

Advanced Oxidation of Caffeine in Water: On-Line and Real-Time Monitoring by Electrospray Ionization Mass Spectrometry

ILZA DALMÁZIO,[†]
LEONARDO S. SANTOS,[‡]
RENATA P. LOPES,[†]
MARCOS N. EBERLIN,[‡] AND
RODINEI AUGUSTI^{*,†}

Chemistry Department, Federal University of Minas Gerais—UFMG, Belo Horizonte, MG, 31270-901, Brazil, and Thomson Mass Spectrometry Laboratory, Institute of Chemistry, State University of Campinas, 13083-970, Campinas, SP, Brazil

High performance liquid chromatography (HPLC), ultraviolet spectroscopy (UV), and total organic carbon (TOC) analyses show that caffeine is quickly and completely degraded under the oxidative conditions of the UV/H₂O₂, TiO₂/UV, and Fenton systems but that the organic carbon content of the solution decreases much more slowly. Continuous on-line and real-time monitoring by electrospray ionization mass (ESI–MS) and tandem mass spectrometric experiments (ESI–MS/MS) as well as high accuracy MS measurements and gas chromatography–mass spectrometry analysis show that caffeine is first oxidized to *N*-dimethylparabanic acid likely via initial OH insertion to the C4=C8 caffeine double bond. A second degradation intermediate, di(*N*-hydroxymethyl)parabanic acid, has been identified by ESI–MS and characterized by ESI–MS/MS and high accuracy mass measurements. This polar and likely relatively unstable compound, which is not detected by off-line GC–MS analysis, is likely formed via further oxidation of *N*-dimethylparabanic acid at both of its *N*-methyl groups and constitutes an unprecedented intermediate in the degradation of caffeine.

Introduction

Concern about the effects of accumulation of pharmaceutical compounds in the environment has greatly increased in recent years. Such accumulation depends mainly on the environmental bio- and phototransformation and natural degradation of these harmful contaminants (1–3). The photolytic degradation of chemical compounds in the environment may be caused by direct sunlight absorption or by reaction with strong oxidizing species such as hydroxyl radicals and singlet oxygen. Various photolysis systems have been used to evaluate abiotic degradation of drugs, such as sunlight irradiation (4), UV/H₂O₂ (5), photo-Fenton reactions (6), and TiO₂/UV (7). Despite the growing number of studies on abiotic degradation of drugs in aqueous solution, only

scarce information on the intermediates and products arising from these processes is available (8).

Caffeine is ranked as the number one drug worldwide, with a massive production of hundreds of tons (9). Usually employed as a stimulant, caffeine is commonly found in coffee, tea, chocolate, cocoa, and soft drinks. It is also a component of hundreds of prescription drugs, ranging from analgesics to cold medicines. Caffeine is therefore introduced continuously into the sewage system through many anthropogenic sources and is likely to persist in water because of its high solubility (21.7 g L⁻¹) and negligible volatility (10). The widespread occurrence of caffeine in sewage, soil, and wastewaters has recently been documented (9, 11–12). Caffeine has also been reported (13–16) to scavenge highly reactive free radicals, including hydroxyl radicals and excited states of oxygen, and to protect crucial biological molecules against these species. The antioxidant activity of caffeine is similar to that of the established biological antioxidant glutathione and significantly higher than that of ascorbic acid (17).

Owing to outstanding sensitivity, speed, and selectivity, mass spectrometry (MS) has been used extensively to monitor the composition and degradation of organic compounds in the environment, but owing to the limitations of classical ionization techniques, MS monitoring has been commonly restricted to the more volatile and lighter components (18–28). Molecular analysis by MS has, however, profited greatly from the development of electrospray ionization (ESI) (29, 30), which has enabled the ionization of most polar molecules and supramolecules (31, 32). ESI has also shown remarkable capability to gently transfer key ionic reaction intermediates to the gas phase with high sensitivity without inducing undesirable side reactions (33–39), and the composition of ESI-generated ions has been found to closely reflect that of the solution (40–44). ESI–MS(/MS) with its unique characteristics is therefore becoming a major technique to elucidate reaction mechanisms especially in solutions of polar solvents including water via the detection and identification of reactants, products, and intermediates, even the short-lived ones occurring at very low concentrations (31–44).

Gas chromatography–mass spectrometry (GC–MS) has often been the technique of choice for product detection and structural elucidation (5, 45). But because GC–MS is an off-line technique that demands extraction and sometimes derivatization pre-steps, transient, polar, or relatively unstable compounds may be missed. Using three different oxidation systems: UV/H₂O₂, TiO₂/UV, and Fenton, we have then performed on-line and real-time ESI–MS and ESI–MS/MS monitoring of advanced oxidation of caffeine in water to directly screen for intermediates and products of this environmentally important process. We also used gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC), ultra violet spectroscopy (UV), and total organic carbon (TOC) analyses to collect additional data on substrate degradation rates and mineralization and to investigate the presence of residual organic compounds in solution.

Experimental Procedures

Chemicals. Caffeine (purchased from Aldrich), TiO₂ P25 (Degussa), H₂O₂ 32% (Merck), ferrous sulfate heptahydrate (Aldrich), sodium thiosulfate (Merck), and HPLC grade methanol (Merck) were used as received without further purification. Doubly distilled water was used to prepare the solutions in all experiments.

* Corresponding author phone: 55-31-34995725; fax: 55-31-34995700, e-mail: augusti@ufmg.br.

[†] Federal University of Minas Gerais.

[‡] State University of Campinas.

Degradation Procedures. TiO₂/UV System. A 100 mL caffeine (1.6×10^{-4} mol L⁻¹) and TiO₂ (0.1 g L⁻¹) aqueous solution was irradiated using a monochromatic UV lamp (254 nm, 15 W, Philips TUV G5T8). Aliquots (10 mL) were taken at several reaction times and submitted to centrifugation (Sigma) at 2600 rpm/10 min. All the samples were kept protected from light during 12 h in a refrigerator at 4 °C prior to the ESI-MS and HPLC analysis.

