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Isolation and structure elucidation of an intermediate in the photodegradation of ciprofloxacin

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Abstract

Ciprofloxacin decomposes photochemically in aqueous solutions at acidic pH forming two major degradation products. One of the products, isolated from irradiated solutions by flash chromatography, was 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. The compound was an intermediate in the photochemical process, which degraded after longer exposure with a high-pressure mercury lamp to an aromatic amino-compound, 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. The structure of the intermediate was elucidated on the basis of information from ultraviolet, mass and nuclear magnetic resonance spectra. © 1997 Elsevier Science B.V.

Keywords: Ciprofloxacin; Fluoroquinolone; Photodegradation; Nuclear magnetic resonance spectroscopy

1. Introduction

The synthetic antibiotic ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-3-quinoline carboxylic acid (Fig. 1a), possesses an expanded spectrum of microbiological activity and during the 1990s has been the most widely used fluoroquinolone in the world [1]. In aqueous solutions, both irradiation with a high-pressure mercury lamp and exposure to daylight causes photochemical degradation. Several degradation products are formed and can be detected by thin-layer (TLC) [2] and high-performance liquid chromatography (HPLC) [3,4]. The rate of photodegradation is pH-dependent, slow at pH 3–4 and considerably accelerated at higher pH. Increase in the pH of the reaction medium also leads to an increase in the number of degradation products [5].

We recently identified one of the degradation products as 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (Fig. 1a: compound II) [6]. The aim of this sequel study was to isolate the other major degradation product, an intermediate to compound II and to elucidate its structure (Fig. 1a: compound I). The analytical methods selected for the study were

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Table 1
^{1}H- and ^{13}C-NMR data of compound I, solvent D_2O (containing one drop of 2 M DCl), sodium salt of 3-(trimethyl)silylpropane sulfonic acid as internal standard (s = singlet, d = doublet, t = triplet, m = multiplet)

Number of H or C atoms	$\delta^{1}H$ (ppm) (multiplicity)	$J_{H,H}$ (Hz)	$J_{H,F}$ (Hz)	$\delta^{13}C$ (ppm)	$J_{C,F}$ (Hz)
2	8.44(s)			150.6	
3				107.6	
4				176.7	$^{4}J < 1$
4a				116.2	^{3}J 8.2
5	7.33(d)		^{3}J 11.5	111.0	^{2}J 20.2
6				153.3	J 248.8
7				145.6	^{2}J 14.4
8	7.02(d)		^{4}J 7.2	99.4	^{3}J 7.9
8a				143.1	
9				172.0	
10	3.63(m)			39.1	
10,11 (10,12) *cis*		7.1			
10,11 (10,12) *trans*		4.0			
11,11 (12,12) gem		−6.7		10.2	
11,12 (12,11) *cis*	1.43(m)	10.3			
11,12 (12,11) *trans*	1.16(m)	5.9			
13	3.72(t)			42.4	
14	3.40(t)			40.3	

ultraviolet (UV) and nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS).

2. Materials and methods

2.1. Materials

The identity and purity of ciprofloxacin hydrochloride Ph. Eur. were verified by measuring the melting point and by TLC, HPLC and UV and IR spectroscopy. All other reagents and solvents were of analytical or HPLC grade. The solid chemicals were purchased from E. Merck (Darmstadt, Germany) and the solvents from Rathburn Chemicals (Walkerburn, UK).

2.2. Apparatus

A high-pressure mercury lamp TQ 718 at 500 W (Hanau, Germany), equipped with a quartz glass cooling mantle, served as radiation source.

The melting point of ciprofloxacin was measured with an electrothermal digital melting point apparatus (Southend, UK). The UV spectra were recorded with a Philips PU 8740 UV/VIS spectrometer from Pye Unicam (Cambridge, UK). The mass spectra were run at room temperature on a VG ZABSPEC OATOF (Manchester, UK) mass spectrometer in liquid secondary ion mass spectrometry (SIMS) mode. The matrix was glycerol–3-nitrobenzyl alcohol (1:1) containing 1% of trifluoroacetic acid as internal standard.

The NMR spectra were recorded at ambient temperature with a JEOL JNM-A-500 spectrometer (Tokyo, Japan) operating at 500.16 MHz for ^{1}H and at 125.65 MHz for ^{13}C, respectively.

