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Structure elucidation of a photodegradation product of ciprofloxacin

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Abstract

In the photochemical degradation of ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-3-quinolone carboxylic acid, two major decomposition products are formed in acidic solution. The main degradation product, after both artificial and daylight exposure, was 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid. This product was also the dominating compound after more than 5 h irradiation with a high-pressure mercury lamp in aqueous solutions at pH ≤ 2 when the solvent additionally contained water-miscible organic solvent. The structure of the isolated compound was elucidated on the basis of the chemical behaviour in thin-layer and high-performance liquid chromatography, and of information from infrared, ultraviolet, mass and nuclear magnetic resonance spectra. © 1997 Elsevier Science B.V.

Keywords: Ciprofloxacin; Photodegradation; Mass spectrometry; Nuclear magnetic resonance spectroscopy

1. Introduction

Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-3-quinolone carboxylic acid (Fig. 1), is a broad-spectrum chemotherapeutic agent which in 1994 was the most frequently used fluoroquinolone in the world [1]. This antimicrobial degrades in aqueous solutions both after irradiation with a high-pressure mercury lamp and after exposure to daylight. 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 slow at pH 3–4 and increases considerably at higher pH values. The number of the degradation products is likewise affected by the pH of the reaction medium, the number evidently increasing with increasing pH [5].

One degradation product has been detected in all irradiated aqueous solutions of ciprofloxacin hydrochloride. In our previous articles [4,5] this product is designated, compound II, and it is one of two main degradation products at acidic pH. The aim of the present study was to elucidate the structure of compound II, 7-amino-1-cyclopropyl-

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6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid (Fig. 1). TLC, HPLC, ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) were utilized in the investigation. A comparison with the chromatographic, spectroscopic and spectrometric data of the parent compound is included.

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.

2.2. Apparatus

The melting points were measured with an electrothermal digital melting point apparatus (Southend, UK). The UV and IR spectra were recorded on a Philips PU 8740 UV/VIS spectrometer and a Unicam SP3-200 infrared spectrometer (KBr disc), both from Pye Unicam (Cambridge, UK). The mass spectra were run on a VG 7070 E (Manchester, UK) mass spectrometer with direct inlet (electron energy 70 eV).

The NMR spectra were recorded at room 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, and with a JEOL JNM-L-400 spectrometer (Tokyo, Japan) operating at 399.78 MHz for ^{1}H and at 100.40 MHz for ^{13}C.

TLC experiments were performed on precoated 0.25-mm silica gel $60F_{254}$ aluminium sheets (E. 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 7 cm and the spots were detected under UV light (254 and 366 nm).

The HPLC equipment consisted of a Waters (Milford; MA, USA) 501 solvent-delivery pump coupled to a 20-μl Rheodyne 7125 manual injector, a Model 484 variable-wavelength UV detector and a Model 741 data module printer. A Nova-Pak C_{18} Guard-Pak as precolumn and a stainless steel Nova-Pak C_{18} column (4 μm, 15 × 0.39 cm I.D.) served for chromatographic separations, which were performed at room temperature. The mobile phase; acetonitrile–phosphoric acid (pH 2.3; 20 mM) (15:85, v/v) containing 2.5 mM 1-heptanesulphonic acid sodium salt, was vacuum filtered with a Waters Associates (Milford; MA, USA) filtering kit, Ø = 0.45 μm. Helium was used for degassing before pumping of the mobile phase at 1.5 ml/min, and the stabilisation period at the beginning of the runs was 30 min. Detection was at 278 nm.

