Journal of Photochemistry and Photobiology A: Chemistry 265 (2013) 10–19

Contents lists available at SciVerse ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Photo-degradation of *trans*-caffeic acid in aqueous solution and influence of complexation by metal ions

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ARTICLE INFO

Article history:
Received 13 November 2012
Received in revised form 12 April 2013
Accepted 11 May 2013
Available online 27 May 2013

Keywords:
Caffeic acid
Humic substances
Photo-degradation
Metal complex
Mechanism
Kinetics

ABSTRACT

The photo-degradation of metal complexes of caffeic acid was compared to the photo-degradation of free caffeic acid by using UV–vis spectroscopy and HPLC–ESI-mass spectrometry. This article reports first the determination of the products that are formed from the photo-degradation of *trans*-caffeic acid in aqueous solution and the investigation of the mechanism by a kinetic approach. The good fit between the model and the experimental concentration profiles confirms the photo-isomerization route of the molecule to *cis*-caffeic acid which then undergoes a cyclization to form the esculetin photo-product. In addition, it reveals, for the first time, another route of major importance leading to the product vinylcatechol. The presence of oxygen leads to an increase of the photo-isomerization rate. Then we report that metallic cations such as Al(III), Pb(II) and Cu(II) can influence the rate and mechanism of caffeic acid photo-degradation. Al(III) ions slow down the photo-degradation whereas Pb(II) and Cu(II) ions have a promoter effect on the production of esculetin. In all cases, the photo-isomerization is reduced by the presence of metal ions and the formation of vinylcatechol does not occur.

1. Introduction

The organic matter of soils is formed from the degradation of plants and animals and is mainly composed of humic substances. These are aromatic and aliphatic macromolecules constituting a heterogeneous and complex system [1,2] and playing an important role in the retention and transport of many metal ions in soils [3]. Most of metal ions are known to be toxic, in particular if they are in free form, and their accumulation is responsible for many environmental concerns [4]. In general, their toxicity and availability diminish with their retention in soils [5]. To reach a better understanding of the interactions between metal ions and humic substances (HS), the approach chosen for few years in our research group consists in studying model systems of HS, because of the complexity and the poly-functionality of these substances. One possible model molecule is caffeic acid (*trans*-3-(3,4-dihydroxyphenyl)prop-2-enoic acid), naturally omnipresent in plant kingdom. When plants degradation occurs, this compound is released and constitutes a precursor of HS [6]. Indeed, this molecule exhibits two competing metal coordination sites, the carboxylic and the catechol groups that are typical functions in HS. This molecule also displays antioxidant properties [7] and is used as photo-protective substance in preparations and sun-creams [8].

The complexation of metal ions such as Al(III) and Pb(II) by caffeic acid has already been investigated by a double approach combining spectroscopic measurements and quantum chemical calculations [9–13]. The structures and stoichiometries of the complexes and their equilibrium constants were obtained. The complexation in solution of Al(III) [10] shows that the 1:1 complex, formed via the catecholate group, largely predominates at pH = 5 for low amount of metal. Small quantities of metal ions also allow the formation of a minor 2:1 binuclear complex, for which the two sites (catechol and carboxylic functions) are involved. At pH = 6.5, only the catecholate site coordinates to Al(III) leading to the formation of 1:2 and 1:3 complexes. Thus, it was concluded that the catechol group presents a chelating power which is higher than that of carboxylic or carboxylate functions toward Al(III) ions. The trend is different with Pb(II) since these ions coordinate first with the carboxylate function [12].

Humic substances are also sensitive to solar light, leading to a potential photo-degradation, as previously reported in the literature [14–17]. However little is known about the photochemistry of their association with metal ions. To our knowledge, we can only report few studies devoted to the photo-degradation of metal complexes of organic molecules [18–20]. This paper focuses on the photo-degradation of caffeic acid and on the influence of metal

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http://dx.doi.org/10.1016/j.jphotochem.2013.05.004

ions on this process. Previous studies already suggested that solar or UV irradiation could modify the behavior of this molecule in the environment [21,22]. Depending on the experimental conditions, different products and mechanisms were proposed for the photo-degradation of caffeic acid but they are not all in accordance. On the one hand, most of the authors [23–28] reported *cis*-caffeic acid and esculetin (6,7-dihydroxy-2H-1-benzopyran-2-one) species as products of the degradation. Despite they suggest different intermediates, they agree about a mechanism based on the initial isomerization of *trans*-caffeic to *cis*-caffeic acid which then undergoes a cyclization to form esculetin. Nevertheless, this mechanism was not confirmed by a kinetic study. On the other hand, some authors [29,30] provided evidence of the formation of various compounds such as 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid (or protocatechuic acid), maleic acid and oxalic acid that do not support the previous mechanism.

In that context, the aim of this work is first to assess the products that are formed from the photo-degradation of caffeic acid in water without any added substance. Based on these products, a mechanism and a kinetic model will be proposed. A good fit between the calculated (with the kinetic model) and the experimental concentration profiles will allow the validation of this mechanism.

Borges and Pinto [26] showed previously the accelerator role of oxygen for the caffeic acid photo-degradation in ethanol. Consequently, we propose to check this parameter in our experimental conditions. Then the influence of the coordination to metal ions such as Al(III), Pb(II) and Cu(II) on the photo-degradation of caffeic acid is investigated. Both electronic spectroscopies and HPLC–ESI-MS analyses are used to reach all these objectives.

2. Methods

2.1. Chemicals

Trans-caffeic acid ((E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid) was obtained from Sigma Aldrich (99%) and used as received without any purification. Complexes were formed by mixing caffeic acid and hexahydrated aluminum chloride ($AlCl_3(H_2O)_6$), lead chloride ($PbCl_2$) or copper chloride ($CuCl_2$) in deionized water at a given molar ratio (R = [metal]/[ligand]). Initial concentrations of caffeic acid before irradiation and/or complexation were in the range of $5.0–5.6 \times 10^{-5}$ M and the pH before irradiation was fixed at 5 or 6.5, depending on the experiments.

2.2. Irradiation setup

The solutions of *trans*-caffeic acid or metal-caffeic acid complexes were placed into a cell (Suprasil Quartz) also aimed at spectroscopic measurements and were kept under stirring upon irradiation. The main source used for irradiation is a 200 W mercury–xenon lamp (LC8 Hamamatsu) connected with a light guide and directed toward the cell. Band-pass filters were used in order to get a light beam centered at 312 ± 10 nm and with a ~10 μW power at the sample. The cell was irradiated directly in the UV–vis spectrometer. A more powerful lamp (1000 W Xenon from Oriel) equipped with a monochromator (wavelength fixed at 312 nm) was also used for one experiment to get a faster kinetic. HPLC–ESI-MS analyses (see next section) were performed by sampling a small volume (100 μL) of the solution directly inside the cell.

2.3. Instrumentation

UV–vis spectra were recorded using a double-beam spectrometer (Cary 100-Varian), in the 200–700 nm region with cells of 1 cm path length. Fluorescence spectra were acquired using a Fluorolog (Jobin-Yvon) spectrofluorimeter with slit width varying from 2 to 4 nm. This apparatus is equipped with multichannel detector that allows the limitation of the recording times and consequently of the illumination times. Three-dimensional spectra were also acquired to obtain excitation–emission matrix (EEM) plots, where the excitation wavelengths are plotted on the *y*-axis, the emission wavelengths on the *x*-axis and the third dimension represents the relative intensity.