Fenton System. The pH of a caffeine aqueous solution (100 mL at 1.6×10^{-4} mol L⁻¹) was adjusted to 3 with 0.1 mol L⁻¹ H₂SO₄. Under stirring, 0.5 mL of H₂O₂ 32% (4.7 mmol) and 10 mg of ferrous sulfate heptahydrate (0.3 mmol) were added. Aliquots (10 mL) were taken at different times, and the reaction was stopped by the addition of an excess of sodium thiosulfate. The aliquots were then maintained protected from light during 12 h in a refrigerator at 4 °C prior to the ESI-MS and HPLC analysis.

UV/H₂O₂ System. A 100 mL caffeine (1.6×10^{-4} mol L⁻¹) and H₂O₂ (1.88 mmol L⁻¹) aqueous solution was irradiated by a monochromatic UV lamp (254 nm, 15 W, Philips TUV G5T8). The reaction was stopped by the addition of an excess of sodium thiosulfate. Aliquots (10 mL) were taken at different times and maintained protected from light during 12 h in a refrigerator at 4 °C prior to the ESI-MS and HPLC analysis.

Analytical Methods. HPLC analyses were carried out on an SPD-10A (Shimadzu) instrument using a LC-18 Supelcosil column (250 mm length \times 4.6 mm i.d., 5 μ m particle size). The following operating conditions were employed: isocratic elution of MeOH/H₂O (1:9), flow rate of 1 mL min⁻¹, injection volume of 20 μ L, and UV-vis detector set up at 215 and 274 nm.

Ultraviolet (UV) absorbance measurements were performed using a Cary 50 Conc instrument (Varian) equipped with a quartz cell with a 1 cm path length. Spectral absorbance was measured with a baseline correction, scan rate of 300 nm min⁻¹, and data point interval of 0.5 nm.

Total organic carbon (TOC) experiments were carried out in a TOC 5000A (Shimadzu) instrument at 680 °C using a platinum catalyst.

ESI-MS and ESI-MS/MS analyses were conducted in a high resolution hybrid quadrupole (Q) and orthogonal time-of-flight (TOF) mass spectrometer (Q-Tof, Micromass UK) with constant nebulizer temperature of 50 °C. The ESI source and the mass spectrometer were operated in the positive-ion mode, and the cone and extractor potentials were set to 40 and 10 V, respectively, with a scan range of m/z 50–1000. Samples were directly infused into the ESI source at flow rates of 10 μ L min⁻¹ via a microsyringe pump. MS/MS experiments were carried out by mass selection of a specific ion in Q1 and then submitted to collision-induced dissociation (CID) with argon in the collision chamber. The product ion MS analysis was accomplished with the high-resolution orthogonal TOF analyzer.

GC-MS analyses were carried out in an HP5989A series II gas chromatograph coupled with an HP5973 mass spectrometer with an HP-5 capillary column (30 m length \times 0.25 mm i.d., 0.25 μ m film thickness) and helium as the carrier gas at 1 mL min⁻¹. For analyses, 1 μ L of sample was injected using the splitless mode. The temperature program was as follows: 100 °C for 1 min, 10 °C min⁻¹ up to 280 °C, hold time of 5 min. The injector and MS ion source temperatures were kept at 270 and 280 °C, respectively. The MS detector was operated in the EI mode at 70 eV, with a scanning range of m/z 50–500. Prior to injection, the aliquots were handled and reduced to a final volume of 2 mL according to the following steps: (i) complete water evaporation under vacuum at 50 °C; (ii) addition of 10 mL of methanol followed by 1 g of Na₂SO₄ to eliminate residual water; (iii) filtration

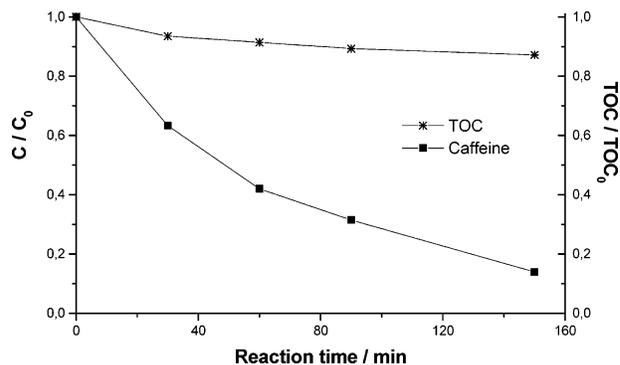


FIGURE 1. Normalized caffeine concentration (C/C_0) as a function of time as monitored via HPLC analyses and total organic carbon content (TOC/TOC_0) for TiO₂/UV aqueous degradation. C_0 of 1.6×10^{-4} mol L⁻¹ for 15.3 mg L⁻¹ TOC₀.

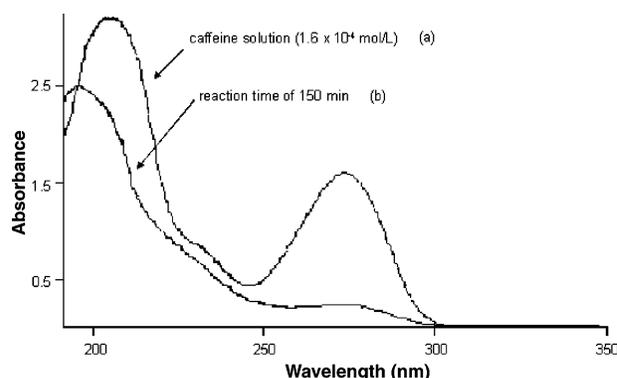


FIGURE 2. UV spectra of (a) an aqueous solution of caffeine at 1.6×10^{-4} mol L⁻¹ and (b) an aliquot of the reaction mixture withdrawn after 150 min of exposure to the TiO₂/UV system.

followed by extractions with six portions of 5 mL of CH₂Cl₂; and (iv) evaporation under vacuum to a final volume of 2 mL of CH₂Cl₂.