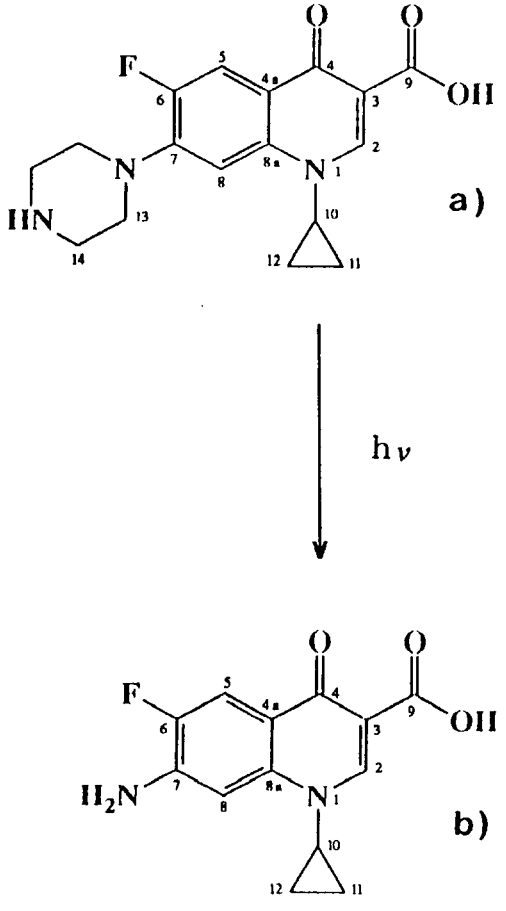

Fig. 1. Structures of ciprofloxacin (a) and compound II (b).

Table 1
^{1}H- and ^{13}C-NMR data of compound II, solvent D_2O (containing one drop of 40% NaOD), TMS external standard (s = singlet, d = doublet, m = multiplet)

Number of H or C atoms	δ ^{1}H (ppm) (multiplicity)	$J_{H,H}$ (Hz)	$J_{H,F}$ (Hz)	δ ^{13}C (ppm)	$J_{C,F}$ (Hz)
2	8.16(s)			146.2	
3				116.3	
4				175.5	^{4}J 2.6
4a				118.7	^{3}J 5.9
5	7.52(d)		^{3}J 11.8	110.1	^{2}J 20.4
6				150.2	J 242.6
7				141.0	^{2}J 15.5
8	7.14(d)		^{4}J 7.7	101.8	^{3}J 3.7
8a				139.3	
9				173.1	
10	3.25(m)			34.8	
10,11 (10,12) *cis*		7.1			
10,11 (10,12) *trans*		4.0			
11,11 (12,12) *gem*		−6.7		7.4	
11,12 (12,11) *cis*	1.05(m)	10.3			
11,12 (12,11) *trans*	0.84(m)	5.9			

2.3. Photodegradation of ciprofloxacin

A total of 50 ml of a ciprofloxacin hydrochloride solution (1 mg/ml) in ethanol—0.1 M hydrochloric acid (1:1, v/v) was charged into 10 ml glass vials. The vials were exposed to radiation from a high-pressure mercury lamp TQ 150 (Hanau, Germany) at a distance of 2 cm from the lamp. The reaction was followed by TLC. After disappearance of the parent compound, which occured after about 5 h irradiation, the solvent was evaporated under reduced pressure until turbidity appeared in the solution. The solution was allowed to stand over night, whereafter compound II precipitated as small brownish crystals. The crystals were filtered, washed with distilled water and dried in an exsiccator.

2.4. NMR studies

The NMR samples were prepared in 5 mm o.d. tubes by dissolving about 10 mg of compound in 0.5 ml deuterium oxide (D_2O). For compound II, one drop of 40% deuterated sodium hydroxide (NaOD) was added to dissolve the sample. Tetramethylsilane (TMS) was used as the external standard. The measurements were performed using JEOL spectrometers and the pulse sequences were in general according to JEOL Application Note [6] and additionally in heteronuclear correlation spectra (HMQC) according to Lerner and Bax [7] and in heteronuclear multiple bond coherence spectra (HMBC) according to Bax and Summers [8].

