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Photosensitized degradation of losartan potassium in an extemporaneous suspension formulation

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Abstract

During development of an extemporaneous suspension formulation for losartan potassium, previously unknown degradation products were observed in experimental suspensions prepared in a commercial cherry syrup vehicle. These degradates increased rapidly when analytical solutions prepared from that suspension were exposed to ambient light. The structures of the degradates were determined using a combination of preparative HPLC, LC/MS, ^{13}C and ^{1}H NMR (1D and 2D), and mechanistic chemistry. Each degradate results from destruction of the imidazole ring of losartan. Formation of the two major degradates required exposure to light (UV or visible) and the presence of oxygen. Experiments using Rose Bengal (a singlet oxygen photosensitizer) and 1,4-diazabicyclooctane (DABCO; a singlet oxygen quencher) established that the major photodegradates are formed via the intermediacy of singlet oxygen. The identity of the photosensitizer in the formulation was not unequivocally determined; however, the experiments implicated the artificial flavoring in fulfilling this role.

Keywords: Losartan; Suspension; Formulation; Photosensitized; Degradation; Singlet oxygen

1. Introduction

Losartan potassium is a highly specific, non-peptide angiotensin II antagonist indicated for hypertension [1]. Losartan blocks the renin–angiotensin system by suppressing the effects of angiotensin II at its receptors [2–4], and was the first antihypertensive introduced that acts by this mechanism. Losartan potassium is a salt of 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole (**1**, Fig. 1). Losartan has been formulated in tablets as COZAAR™ and, in combination with hydrochlorothiazide, as HYZAAR™.

An extemporaneous suspension formulation of losartan potassium was recently developed. For this formulation, one COZAAR™ tablet is shaken and dissolved in 1 mL of water, then an excess of a suspending vehicle is added. Because the vehicle is acidic, precipitation of losartan occurs and a suspension results (losartan $pK_a = 4.9$ [5]). Identification of actual and potential degradation products of drug substances, neat and in the presence of excipients is an important endeavor in pharmaceutical laboratories. Stability studies under stress conditions and degradate screening experiments are designed to achieve this goal [6]. During stability studies, an unknown degradate was observed by HPLC at ca. 0.4% relative to losartan, in an experimental cherry syrup formulation stored at 25 °C for 4 weeks. This degradate grew significantly in the analytical solution under ambient laboratory conditions.

The formation of degradation products from losartan in severely stressed COZAAR™ tablets (40 °C/75% RH/3 years) has been reported [7,8]. Three degradation products (two isomeric dimers and an aldehyde oxidation product) were identified by LC/MS and LC/MS/MS. The chromatographic retention of the degradate observed in the cherry syrup suspension formulation did not correspond to that of any of these three known degradates. The major metabolite of losartan is the carboxylic acid analog resulting from oxidation of the hydroxyl group [9–16]. To date, it has not been observed as a degradate in dosage forms; it also could not reasonably be assigned as the degradate in the suspension. Oxidation is common in pharmaceuticals and can proceed through a variety of mechanisms. These include autoxidation and other free radical processes, electron transfer, and nucleophilic or electrophilic reactions of peroxides [17,18].

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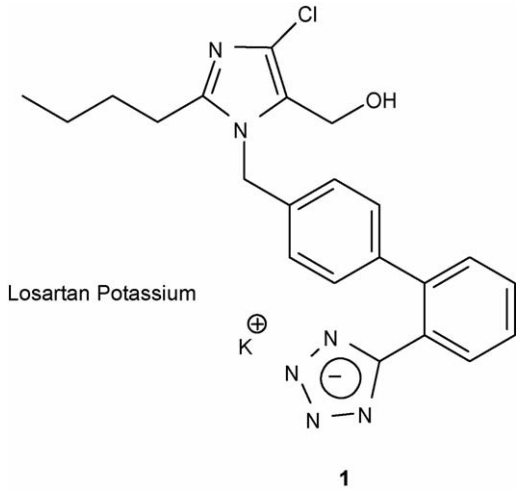

Fig. 1. Structure of losartan potassium (**1**).

For cases in which trappable free radicals are present, radical-scavenging antioxidants (e.g., BHA) have been employed to prevent oxidation. An oxidation mechanism not often reported in pharmaceutical products involves reaction of singlet-state oxygen with drug substances. That mechanism is not suppressed by conventional, radical-scavenging antioxidants. This paper documents the investigation of a novel, singlet oxygen degradation mechanism for losartan in a developmental cherry syrup suspension.

2. Experimental

2.1. Materials

Potassium monohydrogen phosphate, potassium dihydrogen phosphate, and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Fair Lawn, NJ). Trifluoroacetic acid, Rose Bengal (90%), and 1,4-diazabicyclo[2.2.2]octane (DABCO, 98%) were purchased from Aldrich (St. Louis, MO). FD & C Red No. 40 (90%) was from Chr. Hansen (Milwaukee, WI). All reagents were used without further purification. All buffer solutions were made using USP water or HPLC grade water from Fisher Scientific (Fair Lawn, NJ). Cherry syrup for compounding was obtained from Humco, Inc. (Texarkana, Texas). Bulk losartan potassium was provided by Merck Research Laboratories. Losartan cherry syrup formulation (2.5 mg/mL potency) was prepared by dissolving a single COZAAR^{TM} tablet (50 mg) in 1 mL of water, with shaking, followed by addition of Humco^{TM} cherry syrup (19 mL) to cause precipitation of the losartan. Alternatively, suspensions were prepared by dissolving a known amount of losartan potassium bulk drug in water and adding an appropriate volume of cherry syrup suspending vehicle, with stirring, to achieve a 2.5 mg/mL suspension of losartan. Analytical solutions were prepared from suspensions by diluting aliquots of suspension (2 mL) to 100 mL with aq. 50 mM K_2HPO_4 (pH 9.3). The resulting solutions were 0.05 mg/mL in losartan, with a pH of ca. 8.4.

2.2. HPLC analysis of losartan

Analytical HPLC employed a Waters Alliance system, comprising a 2690 separations module and a model 996 photodiode array detector. Quantitative data were collected using the Multichrom version 2.11 data acquisition system (Lab Systems, Beverly, MA). UV/vis spectra were collected using the photodiode array capability of the detector and the Waters Millennium32 software system. Two methods were used for HPLC analysis.

2.2.1. HPLC method 1

Initial experiments employed an Alltech Platinum EPS C8 column (250 mm × 4.6 mm; 5 μm particle size) held at 45 °C with UV detection at 240 nm. The mobile phase comprised aq. 10 mM phosphate buffer (pH 2.3) and acetonitrile at a flow rate of 1.0 mL/min. The following gradient program was applied, %-organic (time, min): 38 (0), 38 (8), 62 (18), 38 (19), 38 (25).

2.2.2. HPLC method 2

A second method was developed to resolve as many of the new degradates as possible, and to be compatible with LC/MS instrumentation. Chromatographic conditions were the same as method 1, except that (a) the mobile phase was composed of aq. 0.1% trifluoroacetic acid and 0.1% trifluoroacetic acid in acetonitrile, and (b) the following gradient program was applied, %-organic (time, min): 15 (0), 15 (20), 50 (35), 50 (40), 70 (43), 70 (48), 15 (49), 15 (55).