Results and Discussion

Degradation of Caffeine. Figure 1 shows TOC and HPLC data for the monitoring of the degradation of caffeine by the TiO₂/UV system. Note that whereas ca. 90% of caffeine is degraded after a reaction time of 150 min, only ca. 13% of TOC removal is observed. These results clearly suggest that although caffeine is almost fully consumed, its mineralization leading to CO₂, H₂O, and NH₃ is rather slow under TiO₂/UV oxidative conditions. Therefore, degradation of caffeine is likely to generate persistent organic intermediates that are not so efficiently oxidized as compared to caffeine. We obtained similar results (not shown) for the UV/H₂O₂ and Fenton degradation of caffeine.

The same conclusions just outlined are reached when examining Figure 2, which shows the UV spectra of the following solutions: (a) caffeine at 1.6×10^{-4} mol L⁻¹ and (b) an aliquot of the reaction mixture withdrawn after 150 min of exposure to the radiation. The UV spectrum of Figure 2a shows the two characteristic absorption bands of caffeine at ca. 274 and 205 nm. In the UV spectrum of the reaction solution, the intensity of both absorption bands is greatly reduced, and a band at ca. 195 nm becomes prominent. This finding confirms that caffeine is quickly degraded, but not mineralized as quickly, and that persistent organic intermediates resist further oxidation.

ESI-MS Monitoring and ESI-MS/MS Structural Elucidation. Figure 3 displays representative ESI mass spectra in the positive ion mode acquired after 0, 90, and 150 min of TiO₂/UV degradation of caffeine (1) in water. Similar

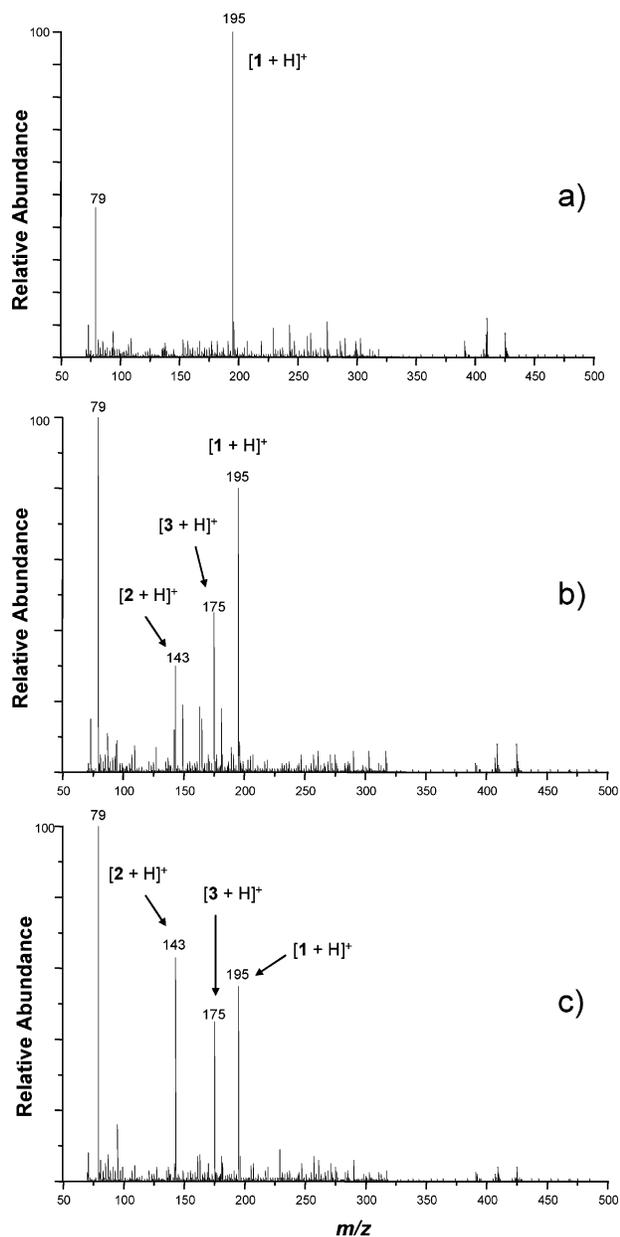


FIGURE 3. ESI(+) mass spectra acquired for aliquots of the reaction mixture after degradation of caffeine (1) with the TiO_2/UV system for (a) 0 min; (b) 90 min; and (c) 150 min. The ion of m/z 79 was detected in all cases likely due to the presence of a contaminant inside the mass spectrometer ion source.

spectra (not shown) were acquired when using the $\text{H}_2\text{O}_2/\text{UV}$ and Fenton systems.

The ESI mass spectrum at zero irradiation time (Figure 3a) detects, as expected, an intense ion of m/z 195 corresponding to protonated caffeine. The ESI tandem mass spectrum (Figure 4a) for collision induced dissociation of protonated caffeine of m/z 195 shows a series of major and structurally diagnostic fragment ions arising from the loss of CH_3 (m/z 180), H_3CNCO (m/z 138), CH_3 plus H_3CNCO (m/z 123), and CO plus H_3CNCO (m/z 110). High accuracy measurement shows excellent agreement between experimental (195.0869 Da) and calculated (195.0883 Da) m/z values for $[\text{I} + \text{H}]^+$ of $\text{C}_8\text{H}_{11}\text{N}_4\text{O}_2$ composition with an error of 7 ppm.