2.5. Photodegradation product

Compound II. 7-Amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid, brownish crystals, mol.wt. 262.2, m.p. 287°C. UVλ_{max} (log ϵ) (0.1 M HCl): 271 (4.63) and 312 (3.94) nm; (0.01 M NaOH): 266 (4.51), 322 (4.08) and 330 (4.07) nm. IRυ_{max}: 3480, 3430, 3380, 3240, 3100, 2950, 1720, 1650–1630, 1520, 1480–1460, 1400, 1320, 1265, 1195, 1035, 900, 815, 755, 730 cm^{-1}. ^{1}H- and ^{13}C-NMR data are collected in Table 1. MS m/z (% rel.int.): 262 (22, M^+), 218 (100, M-44), 203 (6), 189 (8), 163 (5), 149 (6), 122 (6), 109 (7), 41 (10).

2.6. Parent compound, ciprofloxacin hydrochloride

Pale yellow crystals, mol.wt. 385.8 and m.p. 307°C (decomposition). UVλ_{max} (log ϵ) (0.1 M

Table 2
^{1}H- and ^{13}C-NMR data of ciprofloxacin hydrochloride, solvent D_2O, TMS external standard (s = singlet, d = doublet, m = multiplet)

Number of H or C atoms	δ ^{1}H (ppm) (multiplicity)	$J_{H,H}$ (Hz)	$J_{H,F}$ (Hz)	δ ^{13}C (ppm)	$J_{C,F}$ (Hz)
2	8.41(s)			147.0	
3				105.4	
4				175.4	^{4}J 2.6
4a				118.2	^{3}J 8.0
5	7.19(d)		^{3}J 13.0	110.3	^{2}J 23.3
6				153.1	J 251.3
7				144.5	^{2}J 10.1
8	7.33(d)		^{4}J 7.5	106.3	^{3}J 2.6
8a				138.7	
9				168.4	
10	3.55(m)			36.1	
10,11 (10,12) *cis*		7.2			
10,11 (10,12) *trans*		4.0			
11,11 (12,12) *gem*		−6.9		7.5	
11,12 (12,11) *cis*	1.33(m)	10.5			
11,12 (12,11) *trans*	1.05(m)	6.1			
13 *gem*		−15.8		46.2	^{4}J 4.8
13 *a*	3.525(m)	[a]			
13 *e*	3.518(m)	[a]			
14 *gem*		−14.8		43.1	
14 *a*	3.431(m)	[a]			
14 *e*	3.420(m)	[a]			

[a]Proton couplings (Hz) between H-13 and H-14: $J_{a,a}$ 8.7; $J_{a,e}$ 4.1; $J_{e,e}$ 2.0; $J_{e,a}$ 6.4.

HCl): 277 (4.68) and 316 (4.19) nm; (0.01 M NaOH): 272 (4.53), 323 (4.13) and 335 (4.12) nm. ^{1}H- and ^{13}C-NMR data are in Table 2. MS *m/z* (% rel.int.): 331 (82, M^+), 289 (100, M-42), 287 (88, M-44), 245 (70, M-86), 230 (7), 157 (6), 56 (31), 41 (18).

3. Results and discussion

Two major degradation products, designated compounds I and II in a previous article [4], were formed in acidic ciprofloxacin hydrochloride solutions irradiated with a high-pressure mercury lamp ($\lambda > 300$ nm). The same products were formed under the influence of daylight. During longer exposure to artificial radiation, the formation of compound II became dominant in solutions at pH ≤ 2 when the solvent additionally contained water-miscible organic solvent, e.g. methanol, ethanol, acetone (Fig. 2). Compound II precipitated as small brownish crystals when the irradiated solution was concentrated and allowed to stand over night. The best yield was obtained when the irradiation was carried out in a mixture of 0.1 or 0.2 M hydrochloric acid and ethanol (1:1, v/v). Recrystallization of compound II failed owing to its poor solubility in most organic solvents. The purity of the isolated compound was confirmed by HPLC.

The UV absorption maxima of compound II were slightly shifted to lower wavelengths compared with those of ciprofloxacin, indicating only minor changes in the chromophoric structure of the degradation product. During the HPLC method development, the chromatographic behaviour of compound II indicated that the degradation product had considerably less basic character than the parent compound [4]. A positive colour reaction with dimethylaminobenzaldehyde on TLC plate suggested that compound II contained a primary amino group. Further evidence of the primary amine structure was obtained from the IR spectrum, which showed

asymmetric and symmetric absorptions of the NH_2 group between 3500 and 3300 cm^{-1}.