2.3. LC/MS and LC/MS/MS analysis

A Perkin-Elmer Series 200 HPLC system (autosampler, LC pump, 235C diode array detector) was interfaced with a Finnigan MAT LCQ ion trap mass spectrometer via an ESI probe. All data were collected in the positive ion mode. Instrument parameters were: heated capillary, 200 °C; sheath gas (N_2), 70; auxiliary gas (N_2), 30; total three microscans; maximum injection time, 200 ms. For MS experiments, the mass range scanned was *m/z* 150–850. MS/MS parameters were: IW 1.5; RCE 30%. Chromatography was performed using method 2.

2.4. Isolation of degradate **2** by preparative HPLC

Degradate **2** was isolated by preparative HPLC on a Waters preparative/analytical system (Delta 600 separations module, model 996 photodiode array detector). Losartan potassium (1 mg/mL) was dissolved in aq. 50 mM K_2HPO_4, containing 1×10^{-4} M Rose Bengal. The resulting solution was exposed to ambient light for 18 h, then refrigerated in the dark at 5 °C. An Alltech Platinum EPS C8 preparative column (250 mm × 22 mm; 5 μm particle size) was used for separation and isolation. Other chromatographic conditions were the same as method 2 with the following exceptions (a) the flow rate was 10 mL/min, (b) injection volume was 2 mL, (c) column temperature was ambient, and the hold times in the gradient elution program were extended somewhat. Fractions containing degradate **2** were collected, combined, and lyophilized. The isolated

Table 1
Extent of degradation of losartan in cherry syrup suspensions and analytical solutions: effect of ambient light[a]

Sample	Mass % by HPLC (% losartan label claim)						
	4 (RRT 0.28)	**3** (0.35)	**5** (0.92)	**6** (0.96)	**7** (0.97)	Losartan	**2** (1.05)
Cherry Suspension, *hν* (28 h)	–	<0.05	<0.05	–	–	100.2	0.49
Cherry Suspension, dark	–	<0.05	–	–	–	102.3	0.08
Analytical Solution, *hν* (36 h)	2.48	10.1	1.30	0.52	0.77	29.8	34.9
Analytical Solution, dark	–	0.25	–	–	–	95.9	1.22

[a] Suspensions were 2.5 mg/mL losartan and analytical solutions were 0.05 mg/mL losartan, prepared as described in Section 2. Analysis using HPLC method 2.

material was characterized by UV/vis spectroscopy and by spiking into HPLC method 2, which confirmed its identity as the primary degradate **2**. The remaining white powder was refrigerated until used for NMR spectroscopy or other experiments.

2.5. NMR spectroscopy

NMR experiments were performed using a Varian Unity Inova 600 MHz spectrometer. All spectra were acquired on samples dissolved in CD_3CN at 25 °C. 2D-experiments included the following: double quantum filter correlation spectroscopy (DQF-COSY) was used to establish the H–H network; heteronuclear single quantum correlation spectroscopy (HSQC) revealed the C–C connectivity; heteronuclear multiple-bond correlation spectroscopy (HMBC) was used to establish the C–C–H and C–C–C–H connectivity.

3. Results and discussion

3.1. Defining the formulation instability

To define the extent of the losartan instability, an experiment was performed in which analytical solutions were prepared from two losartan cherry syrup suspensions. These solutions were 0.05 mg/mL in losartan, with a pH of 8.4. One solution was exposed to ambient light for 48 h while the other was covered with aluminum foil. HPLC analysis (method 1) showed that two peaks with relative retention time (RRT) of 0.42 and 0.51 grew significantly in the solution exposed to light, about 5% and 8% of losartan label claim, respectively. These values are approximate because of partial coelution with excipient peaks. A number of minor peaks eluting in the range RRT 0.69–0.81 also appeared. The losartan peak decreased by about 20%, and it appeared to broaden slightly, possibly indicating the presence of a coeluting compound. A new HPLC method (method 2) was developed to effect resolution of the degradates from the excipients and from losartan. For method 2, the phosphate buffer mobile phase of method 1 was replaced with 0.1% trifluoroacetic acid, for compatibility with LC/MS instrumentation. After the gradient and runtime were adjusted appropriately, it was confirmed that a second peak (the most prevalent degradate) had been partially coeluting with losartan in photodegraded solutions. Therefore, another experiment was performed to assess more carefully the extent of degradation in losartan cherry syrup suspensions and in the analytical solutions prepared from them by dilution with phosphate buffer (see Section 2). Samples of both types were exposed to ambient laboratory light for 28 and 36 h, respectively, with foil-covered samples serving as controls. The HPLC results, presented in Table 1, clearly show that the degradation is induced by light, and that the losartan suspension is much more stable than the analytical solution. Nevertheless, the 0.5% of degradate **2** that is formed in the suspension surpasses the ICH safety qualification threshold [19] for a 50 mg daily dose, in just over 1 day under ambient light. The dilutions for sample preparation for HPLC analysis were performed in the dark to ensure that the observed degradation had occurred in the suspension only. The instability of losartan in the analytical solution is truly remarkable, with a 70% loss after 36 h of ambient light exposure (see Fig. 2). The degradates **2** and **3** predominate; degradates **4**–**7** are relatively minor, but still significant.

Mass balance in the degraded analytical solution is about 80%, assuming the same relative response for all species. Because photodegradation was seen in the cherry syrup formulation only, and not in another suspension formulation that did not contain the cherry syrup (nor in the analytical solutions derived from it), one or more of the cherry syrup ingredients must have been directly involved. A comparison of the listed ingredients of the two suspending vehicles showed that the species exclusive to the cherry syrup vehicle are FD & C Red No. 40, artificial flavors, and inert ingredients. FD & C Red No. 40 is an azo dye, commonly used in foods and pharmaceuticals [20]. It is well known that many dyes act as efficient photosensitizers, generating singlet-state oxygen by energy transfer from their photoexcited states [21,22], and this became our initial working

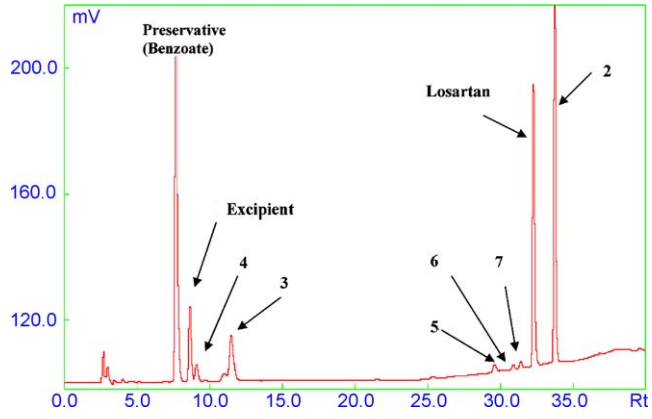

Fig. 2. HPLC chromatogram (method 2) showing the conversion of losartan to degradates **2**–**7**, in an analytical solution derived from losartan cherry suspension (0.05 mg/mL losartan) upon exposure to ambient light (36 h).

Table 2
Photodegradation of losartan in analytical solutions prepared from the cherry syrup formulation: investigation of wavelength of irradiation

Irradiation condition	Relative area % by HPLC				
	4	**3**	**2**	Losartan **1**	Other degradates[a]
UV (320–400 nm) (41 h)	7.6	18.4	40.2	28.6	5.3
UV, covered (41 h)	0.8	0.2	–	99.0	–
Vis (>400 nm) (28 h)	6.8	19.3	38.9	31.0	4.0
Vis, covered (28 h)	0.9	0.3	–	98.8	–

[a] HPLC analysis using method 1. Other Degradates is the sum of other minor species that grow during irradiation. They cannot be assigned due to elution differences between method 1 and the LC/MS-compatible method 2. Losartan and degradate **2** are not fully resolved, and degradates **3** and **4** have minor interferences from excipient peaks; therefore, their relative area% values should be regarded as approximate.

hypothesis. The proprietary components of the artificial flavoring and the inert ingredients remain unknown to us.