After irradiation for 90 min (Figure 3b) as well as 150 min (Figure 3c), ESI is able to gently and efficiently fish directly from the solution to the gas phase for MS analysis two additional and increasingly abundant ions of m/z 143 and

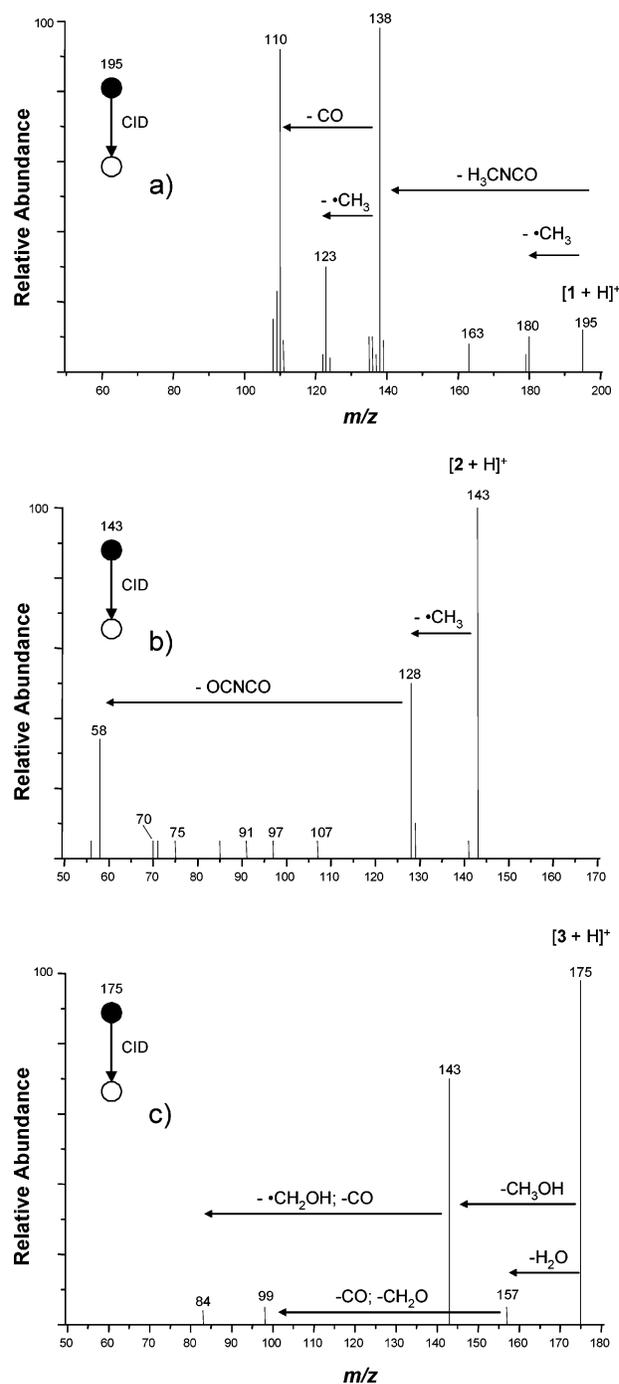
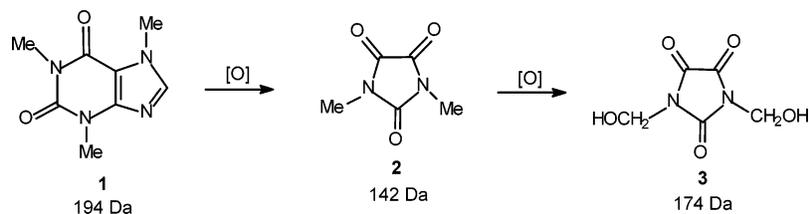


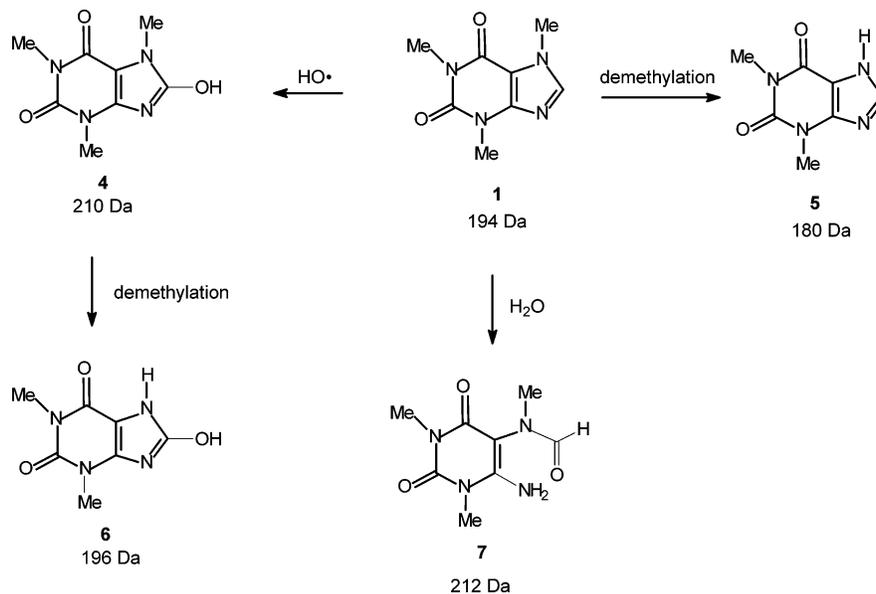
FIGURE 4. ESI tandem mass spectra of (a) protonated caffeine ($[\text{I} + \text{H}]^+$) of m/z 195; (b) the ion of m/z 143 attributed to $[\text{2} + \text{H}]^+$; and (c) the ion of m/z 175 attributed to $[\text{3} + \text{H}]^+$.