In the mass spectrum of compound II the molecular peak appeared at an even mass number 262 m/z, confirming the even number of nitrogens. The base peak in the spectrum was at 218 *m/z* (M-44, M-CO_2) and it was due to the loss of a CO_2 group from the molecule. This finding confirmed that the carboxyl group in the quinolone ring was intact in the degradation product. The molecular ion corresponded to the formula $C_{13}H_{11}N_2O_3F$, where the piperazine ring has been replaced by a primary amino group. Furthermore, in the mass spectrum of compound II, there was no sign of the fragments (M-42) and (M-86), which are prominent in the mass spectrum of ciprofloxacin and which according to Gau and co-workers indicate fragments (M-C_2H_4N) and (M-CO_2-C_2H_4N) and the presence of piperazine side chain [9].

The proposed structure of 7-amino-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolone carboxylic acid was supported 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 ppm regions (around 1, 3, 7 and 8 ppm). The multiplet signal pattern over 3 ppm, in the region typical of piperazine protons, was clearly detected in the ^{1}H-NMR spectrum of ciprofloxacin but was totally lacking in the ^{1}H-NMR spectrum of compound II. The homonuclear correlation spectrum (COSY, Fig. 3) verified the coupling patterns of protons at around 1 and 3 ppm to correspond to the protons H11, H12 and H10 in the cyclopropane substituent. Additionally, the heteronuclear correlation spectrum (HMQC), which associates the signals from directly bonded ^{1}H and ^{13}C, indicated a clear correlation between two protons H11 and carbon C11 at 7.4 ppm and a similar correlation between two protons H12 and carbon C12 at 7.4 ppm, plus a correlation between H10 and C10 at 34.8 ppm.

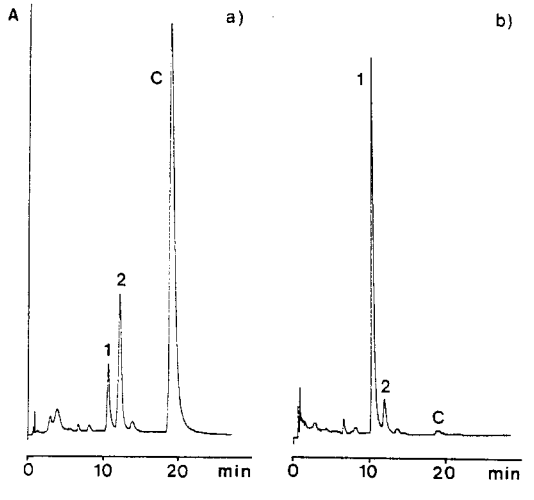

Fig. 2. High-performance liquid chromatograms of ciprofloxacin hydrochloride in 0.1 M HCl–ethanol after high-pressure mercury lamp irradiation. After 1 h (a) and 5 h (b) exposure. Peak identification: C = ciprofloxacin, 1 = compound II, 2 = compound I.

Of the three remaining aromatic protons, the most deshielded apparently is at position 2 (8.16 ppm). HMQC gave, for the quinolone moiety, prominent correlations of the three aromatic protons at 8.16, 7.52 and 7.14 ppm with carbons at 146.2, 110.1 and 101.8 ppm, respectively. The presence of fluorine was supported by the carbon signals at 110.1 and 101.8 ppm, which were split by CF couplings of 20.4 and 3.7 Hz, respectively. The signal at 110.1 ppm with higher $J_{C,F}$ value was assigned to the nearer C5, at a distance of two bonds. With these results, the positions of protons H2, H5 and H8 and also of carbons C2, C5 and C8 were confirmed. The very large (242.6 Hz) one-bond CF coupling at 150.2 ppm was assigned to carbon C6.

