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Chemosphere 54 (2004) 497–505

CHEMOSPHERE

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Kinetic and chemical assessment of the UV/H_2O_2 treatment of antiepileptic drug carbamazepine

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Received 11 February 2003; received in revised form 18 July 2003; accepted 29 July 2003

Abstract

The UV/H_2O_2-induced degradation of carbamazepine, a worldwide used antiepileptic drug, recently found as contaminant in many municipal sewage treatment plant (STP) effluents and other aquatic environments, is investigated. The oxidation treatment caused an effective removal of the drug. At complete abatement of the substrate after 4 min treatment, a 35% value of removed total organic carbon (TOC) was obtained. A kinetic constant of $(2.05 \pm 0.14) \times 10^9$ $1\,mol^{-1}\,s^{-1}$ was determined for OH radical attack to carbamazepine in the UV/H_2O_2 process. Preparative TLC of the reaction mixture led to the isolation of acridine-9-carboxaldehyde as a reaction intermediate. HPLC and GC/MS analysis indicated formation of small amounts of acridine, salicylic acid, catechol and anthranilic acid among the reaction products. Under the same reaction conditions, synthetically prepared 10,11-epoxycarbamazepine was easily degraded to acridine as main product, suggesting that this epoxide is a likely intermediate in the oxidative conversion of carbamazepine to acridine. Under sunlight irradiation, carbamazepine in water underwent slow degradation to afford likewise acridine as main product. In view of the mutagenic properties of acridine, these results would raise important issues concerning the possible environmental impact of carbamazepine release through domestic wastewaters and support the importance of prolonged oxidation treatments to ensure complete degradation of aromatic intermediates.

Keywords: Acridine; AOP; Carbamazepine; Mineralization; Oxidation; UV/H_2O_2

1. Introduction

Pharmaceutical substances and personal care products have recently been recognized as an important class of organic pollutants increasingly found in wastewaters from urban areas, including effluents from sewage treatment plants (STP) (Daughton and Ternes, 1999; Zuccato et al., 2000; Heberer, 2002). In most industrialized countries during the past decades many medicinal classes, including betablockers, sympathomimetics, antiphlogistics, lipid regulators, antibiotics, vasodilators and so on, have been detected at concentration levels up to micrograms per liter in various aquatic environments. A typical case in point is that of carbamazepine, a worldwide used antiepileptic drug currently prescribed for treatment of a range of other medical conditions, such as chronic pain, and for psychopharmacotherapy, which is consumed in England in quantities of more than 40 t/yr (Jones et al., 2002). Carbamazepine has been identified along with other pharmaceuticals in German feeding waters for waterworks (rivers, bank filtrates and

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0045-6535/$ - see front matter
doi:10.1016/S0045-6535(03)00757-4

groundwater) (Ternes, 1998), in river and lake waters at concentrations varying from 35 $ng\,l^{-1}$ up to 800 $ng\,l^{-1}$ (Ollers et al., 2001), as well as in many municipal sewage treatment plant effluents in Germany, Switzerland (Daughton and Ternes, 1999; Ollers et al., 2001), Italy, France, Greece and Sweden (Andreozzi et al., 2003) and was also found with a concentration of 30 $ng\,l^{-1}$ in drinking water (Ternes, 2001).

Although up to now no specific adverse effects on human health can be ascribed to carbamazepine and other pharmaceuticals as water contaminants and their environmental impact is still to be defined, the precautionary principle warrants current efforts aimed at developing efficient treatment methodologies for removing pharmaceutical contaminants from aquatic environments.

Sand filtration and flocculation by iron(III) chloride proved to be unsuitable for the efficient removal of carbamazepine and other selected contaminants (Ternes et al., 2002). Ozonation, on the other hand, was found to be very effective for the degradation and partial mineralization of carbamazepine (Andreozzi et al., 2002), but the potential offered by advanced oxidation processes (AOPs) has not yet been fully assessed, and no detailed analysis of intermediate and degradation products of carbamazepine during AOP is available. This is yet an important gap since abatement of contaminants provides only a partial indication of the efficiency of the treatment, and the generation of toxic intermediates more resilient to degradation should not be overlooked when comparing different methodologies.

In the present paper the authors report a detailed investigation of the advanced oxidation process of carbamazepine with the UV/H_2O_2 system. The kinetics of the process were investigated and the nature of the primary intermediates and by-products assessed by different analytical techniques.

