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# Defluorinated Sparfloxacin as a New Photoproduct Identified by Liquid Chromatography Coupled with UV Detection and Tandem Mass Spectrometry

MICHAEL ENGLER,[1] GUIDO RÜSING,[2] FRITZ SÖRGEL,[2] AND ULRIKE HOLZGRABE[1]*

*Pharmazeutisches Institut der Universität Bonn, D-53115 Bonn,[1] and Institute for Biomedical and Pharmaceutical Research, D-90562 Nürnberg-Heroldsberg,[2] Germany*

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**Photodegradation of sparfloxacin was observed by means of high-pressure liquid chromatography with UV detection and liquid chromatography coupled with UV detection and tandem mass spectrometry (LC-MS/MS). Three products were detected. Comparison with an independently synthesized derivative of sparfloxacin revealed the structure of one product which is believed to be 8-desfluorosparfloxacin. The second product is likely to be formed by the splitting off of a fluorine and a cyclopropyl ring. Thus, photodefluorination of quinolone antibacterial agents is found and proved for the first time by LC-MS/MS.**

Phototoxicity is one of the major adverse effects of modern fluoroquinolone antibacterial agents (9, 12, 17). Two alternative models (5) have been discussed as being the reason for phototoxicity: first, the formation of stable toxic photoproducts leading to skin reactions (15), and second, the formation of singlet oxygen (16), which nonspecifically injures the body. In 1975, Detzer and Huber (1) first isolated dimeric photoproducts of nalidixic acid, a prototype quinolone antibacterial agent. Many papers concerning the photolability of modern fluoroquinolones appeared, and these mostly described the loss of antibiotic activity during the course of irradiation (5–8, 13). It was hypothesized that a high degree of fluorination may result in a low photostability and, in line with this, in the formation of various photoproducts, which might cause adverse effects. However, little is known about the structures of these photodegradation products.

According to the hypothesis that low stability is connected with a high degree of fluorination, we have chosen sparfloxacin with a fluorine substituent at the 6 and the 8 positions as a representative of the highly fluorinated gyrase inhibitors of the newest class of drugs. The aim of the present study is to gain more insight into the process of photodegradation. Therefore, sparfloxacin has been irradiated in aqueous solution, and the structures of the photoproducts that were obtained were elucidated by means of liquid chromatography coupled with UV detection (photodiode array detector) and tandem mass spectrometry (LC-MS/MS). Additionally, an analog compound which is missing a fluorine atom at position 8 has been synthesized and spectroscopically characterized.

Part of this work was already presented as a poster at the ACS Conference, New Orleans, La., 1996.)

## MATERIALS AND METHODS

**Materials.** Sparfloxacin was generously provided by Rhône-Poulenc Rorer GmbH. Acetonitrile (Acros) was of high-pressure liquid chromatography (HPLC) grade, and all other reagents used were of analytical grade.

**HPLC with UV detection.** HPLC was performed with a Kontron HPLC pump 420 equipped with a Perkin-Elmer PE LC 480 Autoscan diode array detector. The chromatographic conditions were as follows: column, Merck LiChrosorb RP-18 (7 μm by 125 mm); loop, 20 μl; mobile phase, acetonitrile-formic acid (0.2% in water; 50:50), isocratic; flow rate, 1 ml/min. Running of the detector in the auto-spectrum mode gave UV spectra every second.

**LC-MS/MS.** LC-MS/MS experiments were performed on a Perkin-Elmer Sciex API III Plus biomolecular mass analyzer equipped with an IonSpray interface. Adjustments were as follows: orifice 60 volts; split, 5/1; collision energy, 25 V for product ion scan; collision gas thickness, $280 \times 10^{13}$ atoms/cm$^2$; nebulizer pressure, 50 lb/in$^2$; curtain gas flow, 0.6 liters/min. Chromatographic conditions were as follows: loop, 200 μl; other conditions, see above.

**Sample preparation and irradiation procedure.** Two milligrams of sparfloxacin was dissolved in 100 μl of 0.1 M NaOH, and this solution was diluted with 900 μl of water. This solution was irradiated for 8 h in a quartz cuvette placed at a distance of 1 cm from a high-pressure mercury lamp (Philips HPK 125 W with solidex glass filter; λ = 248.2 to 578.0 nm; energy = 1.63 to 60.89 W at different wavelengths). For observation of the degradation process, a sample of 20 μl was taken every hour and was diluted with water to a final concentration of 20 μg/ml for HPLC with UV detection and 40 μg/ml for LC-MS/MS.

**LC-MS/MS experiments.** First, a Q1 scan was recorded, i.e., masses from *m/z* 50 to *m/z* 1,000 were registered over a certain period of time (the recording is comparable to a chromatogram). The contour plot of the Q1 scan and the extracted spectra revealed the *m/z* values and retention times of the chromatographically separated compounds. For each compound, a new run (product ion scan of the pseudomolecular ion) was performed, giving the fragmentation pattern of each compound.

**Synthesis of the reference substance, 8-amino-1-cyclopropyl-1,4-dihydro-7-(2,6-dimethyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid, compound 10.** The reference substance, compound 10, has been synthesized by the methods described in the literature (3, 11) (Fig. 1). $^1$H nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM 360A spectrometer (60 MHz; tetramethylsilane was used as the internal standard) or a Varian XL 300 spectrometer (299,956 MHz). Melting points were determined (with a Gallenkamp melting point apparatus) in capillary tubes and are uncorrected. Reagents were purchased from common commercial suppliers and were used as received. All solvents have been distilled and dried by appropriate methods. Organic solutions were dried over anhydrous magnesium sulfate and were concentrated with an IKA rotary evaporator at low pressure. Infrared (IR) spectra were measured in KBr or Nujol on a Perkin-Elmer PE-298 instrument.

**2,4-Dichloro-5-fluoro-3-nitrobenzoic acid, compound 1.** A solution of 30 g (144 mmol) of 2,4-dichloro-5-fluorobenzoic acid (3) in 360 ml of concentrated sulfuric acid was heated at 70°C. Sixty milliliters of fuming nitric acid was added dropwise over a period of 3 h. After the addition of the acid, the reaction mixture was stirred for 2 h at 70°C and at room temperature overnight. The mixture was poured into ice and the white precipitate was filtered, washed with water, and dried over $P_2O_5$ to yield 31.5 g (86%) of compound 1; melting point, 183°C; $^1$H NMR (60 MHz, methanol-$d_4$) (ppm) δ 8.0 (d, 1 H, 9 Hz); IR (cm$^{-1}$) 3000, 1720, 1560, 1240, 1120.