175. The ion of m/z 143 indicates mass reduction of 52 Da (from caffeine of 194 Da) and is likely to be the protonated form of dimethylparabanic acid **2** (Scheme 1), a known oxidation product of caffeine (1) (46). The other ion of m/z 175 indicates a mass increase of 32 Da from **2** (incorporation of two oxygens) and therefore that **2** has been further oxidized likely at both of its *N*-methyl groups yielding **3** (Scheme 1), that is, di(hydroxymethyl)parabanic acid detected in its protonated form of m/z 175. High accuracy measurements also show excellent agreement between experimental (143.0511 and 175.0365) and calculated (143.0457 and 175.0355) m/z values for both $[\text{2} + \text{H}]^+$ and $[\text{3} + \text{H}]^+$ of $\text{C}_5\text{H}_7\text{N}_2\text{O}_3$ and $\text{C}_5\text{H}_7\text{N}_2\text{O}_5$ compositions, respectively. The differences between calculated and experimental high ac-

SCHEME 1



SCHEME 2



curacy values for $[2 + H]^+$ and $[3 + H]^+$ are, therefore, 37.8 and 5.6 ppm, respectively.

The conversion of **1** to **2** and then to **3** (an unprecedented intermediate in the oxidation of caffeine) is further supported by the ESI tandem mass spectra of their protonated molecules, which display a series of structurally diagnostic fragment ions. The mass selected $[2 + H]^+$ of m/z 143 (Figure 4b) dissociates mainly by the loss of CH_3 (m/z 128) and CH_3 plus a neutral molecule (or molecules) of NC_2O_2 composition (m/z 58). These dissociations are consistent with both the presence of *N*-methyl group and the OCNCO connectivity of **2**. The mass-selected $[3 + H]^+$ of m/z 175 dissociates mainly by the losses of H_2O (m/z 157) and CH_3OH (m/z 143), which are consistent with the presence of *N*- CH_2OH groups for **3** (Figure 4c).

It was previously reported that the reaction of caffeine with free hydroxyl radicals yields mainly 1,3,7-trimethyluric acid (**4**) resulting from site-specific hydroxylation on C8. However, products from *N*-demethylation such as theophylline (**5**) were also detected in small amounts (47). Under Fenton conditions, both demethylation and hydroxylation at C8 occur, yielding mainly 1,3-dimethyluric acid (**6**) (47), whereas another product, 6-amino-5-(*N*-formylmethylamino)-1,3-dimethyluracil (**7**) from opening of the imidazole ring, was also detected (Scheme 2) (48). Note that whereas the products **4**, **6**, and **7** were not detected in their protonated forms by ESI-MS analysis, the presence of the ion of m/z 181 in the mass spectrum of Figure 3b could indicate the formation of *N*-demethylated products such as **5** under these reaction conditions. However, the low intensity of this ion prevented accurate mass measurements and MS/MS CID experiments. Thus, the formation of **5** could not be unequivocally confirmed.

GC/MS Analysis. We also analyzed the organic material after caffeine degradation by GC-MS. Figure 5 shows, as an

example, the chromatogram obtained after caffeine degradation by the Fenton system. Very similar chromatograms (not shown) were obtained using TiO_2/UV and UV/H_2O_2 . The major chromatographic peak is from dimethylparabanic acid (**2**) as the excellent agreement between the experimental 70 eV electron ionization (EI) mass spectrum (Figure 5) and the mass spectrum of authentic **2** (49) indicates. Curiously, no evidence for detection of the oxidized product **3** (nor for **4**–**7**), even after sample derivatization with $Si(Me)_3Cl$, is provided by the GC-MS data. This finding appears therefore to explain why **3** has not yet been detected in previous oxidation experiments with caffeine (46, 50). Likely, owing to its high polarity or thermal instability, or even degradation

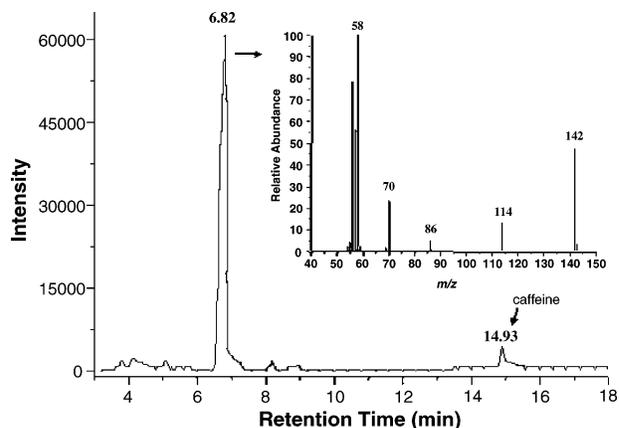
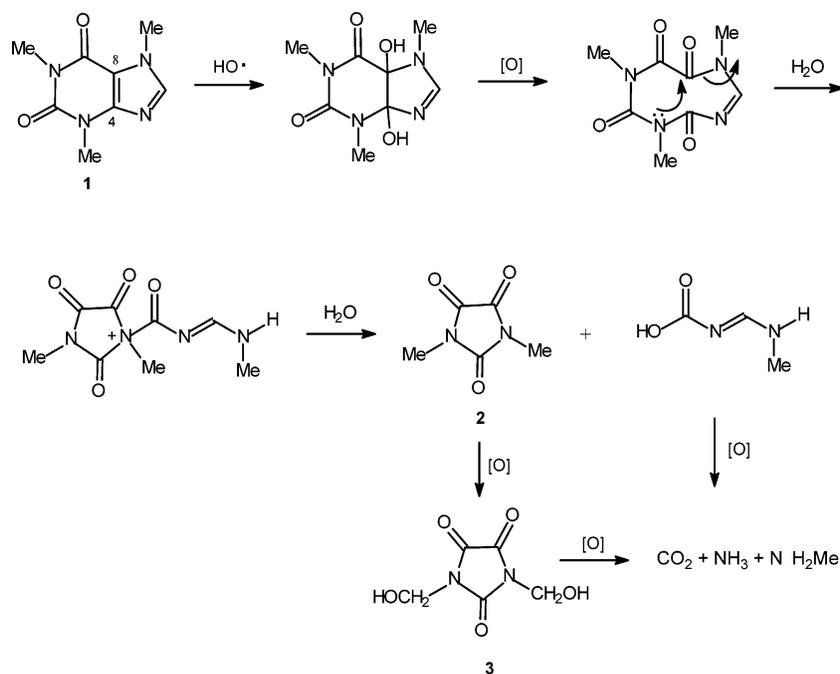


FIGURE 5. GC-MS chromatogram of an aliquot of the reaction mixture after 150 min of Fenton's degradation. The inset shows the 70 eV EI mass spectrum of the compound eluting at 6.82 min. Note the low-intensity caffeine peak at 14.93 min, which demonstrates its almost full consumption.