2. Materials and methods

2.1. Chemicals

Hydrogen peroxide (30% w/w) and humic acid (technical grade) were purchased from Fluka. Carbamazepine, 3-chloroperbenzoic acid, acridine, acridone, iminostilbene, salicylic acid, catechol and anthranilic acid were from Sigma-Aldrich. All other chemicals were of the highest grade commercially available and were used as obtained. Doubly glass-distilled water was used throughout this study.

2.2. UV/H_2O_2 oxidation

Carbamazepine or benzoic acid solutions in water were irradiated with a nominal 17 W low-pressure mercury monochromatic lamp emitting at 254 nm (Helios Italquartz) in a 0.420 l photoreactor. The concentration of hydrogen peroxide solution was determined by iodometric titration (Treadwell and Hall, 1935).

The power output of the lamp was 2.7×10^{-6} $E\,s^{-1}$ as measured by hydrogen peroxide actinometry (Nicole et al., 1990). The pH of reaction mixtures was adjusted to the desired values with perchloric acid and/or sodium hydroxide. When required humic acids at different concentrations were included in the carbamazepine solutions.

2.3. Sunlight irradiation

Solar photodegradation experiments were carried out during Spring at Naples in the South of Italy (40°N–14°E) in 0.5 dm^3 annular magnetically stirred glass reactors thermostated at 25 °C. Aqueous solutions of carbamazepine for irradiation included humic acids or nitrate salts at appropriate concentrations.

2.4. Analytical methods

GC–MS analyses were carried out on a Saturn 2000 apparatus (Varian) equipped with an Ion Trap detector. A DB5-MS fused silica column (Zorbax, 30 m × 0.25 mm ID, 0.25 μm film thickness) was used. Helium was the carrier gas with a 1 $ml\,min^{-1}$ flow rate. The temperature program was as follows: 100 °C for 2 min, 10 $°C\,min^{-1}$ up to 250 °C, hold time 10 min. The injector and GC/MS transferline were taken 250 and 170 °C, respectively. The MS detector was operated in the EI mode, scanning in the range 40–640 amu.

HPLC analyses were performed on a Hewlett Packard 1100 liquid chromatograph using a UV diode array detector. For carbamazepine and acridine analysis a Sinergy RP-max (4.6 × 25 mm) column was used. Elution conditions were as follows: 0.07 M phosphate buffer pH = 2.0 containing 5% CH_3OH (solvent A), CH_3CN (solvent B) 0–5 min: 5% solvent B; 5–25 min: from 5% to 25% solvent B gradient; 25–35 min: 25% solvent B. The flow rate was 1.0 $ml\,min^{-1}$, and the detection wavelength was set at 254 and 300 nm.

Total organic carbon (TOC) was monitored by a TOC analyzer (Shimadzu 5000 A). ^1H and ^{13}C-NMR spectra were recorded at 400.1 and 100.6 MHz, respectively, on a 400 MHz Bruker spectrometer or at 200.2 and 50.1 MHz, respectively on a 200 MHz Gemini Varian spectrometer.

2.5. Product analysis

Aliquots (5 ml) of the oxidation mixture of carbamazepine were periodically withdrawn and directly injected on HPLC. For GC–MS analysis withdrawals were lyophilised and the residue dissolved in methanol

and directly injected into GC–MS instrument. When required the residue was treated with 1,1,1,3,3,3-hexamethyldisilazane (200 μl), anhydrous pyridine (200 μl) and chlorotrimethylsilane (50 μl) (Li et al., 1999). The resulting mixture was shaken vigorously for 1 min and centrifugated to separate the precipitate formed prior to injection into the chromatograph.

National Institute of Standards and Technology (NIST) Library searching was used for compound identification. Identification of the most abundant components was secured by comparison of the chromatographic behaviour and fragmentation patterns with those of authentic samples.

2.6. Product isolation

A solution of carbamazepine (118 mg, 0.5 mmol) and hydrogen peroxide (100 mM) in water (500 ml) was irradiated by UV light for 1 h. The mixture was extracted with ethyl acetate (3×300 ml) and the combined organic layers were dried over anhydrous sodium sulphate, and taken to dryness under vacuum at 25 °C. The residue (40 mg) was analyzed by preparative TLC on silica gel using toluene/chloroform/methanol 8:5:1 as the eluant to afford pure 9-acridincarboxaldehyde ($R_f = 0.82$, 2 mg) identified by comparison of the spectral properties with those of a synthetic sample prepared according to Furst and Uetrecht (1993).