HPLC–ESI-MS analyses were performed on a triple quadrupole mass spectrometer (Quattro II Micromass-Waters) equipped with an electrospray ionization source (ESI) coupled with high pressure liquid chromatography (HPLC) system (HP1100 Agilent). Separations were achieved on a 250 mm × 2.0 mm i.d. column packed with 5 μm Kromasil C18 stationary phase (Interchim) protected by a 10 mm × 2.0 mm C18 precolumn (Interchim) and heated to 25 °C. The flow rate was set at 150 μL/min and the injected volume was 20 μL. The mobile phase was a mixture of solvent A (0.5% formic acid in water) and solvent B (acetonitrile). The proportion of solvent B was increased linearly from 10% to 43% in 25 min, then 43% to 90% in 5 min. After each injection the column was allowed to reequilibrate with 10% solvent B for 13 min. The column eluent was first directed to a UV detector set at 280 nm and then without splitting to the electrospray interface. The mass spectrometer was operated in negative ion mode. For phenolic compounds, this mode leads to a better sensitivity and lower background noise than the positive mode [31,32]. The source parameters were the following: capillary voltage of – 3 kV, cone voltage of – 25 V, source temperature of 120 °C. Nitrogen was used as nebulization and drying gas at flow rates of 10–15 and 250–300 L/h respectively. Data were acquired in full scan MS mode over the range m/z 220–500 in 2 s.

2.4. Calculations

Calculations were performed at the density functional level of theory with the PBEO global hybrid functional [33,34], using the Gaussian (G09) program package [35]. Geometry optimizations were carried out without any symmetry constraints using the 6–311++G(d,p) basis set. Vibrational frequency calculation was performed to ensure that the optimized structure corresponds to an energy minimum. The low-lying excited states were treated within the adiabatic approximation of time dependent density functional theory (TD-DFT) [36] with the PBEO hybrid functional. Vertical excitation energies were computed for the first 50 singlet excited states, in order to estimate the UV–vis spectra of the molecule. As it is well known that UV–vis spectra are very sensitive to the solvent effects, these latter were introduced by the SCRF method, via the polarized continuum model (PCM) [37,38] implemented in the Gaussian program.

3. Results and discussion

3.1. Photo-degradation of trans-caffeic acid followed by electronic spectroscopies

3.1.1. Absorption spectra

The photo-degradation of *trans*-caffeic acid was followed by UV–vis absorption spectroscopy. As seen in Fig. 1a, *trans*-caffeic acid at pH = 6.5 absorbs light below 350 nm and presents two absorption bands centered at 285 and 312 nm. Upon irradiation, the band intensity decrease clearly shows that caffeic acid is degraded. This consumption is also accompanied by a hypsochromic shift. The UV spectrum after 196 min of irradiation exhibits an absorption band at 280 nm, higher than the small contribution around 312 nm. In parallel to this feature, the baseline in the 350–450 nm range is

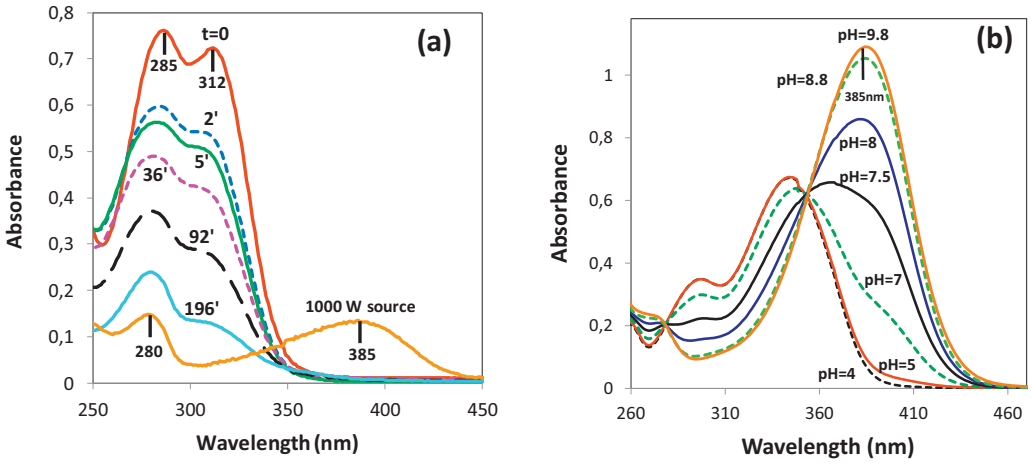

Fig. 1. (a) UV–vis spectra (pH = 6.5) obtained for various times of irradiation (0, 2, 5, 36, 92 and 196 min) of *trans*-caffeic acid with the Hg–Xe lamp (initial concentration of 5.0×10^{-5} M) and UV–vis spectrum obtained after a longer time of irradiation of *trans*-caffeic acid from the 1000 W Xe light source; (b) UV–vis spectra of pure solutions of esculetin in water (concentration of 5.7×10^{-5} M) at various pH.

not equal to zero and a zoom on the spectral part shows a very weak absorbance. For this reason, the solution has been irradiated with a much more intense source (1000 W) and an absorption band with a maximum around 385 nm (Fig. 1a) is clearly observed. Based on the UV–vis spectra of pure solutions of esculetin acquired at different pH (Fig. 1b), this absorption can be attributed to this product at pH ~8, indicating an increase of the pH upon photo-degradation. A one unity pH increase was also directly measured in the solution. This increase in absorption in the visible range was also observed by Cilliers and Singleton [39] for the oxidation of caffeic acid but the product was not identified. Thus our spectral data let us suggest that esculetin formation is due to *trans*-caffeic acid photo-degradation. Unfortunately, it is not possible to quantify the concentrations of esculetin in our irradiation conditions by UV–vis spectroscopy. Furthermore, the determination of *cis*-caffeic acid concentrations from these spectra was not possible for two main reasons: (i) due to very close structures, *trans* and *cis* caffeic acids have very similar absorption spectra so it is hard to discriminate them, (ii) the band observed at 280 nm could be partially assigned to this isomer but also to esculetin and to other eventual photo-products.

3.1.2. Fluorescence measurements

Fluorescence spectroscopy is a more sensitive technique than UV–vis spectroscopy. For this reason, fluorescence measurements were performed in order to highlight the different species involved in the mechanism of *trans*-caffeic acid photo-degradation.

3.1.2.1. Excitation emission matrix. Three-dimensional spectra also called excitation emission matrix (EEM) were acquired by varying the excitation and the emission wavelengths. Before photo-degradation ($t = 0$), a fluorescence band detected at 312/415 nm ($\lambda_{ex}/\lambda_{em}$) is characteristic of *trans*-caffeic acid (Fig. 2). After 6 min of irradiation, the additional fluorescence which appears at 385/468 nm is attributed to esculetin, with respect to the spectral data obtained by UV–vis absorption. Then, the emission of *trans*-caffeic acid continues to decrease while that of esculetin increases from 6 to 180 min. By comparison with the UV spectra where the absorbance related to the formation of esculetin is relatively low, the quantum yield of esculetin fluorescence is assumed to be high, since it was detected in the first minutes of photo-degradation. On the contrary, *cis*-caffeic acid which is known to be formed from *trans*-caffeic acid in the first step of the mechanism could not be observed on the EEM since no other fluorescence band appeared in the first minutes of photo-degradation. One can conclude that fluorescence emission features of *cis*- and *trans*-caffeic acid are too close to be discriminated, as previously observed for absorption. Another fluorescence band also appears at 280/310 nm (56 min of irradiation). This feature could be assigned to protocatechuic acid because the matrix obtained from pure protocatechuic acid is closed to this broad band (Fig. 2). However the fluorescence of this compound does not wholly reproduce the band shape observed in the photo-degradation process. The presence of vinylcatechol product could be another hypothesis. Unfortunately, no experimental proof of its presence could be given since this compound is not available from chemical suppliers. Nevertheless, we have been able to calculate the absorption and fluorescence spectra of this molecule by TD-DFT/PBEO/6-311+G(d,p)/PCM. The theoretical wavelengths of vinylcatechol are found to be 225, 253 and 284 nm for the absorption electronic transitions and 340 nm for the fluorescence emission. The spectroscopic properties of this compound are thus also close to the broad band observed from the photo-degradation of caffeic acid. Both protocatechuic acid and vinylcatechol were identified by using mass spectrometry (see Section 2.2).