The heteronuclear multiple bond coherence technique (HMBC) (Fig. 4), which reveals the long-range couplings, was used to assign the six remaining carbon signals. The carbon at 139.3 ppm exhibited a three-bond coupling to protons H2 and H5, which allowed it to be identified as carbon C8a. Three-bond CH couplings were also observed between the proton H2 and carbons at 173.1 and 175.5 ppm. Of these two typical carbonyl signals, the one at lower field was assigned to carbon C4 on the basis of the detected fluorine coupling, and the other long-range proton–carbon coupling was then attributed to carbon C9. These two assignments were reversed relative to assignments in the literature for a metabolite of

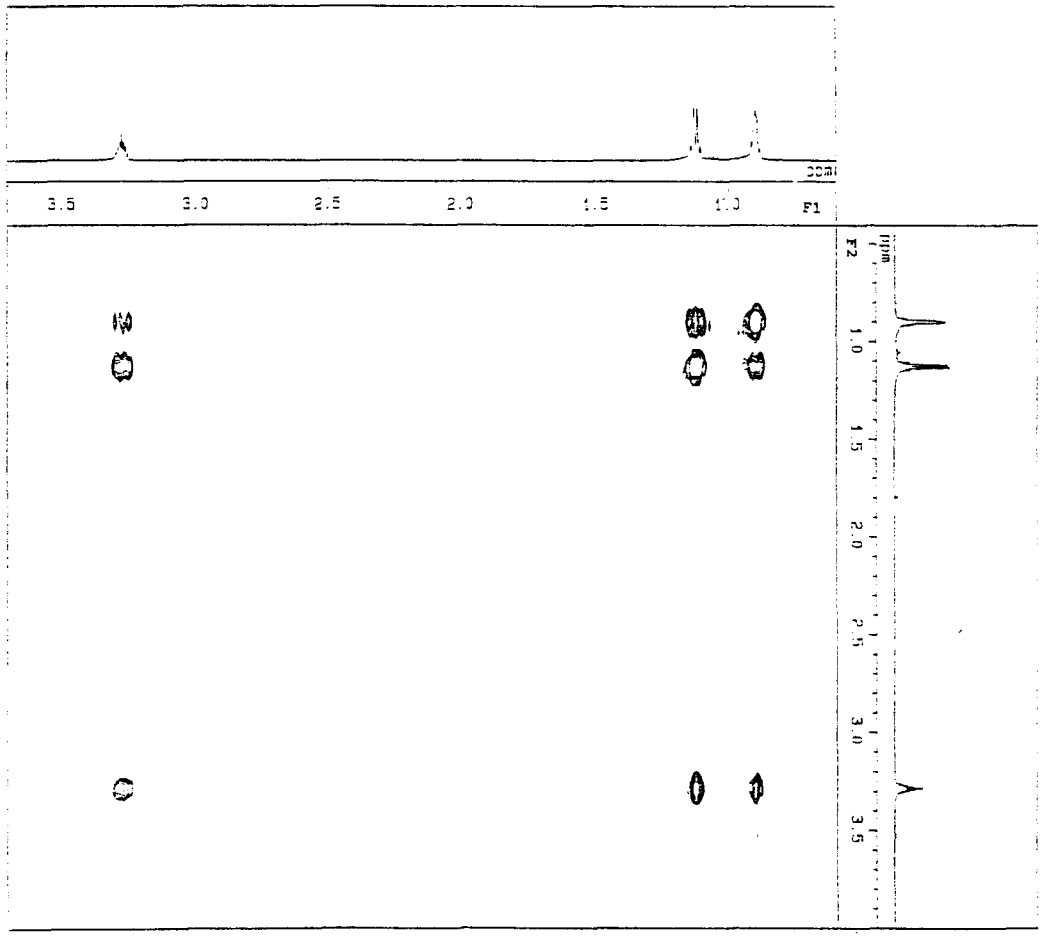

Fig. 3. The cyclopropane side chain region of the two-dimensional 500 MHz COSY-NMR spectrum of compound II.

ciprofloxacin [9] and for norfloxacin [10], but were in good agreement with a recent report of Riley and co-workers [11] on the structure of norfloxacin. Two additional three-bond CH couplings were detected in HMBC of compound II, one between carbon at 141.0 ppm and proton at 7.52 ppm and the other between carbon at 118.7 ppm and proton at 7.14 ppm. The ^{13}C signals were assigned to carbons C7 and C4a, respectively. Confirmation for these assignments was provided by the fluorine couplings, 15.5 Hz for the lower field carbon signal and 5.9 Hz for the higher field carbon, which is at greater distance from fluorine. The last carbon signal, at 116.3 ppm, was assigned to carbon C3.