**2,4-Dichloro-5-fluoro-3-nitrobenzoyl chloride, compound 2.** A mixture of 21 g (83 mmol) of compound 1 and 70 ml of thionylchloride was refluxed for 1 h. The excess thionylchloride was removed in vacuo, and the yellow residue was crystallized overnight at room temperature to give 21.8 g (91%) of compound 2, which was used without further purification for the next step.

**Ethyl (2,4-dichloro-5-fluoro-3-nitrobenzoyl)acetate, compound 4.** A total of 1.82 g (76 mmol) of magnesium was treated with 100 ml of ethanol and 1 ml of tetrachloromethane. After the reaction started, 12.16 g (76 mmol) of malonic

* Corresponding author. Mailing address: Pharmazeutisches Institut der Universität Bonn, Kreuzbergweg 26, D-53115 Bonn, Germany. Phone: 49-228-732-845. Fax: 49-228-739-038. E-mail: holzgrabe@uni-bonn.de.

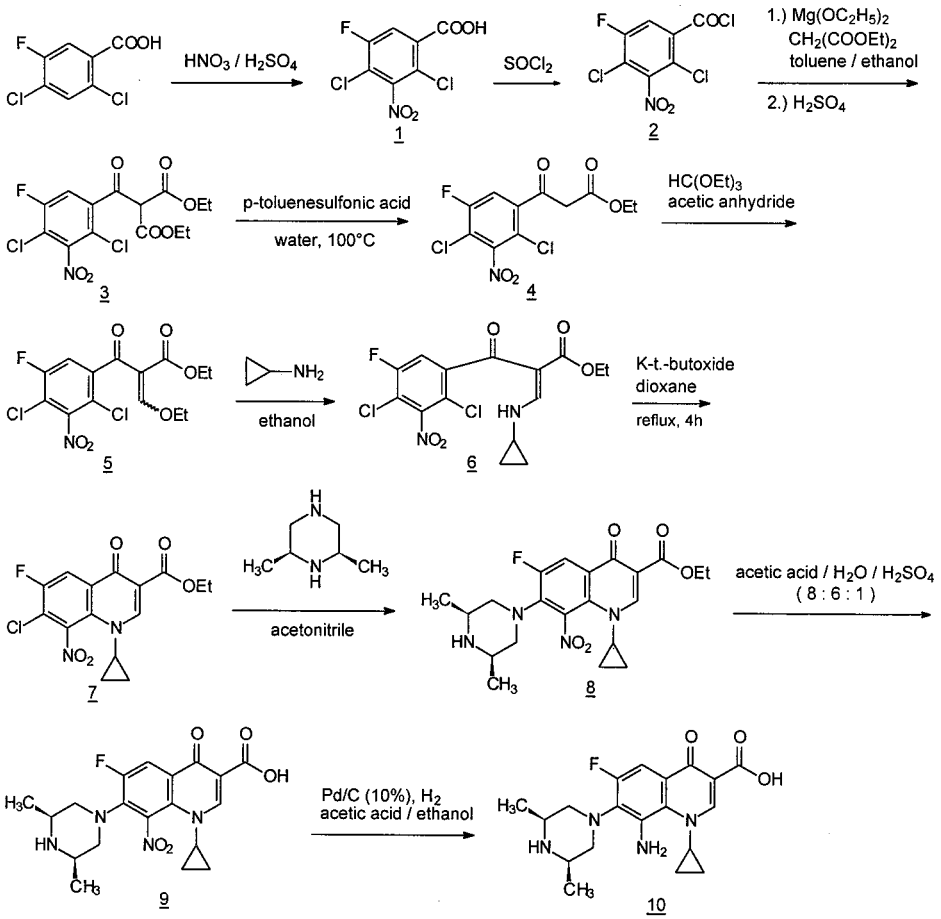


FIG. 1. Synthesis of the reference compound, compound 10. Et, ethyl.

acid diethylate, dissolved in a mixture of 110 ml of ethanol and 300 ml of toluene, was added dropwise. After the addition was completed the mixture was stirred at 70°C for 2 h and cooled to −10°C, and 21.8 g (76 mmol) of acid chloride, compound 2, dissolved in 110 ml of toluene was added slowly. Stirring for 2 h at 0°C and overnight at room temperature completed the reaction. The reaction mixture was cooled to 0°C, and under vigorous stirring, an ice-cold mixture of 200 ml water and 240 ml of concentrated sulfuric acid was added. After extraction with toluene, washing of the combined organic layers with saturated sodium

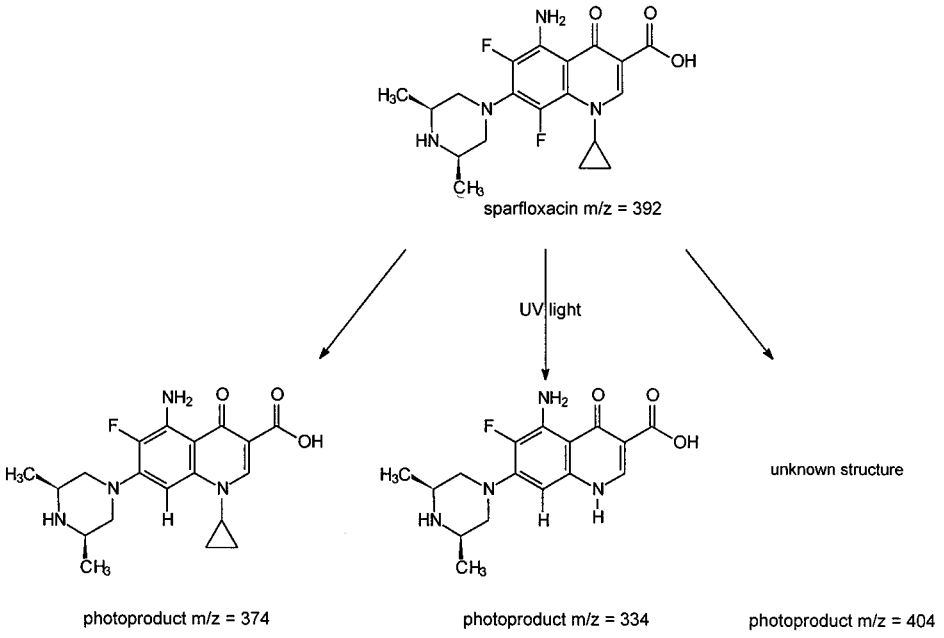


FIG. 2. Photoproducts of sparfloxacin.