3.1.2.2. Fluorescence spectra. Emission fluorescence spectra (Fig. 3) were recorded by exciting the reactant mixture at the maximum absorption wavelengths of the different species (312, 385 and 280 nm) and for different times of irradiation. By exciting at 312 nm, we clearly show the intensity decrease of *trans*-isomer fluorescence band at 415 nm with the irradiation time (Fig. 3a). Concomitantly, a fluorescence band at 468 nm (Fig. 3a) due to esculetin formation grows, despite the low absorption of this product at 312 nm. This puts forward the high fluorescence quantum yield of esculetin. As expected, the fluorescence band observed at 468 nm is enhanced by exciting at the maximum absorption band of esculetin (385 nm), as seen in Fig. 3b. According to the data previously obtained from excitation emission matrixes, the emission spectra recorded with an excitation at 280 nm (Fig. 3c) exhibit a fluorescence band around 310 nm which was previously suggested to be due to the formation of protocatechuic acid and/or vinylcatechol. The continuous increase of this fluorescence with irradiation time confirms that it cannot be attributed to *cis*-caffeic acid. This isomer can still not be detected here. Profiles of fluorescence intensities (Fig. 3d) were extracted at 415, 468 and 310 nm from the three emission fluorescence spectra. The fluorescence intensity evolutions are directly linked to the concentrations of the different species. The rapid decrease of the fluorescence intensity of *trans*-caffeic

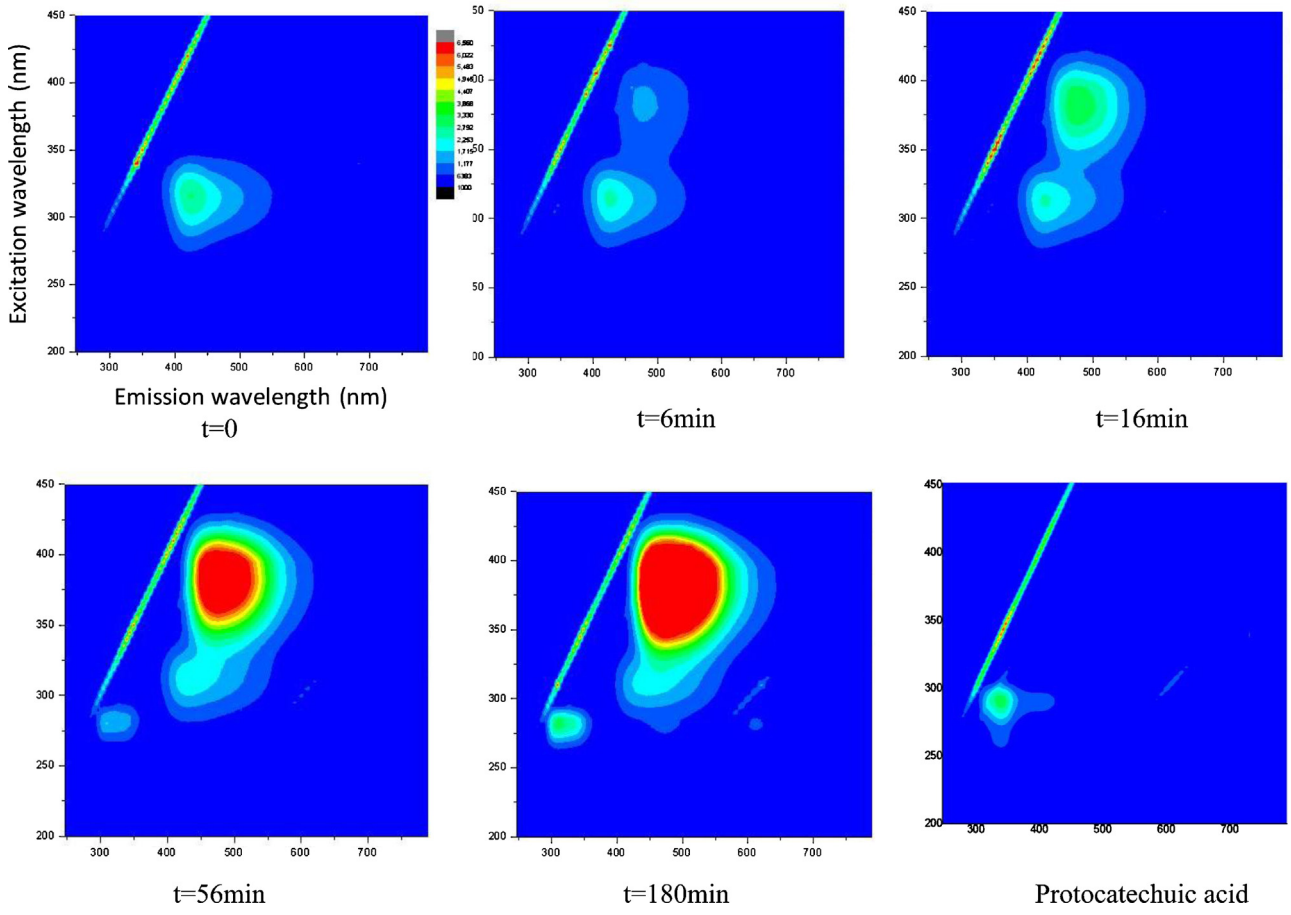

Fig. 2. Excitation emission matrix (EEM) obtained at different times of irradiation (0, 6, 16, 56 and 80 min) of *trans*-caffeic acid (initial concentration of 5.0×10^{-5} M) and EEM of pure protocatechuic acid (concentration of 2.0×10^{-5} M).

observed in the first 2 min can be connected to the fast conversion to the *cis*-isomer. At 200 min, *trans*-caffeic acid is almost completely consumed whereas the concentration of esculetin reaches a maximum. On the contrary, the emission at 310 nm does not show the same behavior since it seems to reach a plateau value after 250 min. Consequently, the formation pathways of the different observed photo-products are not linked and then protocatechuic acid and/or vinylcatechol are not formed from esculetin degradation, as expected.

3.2. Kinetic investigation of the photo-degradation by mass spectrometry

High performance liquid chromatography coupled to mass spectrometry was used to separate the different species involved in the mechanism, in order to get information about the kinetics and the mechanism of the photo-degradation. The *trans*-caffeic acid solution was analyzed by HPLC–ESI-MS for different irradiation times with the mercury–xenon lamp. The initial extracted ion chromatogram (Fig. 4) obtained in the absence of irradiation underlines the main occurrence of *trans*-isomer of caffeic acid and a very small contribution detected at the same mass ($M = 180\,g\,mol^{-1}$) attributed to *cis*-isomer. Upon irradiation, *trans*-caffeic acid is consumed while *cis*-caffeic acid ($M = 180\,g\,mol^{-1}$) is formed and reaches a maximum concentration after ~5 min under these experimental conditions. Then, its concentration decreases and is followed by the formation of esculetin ($M = 178\,g\,mol^{-1}$) which is detected after 20 min of irradiation. The presence of protocatechuic acid was confirmed here from the standard compound. Vinylcatechol is also proposed since a peak was detected at its corresponding $M = 136\,g\,mol^{-1}$. The concentration of protocatechuic acid cannot be obtained because it remains too low during the degradation process to be quantified. The concentration of vinylcatechol cannot be determined as well since this compound is not commercially available. Nevertheless, it can be noted that their concentrations both increase upon photo-degradation.