2.7. Synthesis of 10,11-epoxycarbamazepine

To a solution of carbamazepine (100 mg, 0.42 mmol) in anhydrous ethyl ether (10 ml) 3-chloroperbenzoic acid (73 mg, 0.42 mmol) was added under stirring. After 24 h the mixture was taken to dryness. The residue was purified by column chromatography on alumina using chloroform/methanol 98:2 as the eluant to afford 10,11-epoxycarbamazepine (70 mg, 66%) homogeneous to TLC analysis on silica gel (chloroform/methanol 95:5, R_f 0.51). ^{1}H NMR ($CDCl_3$) δ (ppm): 4.28 (s), 4.51 (bs), 7.34 (m), 7.42 (m), 7.50 (d, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ (ppm): 51.3 (CH), 121.0 (CH), 122.8 (CH), 123.1 (CH), 124.1 (CH), 124.3 (C), 130.9 (C), 150.7 (C).

10,11-Epoxycarbamazepine was converted to acridine-9-carboxaldehyde, $R_t = 10.52$ min, m/z 207 (M^+, 100) under the conditions of GC–MS analysis as described (Frigerio et al., 1973).

3. Results and discussion

3.1. Carbamazepine degradation with UV/H_2O_2

UV radiation at 254 nm causes the photolysis of H_2O_2 into OH radicals as a result of the primary photolytic process. OH radical exhibits a high oxidation potential ($E_o = 2.8$ V) (Juang et al., 1998) and can bring attack to a broad range of organic compounds causing eventually their mineralization through sequential hydroxylation–dehydrogenation–breakdown steps (Gulyas, 1997; Andreozzi et al., 1999).

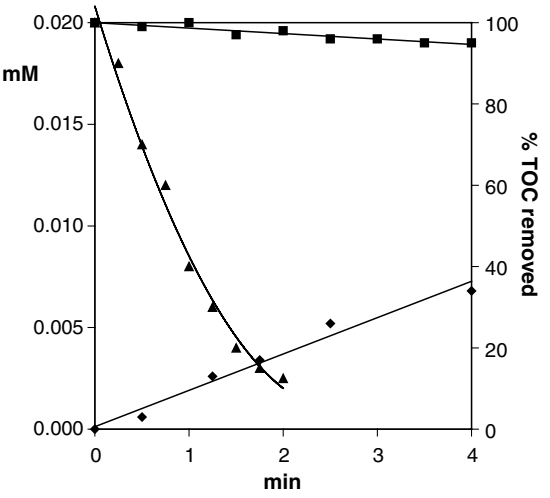


Fig. 1. Carbamazepine decay by UV/H_2O_2 and TOC removed. Carbamazepine was at 2.0×10^{-2} mM, H_2O_2 5.0 mM, pH of the solution was adjusted to 5.0. Left axis: carbamazepine consumption by UV in the presence (▲) or in the absence (■) of H_2O_2; right axis: TOC removed in the UV/H_2O_2 reaction (◆).

Fig. 1 reports the consumption of carbamazepine in aqueous solution (2.0×10^{-2} mM) by UV/H_2O_2 treatment and the TOC removed. The rate of carbamazepine decay was relatively high, with an associated removed total organic carbon (TOC) of about 35% when substrate degradation was complete (≈4 min). Over the same reaction time, direct photolysis of carbamazepine was negligible in the absence of H_2O_2 (full squares in the figure).

Carbamazepine degradation by UV/H_2O_2 treatment was not affected by changing the pH of the solution in the range of 2.0–8.0 (data not shown). The influence of humic acids (4–30 mg l^{-1}), typical constituents of natural aquatic environments, on carbamazepine abatement was also investigated (Fig. 2).

The results indicated a decrease of the system reactivity for humic acid concentrations higher than 4.0 mg l^{-1}, mainly due to the capability of these species to consume hydroxyl radicals (Gao and Zepp, 1998). In fact a simplified analysis based on an approximate rate constant of HO radical attack on humic acid, 2.0×10^4 mg^{-1} l s^{-1} (Hoigné, 1998), allows to calculate ascavenging term in the range of 8×10^4–6×10^5 s^{-1} at concentrations varying from 4.0 to 30 mg l^{-1}. These results indicate that for humic acid concentrations equal to 12 or 30 mg l^{-1} a scavenging effect higher than that due to