TABLE 1. UV data obtained from the HPLC run in acetonitrile-formic acid (0.2%; 50:50) showing the hypsochromic shift of the absorption maximum

| Compound | $\lambda_{max1}$ (nm) | $\lambda_{max2}$ (nm) | $\lambda_{max3}$ (nm) |
|---|---|---|---|
| Sparfloxacin | 364 | 292 | 218 |
| Photoproduct $[M+H]^+$ with a mass of 375 Da | 353 | 286 | 227 |
| Compound 10 | 350 | 288 | 242 |
| Photoproduct $[M+H]^+$ with a mass of 335 Da | 341 | 284 | 227 |

chloride solution, and drying of the layers over magnesium sulfate, the evaporation of the solvent yielded 35.6 g of a mobile oil. This oil was treated with 300 ml of water and 800 mg of *p*-toluenesulfonic acid, and the mixture was refluxed for 3 h. The mixture was stirred overnight at room temperature and was extracted with dichloromethane. The combined organic layers were dried and evaporated to yield 23.4 g (95%) of compound 4 as a yellow oil, which was used without further purification.

**Ethyl 2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)-3-(cyclopropylamino)-acrylate, compound 6.** A solution of 10 g (31 mmol) of compound 4, 8.8 g (60 mmol) of triethyl *ortho*-formate, and 10.2 g (100 mmol) of acetic anhydride was refluxed for 4 h. The solvent was removed under reduced pressure, with the residue been dissolved in 50 ml of ethanol. After cooling to 0°C, a solution of 1.8 g (31 mmol) of cyclopropylamine dissolved in 20 ml of ethanol was added dropwise. The mixture was stirred for 36 h and the precipitate was filtered and recrystallized from ethanol to give 3.36 g (28%) of a yellow powder, compound 6; melting point, 142°C; $^1$H NMR (300 MHz, $CDCl_3$) (ppm) mixture of E and Z isomers 11.01 (d, 1 H, 13.7 Hz, NH; E isomer), 9.78 (d, 1 H, 13 Hz, NH; Z isomer), 8.36 (d, 1 H, 14.5 Hz, ═CH; Z isomer), 8.27 (d, 1 H, 14.7 Hz, ═CH; E isomer), 7.17 (d, 1 H, 8.1 Hz, aromatic H; Z isomer), 7.11 (d, 1 H, 7.9 Hz, aromatic H; E isomer), 3.99 (q, 2 H, 7 Hz, O—$CH_2$; E isomer), 3.92 (q, 2 H, 7 Hz, O—$CH_2$; Z isomer), 3.01 (m, 1 H, cyclopropyl H; E isomer), 1.03 (t, 3 H, 7.1 Hz, $CH_3$; E isomer), 0.97-0.81 (m, 4 H, cyclopropyl $CH_2$); IR ($cm^{-1}$) 3450, 3180, 3030, 1670, 1620, 1560, 1540, 1360.

**Ethyl 7-chloro-1-cyclopropyl-1,4-dihydro-6-fluoro-8-nitro-4-oxo-3-quinolinecarboxylate, compound 7.** Ten grams (25.6 mmol) of compound 6 and 2.9 g (25.6 mmol) of potassium *tert*-butoxide dissolved in 250 ml of dioxane were refluxed for 4 h. The mixture was evaporated to half of its volume and was poured into a mixture of 200 ml of ice water, 40 ml of concentrated hydrochloric acid, and 75 ml of dichloromethane. The organic layer was separated, washed with water, dried, and evaporated. Recrystallization of the residue from ethanol gave 7.3 g (80%) of brown crystals, compound 7; melting point, 172°C; $^1$H NMR (60 MHz, dimethyl sulfoxide [DMSO]-$d_6$ + trifluoroacetic acid) (ppm) 8.75 (s, 1 H, ═CH), 8.37 (d, 1 H, 10 Hz, aromatic H), 4.30 (q, 2 H, O—$CH_2$), 2.70 (m, 1 H, cyclopropyl CH), 1.35 (t, 1 H, $CH_3$), 1.30-1.0 (m, 4 H, cyclopropyl $CH_2$); IR ($cm^{-1}$) 3090, 2990, 1730, 1600, 1540, 1340.

**Ethyl 1-cyclopropyl-1,4-dihydro-7-(2,6-dimethyl-1-piperazinyl)-6-fluoro-8-nitro-4-oxo-3-quinolinecarboxylate, compound 8.** A mixture of 2.8 g (7.9 mmol) of compound 7 and 3.6 g (32 mmol) of 2,6-dimethylpiperazine was refluxed in 100 ml of acetonitrile for 2 h. After stirring overnight at room temperature, the yellow precipitate was filtered and the mother liquid was concentrated. Recrystallization of both residues from ethanol yielded 3 g (88%) of compound 8; melting point, 195°C; $^1$H NMR (300 MHz, $CDCl_3$) (ppm) 8.56 (s, 1 H, ═CH), 8.20 (d, 1 H, 11.3 Hz, aromatic H), 4.34 (q, 2 H, 7 Hz, O—$CH_2$), 3.85 (m, 1 H, cyclopropyl CH), 2.94-2.72 (m, 6 H, piperazinyl CH and piperazinyl $CH_2$), 1.36 (t, 3 H, 7.1 Hz, $OCH_2\underline{CH_3}$), 1.13-1.02 (m, 10 H, piperazinyl $CH_3$ and cyclopropyl $CH_2$); IR ($cm^{-1}$) 3080, 2960, 1730, 1700, 1540, 1320.

**1-Cyclopropyl-1,4-dihydro-7-(2,6-dimethyl-1-piperazinyl)-6-fluoro-8-nitro-4-oxo-3-quinolinecarboxylic acid, compound 9.** A total of 1.57 g (3.6 mmol) of compound 8 was treated with a solution of 40 ml of acetic acid, 30 ml of water, and 5 ml of concentrated sulfuric acid. The mixture was heated at 100°C for 2 h and poured onto ice. The pH was adjusted to 4 to 5 with 2 M NaOH, and the solution was extracted with chloroform. The combined organic layers were dried and concentrated, and the yellow oil was triturated with benzine for crystallization. Subsequently, the yellow solid was filtered and washed with benzine to give 1.46 g (94%) of compound 9; melting point, 255 to 260°C; $^1$H NMR (300 MHz, DMSO-$d_6$ + trifluoroacetic acid) (ppm) 8.78 (s, 1 H, ═CH), 8.28 (d, 1 H, 11 Hz, aromatic H), 3.71 (m, 1 H, cyclopropyl CH), 3.28-3.15 (m, 6 H, piperazinyl CH