TLC experiments were performed on precoated 0.25 mm silica gel $60F_{254}$ aluminium sheets (Merck, Darmstadt, Germany) with an eluent system of acetonitrile—10% ammonia containing 0.3 M ammonium chloride (6.5:3.5, v/v). The migration distance was 8 cm and the spots were detected under UV light (254 and 366 nm).

The HPLC equipment and conditions were as reported earlier [6].

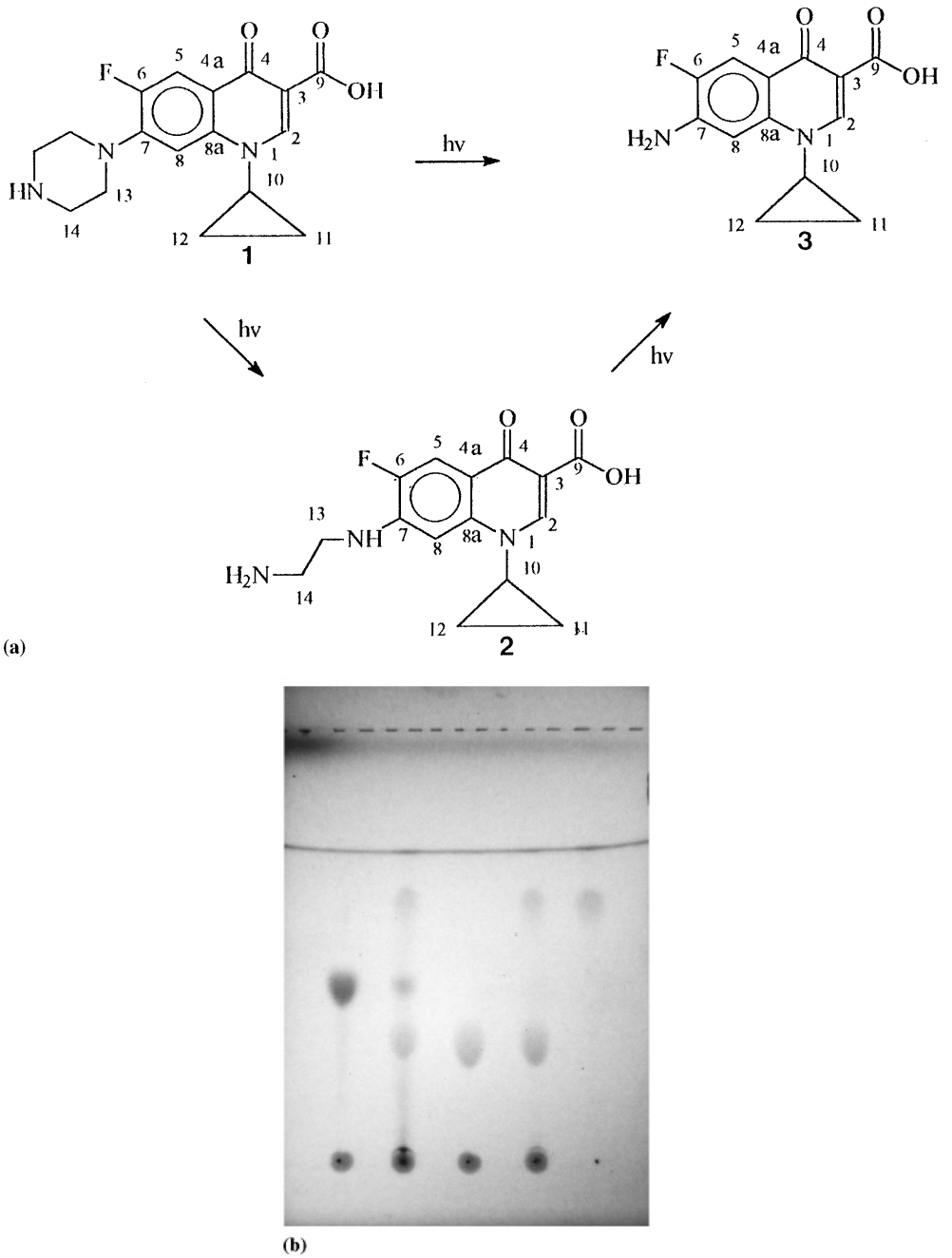

Fig. 1. (a) Scheme of the photodegradation of ciprofloxacin. Compound identification: ciprofloxacin (1), compound I (2) and compound II (3); (b) TLC studies of the photodegradation