The concentration profiles of *trans*-caffeic acid, *cis*-caffeic acid and esculetin and the estimated or calculated concentration profiles of protocatechuic acid and vinylcatechol are reported in Fig. 5. These data were compared to the model mechanism presented in Fig. 6. This model takes into account (i) the reversible *trans*/*cis* isomerization characterized by the kinetic parameters k_1 and k_{-1}, (ii) the formation of esculetin from *cis*-isomer with a rate constant k_2 and (iii) the two other routes leading to vinylcatechol and protocatechuic from *trans*-caffeic acid and characterized by two independent rate constants k'_1 and k''_1, respectively. Although the concentration of vinylcatechol remains unknown all along the degradation process, the areas of the peaks related to this product are available and proportional to the concentrations. The k'_1 rate constant was then adjusted in order to get the same trend between the experimental areas profile and the calculated concentration profile, i.e. in considering a constant ratio between the experimental and calculated profiles. Then a scale factor was applied to the experimental data to obtain the real concentrations of vinylcatechol. Furthermore, the concentrations of protocatechuic acid are also not precisely determined but they are below the quantification limit of $1 \times 10^{-6}\,mol\,L^{-1}$. This limit was obtained from HPLC–ESI-MS measurements of protocatechuic acid standard and

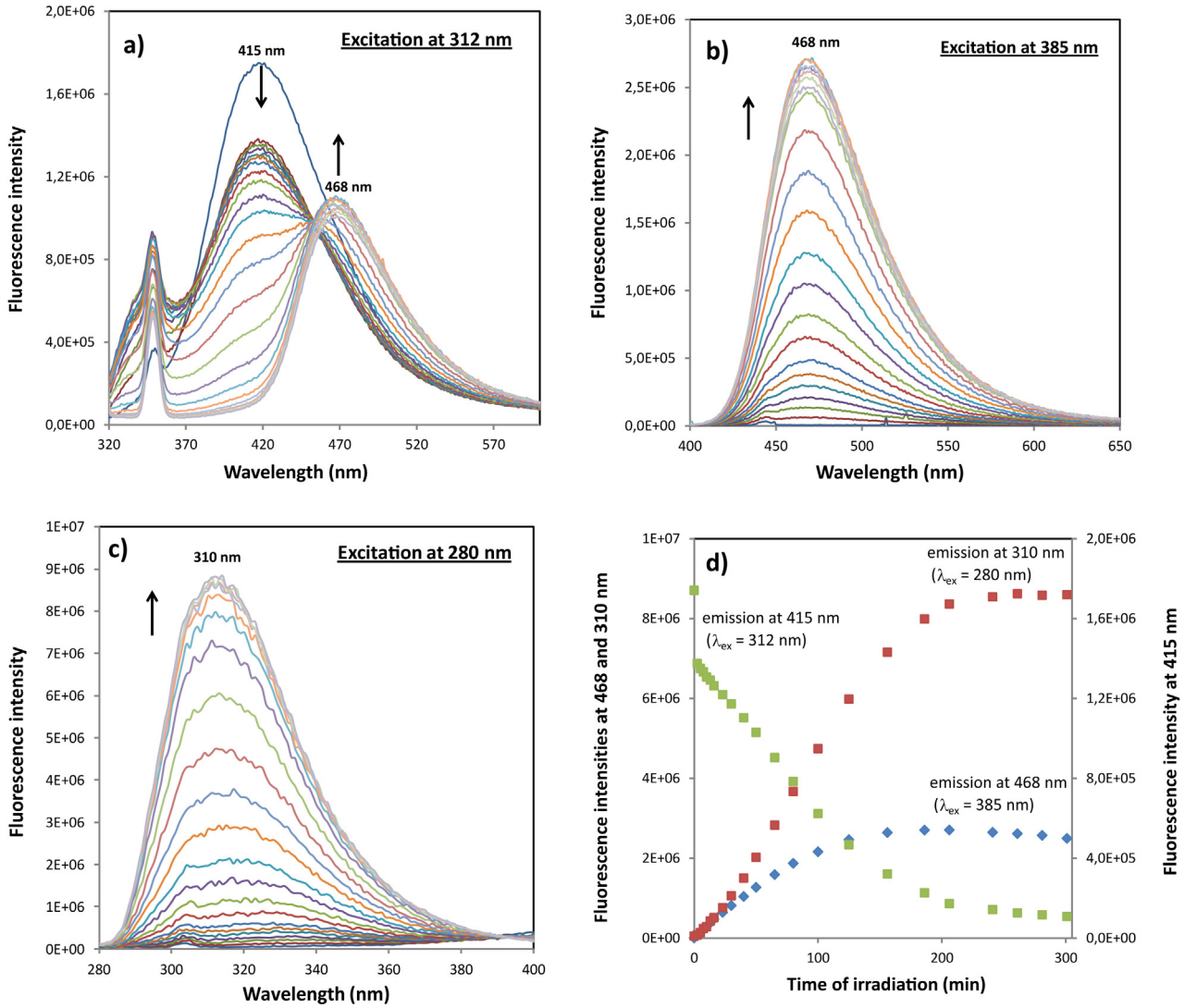

Fig. 3. Emission fluorescence spectra obtained at different times of irradiation of *trans*-caffeic acid from 0 to 5 h by using the spectrofluorimeter as the source of irradiation (initial concentration of 5.6×10^{-5} M). (a) Excitation at 312 nm and slit width of 4 nm; (b) excitation at 385 nm and slit width of 2 nm; (c) excitation at 280 nm and slit width of 2 nm; (d) profiles of fluorescence obtained from the emission spectra a, b and c.

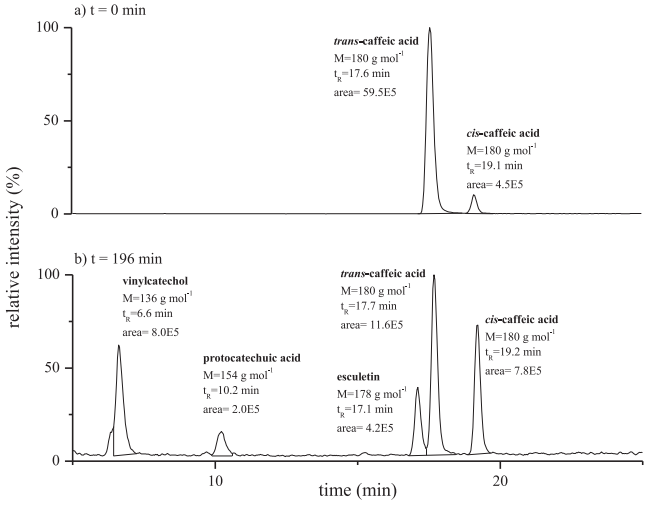

Fig. 4. Extracted ion chromatograms obtained before irradiation and at 196 min of irradiation of *trans*-caffeic acid (initial concentration of 5.0×10^{-5} M) by using the mercury–xenon lamp.

corresponds to the concentration for which the ratio between the height of the corresponding peak and the noise is 10. An upper limit of the k_1'' rate constant was thus estimated in order to get a maximum concentration of this product below this limit. With the hypothesis of pseudo-first order reactions, the simulated data fit well with experimental ones (Fig. 5) by optimizing the kinetic parameters to the following values: $k_1 = 3.8 \pm 0.1 \times 10^{-3}\ s^{-1}$, $k_{-1} = 7.0 \pm 0.1 \times 10^{-3}\ s^{-1}$, $k_2 = 1.5 \pm 0.1 \times 10^{-5}\ s^{-1}$, $k_1' = 1.5 \pm 0.1 \times 10^{-4}\ s^{-1}$ and $k_1'' = 2.5 \pm 0.1 \times 10^{-6}\ s^{-1}$. The pseudo-first order rates are in disagreement with the work of Carlotti et al. [21] who observed a degradation of caffeic acid in aqueous solutions upon UV irradiation following a pseudo-zero order kinetic. It can then be noticed that the sum of the concentrations of the species present at a given time are roughly equal to the initial concentration of caffeic acid (5×10^{-5} mol L^{-1}). This means that possible other products that could not be detected might have lower concentrations. The values of rate constants depend on experimental conditions and notably on irradiation wavelength range. The most important results of this study rely on the mechanistic aspect of the caffeic acid photo-degradation. We show that the photo-isomerization and the intramolecular cyclization really occur but are not the major routes,