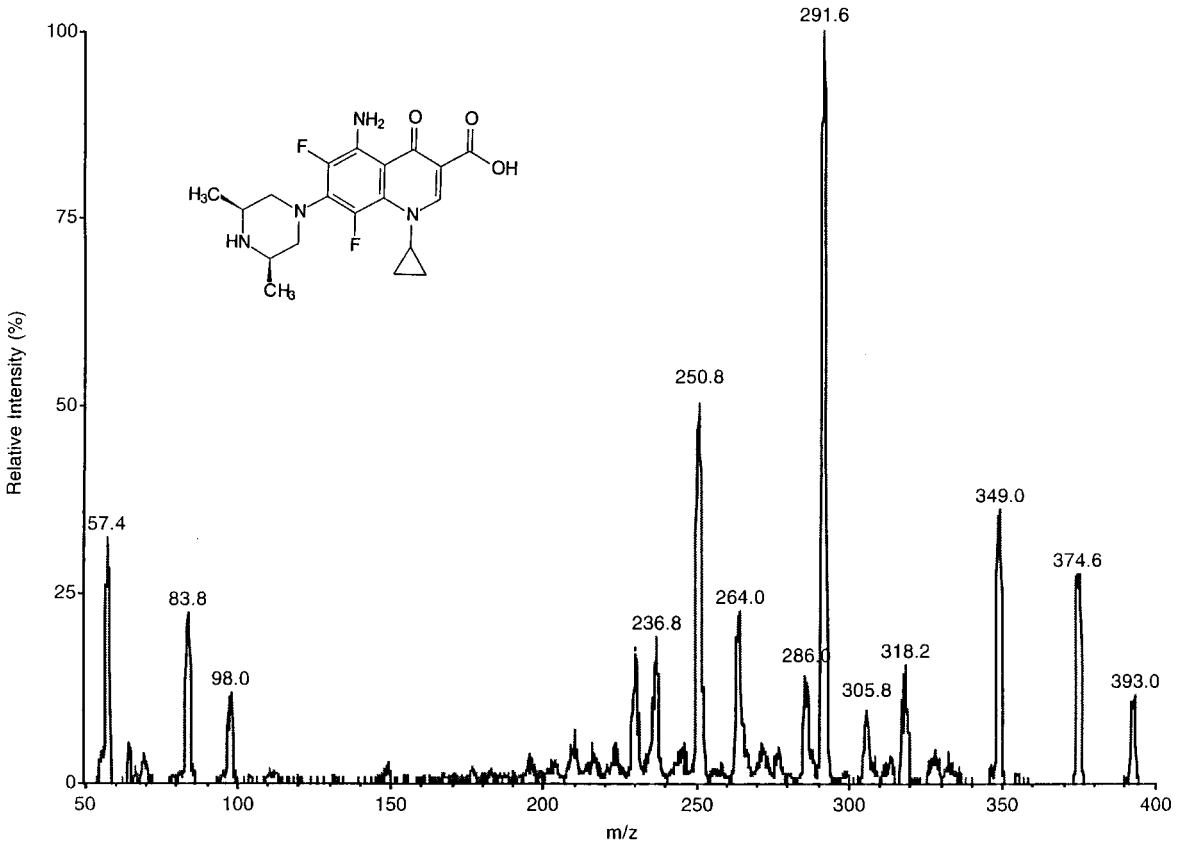


FIG. 3. Product ion spectrum of sparfloxacin.

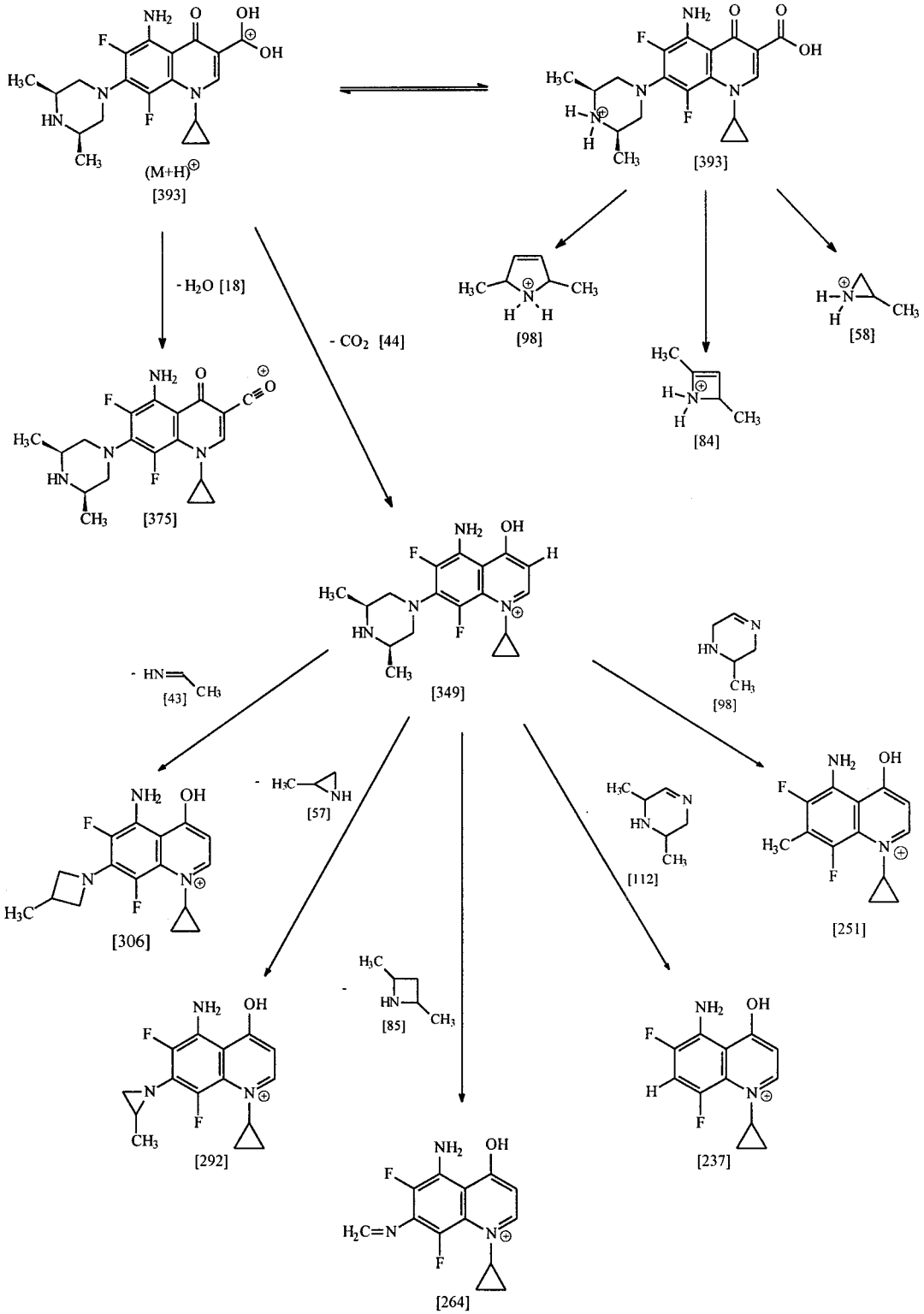


FIG. 4. Fragmentation pathway of sparfloxacin under MS/MS conditions. Numbers in brackets are *m*/*z*'s.