SCHEME 3



or loss during extraction and sample preparation steps, or both of these complications, GC–MS analysis has missed this important degradation product of caffeine.

Oxidation Mechanism. The degradation of organic compounds by the UV/TiO₂ (51–54), H₂O₂/UV (55–56), and Fenton (57) systems is known to occur via the in situ generation of free hydroxyl radicals. Thus, we propose that the mechanism for the conversion of caffeine (1) to dimethylparabanic acid (2) involves initially the (fast) attack of the hydroxyl radicals to the C4=C8 double bond of caffeine (Scheme 3). After successive hydroxylations and oxidations, 2 and 3 are formed, whereas 3 is slowly mineralized to CO₂, NH₃, and NH₂Me. Note also that the oxidation products 4–7 (Scheme 2) could also be converted to dimethylparabanic acid (2) via a mechanism similar to that depicted in Scheme 3. Thus, based on this assumption, it can be postulated that caffeine (1) is initially oxidized by free hydroxyl radicals to yield not only dimethylparabanic acid (2) but also 4–7, which are subsequently converted to 2 under these strong oxidizing reaction conditions.

As exemplified herein for caffeine, ESI mass spectrometry and tandem mass spectrometry are suitable techniques for on-line and real-time monitoring of advanced oxidation processes of drugs and other environmentally relevant compounds. The extraordinary ability to directly fish reactants, products, and intermediates (either ionic species or neutral species in their protonated or deprotonated forms) directly from the solution to the gas phase with gentleness, speed, and high sensitivity allows a detailed overview of the process and the interception and structural characterization of polar players of the oxidation process. Whereas classical off-line approaches such as GC–MS analysis can handle the more volatile and lighter species, the more polar, relatively unstable, or even transient intermediates or products, which other off-line techniques or on-line techniques based on volatile species are unable to detect, may be intercepted and structurally characterized by on-line and real-time ESI–MS and ESI–MS/MS monitoring, as exemplified herein for di(*N*-hydroxymethyl)parabanic acid (3), an unprecedented intermediate detected in the degradation of caffeine in water.

Acknowledgments

We thank the Minas Gerais State Science Foundation (FAPEMIG), the São Paulo State Science Foundation (FAPESP), and the Brazilian National Research Council (CNPq) for financial support and research fellowships. The authors also thank Drs. Adriano Pimenta and Francisco A. R. Barbosa (ICB-UFMG) for their help during the acquisition of preliminary ESI data and TOC analyses, respectively.

Literature Cited

- Daughton, C. G.; Ternes, T. A. Pharmaceuticals and personal care products in the environment: agents of subtle change? *Environ. Health Perspect.* **1999**, *107*, 907–938.
- Halling-Sorensen, B.; Nielsen, S. N.; Lanzky, P. F.; Ingerslev, F.; Lutzhoft, H. C. H.; Jorgensen, S. E. Occurrence, fate, and effects of pharmaceutical substances in the environment: a review. *Chemosphere* **1998**, *36*, 357–394.
- Heberer, T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.* **2002**, *131*, 5–17.
- Andreozzi, R.; Raffaele, M.; Nicklas, P. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. *Chemosphere* **2003**, *50*, 1319–1330.
- Vogna, D.; Marotta, R.; Andreozzi, R.; Napolitano, A.; d'Ischia, M. Kinetic and chemical assessment of the UV/H₂O₂ treatment of antiepileptic drug carbamazepine. *Chemosphere* **2004**, *54*, 497–505.
- Ravina, M.; Campanella, L.; Kiwi, J. Accelerated mineralization of the drug diclofenac via Fenton reactions in a concentric photoreactor. *Water Res.* **2002**, *36*, 3553–3560.
- Doll, T. E.; Frimmel, F. H. Kinetic study of photocatalytic degradation of carbamazepine, clofibric acid, iomeprol, and iopromide assisted by different TiO₂ materials: determination of intermediates and reaction pathways. *Water Res.* **2004**, *38*, 955–964.
- Boreen, A. L.; Arnold, W. A.; McNeill, K. Photodegradation of pharmaceuticals in the aquatic environment: a review. *Aquat. Sci.* **2003**, *65*, 320–341.
- Gardinali, P. R.; Zhao, X. Trace determination of caffeine in surface water samples by liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry (LC–APCI–MS). *Environ. Int.* **2002**, *28*, 521–528.
- Seiler, R. L.; Zaugg, S. D.; Thomas, J. M.; Howcroft, D. L. Caffeine and pharmaceuticals as indicators of wastewater contamination in wells. *Groundwater* **1999**, *37*, 405–410.