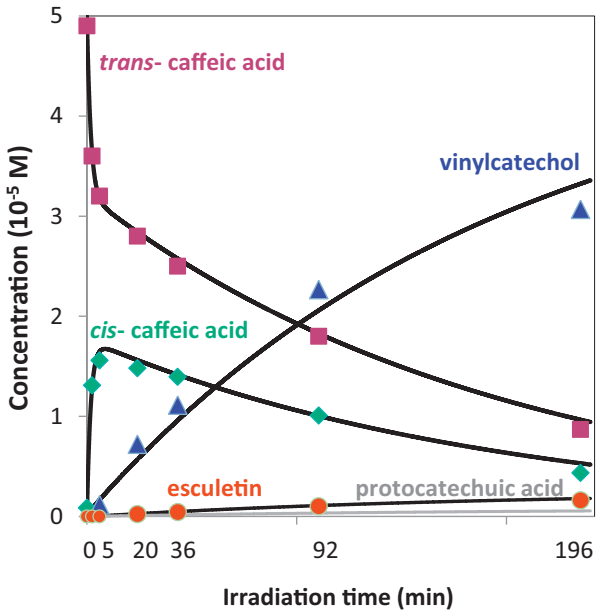

Fig. 5. Experimental (HPLC) and calculated or estimated concentration profiles of *trans*-caffeic acid, *cis*-caffeic acid, esculetin, vinylcatechol and protocatechuic acid at different times of irradiation (0, 2, 5, 20, 36, 92 and 196 min) by using the Hg–Xe lamp.

since the formation of vinylcatechol seems to be important. So far, this latter compound was never detected as a product of caffeic acid photo-degradation. We can just mention that Grimes et al. [29] observed an increase of the total amount of carbon dioxide upon photo-degradation. This could be linked to the loss of the caffeic acid carboxylic group, thus leading to the formation of vinylcatechol species. It was also shown that this compound can be formed by thermal decarboxylation of caffeic acid [40–42] and it was also detected in roasted coffee [43]. Finally, the pathway leading to the protocatechuic acid product, also observed by Grimes et al. [29] and Amat et al. [30], is minor.

3.3. Influence of oxygen on the photo-degradation of trans-caffeic acid

The results presented above were obtained from solutions containing air. Complementary experiments were carried out to test the real influence of oxygen in our experimental conditions by

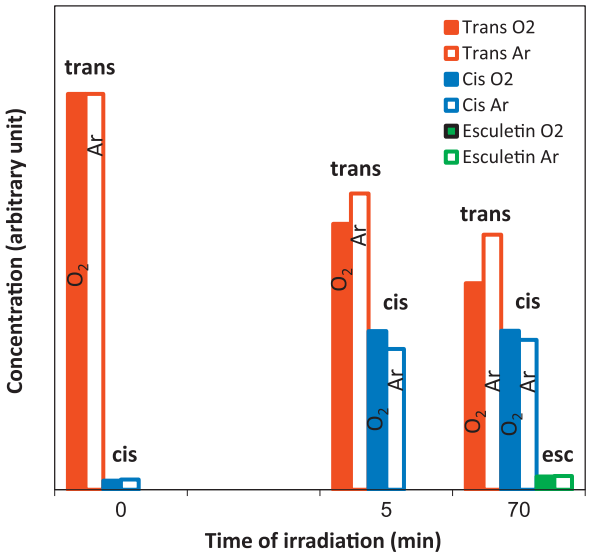

Fig. 7. Comparison between the compositions of the oxygen containing solution and the deoxygenated solution (*trans*-caffeic acid, *cis*-caffeic acid and esculetin concentrations in arbitrary units obtained from extracted ion chromatograms).

using two cells: one containing air and the other deoxygenated by degassing 30 min with a slow Ar stream before irradiation. The concentrations of *trans*-caffeic acid and *cis*-caffeic acid were then compared for similar irradiation times (5 and 70 min), as seen in Fig. 7. The decrease of *trans* to *cis* conversion in the presence of Ar compared to oxygen is clear. One can thus conclude that the photo-isomerization rate is higher in the presence of air. Borges and Pinto [26] showed that the production of esculetin decreases in deoxygenated solutions. This formation that occurs in the absence of oxygen was explained by an auto-oxidation as suggested by these authors. In our experiments, the concentrations of esculetin are similar in both conditions but the very low content of this product for the studied irradiation time does not permit to conclude. Nevertheless, the results reported by Borges and Pinto [26] are also consistent with an increase of the photo-isomerization. We underline the fact that the concentrations of vinycatechol and protocatechuic acid are equal in both conditions.

3.4. Influence of the complexation on the photo-degradation

Photo-degradation of metal complexes of caffeic acid was compared to the photo-degradation of free caffeic acid using the

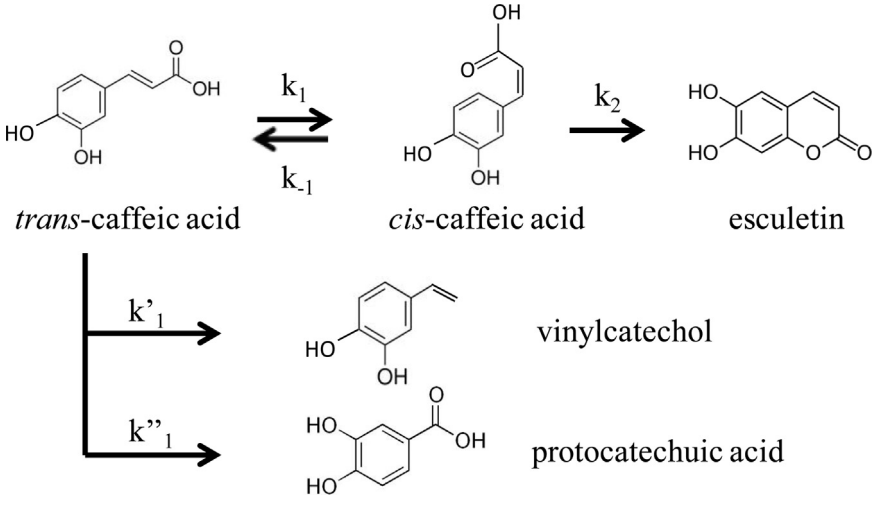

Fig. 6. Proposed mechanism of the photo-degradation of *trans*-caffeic acid.

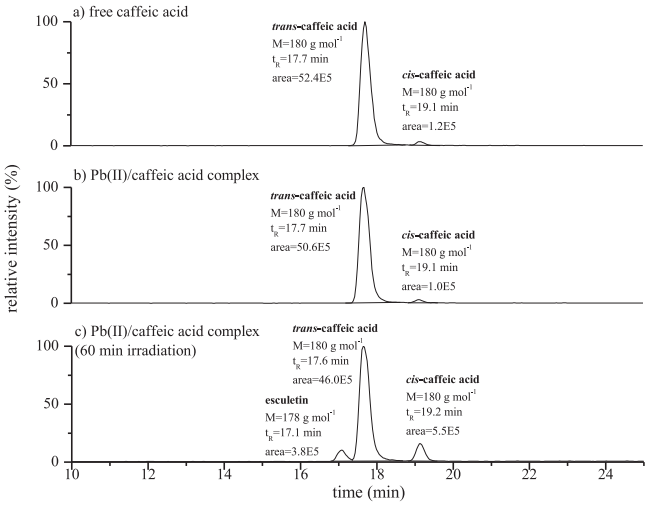

Fig. 8. Extracted ion chromatograms obtained with free *trans*-caffeic acid (a) and with Pb(II) ions complexed by caffeic acid before irradiation (b) and after 60 min of irradiation (c).

same experimental conditions. Samples were first analyzed before degradation: the extracted ion chromatograms obtained from a metal–caffeic acid complex are similar to those obtained from free caffeic acid, in terms of retention time (17.7 min) and peak area, showing that complexes dissociate in the HPLC device (Fig. 8). Therefore, the peaks observed at this retention time are due to the total contribution of free and complexed caffeic acid. HPLC–ESI-MS can still be used for the photochemical study of complexed caffeic acid since the photo-degradation is prior to analysis. The comparison with the photo-degradation of free caffeic acid is based on HPLC–ESI-MS and UV–vis results. Vinylcatechol and protocatechuic acid were not detected upon irradiation of metal complexes.