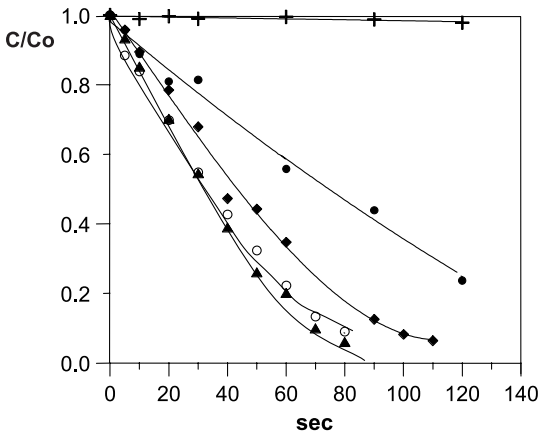


Fig. 2. Effect of humic acids on carbamazepine decay. Carbamazepine was at 8.0×10^{-3} mM, H_2O_2 at 5.0 mM in solutions at pH 5.5. Experiments were run in the presence of humic acids, 4 mg l^{-1} (▲), 12 mg l^{-1} (◆), 30 mg l^{-1} (●); or without H_2O_2 and with humic acids at 4 mg l^{-1} (+); or without humic acids and in the presence of H_2O_2 (○).

hydrogen peroxide at a concentration of 5.0 mM ($k_h\cdot[H_2O_2]=2.7\times10^7$ l mol^{-1} s$^{-1}\cdot5.0\times10^{-3}$ mol l$^{-1}=1.35\times10^5$ s^{-1}) is expected.

3.2. Kinetic analysis

Reaction kinetics were assessed by following the approach successfully proposed by Alnaizy and Akergman (2000) for phenol.

A simplified kinetic scheme (r_1–r_4) was adopted to describe the kinetics of the reaction behaviour of carbamazepine by UV radiation in the presence of hydrogen peroxide. This assumes that the photolysis of hydrogen peroxide at 254 nm generates highly reactive species (hydroxyl radicals)which attack the substrate and hydrogen peroxide.

$$H_2O_2 \xrightarrow{h\nu} 2HO^{\cdot} \quad (r_1)$$

$$HO^{\cdot} + S \xrightarrow{k_p} S^{\cdot} \quad (r_2)$$

$$HO^{\cdot} + H_2O_2 \xrightarrow{k_h} H_2O + HO_2^{\cdot} \quad (r_3)$$

$$2HO_2^{\cdot} \xrightarrow{k_t} H_2O_2 + O_2 \quad (r_4)$$

Considering that UV direct photolysis of the substrate is negligible during its complete removal and taking the steady-state hypothesis for the HO and HO_2 radicals, the rates of consumption of carbamazepine and hydrogen peroxide can be expressed as follows:

$$\frac{d[S]}{dt} = -k_p \cdot \frac{2\phi_{H_2O_2}}{V_{sol}} \cdot \frac{I_o \cdot [1-\exp(-2.3l(\varepsilon_{H_2O_2}\cdot[H_2O_2]+\varepsilon_S\cdot[S]))]\cdot[S]}{k_h\cdot[H_2O_2]+k_p\cdot[S]} \cdot f_{H_2O_2} \quad (1)$$

$$\frac{d[H_2O_2]}{dt} = -\frac{\phi_{H_2O_2}}{V_{sol}}\cdot I_o \cdot [1-\exp(-2.3l(\varepsilon_{H_2O_2}\cdot[H_2O_2]+\varepsilon_S\cdot[S]))] \cdot f_{H_2O_2} - k_h \cdot \frac{\phi_{H_2O_2}}{V_{sol}} \cdot \frac{I_o \cdot [1-\exp(-2.3l(\varepsilon_{H_2O_2}\cdot[H_2O_2]+\varepsilon_S\cdot[S]))]\cdot[H_2O_2]}{k_h\cdot[H_2O_2]+k_p\cdot[S]} \cdot f_{H_2O_2} \quad (2)$$

where $\phi_{H_2O_2}$ is the primary quantum yields of the direct photolysis at 254 nm of hydrogen peroxide ($\phi_{H_2O_2}=0.5$ mol E^{-1}) (Baxendale and Wilson, 1957), V_{sol} is the volume of the aqueous solution (0.42 l), I_o is the measured lamp UV-light intensity at 254 nm (2.7×10^{-6} E s^{-1}), l is the optical pathlength (0.201 dm) of the reactor, ε_S and $\varepsilon_{H_2O_2}$ are the molar extinction coefficients at 254 nm for carbamazepine (6025 mol^{-1} l cm^{-1}) and hydrogen peroxide (18.6 l dm^3 cm^{-1}) respectively, $f_{H_2O_2}$ represents the UV fraction absorbed by hydrogen peroxide and k_h (2.7×10^7 mol^{-1} l s^{-1} (Buxton et al., 1988)) is the kinetic constant for HO radical attack to hydrogen peroxide.