3.2. Intermediacy of singlet oxygen in the photodegradation of losartan

The wavelength dependence of the photodegradation was investigated. Covered and uncovered analytical solutions prepared from losartan cherry syrup formulations were placed in photostability chambers. Two irradiation conditions were used, UV-A light for 41 h (ca. 320–400 nm) and visible light for 28 h (>400 nm). The results are presented in Table 2. Both irradiation conditions produced very similar degradation profiles, but the quantitative results in Table 2 should not be overinterpreted. HPLC analysis was by method 1, which does not give full resolution of degradate **2** from losartan, so the results should be considered as approximations. Nevertheless, the gross features and relative intensities of the degradation profile are reliable.

These results indicate that a species absorbing broadly in the visible and UV regions is responsible for the photodegradation. Losartan does not absorb light of wavelength greater than 300 nm, and the control experiment with the alternative formulation exhibited no losartan photodegradation. Therefore, photoexcited losartan can be ruled as the reactive species, and one or more of the excipients is implicated. Because method 1 was employed for this experiment, we were able to demonstrate that neither of the known dimeric degradates of losartan forms under irradiation. Therefore, method 2 (which is not capable of quantitating the dimeric degradates) was used in all subsequent analyses.

In order to identify the formulation component(s) responsible for the photodegradation, binary solutions of losartan with individual components of the cherry syrup (excluding the artificial flavor, which could not be obtained) were exposed to ambient light for 48 h and analyzed by HPLC. An analytical solution prepared from the losartan cherry syrup formulation was included as a control. None of the binary solutions exhibited any degradation, including the solution containing FD & C Red No. 40, which proved inert under these conditions. In contrast, the control solution did degrade significantly (ca. 20%), indicating the likelihood of the artifical flavor or an unspecified impurity playing a role in the photodegradation. The relative unreactivity of FD & C Red No. 40 is not surprising. It has been reported that this azo dye does not initiate singlet oxygen oxidation of soybean oil [23], and others have reported that FD & C Red No. 40, unlike other dyes, does not photoinduce the decarboxylation of peptides [24].

A simple experiment established the necessity of oxygen for the photodegradation. Two analytical solutions of losartan cherry syrup formulation were exposed to ambient light in a fume hood for 16 h. One solution was continuously degassed by bubbling helium through it, to remove oxygen. Table 3 presents the results of subsequent HPLC analyses. The non-degassed solution exhibited significant degradation of losartan, leading to at least six degradates, predominantly **2** and **3**. In the degassed solution, much less degradation of losartan occurred. Fewer degradates were generated (only **2** and **3**), and with significantly reduced intensities (it is likely that the degassing procedure was not successful at removing all of the oxygen).

Two experiments were then performed to confirm that the photodegradation mechanism requires singlet-state oxygen. In the first, DABCO was added to an analytical solution of losartan prepared from the cherry syrup formulation. DABCO efficiently quenches singlet oxygen by charge transfer, with the added advantage that it does not undergo any further reaction, as less structurally constrained tertiary amines do [25,26]. Along with a control analytical solution containing no DABCO, this solution was exposed to ambient laboratory light for 18 h. The presence of DABCO significantly curtailed the loss of losartan, with significantly less of the major degradates **2** and **3** being formed (Table 4). Thus, the major degradation pathway for losartan requires singlet oxygen and leads primarily to **2** and **3**. Interestingly, the presence of DABCO enhanced the formation of two of the minor degradates, **5** and **6**. These two species are likely generated by a mechanism not requiring the presence of free singlet oxygen.

Table 3
Dependence of losartan photodegradation on oxygen[a]

Reaction condition	Relative area % by HPLC						
	4	**3**	**5**	**6**	**7**	Losartan **1**	**2**
O_2 (non-degassed)	0.87	3.01	0.39	0.15	0.25	82.1	13.2
No O_2 (degassed)	–	0.24	–	–	–	98.6	1.16

[a] Solutions were 0.05 mg/mL in losartan and contained cherry syrup ingredients, prepared as described in Section 2. Solutions were exposed to ambient lab light for 16 h in a fume hood. Helium gas was bubbled continuously through one solution to effect degassing.

Table 4
Effect of a singlet oxygen quencher (DABCO) on photodegradation of losartan in analytical solutions of cherry syrup formulation[a]

Reaction condition	Relative area % by HPLC						
	4	**3**	**5**	**6**	**7**	Losartan **1**	**2**
DABCO present (0.009 M)	0.48	0.74	2.36	2.46	0.15	93.6	0.22
No quencher present	2.14	5.68	1.19	0.43	0.90	54.3	35.4

[a] Solutions were 0.05 mg/mL in losartan and contained cherry syrup ingredients, prepared as described in Section 2, and were irradiated with ambient lab light for 18 h. DABCO = 1,4-diazabicyclo[2.2.2]octane.

In the second experiment, singlet oxygen was independently generated using a photosensitizer, Rose Bengal. Two sets of binary solutions of losartan potassium and Rose Bengal were prepared in method diluent (50 mM K_2HPO_4), one at the diluent pH 9.2 and the other adjusted to pH 8.4 (to mimic the pH of the losartan analytical solutions). Two concentrations of sensitizer were investigated, 1×10^{-4} and 5×10^{-4} M. An analytical solution of losartan from the cherry suspension was prepared as a control. Table 5 presents the degradation results after 18 h of exposure to ambient laboratory light. The degradate profiles of the analytical solution and the binary solution of losartan and 1×10^{-4} M Rose Bengal (pH 8.4) are identical in terms of species generated, and though the extent of degradation is different in the two solutions the product ratios for **2**–**7** are similar. This is clear evidence for the intermediacy of singlet oxygen in the losartan photodegradation.

Other mechanistic information can be gleaned from the results of the Rose Bengal experiment. The solution pH, over the relatively narrow range investigated, has little effect on the extent of losartan degradation. However, the relative intensities of the degradates change significantly as a function of pH. At pH 9.2, the ratio of **3** relative to **2** is much higher than at pH 8.4, suggesting that **3** may result from a base-induced reaction of **2**. Degradates **5** and **6** also increase as the pH rises. Increasing the concentration of the sensitizer decreases the extent of losartan degradation, due perhaps to a sensitizer self-filtering effect or to self-quenching. Increased sensitizer concentration does not affect the ratio of **3** to **2**, but it does decrease the sum of **2** and **3** relative to the sum of minor degradates **5** and **6**, which increase significantly at higher sensitizer concentration. These results suggest the existence of a second mechanism leading to degradates **5** and **6**, without the intervention of singlet oxygen. These results are consistent with those of the DABCO-quenching experiment. Minor degradate **7** exhibits the same relationship to pH and sensitizer concentration as **3**, and therefore likely results from base-induced reaction of **2** or some other intermediate in the singlet oxygen mechanism.

To gain further mechanistic information, the photodegradation of losartan in analytical solutions was followed over time. HPLC data were collected over the course of 91 h of continuous irradiation with ambient laboratory light. Fig. 3a and b show the time-course of losartan degradation and the evolution of degradates, respectively. Losartan decreases by 75% in 91 h, while major degradate **2** increases rapidly, reaches a plateau at ca. 65 h, then decreases. The growth of major degradate **3** is very slow initially but accelerates as **2** decreases. These results are consistent with **3** being a secondary degradate that is formed from **2**. The minor degradates **4**–**7** do not appear to exhibit a similar relationship.