3.4.1. *Influence of Pb(II) ions*

Caffeic acid is known to complex Pb(II) ions [12]. These ions first coordinate with the carboxylate function to form a complex of 1:1 stoichiometry. Then both carboxylate and catechol functions are naturally involved in the 2:1 complex [13]. However, the complexation of caffeic acid is not complete for molar ratio below 3, preventing the precipitation of lead hydroxide. UV–vis spectra of Pb(II)/caffeic acid solution were recorded during the photo-degradation at pH = 6.5 (Fig. 9a), in the same conditions as the photo-degradation of the free molecule (Fig. 1a). The UV absorption bands observed in the 260–360 nm spectral range before irradiation are linked to the simultaneous presence of free and complexed (1:1 and 2:1) forms of caffeic acid for a molar ratio of $R = 2$ [13]. Thus the decrease of the absorption bands upon irradiation is due to the photo-degradation of the free molecule and of the two possible complexed forms. Despite the apparent difficulty to distinguish between the three processes, it appears that this decrease is accompanied by the formation of a neat absorption band at 390 nm which can be attributed to the complex formed between esculetin and Pb(II) ions (Fig. 9b). The intensity of this band is much higher than the absorbance measured in the same conditions for the photo-degradation of the free form (Fig. 1a), showing that the formation of esculetin is enhanced by the presence of Pb(II) ions. These results are confirmed by HPLC–ESI-MS measurements. Indeed, the esculetin concentration reached in the

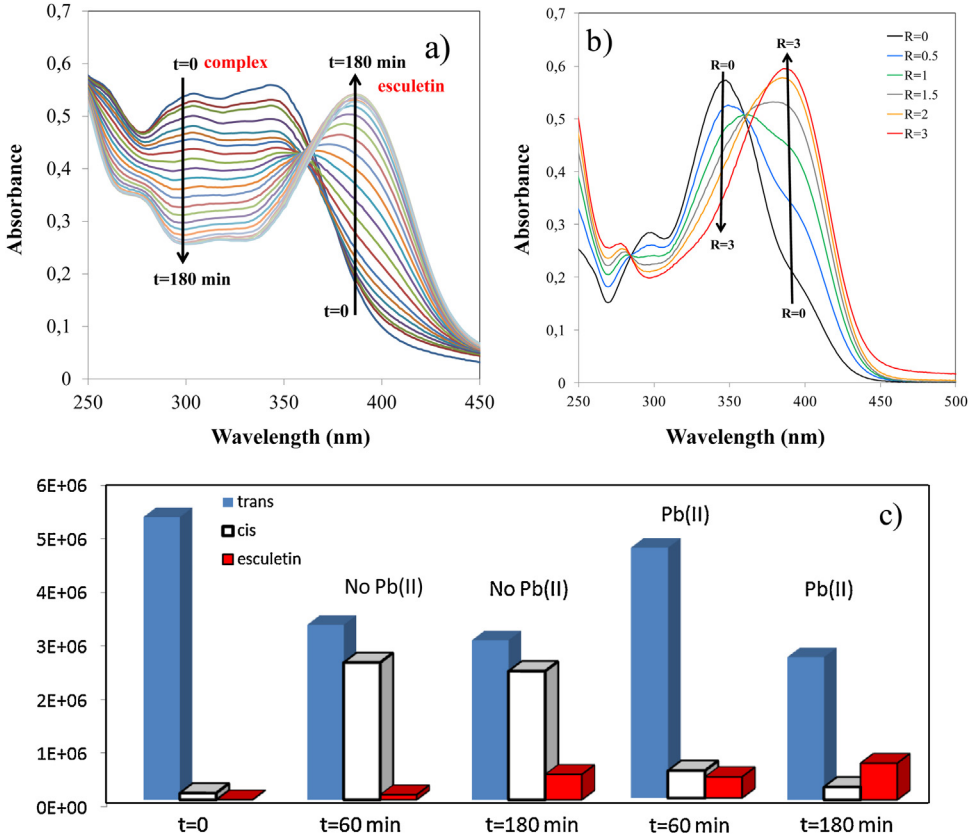

Fig. 9. (a) UV–vis spectra (pH = 6.5) obtained at different times of irradiation (0–180 min) of Pb(II)/caffeic acid complexes (molar ratio of $R = 2$); (b) UV–vis spectra (pH = 6.5) obtained from the complexation of Pb(II) ions by esculetin (molar ratio R = Pb(II)]/[esculetin] from 0 to 3); (c) photo-degradation (pH = 6.5) of free caffeic acid compared to the photo-degradation of Pb(II)/caffeic acid complexes (molar ratio of $R = 2$) for the same time of irradiation (60 and 180 min) obtained from extracted ion chromatograms. The *y*-axis represents the areas of the peaks related to *trans*-caffeic acid, *cis*-caffeic acid and esculetin.

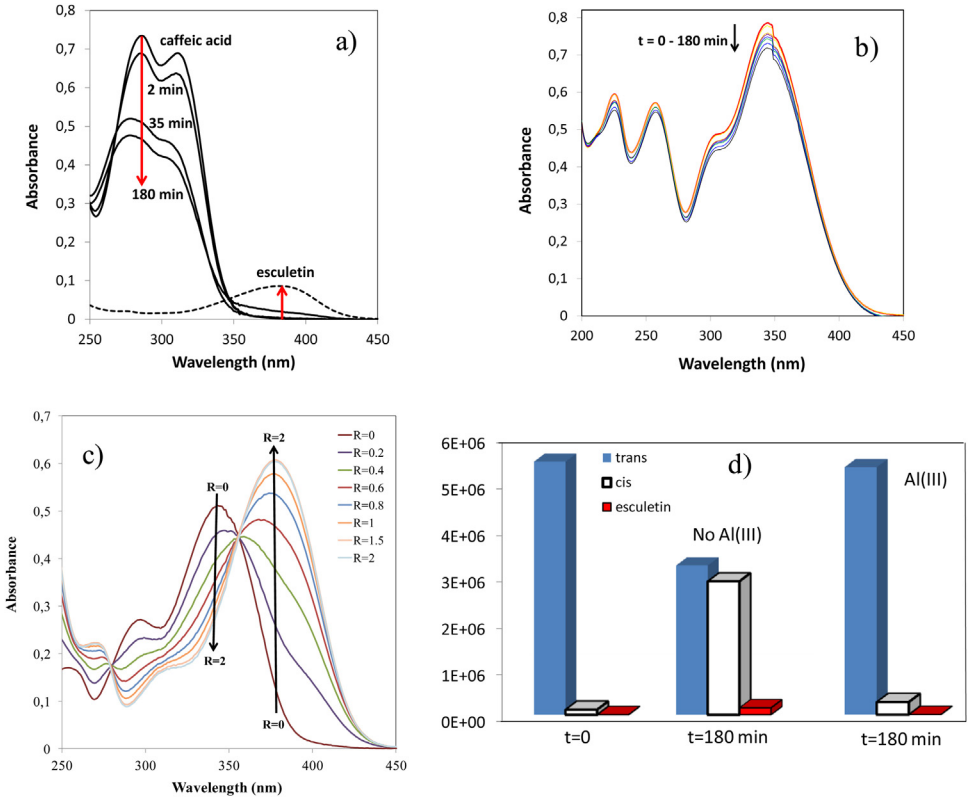

Fig. 10. UV–vis spectra (pH = 5) obtained at different times of irradiation (0–180 min) of (a) free caffeic acid (b) Al(III)/caffeic acid complexes (molar ratio of $R = 10$); (c) UV–vis spectra (pH = 5) obtained from the complexation of Al(III) ions by esculetin (molar ratio R = [Al(III)]/[esculetin] from 0 to 2); (d) photo-degradation (pH = 5) of free caffeic acid compared to the photo-degradation of Al(III)/caffeic acid complexes (molar ratio of $R = 10$) for the same time of irradiation (180 min) obtained from extracted ion chromatograms. The *y*-axis represents the areas of the peaks related to *trans*-caffeic acid, *cis*-caffeic acid and esculetin.

presence of the complex is higher than with the free molecule, for a similar irradiation time (Fig. 9c). As this photo-product is formed from *cis*-caffeic acid, this partly explains that the concentration of the latter isomer is lower in the presence of Pb(II) than in the absence of these ions (Fig. 9c). One can conclude that the rate constant k_2, related to the formation of esculetin from *cis*-isomer, increases in the presence of Pb(II) ions (Fig. 6). As for *trans*-caffeic acid, its concentration is higher with metal ions at 60 min of irradiation but is lower at 180 min. It implies that the isomerization reaction is also modified by the complexation process. For low times of irradiation such as 60 min, the decrease of the conversion from the *trans* form to the *cis* form in the presence of Pb(II) ions can account for a decrease of the k_1/k_{-1} ratio, where k_1 and k_{-1} are the rate constants corresponding to the isomerization process. In contrast, the k_2 rate constant enhancement explains that the amount of caffeic acid that reacted becomes higher after a given irradiation time. Thus we confirm the promoter effect of Pb(II) ions on the caffeic acid photo-degradation leading to the formation of esculetin. This effect is probably linked to the coordination of Pb(II) ions on the carboxylate function of caffeic acid [13].