The best value of k_p constant was estimated by comparing the data collected at pH = 5.0 during experimental runs at different carbamazepine (1.0×10^{-3}, 2.0×10^{-3}, 4.0×10^{-3} and 8.0×10^{-3} mM) and hydrogen peroxide (2.0, 5.0 and 7.5 mM) concentrations with those calculated by means of Eqs. (1) and (2). These were integrated with the following initial conditions: $[S]=[S]_0$ and $[H_2O_2]=[H_2O_2]_0$. The best estimated value for k_p, $(2.05\pm0.14)\times10^9$ l mol^{-1} s^{-1}, was chosen as that which minimizes the sum of the square of the difference between calculated and measured substrate concentrations at different reaction times during a single run.

The value of k_p found in this study is lower than that $(8.8\pm1.2)\times10^9$ l mol^{-1} s^{-1} recently reported by others (Huber et al., 2003). To assess the reliability of the present procedure, in a separate set of experiments the H_2O_2/UV treatment was applied to benzoic acid as a standard compound, for which a kinetic constant of 4.3×10^9 l mol^{-1} s^{-1} for HO attack was expected at pH $\leqslant$ 3.0 (Buxton et al., 1988). An oxidation run was thus carried out at pH = 3.0 by following benzoic acid decay, starting from $[H_2O_2]=2.0\times10^{-2}$ mol l^{-1} and [benzoic acid] $=5.0\times10^{-5}$ mol l^{-1} as previously described. Application of the kinetic model allowed to estimate a kinetic constant of $3.07\pm0.33\times10^9$ l mol^{-1} s^{-1}, which is comparable to the literature datum.

Moreover, it is clear that since

(1) the relevant assumption for the development of the model was that the reaction between HO radical and oxidation by-products was negligible;
(2) and that this assumption strictly holds for a fixed starting $[H_2O_2]$ when the initial substrate concentration goes to zero.

The result obtained can be validated by performing an oxidative run on carbamazepine with an initial concentration equal to 4.2×10^{-8} M. In Fig. 3 the results collected during this run are compared with the decay predicted by using the above-reported model with $k_p = 2.05 \times 10^9$ $1\,mol^{-1}\,s^{-1}$, thus demonstrating the validity of the present kinetic model.

3.3. Mechanism of carbamazepine oxidation by the H_2O_2/UV system and intermediate/product analysis

In another series of experiments the intermediates and products formed by H_2O_2/UV oxidation of carbamazepine were investigated to gain an insight into the mechanism of the reaction. To this aim, carbamazepine oxidation was carried out by treating aqueous solutions of the drug at 0.5 mM concentration with 100 mM H_2O_2 under UV irradiation. At various intervals of time, the mixture was analyzed by GC–MS, HPLC with diode array detection, and TLC. Product analysis at 30 min reaction time indicated the presence of several components in small amounts, eluting between 16 and 21 min, which exhibited chromophores typical of acridine derivatives. The major of these products (eluted at 17.6 min) was identified as the parent acridine by comparison of the chromatographic behaviour and UV spectrum with those of an authentic sample.

Fig. 4 shows acridine formation by degradation of 0.5 mM carbamazepine with UV/H_2O_2. After 20 min acridine reached the maximum concentration of 5 μM. Although it is possible that such low yields denote formation of acridine from a minor degradation path of carbamazepine, the kinetic constant for OH radical attack to acridine (5.8×10^9 $1\,mol^{-1}\,s^{-1}$ (Milano et al., 1995)) would also be compatible with decomposition of this intermediate as soon as it is produced.

Preparative TLC of the ethyl acetate extractable fraction of the mixture allowed isolation of a relatively apolar reaction product which was identified as acridine-9-carboxaldehyde (≈2% isolated yield) by comparison of the ^1H NMR and mass spectrum with that of a synthetic sample prepared by oxidation of iminostilbene with *m*-chloroperbenzoic acid (Furst and Uetrecht, 1993).

GC/MS runs showed well discernible peaks for both acridone and iminostilbene, in addition to acridine and acridine-9-carboxaldehyde. Injection of standard carbamazepine solution not exposed to the UV/H_2O_2 treatment gave the same product pattern, though in lower yield, suggesting that carbamazepine was thermally decomposed to acridone and iminostilbene into the GC apparatus. On the other hand, HPLC analysis did not reveal detectable acridone nor iminostilbene.