of ciprofloxacin after exposure to a high-pressure mercury lamp. Sample identification (from left to right): ciprofloxacin hydrochloride 1 mg ml^{-1} in 0.1 M HCl reference sample, ciprofloxacin hydrochloride reference sample after 7 h irradiation, fraction of compound I after flash chromatographic isolation, compound I (initial concentration 1 mg ml^{-1}) in 0.1 M HCl after 30 min irradiation, fraction of compound II after flash chromatographic isolation.

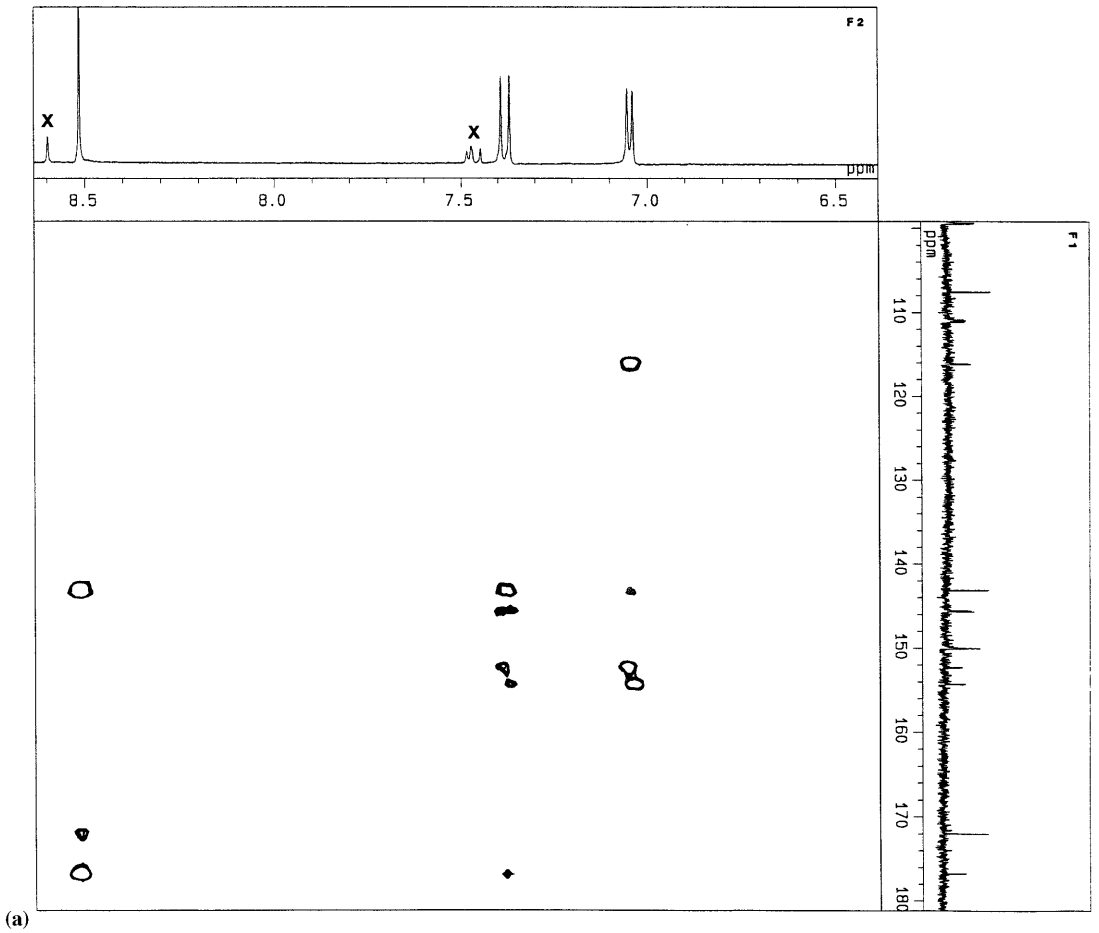

Fig. 2. (a) Aromatic region of the two-dimensional HMBC-NMR spectrum of compound I (X = impurity).