3.3. Structural identification of degradates

From the new degradates formed upon ambient irradiation, **2** and **3** were chosen for particular attention, since these account for the majority of the reaction products. LC/MS analysis provided the primary means for structure determination. Degradate **2** was isolated by preparative HPLC and subjected to 2D ^{13}C and ^{1}H NMR spectroscopy. The structure for degradate **3** was confirmed mechanistically.

Fig. 4 shows the LC/UV chromatogram (trace A) and corresponding total ion chromatogram (TIC; trace B) for a losartan analytical solution (0.05 mg/mL) prepared from the cherry syrup formulation and exposed to ambient light for 33 h. The chromatographic conditions employed for the LC/MS experiment were those of method 2; therefore, correlation of the peaks in the HPLC/UV chromatograms was straightforward. Inspection of the LC/MS data and comparison of trace A with B reveals (i) that degradate **5** exhibits enhanced ionization efficiency relative to the other degradates and to losartan, (ii) there is a species eluting about 7 min before degradate **5** that is not observed via

Table 5
Photodegradation of losartan in binary solutions containing the singlet oxygen sensitizer Rose Bengal[a]

Losartan solution	Mass % by HPLC						
	4	**3**	**5**	**6**	**7**	Losartan **1**	**2**
5×10^{-4} M Rose Bengal, pH 9.2	4.62	15.2	7.31	9.66	0.90	19.3	17.4
1×10^{-4} M Rose Bengal, pH 9.2	5.21	24.4	3.58	2.11	2.21	2.73	33.3
5×10^{-4} M Rose Bengal, pH 8.4	4.35	4.96	4.77	4.52	0.58	20.7	24.4
1×10^{-4} M Rose Bengal, pH 8.4	3.49	7.10	1.58	0.82	1.60	3.89	51.1
Losartan analytical solution (from cherry syrup formulation)	1.50	4.0	0.76	0.23	0.42	54.3	22.1

[a] Binary solutions were 0.05 mg/mL in losartan and were irradiated with ambient lab light for 18 h. Mass percent is relative to 0.05 mg/mL losartan.

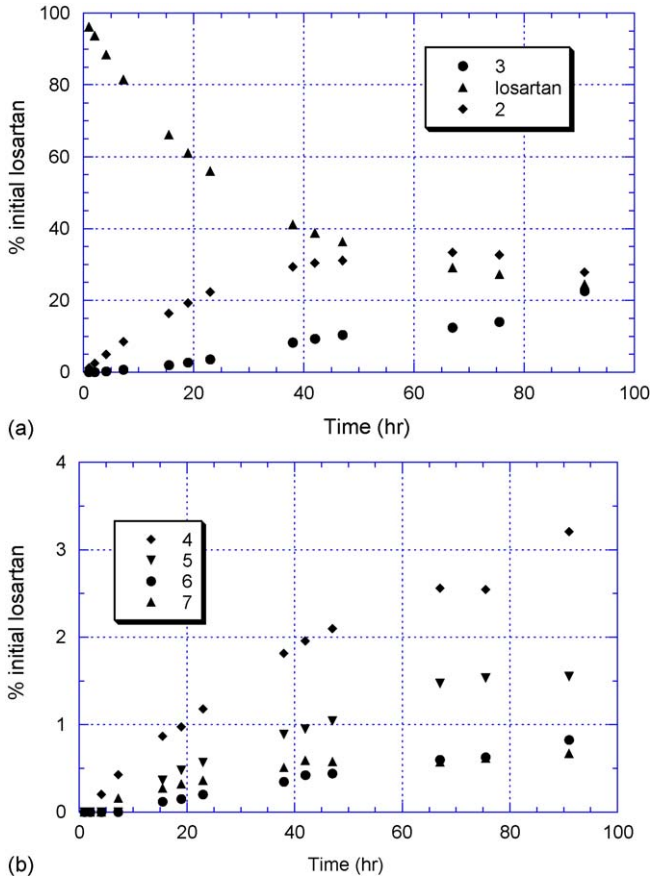

Fig. 3. Time-course for losartan photodegradation in analytical solutions derived from losartan cherry syrup formulation, followed by HPLC assay for 91 h under ambient light.

UV detection and (iii) degradate **6** produces a barely detectable ion signal. The LC/MS and LC/MS/MS data are presented in Table 6. Data from losartan are included to aid in understanding the mass spectra of the degradates. Losartan exhibits $[M+H]^+$ peaks at *m/z* 423 and 425 with a 3:1 relative intensity ratio, as expected for a molecule containing one chlorine atom. The MS/MS spectrum of *m/z* 423 shows, in addition to the three fragment ions already reported for losartan (*m/z* 377, 341, and 207) [7], a predominant fragment ion at *m/z* 405 resulting from expulsion of water. This fragment ion was not observed in the work reported previously because a triple quadrupole mass spectrometer, which typically imparts higher internal energies to the ions than does an ion trap, had been used to generate the MS/MS spectra. The *m/z* 377 fragment ion is presumably formed by losses of H_2O and N_2 from *m/z* 423.

The $[M+H]^+$ ions for most of the degradates were readily determined from the LC/MS spectra (see Table 6). The mass spectrum of degradate **2** is shown in Fig. 5. This species possesses a molecular weight (M_W) of 393 Da, confirmed by the proton-bound dimer signal at *m/z* 787. This M_W requires the presence of an odd number of nitrogens. In addition, the MS data indicate the absence of chlorine from this species. The proposed structure for **2** must account for loss of Cl and at least one N and incorporation of other atoms to bring the M_W to 393 Da. This is accomplished by addition of O_2 to losartan and loss of ClCN. Fig. 6 shows two structures consistent with these constraints for **2**, an ester and an imide. The MS/MS spectrum of *m/z* 394 $[M+H]^+$ is shown in Fig. 7; the fragmentation data cannot easily distinguish between ester **2** and imide **2**. The characteristic fragment ions are at *m/z* 376, 366, 310, 291 and 235. The major fragment ion at *m/z* 310 results from the loss of propylketene from **2**. The *m/z* 366 ion occurs by loss of dinitrogen from the tetrazole ring of **2** (a fragmentation also seen

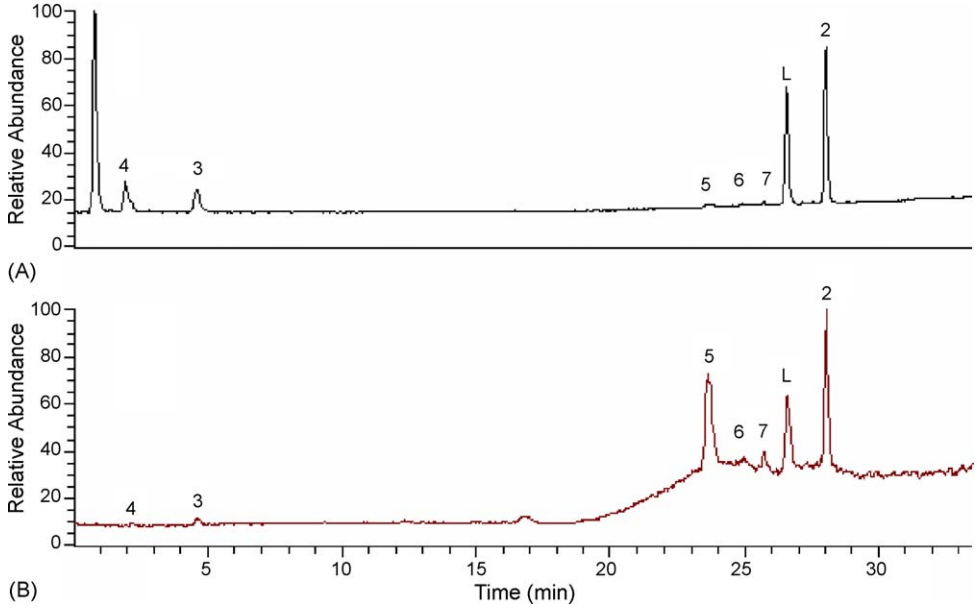

Fig. 4. (A) LC/UV chromatogram (method 2) illustrating losartan photodegradation. (B) Corresponding LC/MS total ion chromatogram for the same analysis. The losartan peak is denoted by "L" and degradates **2**–**7** are indicated.