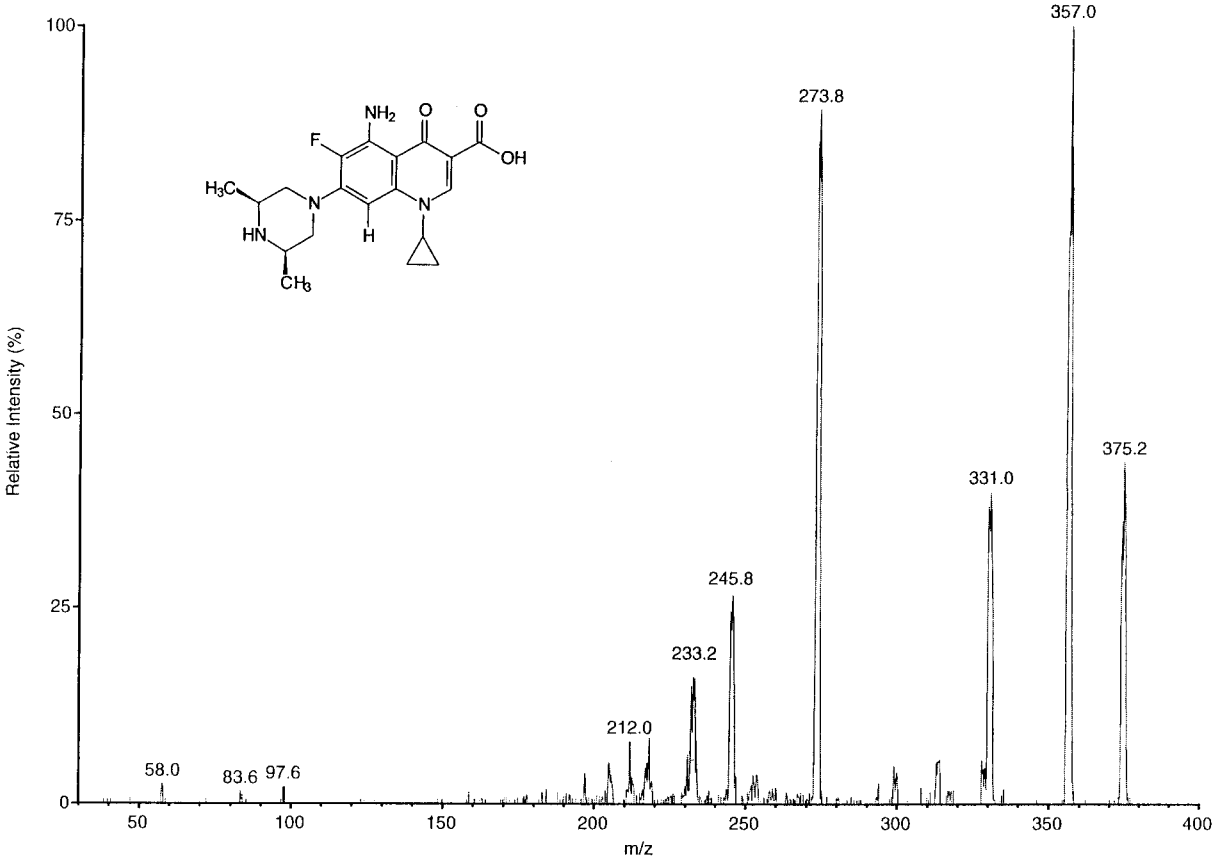


FIG. 5. Product ion spectrum of the photoproduct $[M+H]^+$ with a mass of 375 Da.