- (11) Kolpin, D. W.; Skopec, M.; Meyer, M. T.; Furlong, E. T.; Zaugg, S. D. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. *Sci. Total Environ.* **2004**, *328*, 119–13.
- (12) Ternes, T.; Bonerz, M.; Schmidt, T. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography–electrospray tandem mass spectrometry. *J. Chromatogr. A* **2001**, *938*, 175–185.
- (13) (a) Devasagayam, T. P. A.; Kesavan, P. C. Radioprotective and antioxidant action of caffeine: mechanistic considerations. *Indian J. Exp. Biol.* **1996**, *34*, 291–297.
- (14) Devasagayam, T. P. A.; Kamat, J. P.; Mohan, H.; Kesavan, P. C. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys. Acta* **1996**, *1282*, 63–70.
- (15) Kesavan, P. C.; Powers, E. L. Differential modification of oxic and anoxic components of radiation damage in *Bacillus megaterium* spores by caffeine. *Int. J. Radiat. Biol.* **1985**, *48*, 223–233.
- (16) Kesavan, P. C. Protection by caffeine against oxic radiation damage and chemical carcinogens: mechanistic considerations. *Curr. Sci. India* **1992**, *62*, 791–797.
- (17) Kesavan, P. C.; Sarma, L. *Subcellular biochemistry*; Plenum: New York, 1995.
- (18) Alberici, R. M.; Zampronio, C. G.; Poppi, R. J.; Eberlin, M. N. Water solubilization of ethanol and BTEX from gasoline: on-line monitoring by membrane introduction mass spectrometry. *Analyst* **2002**, *127*, 230–234.
- (19) Alberici, R. M.; Canela, M. C.; Eberlin, M. N.; Jardim, W. F. Catalytic deactivation in the gas phase: destruction of nitrogen-containing organic compounds using TiO₂/UV–vis. *Appl. Catal., B* **2001**, *30*, 389–397.
- (20) Alberici, R. M.; Sparrapan, R.; Jardim, W. F.; Eberlin, M. N. Selective trace level analysis of phenolic compounds in water by flow injection analysis–membrane introduction mass spectrometry. *Environ. Sci. Technol.* **2001**, *35*, 2084–2088.
- (21) Canela, M. C.; Alberici, R. M.; Sofia, R. C. R.; Eberlin, M. N.; Jardim, W. F. Destruction of malodorous compounds using heterogeneous photocatalysis. *Environ. Sci. Technol.* **1999**, *33*, 2788.
- (22) Nogueira, R. F. P.; Alberici, R. M.; Mendes, M. A.; Jardim, W. F.; Eberlin, M. N. Photocatalytic degradation of phenol and trichloroethylene. Online and real-time monitoring via membrane introduction mass spectrometry. *Ind. Eng. Chem. Res.* **1999**, *38*, 1754–1758.
- (23) Alberici, R. M.; Mendes, M. A.; Jardim, W. F.; Eberlin, M. N. Mass spectrometry on-line monitoring and MS² product characterization of TiO₂/UV photocatalytic degradation of chlorinated volatile organic compounds. *J. Am. Soc. Mass Spectrom.* **1998**, *9*, 1321–1327 and references therein.
- (24) Rios, R. V. R. A.; Da Rocha, L. L.; Vieira, T. G.; Lago, R. M.; Augusti, R. On-line monitoring by membrane introduction mass spectrometry of chlorination of organics in water. Mechanistic and kinetic aspects of chloroform formation. *J. Mass Spectrom.* **2000**, *35*, 618–624.
- (25) Lago, R. M.; Silva, A. C. B.; Teixeira, A. C. M.; Augusti, R. Application of membrane introduction mass spectrometry to the study of adsorption of organic compounds on activated carbon and solid-phase extraction experiments. *Analyst* **2003**, *128*, 884–888.
- (26) Lago, R. M.; Nagem, N. F.; Dalmazio, I.; Augusti, R. Catalytic hydrodehalogenation of aromatic halides monitored by membrane introduction mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1507–1510.
- (27) Burgos, A. E.; Sinisterra, R. D.; Augusti, R.; Lago, R. M. Application of the MIMS technique to study the stability constants of small organic guest molecules into cyclodextrin hosts in aqueous medium. *J. Inclusion Phenom. Macroyclic Chem.* **2003**, *45*, 149–154.
- (28) Toledo, L. U.; Silva, A. C. B.; Augusti, R.; Lago, R. M. Application of Fenton's reagent to regenerate activated carbon saturated with organochloro compounds. *Chemosphere* **2003**, *50*, 1049–1054.
- (29) Whitehouse, C. M.; Dreyer, R. N.; Yamashita, M.; Fenn, J. B. Electrospray interface for liquid chromatographs and mass spectrometers. *Anal. Chem.* **1985**, *57*, 675–679.
- (30) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. Electrospray ionization for mass spectrometry of large biomolecules. *Science* **1989**, *246*, 64–71.
- (31) Cooks, R. G.; Zhang, D. X.; Koch, K. J.; Gozzo, F. C.; Eberlin, M. N. Chiroselective self-directed octamerization of serine: implications for homochirogenesis. *Anal. Chem.* **2001**, *73*, 3646–3655.
- (32) Takats, Z.; Nanita, S. C.; Cooks, R. G. Serine octamer reactions: indicators of prebiotic relevance. *Angew. Chem., Int. Ed.* **2003**, *42*, 3521–3523.
- (33) Domingos, J. B.; Longhinotti, E.; Brandao, T. A. S.; Bunton, C. A.; Santos, L. S.; Eberlin, M. N.; Nome, F. Mechanisms of nucleophilic substitution reactions of methylated hydroxylamines with bis(2,4-dinitrophenyl)phosphate. Mass spectrometric identification of key intermediates. *J. Org. Chem.* **2004**, *69*, 6024–6033.
- (34) Koch, K. J.; Gozzo, F. C.; Nanita, S. C.; Takats, Z.; Eberlin, M. N.; Cooks, R. G. Chiral transmission between amino acids: chirally selective amino acid substitution in the serine octamer as a possible step in homochirogenesis. *Angew. Chem., Int. Ed.* **2002**, *41*, 1721–1724.
- (35) Meurer, E. C.; Sabino, A. A.; Eberlin, M. N. Ionic transacetalization with acylium ions: a class-selective and structurally diagnostic reaction for cyclic acetals performed under unique electrospray and atmospheric pressure chemical ionization in-source ion–molecule reaction conditions. *Anal. Chem.* **2003**, *75*, 4701–4709.
- (36) Griep-Raming, J.; Meyer, S.; Bruhn, T.; Metzger, J. O. Investigation of reactive intermediates of chemical reactions in solution by electrospray ionization mass spectrometry: radical chain reactions. *Angew. Chem., Int. Ed.* **2002**, *41*, 2738–2742.
- (37) Sabino, A. A.; Machado, A. H. L.; Correia, C. R. D.; Eberlin, M. N. Probing the mechanism of the Heck reaction with arene diazonium salts by electrospray mass and tandem mass spectrometry. *Angew. Chem., Int. Ed.* **2004**, *43*, 2514–2518.
- (38) Meyer, S.; Metzger, J. O. Use of electrospray ionization mass spectrometry for the investigation of radical cation chain reactions in solution: detection of transient radical cations. *Anal. Bioanal. Chem.* **2003**, *377*, 1108–1114.
- (39) Augusti, D. V.; Carazza, F.; Okuma, A. A.; Augusti, R. Reactivity of some novel multifunctional benzoyl esters towards nucleophiles investigated by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1084–1088.
- (40) Pereira, R. M. S.; Paula, V. I.; Buffon, R.; Tomazela, D. M.; Eberlin, M. N. New iridium(I) complexes with labile ligands: reactivity and structural characterization by atmospheric pressure mass and tandem mass spectrometry. *Inorg. Chim. Acta* **2004**, *357*, 2100–2106.
- (41) Gozzo, F. C.; Santos, L. S.; Augusti, R.; Consorti, C. S.; Dupont, J.; Eberlin, M. N. Gaseous supramolecules of imidazolium ionic liquids: magic numbers and intrinsic strengths of hydrogen bonds. *Chem.–Eur. J.* **2004**, *10*, 6187–6193.
- (42) Toma, S. H.; Nikolaou, S.; Tomazela, D. M.; Eberlin, M. N.; Toma, H. E. Synthesis, spectroscopy, tandem mass spectrometry, and electrochemistry of the linearly bridged μ -{*trans*-1,4-bis(2-(4-pyridyl)ethenyl)-benzene}-{Ru₃O(CH₃COO)₆(py)₂}₂ cluster. *Inorg. Chim. Acta* **2004**, *357*, 2253–2260.
- (43) Hinderling, C.; Adlhart, C.; Chen, P. Olefin metathesis of a ruthenium carbene complex by electrospray ionization in the gas phase. *Angew. Chem., Int. Ed.* **1998**, *37*, 2685–2689.
- (44) Chen, P. Electrospray ionization tandem mass spectrometry in high-throughput screening of homogeneous catalysts. *Angew. Chem., Int. Ed.* **2003**, *42*, 2832–2847.
- (45) Vogna, D.; Marotta, R.; Napolitano, A.; d'Ischia, M. Advanced oxidation chemistry of paracetamol. UV/H₂O₂-induced hydroxylation/degradation pathways and ¹⁵N-aided inventory of nitrogenous breakdown products. *J. Org. Chem.* **2002**, *67*, 6143–6151.
- (46) Kolonko, K. J.; Shapiro, R. H.; Barkley, R. M.; Sievers, R. E. Ozonation of caffeine in aqueous solution. *J. Org. Chem.* **1979**, *44*, 3769–3778.
- (47) Telo, J. P.; Vieira, J. S. C. Mechanism of free radical oxidation of caffeine in aqueous solution. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1755–1757.
- (48) Stadler, R. H.; Richoz, J.; Turezki, R. J.; Welti, D. H.; Fay, L. B. Oxidation of caffeine and related methylxanthines in ascorbate and polyphenol-driven Fenton-type oxidations. *Free Radical Res.* **1996**, *24*, 225–240.
- (49) Lias, S. G.; Bartmess, J. E.; Liebman, J. F.; Holmes, J. L.; Levin, R. D.; Mallard, W. G. In *The NIST Chemistry Webbook*; Linstrom, P. J., Mallard, W. G., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, 2001.