Table 6
LC/MS and LC/MS/MS data for losartan and six degradates, obtained using electrospray ionization (positive ion mode)[a]

Peak	Major ions, MS (*m/z*)	No. of *N* atoms	MS/MS fragment ions (*m/z*)	Comments
4	252 [*M* + H]	Odd	252 ⇒ 235	No Cl
3	348 332 310 [*M* + H] 276 235 207	Odd	310 ⇒ 235, 207	No Cl; *m/z* 332 and 348 are sodiated and potassiated molecular ions, respectively
5	335 [*M* + H] 207	Even	335 ⇒ 307, 235, 207	No Cl; high ionization efficiency
6	391 [*M* + H]	Even	Not generated	No Cl; very weak signal
7	709 693 671 358 336 [*M* + H]	Odd	**336** ⇒ 291, 252, 235, 207	No Cl; *m/z* 671, 693, and 709 are H^+-, Na^+-, and K^+-bound dimers of M, respectively; *m/z* 358 is sodiated molecular ion
Losartan **1**	423, 425 [*M* + H] 405, 407	Even	423 ⇒ 405, 377, 341, 207	*m/z* 423 and 425 are Cl isotope peaks
2	787 394 [*M* + H] 310	Odd	394 ⇒ 376, 366, 310, 291, 235	No Cl; *m/z* 787 is H^+-bound dimer of M

[a] Data was obtained from solutions of losartan (0.05 or 1.0 mg/mL) and cherry syrup in 0.05 M K_2HPO_4 (pH 8.4), exposed to ambient lab light for 33 h.

for losartan). The *m/z* 235 fragment results from cleavage of the C–N bond in the side chain, leaving behind a stable benzylic cation. The *m/z* 376 ion results from loss of H_2O. Finally, the ion at *m/z* 291 corresponds to migration of the $CH_3(CH_2)_3C{=}O$ group to the tetrazole ring followed by loss of dinitrogen and $H_2NC(O)CH_2OH$. One could envision each of these fragmentations occurring from either the ester or the imide structure for **2**. It is more difficult to arrive at *m/z* 291 from the imide **2** structure, but such a rearrangement is not impossible if enough internal energy exists in the precursor ion of *m/z* 394 upon excitation in the ion trap.

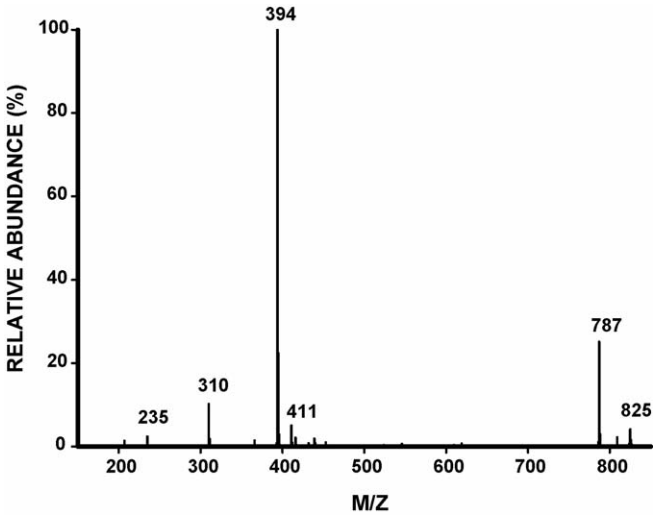

Fig. 5. LC/MS mass spectrum of degradate **2**.

The $[M + H]^+$ ions of degradates **3**–**5**, and **7** are at *m/z* 310, 252, 335 and 336, respectively. The recurrence of the fragment ions *m/z* 235 and 207 in the MS/MS data indicate that each of these species retains an intact tetrazolobiphenyl group. In addition, chlorine is absent from each of these species. Each has an odd number of nitrogen atoms except **5**. Proposed structures are given in Fig. 6. Mechanistically, degradates **3** and **7** could be derived from **2** via hydrolysis, and degradate **4** from **3** and **7**. Structure **5** conserves an even number of nitrogens and by virtue of its amidine functionality would be expected to have a very high electrospray ionization efficiency in the positive ion mode, as is observed. Degradate **6** gave extremely weak signals at *m/z* 391 $[M + H]^+$ and 413 $[M + Na]^+$, consistent with M_W 390 Da (nitrogen atom count even; contains no chlorine). An MS/MS spectrum was not achievable and therefore no structure is proposed for **6**.

Because **2** is the most prevalent degradate and because its structure is not unambiguously assigned from the LC/MS data, it was isolated by preparative HPLC and subjected to NMR analysis. 1D and 2D NMR experiments made it possible to distinguish between ester **2** and imide **2** (numbering in Fig. 6). The ^1H NMR spectrum of **2**, with proton integration values below the axis, is shown in Fig. 8. Resonances from 0.9–2.4 ppm are evidence for a *n*-butyl chain. Resonances at 4.40 (doublet) and 4.51 (singlet) ppm indicate methylene groups, the former of which is split by a neighboring proton. Among the downfield resonances is a broad triplet at 7.18 ppm. This is either an amide or hydroxyl proton, coupled to a neighboring methylene group. The chemical shift of this band is more consistent with an amide hydrogen (ester **2**) than a hydroxyl hydrogen (imide **2**); nevertheless, 2D ^{13}C and ^1H experiments