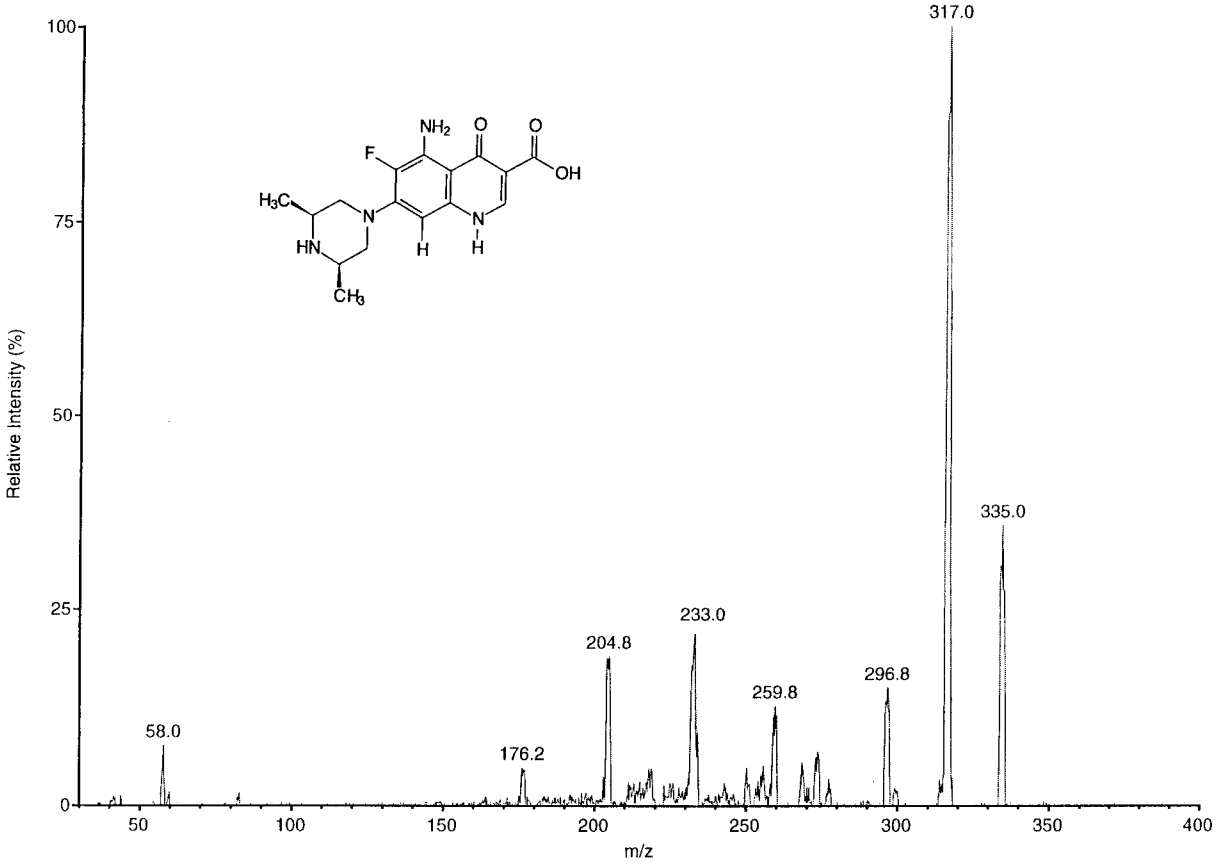


FIG. 6. Product ion spectrum of the photoproduct $[M+H]^+$ with a mass of 335 Da.

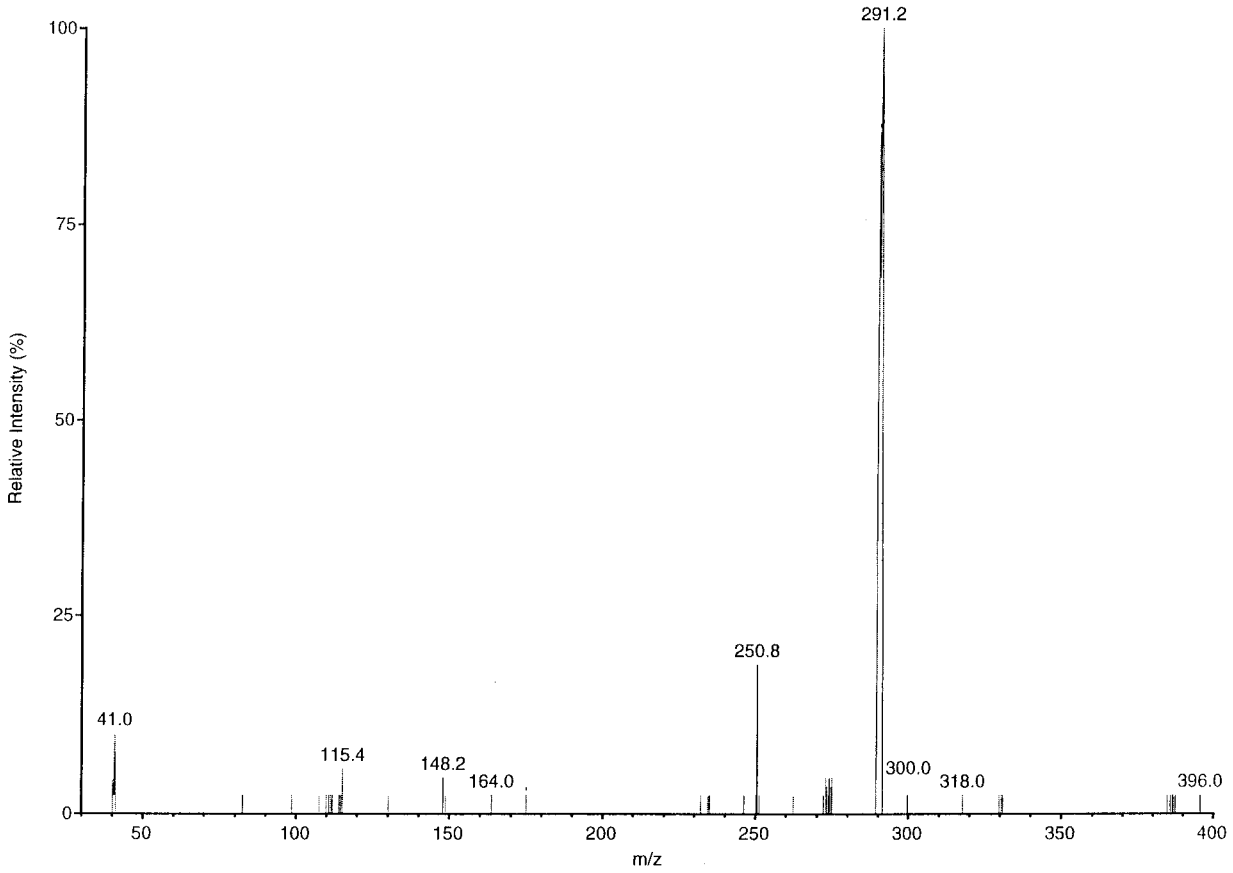


FIG. 7. Product ion spectrum of the photoproduct $[M+H]^+$ with a mass of 405 Da.

and piperazinyl $CH_2$), 1.24-1.04 (m, 10 H, piperazinyl $CH_3$ and cyclopropyl $CH_2$); IR ($cm^{-1}$) 3020, 1720, 1600, 1550, 1320, 1260.

**8-Amino-1-cyclopropyl-1,4-dihydro-7-(2,6-dimethyl-1-piperazinyl)-6-fluoro-4-oxo-3-quinolinecarboxylic acid, compound 10.** One gram (2.5 mmol) of compound 9 was dissolved in a solution of 30 ml of ethanol and 30 ml of acetic acid. After the addition of 40 mg of Pd/C (10%) catalyst, the mixture was hydrogenated at room temperature and 1 bar for 24 h. After filtration and removal of the solvent, a black oil was obtained. Treatment of the oil with benzine gave a black solid, which was recrystallized from isopropanol with activated charcoal. A second recrystallization from isopropanol yielded 312 mg (33%) of compound 10; melting point, 259°C (decomposed); $^1H$ NMR (300 MHz, DMSO-$d_6$) (ppm) 8.70 (s, 1 H, =CH), 7.23 (d, 1 H, 12.1 Hz, aromatic H), 6.03 [s (br), 2 H, $NH_2$], 3.77 (m, 1 H, cyclopropyl CH), 3.05-2.66 (m, 6 H, piperazinyl CH and piperazinyl $CH_2$), 1.25-0.97 (m, 10 H, cyclopropyl $CH_2$ and piperazinyl $CH_3$); IR ($cm^{-1}$) 3340, 3010, 1630, 1540; UV (nm) 242 maximum, 288, 350.