3.4.2. *Influence of Al(III) ions*

The complexation of Al(III) ions by caffeic acid was previously studied [10]. Unlike Pb(II) ions, Al(III) ions are known to coordinate preferentially to the catechol function. The optimal conditions to reach a complete complexation of Al(III) ions by caffeic acid consist in working at pH = 5 and with a [Al(III)]/[caffeic acid] molar ratio (R) higher than 10 [10]. By this way, the concentration of free caffeic acid can be considered as negligible compared to the concentrations of the complexes. The photo-degradation of Al(III)/caffeic acid complex was followed by UV–vis spectroscopy (Fig. 10b) and compared to the photo-degradation of the free molecule at pH = 5 (Fig. 10a). The UV–vis spectrum (Fig. 10b) recorded before irradiation in the presence of Al(III) ions presents an absorption band at 345 nm which fits well with the maxima at 335 and 347 nm of the 1:1 and 2:1 complexes, respectively [10]. Upon irradiation, the decrease of this band is slow (less than 8% after 180 min) and no shift or new absorption band is observed. Moreover, since no absorption band at 350 or 390 nm, corresponding to free or complexed forms of esculetin (Fig. 10c), respectively, is detected, we can assume that the presence of Al(III) ions does not enhance the photo-degradation. The decrease of the photo-degradation of caffeic acid in the presence of Al(III) ions is confirmed by the HPLC–ESI-MS results. The areas of the peaks obtained from the extracted ion chromatograms and related to the concentrations of *trans*-caffeic acid, *cis*-caffeic acid and esculetin are presented on Fig. 10d. Three observations can be deduced from this figure: (i) the degradation rate of *trans*-caffeic acid is much higher in the absence (~40%) than in the presence (less than 2%) of Al(III), after the same irradiation time (180 min). (ii) Conversion to *cis*-isomer is also hampered by the presence of Al(III) ions. (iii) The photo-product (esculetin) is observed by irradiation of free caffeic acid but not by irradiation of the complexes. Thus it is clear that the presence of Al(III) ions inhibits the photo-degradation of the molecule. This behavior can be explained by the preferential fixation site of Al(III) ions on the catechol function of caffeic acid, this function being also involved in the photo-degradation mechanism [23,27,28]. If this latter is blocked by Al(III) ions, the best stability of the molecule toward photo-degradation can be easily understood. It is interesting to note that the isomerization is also inhibited by the presence of this metal ion coordinated to catechol site.

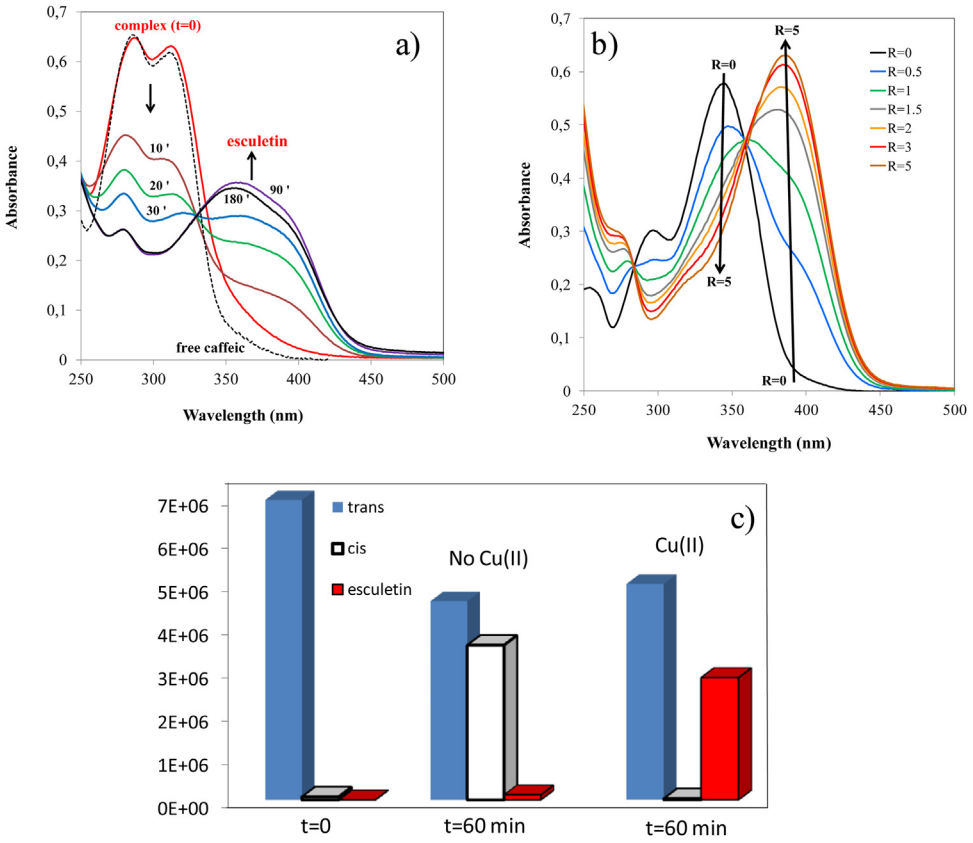

Fig. 11. UV–vis spectra (pH = 5) obtained at different times of irradiation (0–180 min) of (a) Cu(II)/caffeic acid complexes (molar ratio of $R = 2$); (b) UV–vis spectra (pH = 5) obtained from the complexation of Cu(II) ions by esculetin (molar ratio $R = [Cu(II)]/[esculetin]$ from 0 to 5); (c) photo-degradation (pH = 5) of free caffeic acid compared to the photo-degradation of Cu(II)/caffeic acid complexes (molar ratio of $R = 2$) for the same time of irradiation (60 min) obtained from extracted ion chromatograms. The *y*-axis represents the areas of the peaks related to *trans*-caffeic acid, *cis*-caffeic acid and esculetin.

3.4.3. *Influence of Cu(II) ions*

The study of the photo-degradation of the Cu(II)/caffeic acid system has been carried out in the same conditions as those used for Al(III) ions (pH = 5). The photo-degradation of Cu(II)/caffeic acid complex (Fig. 11a) was compared to the photo-degradation of the free molecule (Fig. 10a). The absorption spectra of Cu(II)/caffeic acid complex and free caffeic acid (dotted line in Fig. 11a) are very similar. Only a slight shoulder appears at approximately 350 nm. Upon irradiation, the UV–vis spectra (Fig. 11a) highlight the growth of an absorption band in the 350–450 nm range, which can be attributed to the formation of the complexed form of esculetin (Fig. 11b). Compared to lead ions, cupric ions have a similar and even more marked influence on the photo-degradation of caffeic acid. Indeed, HPLC–ESI-MS results (Fig. 11c) show that the formation of the photo-product is 20 times larger in the presence of Cu(II) ions than in its absence whereas it was only 4 times larger in the presence of Pb(II) ions. For an irradiation time of 60 min, the *cis*-isomer formed was almost completely consumed in the presence of Cu(II) ions contrary to the degradation of the free molecule (Fig. 11c). The consumption of this isomer is more pronounced than what was observed with Pb(II) solutions.

To our knowledge, the coordination site of Cu(II) ions on caffeic acid has not been identified yet. However, the similar influences of Pb(II) and Cu(II) ions on the photo-degradation of caffeic acid could be explained by a common site of fixation of metal ions on the molecule such as the carboxylate function.