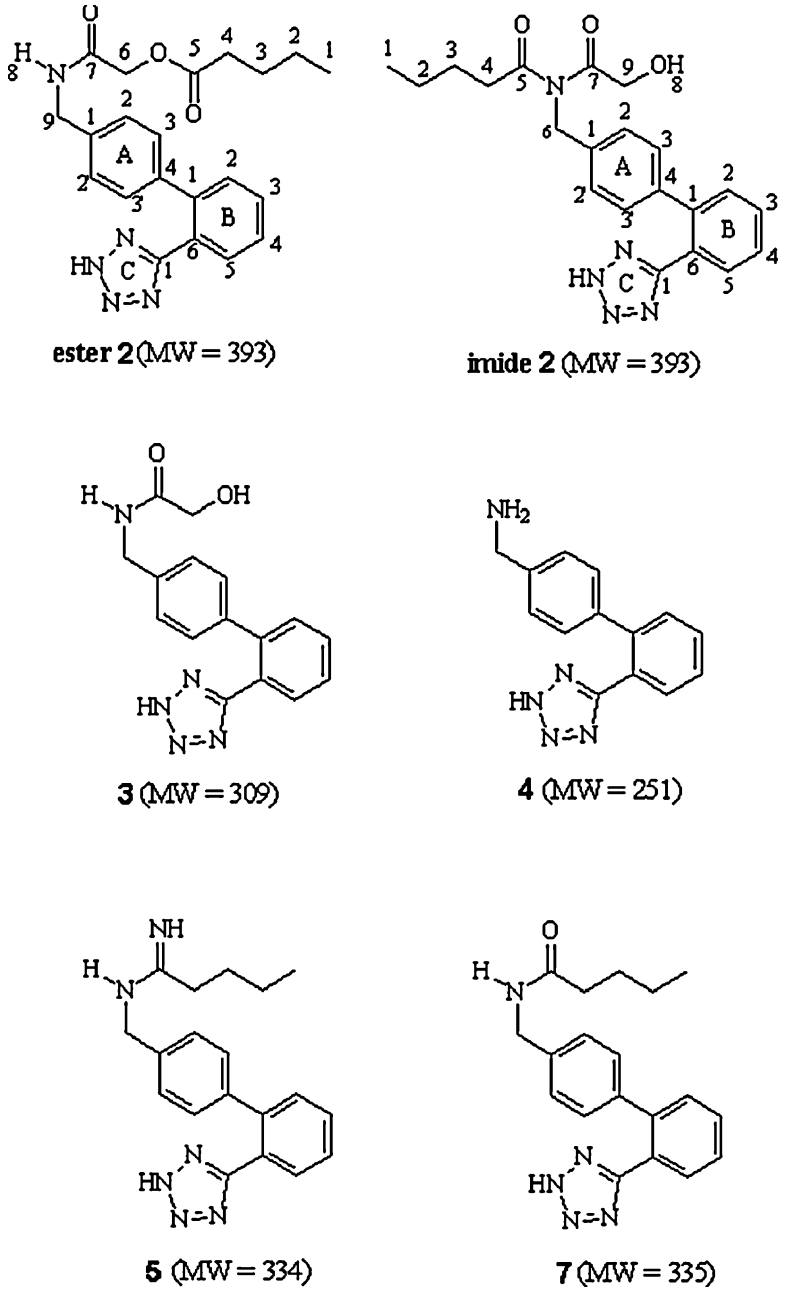

Fig. 6. Proposed structures for degradates **2**–**5** and **7**.

were necessary for full confidence in the structural assignment.

H–H coupling was established in a DQF-COSY experiment, one-bond C–H coupling in an HSQC experiment, and two-bond C–C–H and three-bond C–C–C–H connectivity by performing an HMBC experiment. Table 7 presents the resulting structural assignments for each carbon and hydrogen in degradate **2**. Two carbonyl carbons are evident at 168 and 172 ppm. The key difference between the two regioisomers of **2** is the methylene protons H-6 and H-9, which are located very differently relative to the two carbonyl groups and to the biphenyl moiety. Three-bond C–C–C–H coupling information from the HMBC experiment unambiguously distinguishes between the ester and the imide, as shown in Fig. 9. Methylene protons H-9 at 4.40 ppm couple to only one carbonyl carbon (C-7) and to two types of aromatic carbons, whereas methylene protons H-6 at 4.51 ppm couple to both carbonyls (C-5 and C-7) and to no aromatic carbons. The ester structure for **2** is consistent with this connectivity, whereas the imide structure is not.

The structure of degradate **3** was confirmed mechanistically. Ester **2**, after isolation by preparative HPLC, was subjected to basic hydrolysis in an acetonitrile/aq. buffer solution at pH 8.4 (28 h, dark). Analysis of this solution by HPLC (method 2) indicated significant conversion of the ester to a compound having the same retention time as degradate **3**. At neutral pH, ester **2** does not generate any detectable **3**. Therefore, we conclude that the structure for **3** must be the amide shown in Fig. 6.

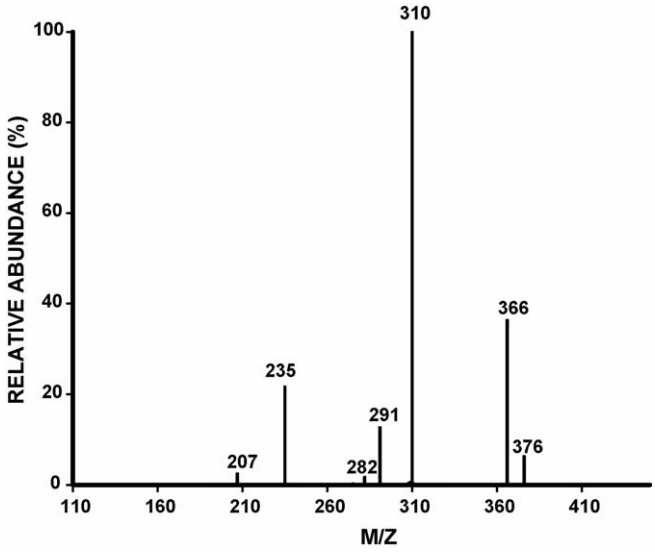

Fig. 7. MS/MS spectrum of molecular ion (*m/z* 394) of degradate **2**.

Table 7
^{13}C and ^{1}H NMR chemical shift assignments for degradate **2**[a]

Assignment	Carbon, δ_C (ppm)	Proton, δ_H (ppm)
1 (CH_3)	13.96	0.907
2 (CH_2)	22.82	1.352
3 (CH_2)	27.58	1.593
4 (CH_2)	34.13	2.416
5 (C)	172.43	
6 (CH_2)	63.46	4.511
7 (C)	168.14	
8 (NH)		7.176
9 (CH_2)	42.68	4.404
A1 (C)	139.69	
A2, A2′ (CH)	128.21	7.229
A3, A3′ (CH)	130.16	7.114
A4 (C)	139.01	
B1 (C)	142.55	
B2 (CH)	131.98	7.546
B3 (CH)	132.27	7.663
B4 (CH)	128.87	7.558
B5 (CH)	131.59	7.728
B6 (C)	123.95	
C1 (C)	155.76	

[a] δ_C referenced to acetonitrile at 1.39 ppm; δ_H referenced to acetonitrile-d_3 at 1.94 ppm.

3.4. Photodegradation mechanism

Each of the major degradate structures is consistent with incorporation of singlet oxygen into losartan. Singlet oxygen has a rich and varied chemistry [27,28]. Common reaction products of singlet oxygen include endoperoxides formed via 1,4-cycloaddition to dienes, dioxetanes from 1,2-cycloaddition to alkenes, and hydroperoxides from formal ene reaction with alkenes [29,30]. Generally, these reactions are electrophilic in nature and do not proceed through radical chain mechanisms.