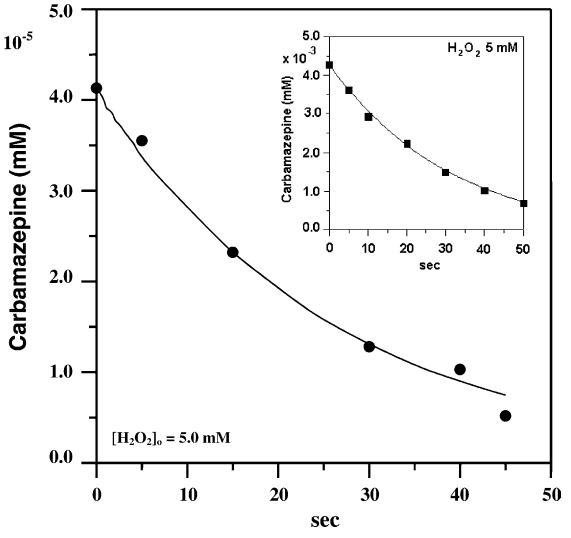


Fig. 3. Comparison between experimental and predicted values for H_2O_2/UV oxidation of carbamazepine at pH = 5.5. Carbamazepine was at (●) 4.2×10^{-5} mM or (■) 4.3×10^{-3} mM, H_2O_2 was at 5.0 mM. All points represent experimental data. The rate of decay determined at concentrations of carbamazepine of 4.3×10^{-3} mM was fitted with the experimental data obtained at carbamazepine concentrations of 4.2×10^{-5} mM.

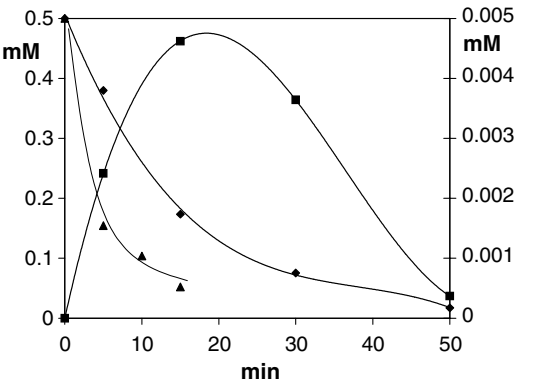


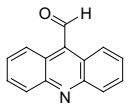
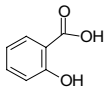
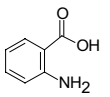
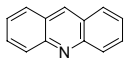
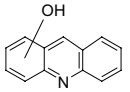
Fig. 4. Carbamazepine and acridine decay by UV/H_2O_2. Carbamazepine was at 0.5 mM, H_2O_2 was at 100 mM, pH of the solution was adjusted to 7.0. Left axis: carbamazepine consumption (◆). Right axis: profile of acridine concentration during carbamazepine degradation, (■); decay of acridine alone at 5.0×10^{-3} mM under the reaction conditions, (▲).

To prevent or limit artifactual product formation by degradation of carbamazepine into the GC injector (Frigerio et al., 1973), GC–MS analyses were carried out only when carbamazepine consumption was far complete. Silylation treatment prior to injection into the gas chromatograph was essential to permit analysis of highly polar polyhydroxylated species and carboxylic acids.

Table 1 shows main aromatic products identified by GC–MS and their fragmentation peaks. Product identification was carried out by comparing chromatographic and mass spectrometric behaviour with that of authentic standards. The products with R_t 17.75 and 17.95 were assigned the structure of hydroxyacridine isomers on the basis of the molecular peaks (m/z 267) and the peak at 177 denoting loss of $(CH_3)_3SiOH$. Anthranilic acid (2-aminobenzoic acid), salicylic acid (2-hydroxybenzoic acid) and catechol (2-hydroxyphenol) were also among the main detectable species, along with hydroxyacetic, oxalic, malonic, oxaloacetic, maleic, fumaric, succinic, tartronic, malic and tartaric acids.

An overall view of carbamazepine degradation pathways is provided in Fig. 5. This scheme is merely indicative and is not intended to provide a comprehensive representation of the actual complexities of the degradation process. One of the initial steps may be hydroxylation of carbamazepine at the 10 position to give a radical intermediate that can evolve to give 10,11-epoxycarbamazepine. Subsequent opening of epoxide ring would give a labile species that suffers facile ring contraction to give 9-acridine-9-carboxaldehyde. This latter would decompose to yield acridine (Furst and Uetrecht, 1993). Support to this mechanism was obtained in separate experiments in which synthetic 10,11-epoxycarbamazepine was exposed to the UV/H_2O_2 system to give acridine among the reaction products. Alternatively, oxidation of carbamazepine by OH radicals may occur through initial attack to the aromatic ring moieties leading to hydroxylated derivatives which may be prone to breakdown via anthranilic acid, salicylic acid, catechol and other intermediates. Anthranilic acid in particular may conceivably arise by oxidative cleavage routes involving the C10–C11 bond of carbamazepine.