2.3. Photodegradation of ciprofloxacin and isolation of the degradation product

For the photodegradation reaction, 100 ml of ciprofloxacin hydrochloride solution (1 mg ml^{-1}) in 0.1 M hydrochloric acid was charged into 10 ml glass vials and exposed at a distance of 3 cm to radiation from a high-pressure mercury lamp TQ 718 (Hanau, Germany). The reaction was followed by TLC. After about 7 h irradiation, the amount of the intermediate was considerable compared with the amounts of compound II and ciprofloxacin. The filtered solution was evaporated to dryness and the residue was dissolved in 4 ml of 0.1 M hydrochloric acid, re-filtered if necessary and subjected to flash chromatography [7].

Before the flash chromatographic separation, the silica gel (Sorbsil C-60) column (length 26 cm i.d. 2 cm) was pre-eluted with acetonitrile. The aqueous sample was transferred to the column and eluted with acetonitrile—5% ammonia (7:3, v/v), fed at a flow rate of 5 cm per 1.5 min. The 1.5 ml fractions collected were qualitatively analyzed by TLC. Successive flash chromatographic procedure was required to isolate pure fractions of the intermediate. To collect the intermediate in maximum amount, the fractions were concentrated under reduced pressure and allowed to stand over night. The light-brownish crystals that precipitated were separated by centrifuging and dried in an exsiccator.

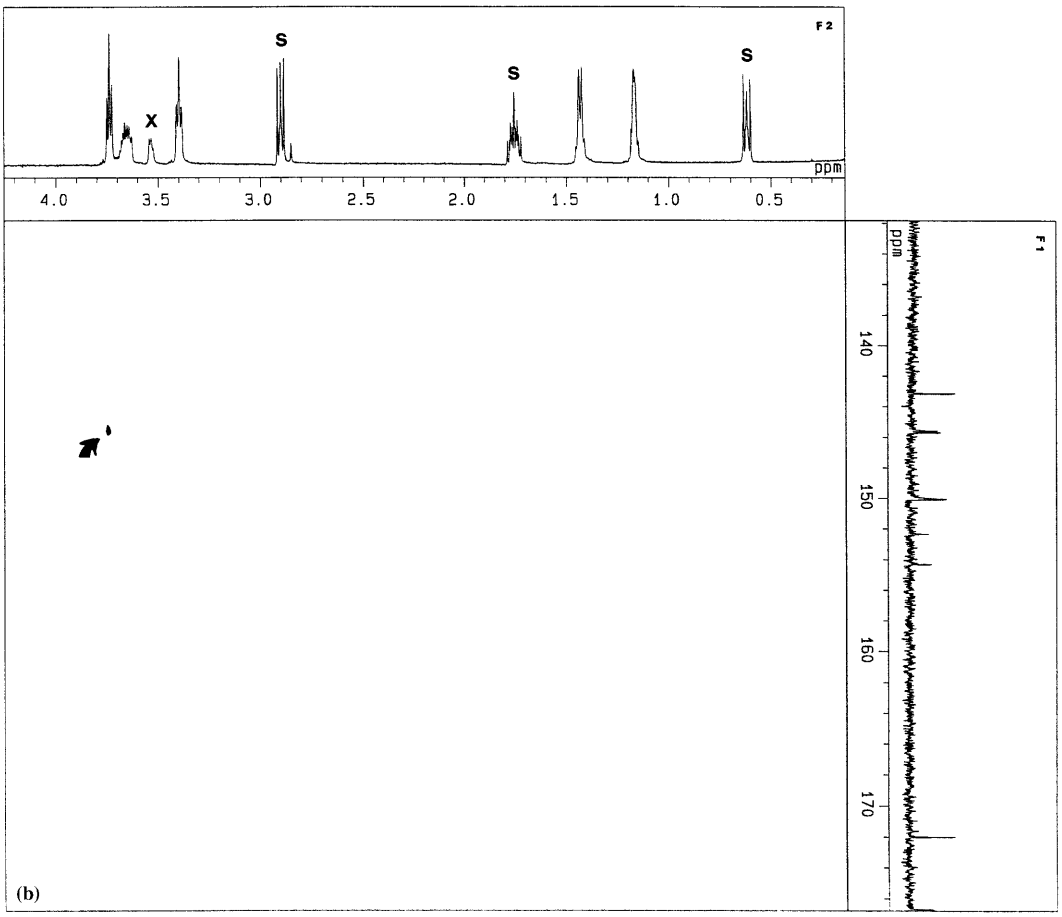

Fig. 2. (b) Aliphatic side-chain region (arrow) of the two-dimensional HMBC-NMR spectrum of compound I (X = impurity, S = internal standard).