Many heterocycles react readily with singlet oxygen, including furans, pyrroles, oxazoles, and thiophenes [27,31]. Reports

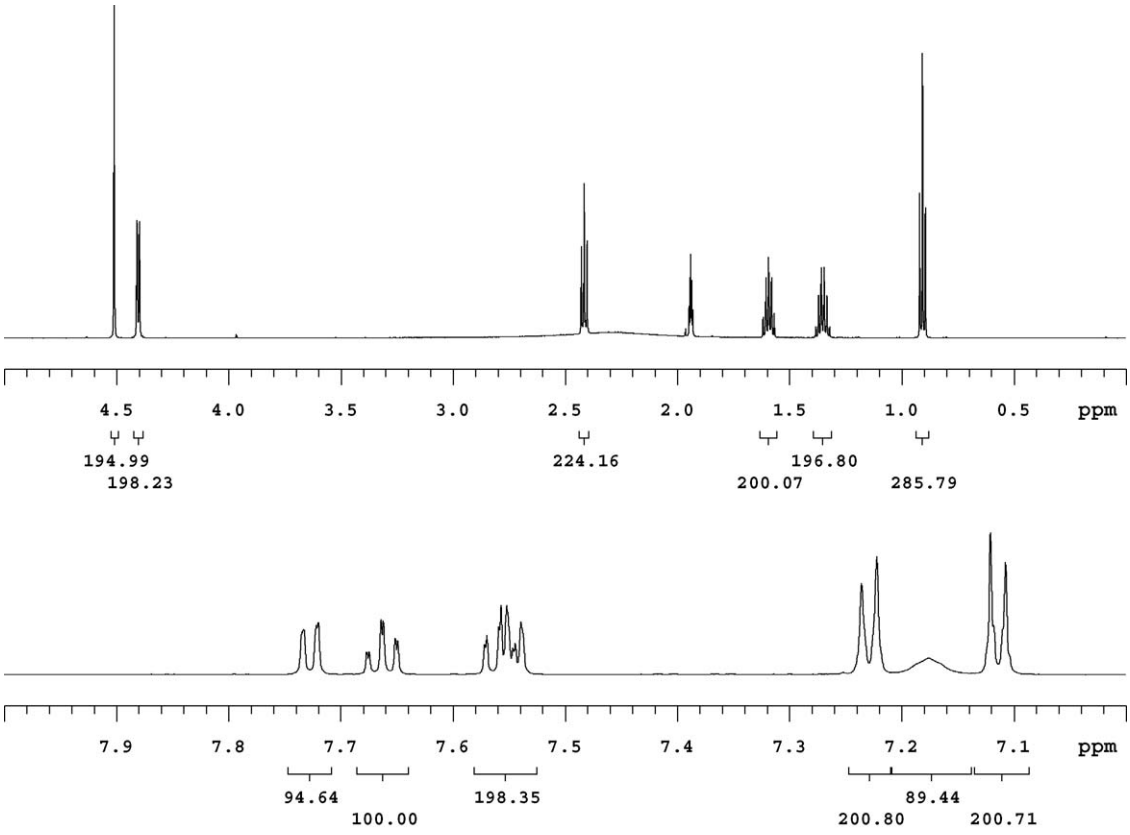

Fig. 8. ^{1}H NMR spectrum of degradate **2** (in CD_3CN at 25 °C). Relative integrations for resonances are indicated below the axis.

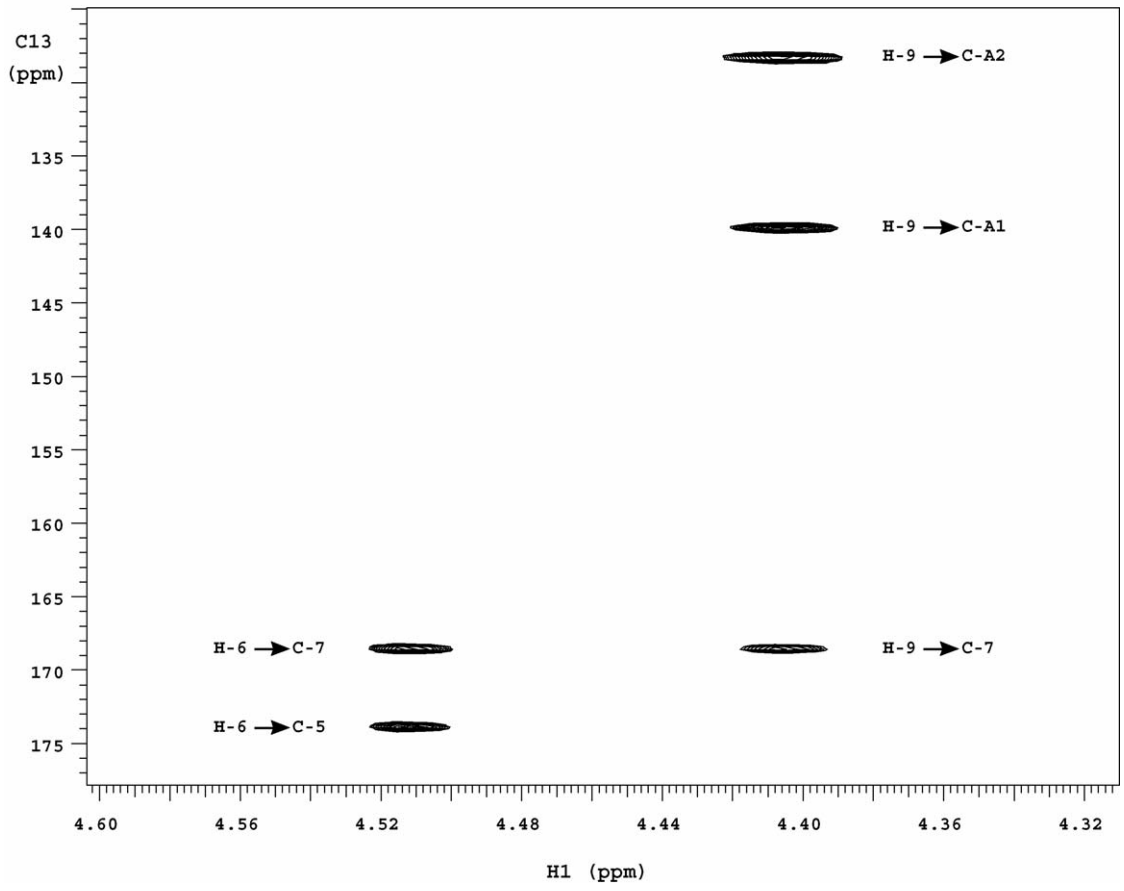

Fig. 9. HMBC 2D NMR spectrum of degradate **2** (in CD_3CN at 25 °C), showing the C–C–H and C–C–C–H connectivity relationships of methylene protons H-6 and H-9 with carbonyl carbons C-5 and C-7 and aromatic carbons C-A1 and C-A2. The *Y*-axis comprises the aromatic and carbonyl region of ^{13}C-chemical shifts. The *X*-axis comprises ^{1}H-chemical shifts.

of singlet oxygen reactions with imidazoles are of direct relevance to the photosensitized degradation of losartan. Photooxidative inactivation of enzymes has been linked to damage of histidine residues [32], which occurs by reaction of the imidazole ring with singlet oxygen [33–35]. Photosensitized cross-linking of proteins at histidine residues is also mediated by reaction with singlet oxygen [36]. Therefore, losartan is expected to be susceptible to photooxidation at the imidazole group. The structurally similar purines also undergo reaction with singlet oxygen [27,37–40]. The reaction intermediates resulting from addition of singlet oxygen to the imidazole or purine rings are rarely observed directly. Evidence exists for three types of intermediates, namely hydroperoxides, 1,2-dioxetanes, and 1,4-endoperoxides. The nature of the intermediates appears to be a function of the structure of the imidazole substrate and the polarity of the solvent.