Table 1
Main aromatic species formed by carbamazepine identified by GC–MS analysis in comparison with authentic samples

R_t, min	Compound	IUPAC name, CAS number	m/z(relative abundance %)
7.39[a]		2-Hydroxyphenol, 120-80-9	254(100), 239(45), 223(10), 147(9), 133(6), 73(15)
10.51		Acridine-9-carboxaldehyde, 885-23-4	207(100), 179(82), 151(18), 125(6), 100(4), 87(6), 75(12)
10.82[a]		2-Hydroxybenzoic acid, 69-72-7	282(30), 267(100), 223(25), 193(35), 177(3), 165(7), 149(6), 126(12), 91(18), 73(70)
11.33[a]		2-Aminobenzoic acid, 118-92-3	281(3), 266(19), 250(3), 232(6), 208(4), 192(6), 178(3), 149(6), 135(9), 118(6), 73(15)
13.74		Acridine, 260-94-6	179(100), 167(3), 152(6), 126(3), 113(2), 89(3), 75(5)
17.75 and 17.95[a,b]		1-Hydroxyacridine, 5464-73-3 and 2-Hydroxyacridine, 22817-17-0	267(100), 252(80), 224(9), 193(4), 177(10), 166(12), 139(5), 129(4), 117(5)

[a] TMS derivative.
[b] Structural formulation based on fragmentation patterns.

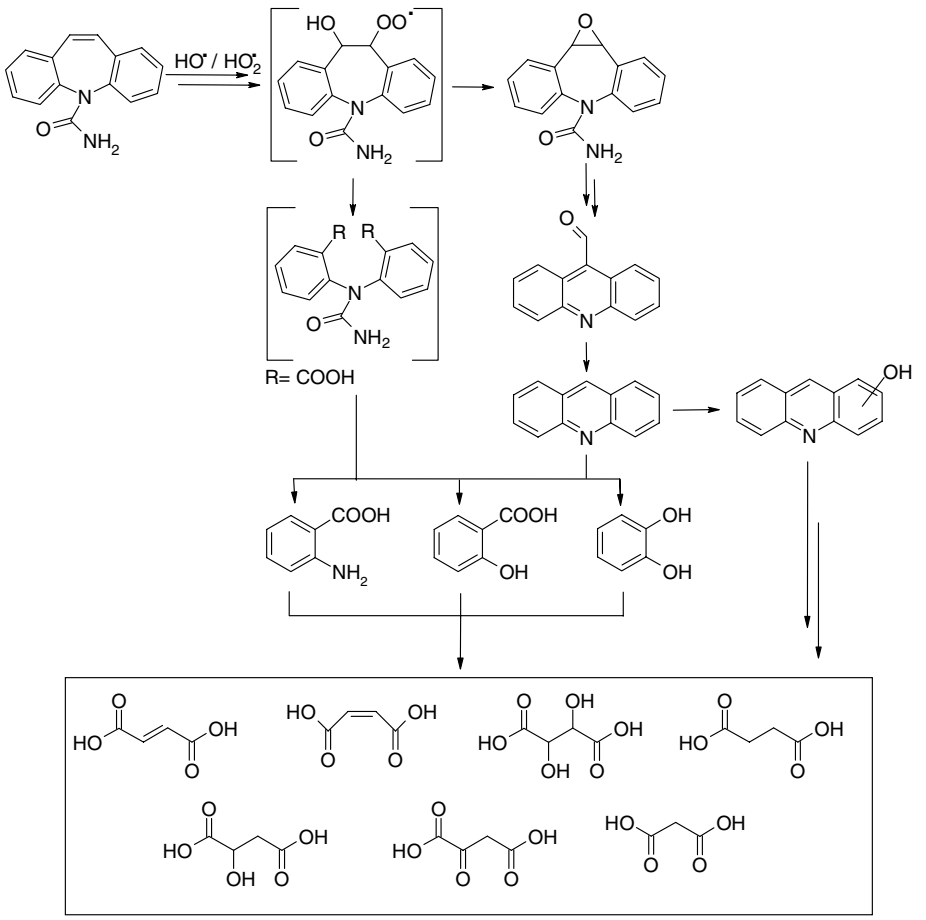


Fig. 5. Carbamazepine degradation pathways induced by UV/H_2O_2 treatment.