## RESULTS AND DISCUSSION

Irradiation of sparfloxacin with UV light results in at least three photoproducts (Fig. 2). As can be detected by HPLC, the best irradiation time was found to be 4 to 8 h. After 4 h, the sparfloxacin peak decreases significantly, whereas the other peaks increase. Irradiation times longer than 8 h did not affect the intensity of the sparfloxacin peak anymore. A total degradation of the quinolone could not be achieved. A reason for this might be the quenching effect in concentrated solutions described by Morimura et al. (7). The UV spectra of all photoproducts recorded during the HPLC run show a hypsochromic shift of 6 to 8 nm of the absorption maximum (Table 1) indicating the loss of a weak chromophoric group, such as a fluorine atom.

In order to find similarities and changes in the MS spectra of the photoproducts, an LC-MS/MS experiment was first performed with the nonirradiated sparfloxacin. The fragmentation of the ion spray ionization technique applied is different from that of electron impact-mass spectrometry. The product ion spectrum of sparfloxacin is displayed in Fig. 3, and the derived fragmentation pathway is shown in Fig. 4. The pseudomolecular ion $[M+H]^+$ (*m/z* 393) can split off of a water molecule (loss of 18 Da) or decarboxylate (loss of 44 Da). The resulting oxoquinoline ion (*m/z* 349) loses different fragments of the piperazine ring. Interestingly, no fragmentations concerning the aromatic ring system and the substituents at N-1 are observed. The peaks *m/z* 98, 84, and 58 can be explained by the presence of a pseudomolecular ion with a positive charge on the piperazine nitrogen atom.

From the Q1 scan of the irradiated samples, a chromatogram was obtained. The chromatogram showed three chromatographically well separated compounds with pseudomolecular ions of 375, 335, and 405 Da. The subsequent product ion scans of these pseudomolecular ions gave the fragmentation patterns of all three photoproducts (Fig. 5 to 7).

As can be seen in Fig. 5, the spectra of the photoproduct $[M+H]^+$ with a mass of 375 Da and sparfloxacin (Fig. 3) exhibit corresponding mass differences. The masses of all fragment ions of the photoproduct are decreased by 18 Da. A loss of water may have occurred upon irradiation. Since the mass difference of 18 Da is still found in the product ion spectrum of the photoproduct $[M+H]^+$ with a mass of 375 Da, a conversion of the carboxylic function is impossible. Thus, the loss of water is unlikely. Alternatively, a photochemical replacement of one fluorine atom of sparfloxacin with a hydrogen must be

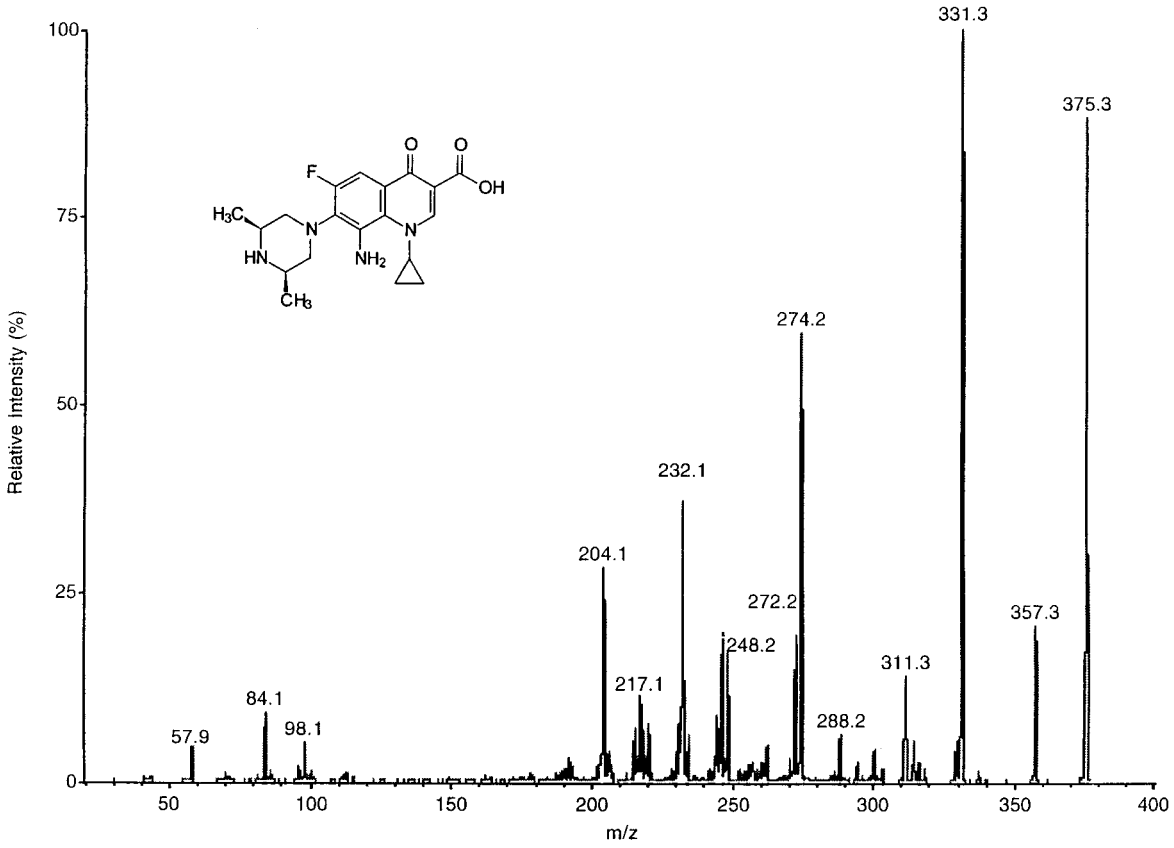


FIG. 8. Product ion spectrum of the reference compound, compound 10.

taken into account, because the UV spectrum of the photoproduct showed a corresponding shift (Table 1). The fact that the masses of all the fragment ions showed a difference of 18 Da further supports the hypothesis of photodefluorination (2). In order to ensure this hypothesis, the 8-desfluoro compound (compound 10) has been synthesized, and mass and UV spectra were recorded by using the conditions described above. As can be seen in Fig. 5 and 8, the spectra of the photoproduct $[M+H]^+$ with a mass of 375 Da and the synthesized compound show nearly the same patterns of fragmentation, indicating similar structures. The differences between the photoproduct and compound 10 (fragment ions of *m/z* 274, 232, 219, 217, and 204) are due to the different positions of the amino group, which results from a kind of "*ortho* effect" of the amino group in the fragmentation pattern of compound 10 (Fig. 9). According to this observation, it seems likely that the fluorine atom in the photoproduct takes the 6 position or, in turn, the fluorine at position 8 was split off upon irradiation. In addition, the UV spectrum of compound 10 shows nearly the same hypsochromic shift ($\lambda_{max}$ = 288 [compound 10], and 292 [sparfloxacin]) as the photoproduct. The finding of defluorination of the difluorinated quinolone supports the observation reported previously (10) that 6-monofluorinated quinolones are found to be more photostable than 6,8-difluorinated quinolones. In addition, the defluorination is in accordance with recently reported results obtained with irradiated lomefloxacin and fleroxacin (4). The defluorinated products were identified by means of $^{19}F$ NMR spectroscopy.