Our experimental results provide strong evidence for photosensitized generation of singlet oxygen, followed by reaction with losartan at the imidazole ring. None of the degradation products contains a chlorine atom and all possess molecular weights significantly less than that of losartan, indicating the destruction of the imidazole ring. The structure of the primary degradate (ester **2**) is consistent with this mechanism, for it has incorporated two oxygen atoms. Presumably, this species results from rearrangement of the breakdown product of the initial singlet oxygen adduct, which is too reactive for observation in our experiments (Fig. 10). The intermediacy of a 2,5-endoperoxide species is proposed, resulting from 1,4-cycloaddition of singlet oxygen to the imidazole. Mechanistically, the structure of the ester degradate is consistent with this intermediate. The intermediacy of a zwitterionic hydroperoxide (at either the 2- or 5-positions of the imidazole), which may be a favorable intermediate because of the polar/protic nature of the solvent, cannot be ruled out; however, the subsequent reactivity likely occurs from the endoperoxide, which would be relatively unstable in the polar environment and be in dynamic equilibrium with the zwitterion. Formation of a dioxetane at the 4,5-double bond is unlikely. The degradate **2** has incorporated two oxygen atoms, whereas the dioxetane decomposition product would likely possess only one additional oxygen (the second leaving with the expelled small molecule containing chlorine).

Reactive 2,5-endoperoxide intermediates resulting from imidazoles and singlet oxygen have been directly observed at low temperature. Foote et al. successfully characterized the 2,5-endoperoxides derived from 1-methyl-, 1,4-dimethyl- and 1,5-dimethylimidazole using ^{1}H NMR spectroscopy [41]. These species were generated at −78 °C in $CDCl_3/CFCl_3$ and at −100 °C in $(CD_3)_2CO/ CFCl_3$ (1:1). The endoperoxides per-

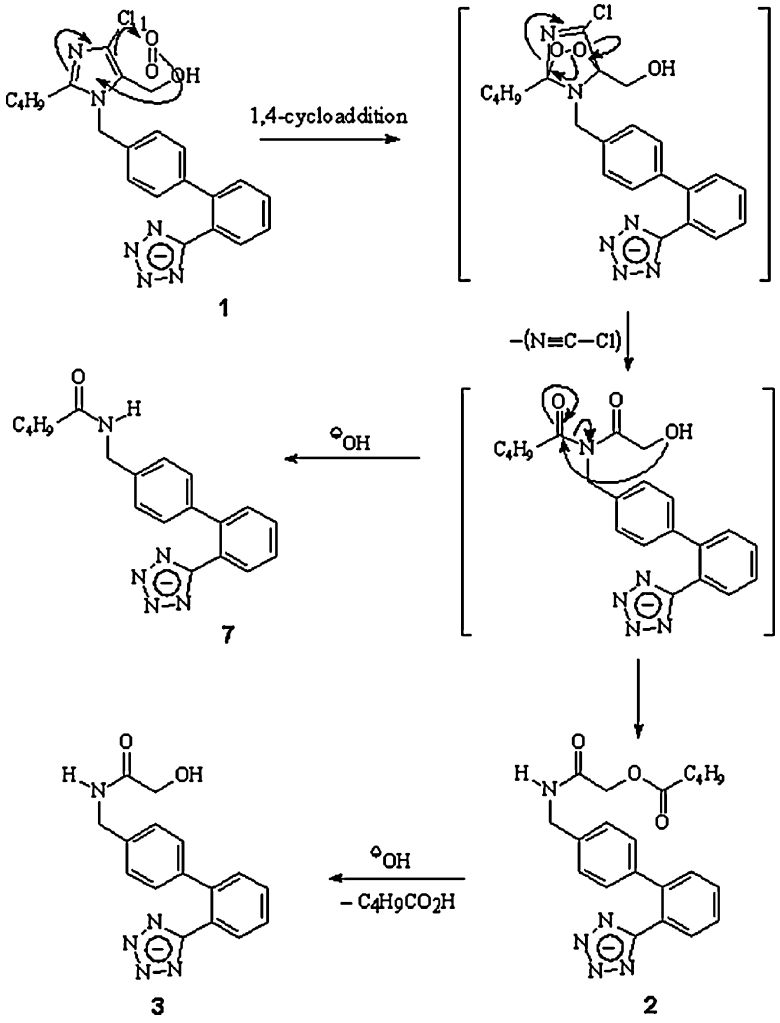

Fig. 10. Proposed mechanism for formation of degradates **2**, **3** and **7**.

sisted at temperatures up to −30 °C in the first solvent system. More recently, the 2,5-endoperoxide of 4,5-diphenylimidazole was characterized by low temperature ^{1}H, ^{13}C and ^{15}N NMR spectroscopy [42]. Foote's group has also observed, by ^{1}H NMR spectroscopy, endoperoxide intermediates obtained from photosensitized reaction of oxygen with a substituted guanosine [43]. These imidazole species are structurally similar to losartan and provide further support for the proposed mechanism. The endoperoxide intermediate decomposes quickly via [4 + 2] cycloreversion (Fig. 10), expelling cyanogen chloride and leading to the unobserved imide **2**. The imide undergoes an intramolecular rearrangement to the primary degradate, ester **2**. This process is 5-*exo*-trig, which is favored according to Baldwin's rules of ring closure [44]. It cannot be concluded whether this rearrangement occurs in the suspension milieu or only in the more basic environment of the analytical solution. The ester **2** is stable at neutral pH. However, under basic conditions (as in the method diluent) this species is susceptible to nucleophilic hydrolysis via hydroxide attack, which leads to the second major degradate, amide **3**. This is consistent with the time-course of degradate evolution shown in Fig. 3b. Hydrolysis of the unobserved imide in competition with intramolecular rearrangement may lead to formation of the minor degradate **7**.

The mechanism for formation of the low-level amidine degradate **5** appears somewhat different than that given above. This species is not generated when oxygen is excluded from the reaction solution, but it does form when the singlet oxygen quencher DABCO is present. Also, in the Rose Bengal experiment, generation of the amidine increased as a function of the sensitizer concentration. One can envisage a number of possible mechanisms, including the formation of an excited state complex (exciplex) between the sensitizer and losartan, which may then react directly with dissolved oxygen. Alternatively, the superoxide ion $O_2^{-\bullet}$ may be the reactive species. Regardless, the structure of the amidine degradate seems to indicate that the immediate reaction product is not an endoperoxide but perhaps a 4,5-dioxetane. Other possible intermediates cannot be ruled out.

4. Conclusions and implications

Losartan undergoes facile reaction with singlet oxygen, resulting in oxidation and subsequent destruction of the

imidazole ring. Singlet oxygen is generated by an unknown photosensitizer present in a commercial cherry syrup suspending vehicle, which was investigated for the development of a suspension formulation of losartan. The photooxidation is very rapid when losartan is dissolved in solution, but is much slower when losartan is suspended. Nevertheless, the photooxidation occurs significantly enough to surpass ICH identification and qualification thresholds for degradates. The degradation proceeds primarily by the photosensitization of oxygen to its singlet state, followed by a formal [4 + 2] cycloaddition of singlet oxygen to the imidazole ring of losartan, leading to multiple degradation products. The structures of two major degradates are established and those of a group of minor degradates are proposed. The identity of the photosensitizer remains unknown; it is likely an unspecified component of the artificial flavoring in the cherry syrup suspending vehicle.

The implications for formulation development and pharmaceutical analysis are important. If a drug substance possesses functionality susceptible to reaction with singlet oxygen (e.g., 1,3-dienes or 1,3-unsaturated heterocycles), one must be wary of selecting excipients for solution or suspension formulations that have the capability for generating singlet oxygen, especially extemporaneous formulations. In particular, flavors and dyes should be carefully evaluated. Photosensitized singlet oxygen reactions also present serious challenges to chemists developing analytical methods. These reactions can make achieving satisfactory solution stability difficult, which undermines the ability of the method to provide an accurate assessment of the degradate content of the formulation itself.

Acknowledgments

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