2.4. NMR studies

The NMR samples were prepared in 5 mm o.d. tubes by dissolving about 3.5 mg of intermediate in 0.5 ml deuterium oxide (D_2O) containing one drop of 2 M deuterated hydrochloric acid. The sodium salt of 3-(trimethyl)silylpropane sulfonic acid was used as the internal standard. The measurements were performed by applying the pulse sequences according to JEOL Application Note [8]. Additionally, in heteronuclear correlation spectra (HMQC) the pulse sequences were according to Lerner and Bax [9], and in heteronuclear multiple bond coherence spectra (HMBC) according to Bax and Summers [10].

2.5. Photodegradation product

7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid. Light-brownish crystals, mol.wt. 305.1. UV λ_{max} (log ϵ) (0.1 M HCl): 275 (4.64) nm and 314 (3.99) nm. ^{1}H- and ^{13}C-NMR data are collected in Table 1. MS m/z (% rel.int.): 306 (100, MH^+).

3. Results and discussion

Ciprofloxacin hydrochloride degrades photochemically in acidic solutions, under irradiation with the high-pressure mercury lamp ($\lambda > 300$ nm)

or exposure to daylight, forming two main degradation products, compounds I and II. Formation of compound I (Fig. 1a) is dominant in solely aqueous acidic solutions at pH $\leq$ 5, whereas compound II (Fig. 1a) is the main degradation product when the solvent (pH $\leq$ 2) additionally contained water-miscible organic solvent [4,6]. After about 7 h irradiation in 0.1 M hydrochloric acid solution, TLC study showed the proportion of compound I to be considerable compared with compound II and ciprofloxacin (Fig. 1b). The brownish-red precipitate, formed during longer irradiation times was identified from its behaviour on TLC plates as compound II and was filtered away.

Still et al. have developed a flash chromatographic technique in which solely organic solvents are used both in samples and as eluents [7]. Further development of this method was required owing to the poor solubility of ciprofloxacin and its degradation products in most organic solvents. Optimization of the flash chromatographic separation required the flow rate to be decreased by 50%. Additionally optimization required the use of ammonia-containing aqueous eluent. For the satisfactory separation of compounds, a pre-elution with acetonitrile was introduced before application of the water-based sample (in 0.1 M HCl) and elution. After a second flash chromatographic run the purity of the isolated intermediate according to NMR was about 95%.

The UV spectra of compound I and ciprofloxacin exhibited absorption maxima in almost the same wavelength areas [6], indicating similar chromophoric structures. The two compounds also behaved very similarly during the HPLC method development, indicating them to have only slightly different chemical structures [4]. A clear violet-red colour reaction with ninhydrin on TLC plate suggested that compound I contained a primary aliphatic amino group [11].

Ciprofloxacin as a zwitterionic compound is a polar molecule, and a similar structure was assumed for compound I. The mass spectrum of compound I was recorded by SIMS-technique, which typically gives MH^+ as the most abundant ion, making molecular weight determination extremely easy. The molecular (MH^+) peak appeared at mass number 306 m/z, suggesting to the formula $C_{15}H_{16}FN_3O_3$, where the piperazine ring of the parent compound has opened and an ethylene group cleaved off.