4. Conclusion

This study corroborates the first route of *trans*-caffeic acid photo-degradation already proposed by several authors. This one is based on the photo-isomerization of the molecule followed by a cyclization to form esculetin as photo-product. However, this report reveals the occurrence of another route of major importance leading to the likely formation of vinylcatechol which was never observed up to now. As shown previously [29,30], protocatechuic acid is also formed but in small amounts. As expected, the presence of oxygen leads to an increase of the photo-isomerization rate. This study provides also clear evidence of the influence of complexation to metal ions on caffeic acid photo-degradation. Note that complexation process with the metal ions was also observed on esculetin when this photo-product is formed. In all cases, the photo-isomerization is reduced by the presence of Pb(II), Al(III) and Cu(II) ions. In addition, the metal type was shown to play a significant role for the esculetin formation. Indeed, the presence of Al(III) ions leads to a very photo-stable complex and inhibits the formation of esculetin, whereas Pb(II) and Cu(II) ions enhance the production of this compound. This phenomenon might be explained by the different coordination sites of the metal ions to the ligand: the preferential fixation site of Al(III) ions is the catechol function and since this function is involved in the photo-degradation mechanism, the complexation blocks the reaction. On the contrary, the coordination of Pb(II) ions on the carboxylate function promotes the formation of esculetin. Finally, one can conclude that, in soils polluted by heavy metals, the possibility of formation of metal complexes with humic substances contributes to significantly change the photo-degradation of these substances.

Acknowledgments

The Mass Spectrometry facility used in this study was funded by the European Community (FEDER), the Région Nord-Pas de Calais

(France), the CNRS, and the Université Lille 1, Sciences et Technologies. The authors are also grateful to Dr. A. Moncomble for his precious help about the quantum chemical calculations.

References

[1] F.J. Stevenson, Humus chemistry: Genesis, Composition Reactions, Wiley, New York, 1982.
[2] H.R. Schulten, M. Schnitzer, Soil Science 162 (1997) 115–130.
[3] R.L. Wershaw, G.R. Aiken, Humic Substances is Soils, Sediments and Water, Wiley Interscience, New York, 1995.
[4] E. Baath, Water Air Soil Pollution 47 (1989) 335–379.
[5] P. Doelman, L. Haanstra, Soil Biology and Biochemistry 11 (1979) 475–479.
[6] R.A. Olsen, J.C. Brown, J.H. Bennet, D. Blume, Journal of Plant Nutrition 5 (1982) 433–445.
[7] I. Gülçin, Toxicology 217 (2006) 213–220.
[8] A. Saija, A. Tomaino, D. Trombetta, A. De Pasquale, N. Uccella, T. Barbuzzi, T. Paolino, F. Bonina, International Journal of Pharmaceutics 199 (2000) 39–47.
[9] C. Lapouge, J.P. Cornard, ChemPhysChem 8 (2007) 473–479.
[10] J.P. Cornard, A. Caudron, J.C. Merlin, Polyhedron 25 (2006) 2215–2222.
[11] J.P. Cornard, C. Lapouge, Journal of Physical Chemistry A 108 (2004) 4470–4478.
[12] J.P. Cornard, C. Lapouge, Chemical Physics Letters 438 (2007) 41–46.
[13] L. Boilet, J.P. Cornard, C. Lapouge, Journal of Physical Chemistry A 109 (2005) 1952–1960.
[14] I.V. Sokolova, O.N. Tchaikovskaya, Atmospheric and Oceanic Optics 19 (2006) 220–222.
[15] D.E. Latch, R. McNeil, Science 311 (2006) 1743–1747.
[16] W.L. Miller, Ecological Studies 133 (1998) 125–143.
[17] R.G. Zepp, G.L. Baughman, P.F. Schlotzhauer, Chemosphere 10 (1981) 109–117.
[18] S. Protti, A. Mezzetti, C. Lapouge, J.P. Cornard, Photochemistry & Photobiological Sciences 7 (2008) 109–119.
[19] J. Buschmann, S. Canonica, L. Sigg, Environmental Science and Technology 39 (2005) 5335–5341.
[20] Ba L. Tran, Seth M. Cohen, Chemical Communications (2006) 203–205.
[21] M.E. Carlotti, E. Ugazio, S. Sapino, E. Peira, L. Battaglia, R. Cavalli, Journal of Dispersion Science and Technology 29 (2008) 1435–1444.
[22] F.J. Benitez, J. Beltran-Heredia, J.L. Acero, M.L. Pinilla, Journal of Chemical Technology and Biotechnology 70 (1997) 253–260.
[23] W.L. Butler, H.W. Siegelman, Nature (London) 183 (1959) 1813–1814.
[24] R.D. Hartley, E.C. Jones, Journal of Chromatography 107 (1975) 213–218.
[25] F. Borges, M. Pinto, Journal of Liquid Chromatography 12 (12) (1989) 2345–2354.
[26] F. Borges, M. Pinto, Helvetica Chimica Acta 75 (1992) 1061–1068.
[27] G. Pandey, A. Krishna, J.M. Rao, Tetrahedron Letters 27 (34) (1986) 4075–4076.
[28] M. Satô, A. Hiraoka, Chemical and Pharmaceutical Bulletin 33 (3) (1985) 1289–1292.
[29] S.M. Grimes, L.K. Mehta, H.C. Ngwang, Journal of Environment Science and Health, Part A Environmental Science 36 (5) (2001) 599–612.
[30] A.M. Amat, A. Arques, M.A. Miranda, Applied Catalysis B: Environmental 23 (1999) 205–214.
[31] S. Perez-Magarino, I. Revilla, M.L. Gonzalez-SanJose, S. Beltran, Journal of Chromatography A 847 (1999) 75–81.
[32] I. Nicoletti, A. De Rossi, G. Giovinazzo, D. Corradini, Journal of Agricultural and Food Chemistry 55 (2007) 3304–3311.
[33] J.P. Perdew, K. Burke, M. Ernzerhof, Physical Review Letters 77 (1996) 3865–3868.
[34] C. Adamo, V.J. Barone, Journal of Chemical Physics 110 (1999) 6158–6169.
[35] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G.A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H.P. Hratchian, A.F. Izmaylov, J. Bloino, G. Zheng, J.L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J.A. Montgomery Jr., J.E. Peralta, F. Ogliaro, M. Bearpark, J.J. Heyd, E. Brothers, K.N. Kudin, V.N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J.C. Burant, S.S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J.M. Millam, M. Klene, J.E. Knox, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, R.L. Martin, K. Morokuma, V.G. Zakrzewski, G.A. Voth, P. Salvador, J.J. Dannenberg, S. Dapprich, A.D. Daniels, Ö. Farkas, J.B. Foresman, J.V. Ortiz, J. Cioslowski, D.J. Fox, Gaussian 09, Revision B.01, Gaussian, Inc., Wallingford, CT, 2009.
[36] R. Bauernschmitt, R. Ahlrichs, Chemical Physics 256 (1996) 454–464.
[37] M. Cossi, G. Scalmani, N. Rega, V.J. Barone, Journal of Chemical Physics 117 (2002) 43–54.
[38] J. Tomasi, R. Cammi, B. Mennucci, C. Cappelli, S. Corni, Physical Chemistry Chemical Physics 4 (2002) 5697–5712.
[39] J.J.L. Cilliers, V.L. Singleton, Journal of Agricultural and Food Chemistry 39 (1991) 1298–1303.
[40] P. Terpinc, T. Polak, N. Segatin, A. Hanzlowsky, N.P. Ulrih, H. Abramovic, Food Chemistry 128 (2011) 62–69.
[41] R. Stadler, H.D. Welti, A. Stämpfli, L. Fay, Journal of Agricultural and Food Chemistry 44 (1996) 898–905.
[42] G. Rizzi, L. Boekley, Journal of Agricultural and Food Chemistry 40 (1992) 1666–1670.
[43] O. Frank, S. Blumberg, C. Kunert, G. Zehentbauer, T. Hofmann, Journal of Agricultural and Food Chemistry 55 (2007) 1945–1954.