An identical NMR study of ciprofloxacin gave results (Table 2) in good agreement with the assignments for compound II. However, the ^{1}H signals for protons at positions 5 and 8 appear in opposite order in ciprofloxacin and compound II. To ensure the correct assignment, a 2D nuclear Overhauser effect spectrum (NOESY) was recorded, and a clear NOE from H8 to the cyclopropane moiety as well as to the piperazine protons was found in ciprofloxacin.

The proton spectrum of the cyclopropane moiety, both in compound II and in ciprofloxacin, formed a highly degenerated spin system. The NMR parameters could be extracted only by computer simulation/iteration. A PERCH pro-

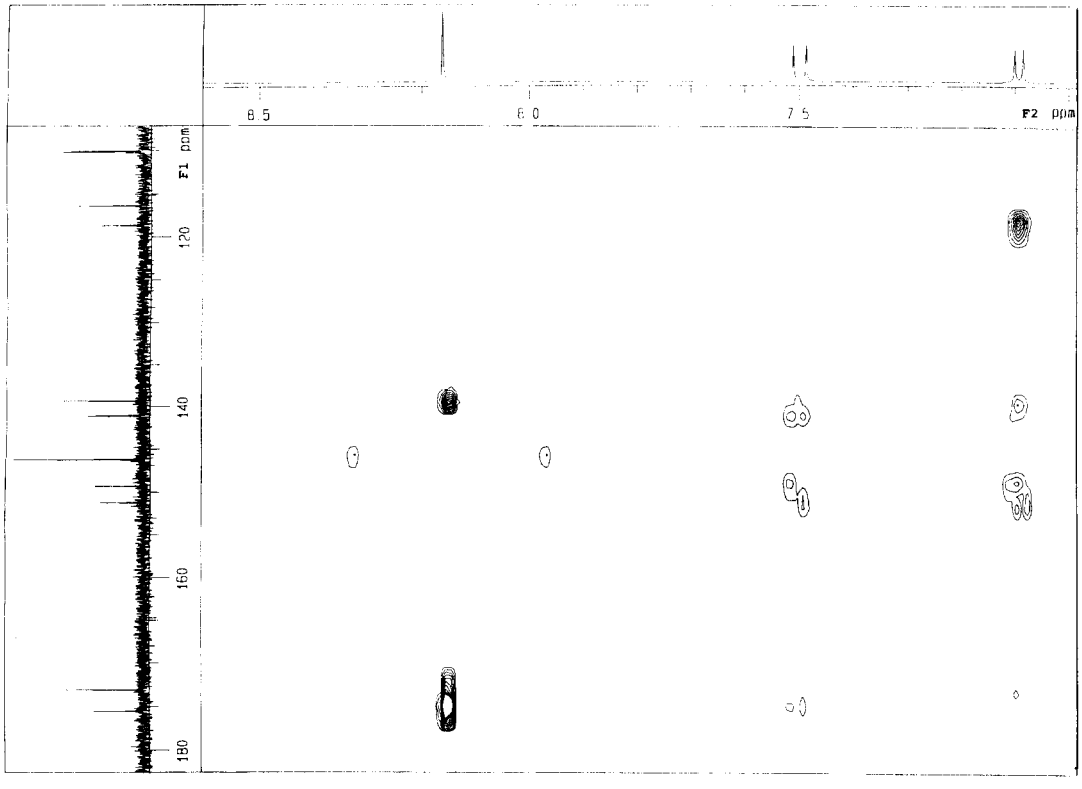

Fig. 4. Aromatic region of the two-dimensional HMBC-NMR spectrum of compound II.