- (50) Delia, T. J.; Brown, G. B. Purine *N*-oxides. 17. Oxidation of guanine at position 7. *J. Org. Chem.* **1966**, *31*, 178–181.
- (51) Hoffmann, M. R.; Martin, S. T.; Choi, W. Y.; Bahnemann, D. W. Environmental applications of semiconductor photocatalysis. *Chem. Rev.* **1995**, *95*, 69–96.
- (52) Turchi, C. S.; Ollis, D. F. Photocatalytic degradation of organic water contaminants: mechanisms involving hydroxyl radical attack. *J. Catal.* **1990**, *122*, 178–192.
- (53) Riegel, G.; Bolton, J. R. Photocatalytic efficiency variability in TiO₂ particles. *J. Phys. Chem.* **1995**, *99*, 4215–4224.
- (54) Barreto, R. D.; Gray, K. A.; Anders, K. Photocatalytic degradation of methyl-*t*-butyl ether in TiO₂ slurries: a proposed reaction scheme. *Water Res.* **1995**, *29*, 1243–1248.
- (55) Huang, C. R.; Shu, H. Y. The reaction kinetics, decomposition pathways, and intermediate formations of phenol in ozonation, UV/O₃, and UV/H₂O₂ processes. *J. Hazard Mater.* **1995**, *41*, 47–64.
- (56) Scheck, C. K.; Frimmel, F. H. Degradation of phenol and salicylic acid by ultraviolet radiation hydrogen peroxide oxygen. *Water Res.* **1995**, *29*, 2346–2352.
- (57) Augusti, R.; Dias, A. O.; Rocha, L. L.; Lago, R. M. Kinetics and mechanism of benzene derivative degradation with Fenton's reagent in aqueous medium studied by MIMS. *J. Phys. Chem. A* **1998**, *102*, 10723–10727.

Received for review December 20, 2004. Revised manuscript received May 30, 2005. Accepted June 1, 2005.

ES047985V