The elucidation of the structure of the photoproducts $[M+H]^+$ with masses of 335 and 405 Da is more complicated and not yet finished. The mass spectrum of the product $[M+H]^+$ with a mass of 335 Da (Fig. 6) exhibits the same fragmentation pattern concerning the piperazine ring (compare with Fig. 3 for sparfloxacin) and a loss of water ($[M+H]^+$ with a loss of 18 Da). It is remarkable that there is no loss of $CO_2$ connected with the loss of water in this case, which indicates the presence of a COOH group. Nevertheless, it seems likely that the piperazine ring and the carboxylic group are still present. The mass difference of 58 Da between sparfloxacin ($[M+H]^+$ with a mass of 393 Da) and the photoproduct might be explained by the loss of two groups: first, a replacement of fluorine with a hydrogen (18 Da), which is in agreement with the hypsochromic shift in the UV spectrum, and second, substitution of the cyclopropyl group with a hydrogen (40 Da) (Fig. 6). Since such a photodegradation has not yet been described, further investigations must be performed. The information which can be extracted from the product ion spectrum of the photoproduct $[M+H]^+$ with a mass of 405 Da is rather poor, because the signals are rather weak because of the high degree of fragmentation (Fig. 7). The base peak of *m/z* 291 can be explained by the loss of a neutral piperazine ring ($[M+H]^+$ with a mass of 405 Da − 114 Da (neutral molecule) = 291 Da). In turn, a peak for a protonated piperazine is found at *m/z* 115. Thus, it is likely that the piperazine ring is still present in this photoproduct.

Taken together, the replacement of a fluorine atom with a hydrogen atom in the quinolone skeleton of gyrase inhibitors induced by UV light could be confirmed by LC-MS/MS as well as with the UV spectra of two photoproducts. The photodegradation of the piperazine ring (levofloxacin [18], ciprofloxacin

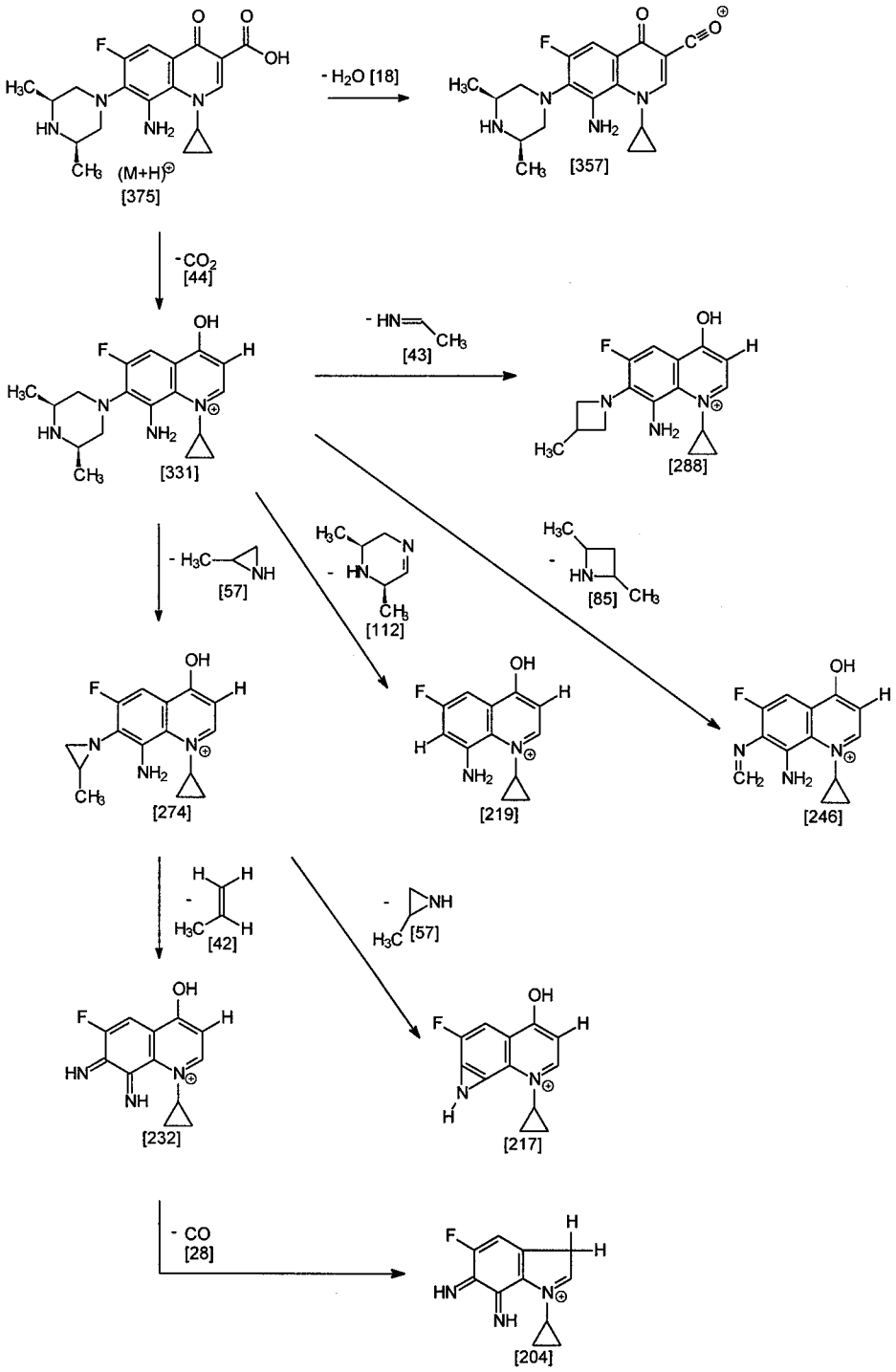


FIG. 9. Fragmentation pathway of the reference compound, compound 10, under MS/MS conditions. Numbers in brackets are *m*/*z*'s.

[14]) and the carboxyl group (cinoxacin [15]) described for other quinolones or the photodimerizations described for nalidixic acid (1) were not found in the present study.

## ACKNOWLEDGMENT

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