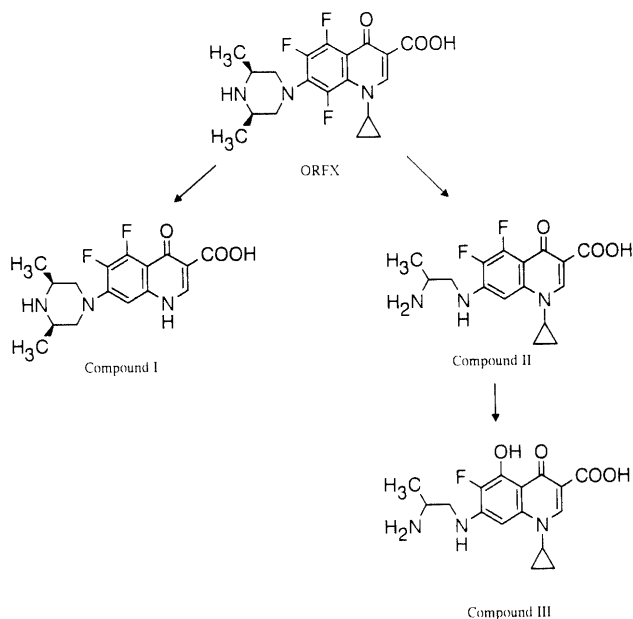


Chart 1. Postulated Photodegradation Pattern of ORFX

peak at  $m/z$  336.

The  $^1\text{H-NMR}$  data of compound III are shown in Table 2. The  $^1\text{H-NMR}$  spectrum of compound III is almost the same as that of compound II. A new signal for an aromatic proton at  $\delta$  6.67 with a coupling constant of 6.6 Hz suggests that a proton is attached at the 8-position. Additionally, the signal at  $\delta$  -163.53, with a coupling constant of 6.0 Hz, is observed from  $^{19}\text{F-NMR}$  spectrometry analysis. These results suggest that the fluorine atoms at the 5 and 8-positions disappear, while a fluorine atom at the 6-position remains.<sup>12)</sup> Moreover, the formula of compound III is assumed to be  $\text{C}_{16}\text{H}_{18}\text{FN}_3\text{O}_4$  from the LSI mass spectral data, so the substituent group at the 5-position is considered to be an OH group.

Then, from the MS and NMR information, it is concluded that compound III is 7-(2-aminopropylamino)-1-cyclopropyl-6-fluoro-1,4-dihydro-5-hydroxy-4-oxoquinoline-3-carboxylic acid, as shown in Chart 1.

The postulated pathway for the photodegradation of ORFX in solution is shown in Chart 1.

**Reaction Mechanism** From the results of the structural elucidation of the photodegradation products, irradiation to an ORFX solution seems to cause two types of reactions and these reactions seem to be dependent on the pH of the solution. One is the photodecomposition of the dimethylpiperazinyl moiety at the 7-position with the cleavage of a C-F bond at the 8-position, and another is the elimination of a cyclopropyl group at the 1-position with the cleavage of a C-F bond at the 8-position. These facts suggest that the elimination of a fluorine atom at the 8-position occurs in the early stage of the photochemical reaction of ORFX.

Thus, it was presumed that the degradation of ORFX had to go through intermediates, AT-4929, AT-6855 or AT-7592, before compound I and II could be produced. However, AT-4929, AT-6855 and AT-7592 were not detected in the chromatogram obtained from the sample solution after irradiation with sunlight or a chemical lamp. Furthermore, the direct photodegradation of AT-4929,

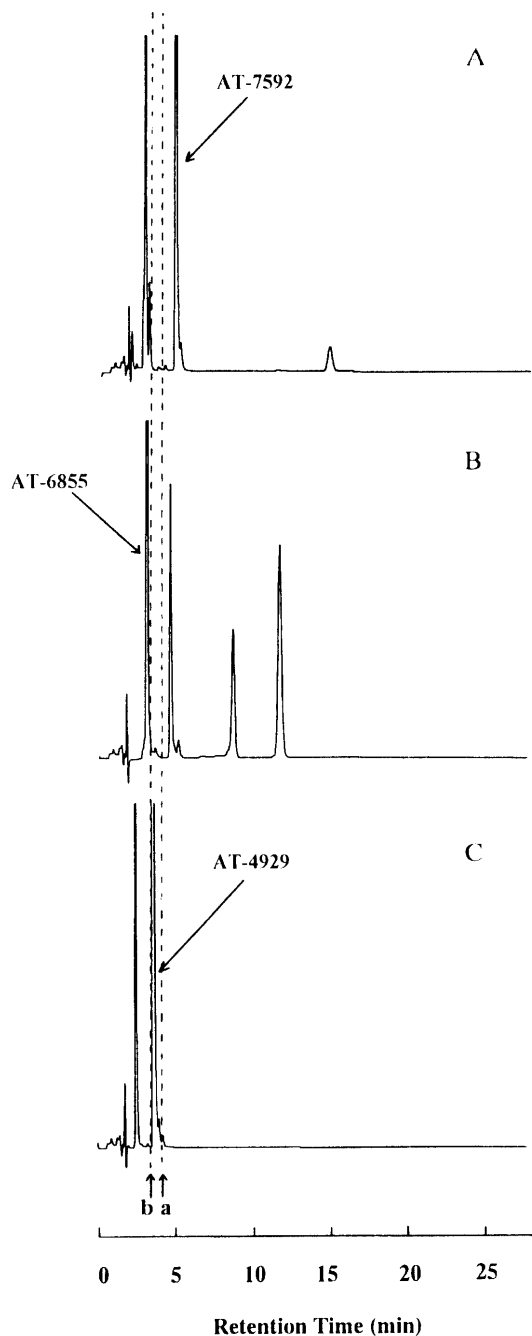


Fig. 4. HPLC Chromatograms of Fluoroquinolones Presumed to be Intermediates of the Photodegradation of ORFX after Irradiation with a Chemical Lamp for 20 min

A: AT-7592, B: AT-6855, C: AT-4929. Arrows a and b represent the retention of compounds I and II, respectively.