gram [12] was used to calculate the shifts and couplings collected in Tables 1 and 2. Fig. 5 shows the excellent correlation between the recorded part of the proton spectra of ciprofloxacin and the calculated proton spectra of the cyclopropane moiety in ciprofloxacin. The proton parameters for the piperazine ring in ciprofloxacin were processed by computer in a similar manner (Table 2). The comments on the spatial structure of the piperazine ring in ciprofloxacin are published here because they could not be found in the literature, even though the compound is well-known. The couplings indicate that the piperazine ring in ciprofloxacin exists in a conformational equilibrium of the two possible chair forms. The explanation for this is the low energy barrier for the nitrogen inversion, due to which the aromatic *N*-substituent is not forced into axial orientation in either conformer.

In conclusion, the photodegradation of ciprofloxacin seems to proceed via partial loss of the piperazine moiety. Further studies on the structural character of compound I, a possible intermediate in the degradation, are in progress.

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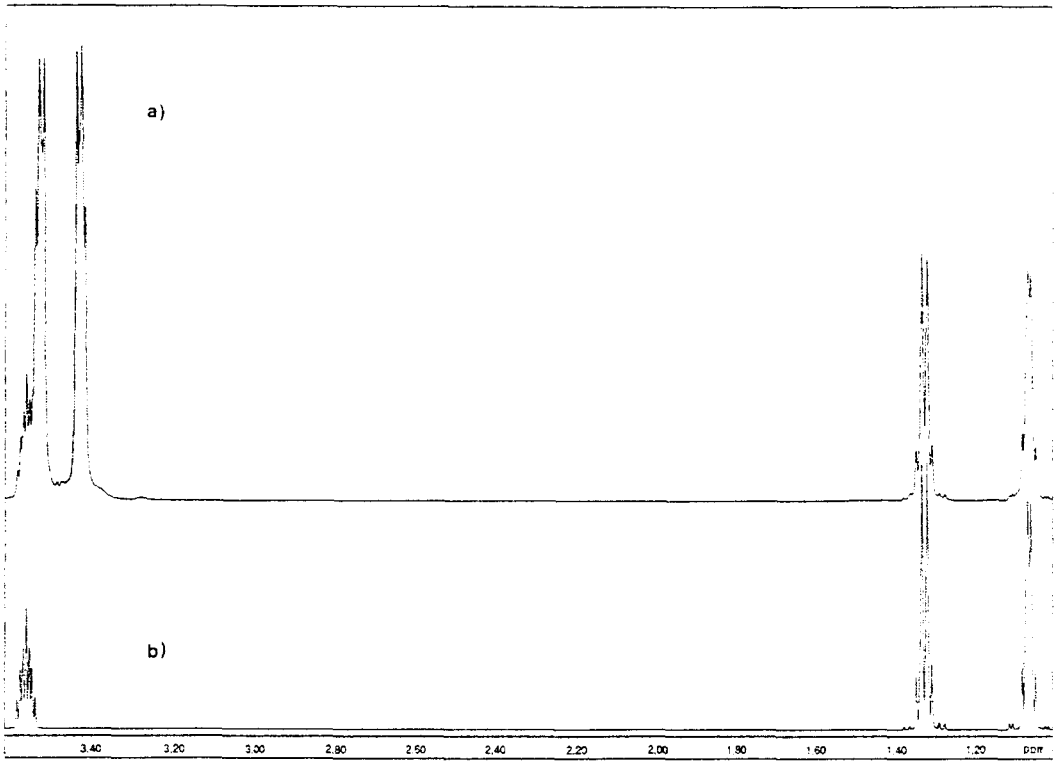

Fig. 5. Part of the recorded ^{1}H NMR spectrum of ciprofloxacin hydrochloride showing the peaks of protons in the cyclopropane moiety and piperazine ring (a). Calculated ^{1}H NMR spectrum for protons of the cyclopropane moiety in ciprofloxacin (b).

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