The proposed structure of compound I, 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid, was confirmed with the data of one- (1D) and two-dimensional (2D) ^{1}H-NMR and ^{13}C-NMR spectra. In the ^{1}H-NMR spectrum, signals were detected in four regions (about 1, 3, 7 and 8 ppm). The multiplet signal pattern over 3 ppm, in the piperazine proton region, was clearly detected in the ^{1}H-NMR spectrum of ciprofloxacin [6], but only two triplets appeared in this region in the spectrum of compound I, indicating the presence of two adjacent methylene groups. The heteronuclear correlation spectrum (HMQC), which associates the signals from directly bonded ^{1}H and ^{13}C, indicated a clear correlation between the two protons at 3.72 ppm and a carbon at 42.4 ppm and a similar correlation between the two protons at 3.40 and a carbon at 40.3 ppm. Accordingly the aliphatic side-chain protons H13 and carbon C13 plus protons H14 and carbon C14 could be assigned.

Evidence for the presence of cyclopropane side-chain was provided by the proton signals at about 1 ppm. HMQC indicated correlation between the protons at 1.16 ppm and carbons at 10.2 ppm, and likewise between the protons at 1.43 ppm and same carbons. The multiplets of these protons could be assigned to two protons at position 11 and two at position 12, respectively, allowing also the assignment of carbons C11 and C12. The single proton in the cyclopropane ring is adjacent to nitrogen atom, which shifts the multiplet signal of the proton towards lower field, to 3.63 ppm. HMQC indicated a correlation between this proton and a carbon at 39.1 ppm, and proton H10 and carbon C10 were assigned. A computer simulation/iteration was needed to extract the NMR parameters from the highly degenerated spin system of the cyclopropane moiety in the proton spectrum. The iterative PERCH program [12] was used to calculate the shifts and couplings collected in Table 1.

Three aromatic protons were left in the quinolone moiety. HMQC gave clear correlations between protons at 8.44, 7.33 and 7.02 ppm and carbons at 150.6, 111.0 and 99.4 ppm, respectively. The carbon signals at 111.0 and 99.4 ppm were split by CF couplings of 20.2 and 7.9 Hz. The signal at 111.0 ppm had a higher $J_{C,F}$ value and was assigned to the carbon C5, at a distance of two bonds from the fluorine atom. The signal at 99.4 ppm was correspondingly assigned to carbon C8 at a distance of three bonds from fluorine. Accordingly the protons H5 and H8 were confirmed. The carbon signal at 150.6 ppm correlating with the signal of the most deshielded proton at 8.44 ppm had no sign of fluorine coupling, giving confirmation to C2 and H2. The assignment of carbon C6 having a signal at 153.3 ppm was based on the typical one-bond CF coupling of 248.8 Hz.

The heteronuclear multiple bond coherence technique (HMBC) reveals the long-range couplings. This technique was used to assign the remaining carbon signals of the quinolone moiety (Fig. 2a). The carbon at 143.1 ppm exhibited a three-bond coupling to protons H2 and H5 and a two-bond coupling to H8, and carbon C8a was confirmed. Additional three-bond CH couplings were observed between the carbons at 172.0 and 176.7 ppm and proton H2. A fifth three-bond coupling was detected between proton H5 and the carbon at 176.7 ppm. Accordingly, on the basis of two detected proton couplings and a weak fluorine coupling, the typical carbonyl signal at lower field was assigned to the carbon C4. The other carbonyl signal was attributed to carbon C9. A HMBC correlation was detected between the carbon at 145.6 ppm and the proton at 7.33 ppm, and between the carbon at 116.2 ppm and the proton at 7.02 ppm. On this basis carbons C7 and C4a were assigned and confirmed by the detected fluorine couplings, 14.4 Hz for the nearer carbon and 8.2 Hz for the carbon at a distance of three bonds from the fluorine atom. The remaining signal at 107.6 ppm was assigned to carbon C3.

The HMBC spectra also provided additional evidence for the presence of the aliphatic side chain in compound I, remaining after partial cleavage of the piperazine ring. A three-bond coupling was observed between carbon C7 at 145.6 ppm and proton H13 at 3.72 ppm (Fig. 2b, see arrow).

The NMR assignments in this study are in good agreement with the results presented for the parent compound, ciprofloxacin [6]. A solution of isolated compound I (initial concentration 1 mg ml^{-1} in 0.1 M hydrochloric acid) was exposed to the irradiation of a high pressure mercury lamp to confirm the intermediate character of compound I. The further degradation of compound I to compound II is clearly to be seen in Fig. 1 b. Direct degradation of ciprofloxacin to compound II cannot, however, be excluded.

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