AT-6855 and AT-7592 was also examined under a chemical lamp. Although photodegradation products were found in the photodegradation study of AT-4929, AT-6855 and AT-7592, respectively, these retentions on HPLC did not correspond with those of compounds I and II (Fig. 4). Therefore, compounds I and II were not formed from the photodegradation of AT-4929, AT-6855 and AT-7592.

Based on these results, it is considered that the photodecomposition of the dimethylpiperazinyl ring at the 7-position and the elimination of the cyclopropyl group at the 1-position always occurs with the release of fluorine at the 8-position.

Photodegradation studies of some fluoroquinolone de-

rivatives have been reported.<sup>13,14)</sup> Two fluoroquinolone derivatives, ciprofloxacin (CPFX) and norfloxacin (NRFX), which have a piperazinyl group at the 7-position and hydrogen at the 8-position, are photodecomposed to produce 7-aminoethylamino derivatives in aqueous solution.<sup>9,13,14)</sup> The structures of the photodegradation products of CPFX, NRFX and ORFX are quite similar, so the photodegradation mechanisms of these compounds seem to be the same. However, no aminopropylamino derivative (AT-6445) was observed in the photodegradation study of AT-4929 (8H ORFX). This fact suggests that ORFX, which has a fluorine atom at the 8-position, is decomposed photochemically through a different pathway from the photodegradation of the 8-unsubstituted fluoroquinolones, such as CPFX, NRFX and AT-4929.

Thus, the photodegradation mechanism is presumed to be as follows. The fluorine atom is eliminated by irradiation and a carbon radical is produced at the 8-position. Then, the hydrogen at the 2'-position of a cyclopropyl group or the hydrogen at the 3'-position of a dimethylpiperazinyl group, these protons are located at a similar distance from the carbon radical at the 8-position, are transferred to the 8-position, and the elimination of cyclopropyl group at the 1-position or the decomposition of the dimethylpiperazinyl moiety at the 7-position may proceed spontaneously. The pH profile for the photodegradation of ORFX reported previously<sup>11)</sup> suggests that the photochemical reaction of ORFX is not catalyzed by a proton or hydroxide ion in the solution, and concerns only an ionic form of the ORFX molecule. ORFX produces two kinds of photodegradation products, and the

degradation pathway may be dependent on the protonation of the ORFX molecule.

**Acknowledgments** The authors thank Mr. Koji Yoshida and Mr. Akira Itokawa of our company for mass spectral and NMR spectral measurements. We also thank Dr. Katsumi Chiba of our company for supplying us with authentic samples of the photodegradation products. We would like to thank Dr. Yasutaka Nagai for his kind support during this work.

#### References

- 1) Przybilla B., Georgii A., Bergner T., Ring J., *J. Dermatol.*, **181**, 98—103 (1990).
- 2) Christ W., Schmidt V., *Fortschr. Antimikrob. Antineoplast.*, **173**, 795—804 (1984).
- 3) Baran R., Brun P., *Dermatologica*, **173**, 185—188 (1986).
- 4) Kochevar I. E., Morison W. L., Lamm J. L., McAuliffe D. J., Western A., Hood A. F., *Arch. Dermatol.*, **122**, 1283—1287 (1986).
- 5) Kochevar I. E., *J. Invest. Dermatol.*, **76**, 59—64 (1981).
- 6) Castell J. V., Gomez L. J., Miranda M. A., Morera I. M., *Photochem. Photobiol.*, **46**, 991—996 (1987).
- 7) Gasparro F. P., *Photodermatology*, **2**, 151—157 (1985).
- 8) Ferguson J., Phillips G., McEwan J., Moreland T., Johnson B. E., *J. Dermatol.*, **4**, 119 (1988).
- 9) Tiefenbacher E. M., Haem E., Przybilla B., Kurz H., *J. Pharm. Sci.*, **83**, 463—467 (1994).
- 10) Morimura T., Ohno T., Matsukura H., Nobuhara Y., *Chem. Pharm. Bull.*, **43**, 1052—1054 (1995).
- 11) Morimura T., Ohno T., Matsukura H., Nobuhara Y., *Chem. Pharm. Bull.*, **43**, 1000—1004 (1995).
- 12) Emsley J. W., Feeney J., Sutcliffe L. H., *Progress in NMR Spectroscopy*, **10**, 99—100 (1976).
- 13) Takahashi Y., Tamura H., Hirako I., Shioyama C., Funaba T., Hiller H., *Iyakuhin Kenkyu*, **17**, 726—735 (1986).
- 14) Nangia A., Lam F., Hung C. T., *Drug Dev. Ind. Pharm.*, **17**, 681—694 (1991).