3.4. Acridine formation by carbamazepine degradation in aqueous solutions under solar irradiation

Acridine belongs to azaarenes, an established class of air and water pollutants with mutagenic and carcinogenic activity (Shinohara et al., 1983; Furst and Uetrecht, 1993). The observed tendency of carbamazepine to give acridine products during UV/H_2O_2 treatment and the documented facility of iminostilbenes to undergo ring contraction (Haque and Proctor, 1968; Haque et al., 1972; Cann and Lezinisky, 1988) prompted us to extend the study to carbamazepine degradation under sunlight exposure in aqueous solution in presence of humic acid and nitrate which are commonly present in natural waters (Fig. 6). The aim of the experiments was to determine whether acridine formation is a general conversion route of carbamazepine under environmentally relevant conditions.

The results showed that carbamazepine was degraded in all mixtures exposed to sunlight, and less then 10% in the dark control experiments. In all cases, acridine was present as detectable product (HPLC) but not in the dark experiments, reaching the maximum concentration of 6 μM after 300 h exposure to sunlight in the presence of humic acids and nitrate. Apparently, humic acids and nitrate in the photolytic process act by enhancing carbamazepine decay, presumably by generation of reactive oxygen species (Hoigné, 1998), without however inducing accumulation of acridine. Thus, it appears that acridine formation is essentially induced by direct photolysis.

4. Conclusions

The results of the present study indicate that UV/H_2O_2 treatment can cause efficient abatement of carbamazepine partly via a series of acridine intermediates. This is a typical case in which intermediate species are more toxic and hazardous than the parent pollutant, and failure to ensure complete mineralization of these species during AOP may even worsen the environmental and

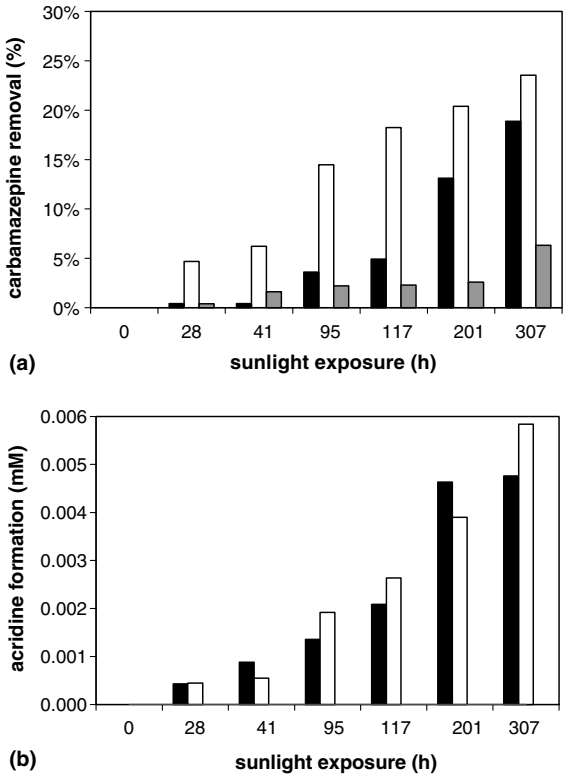


Fig. 6. Effect of nitrate and humic acids on carbamazepine degradation (a) and acridine formation (b) under sunlight exposure. All experiments were carried out with carbamazepine at 0.1 mM. Experimental runs without addition of sodium nitrate and humic acids (black columns) or with sodium nitrate (10 $mg\,l^{-1}$) and humic acids (6 $mg\,l^{-1}$) (open columns). Carbamazepine degradation in dark experiments (grey columns).

health impact of water contamination by carbamazepine. Assuming an aquatic carbamazepine concentration of 100 $ng\,l^{-1}$ (Ternes, 1998), acridine formation yields in the order of $ng\,l^{-1}$ could be grossly estimated. Although these amounts would be far below the reported toxicity levels of acridine (in the μM range, Weigman et al., 2002), the accumulation of acridine products by natural photooxidation or the incomplete oxidation of carbamazepine in water remediation processes might nonetheless be of some environmental relevance.

Acknowledgements

This work was carried out in the frame of the EC project "Ecotoxicological assessments and removal technologies for pharmaceuticals in wastewaters" REMPHARMAWATER (No EVK1-CT-2000-00048). We thank the "Centro Interdipartimentale di Metodologie Chimico–Fisiche of Naples University" for NMR facilities and Dr. Italo Giudicianni and Miss Silvana Corsani for technical assistance.

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