

# A spectroelectrochemical and chemical study on oxidation of hydroxycinnamic acids in aprotic medium

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## Abstract

Electrochemical and chemical oxidation of hydroxycinnamic acids (HCAs) was studied to investigate the mechanisms occurring in their antioxidant activities in a protons poor medium. Electrolyses and chemical reactions were followed on-line by monitoring the UV-spectral changes with time; final solutions were analysed by HPLC–MS. Anodic oxidation of mono- and di-HCAs, studied by cyclic voltammetry and controlled potential electrolyses, occurs via a reversible one-step two-electrons process, yielding the corresponding stable phenoxonium cation. A cyclization product was also proposed, as supported by ESR studies. Chemical oxidation with lead dioxide leads to different oxidation products according to the starting substrate. Di-HCAs like chlorogenic and rosmarinic acids and the ethyl ester of caffeic acid gave the corresponding neutral *o*-quinones, while mono-HCAs like cumaric, ferulic and sinapinic acids yielded the corresponding unstable neutral phenoxyl radical, as supported by the formation of dimerization products evidenced by HPLC–MS. In the case of caffeic acid, traces of the dimerization product suggest that the neutral phenoxyl radical may competitively undergo dimerization or decomposition of the neutral quinone.

Chemical oxidation of HCAs was also followed by ESR spectroscopy: the di-HCAs radical anions were generated and detected, whereas among the mono-HCAs only the phenoxyl radical of the sinapinic acid was recorded.

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## 1. Introduction

The consumption of fruits and vegetables has been shown to decrease the risk of cardiovascular diseases [1] and cancer [2]. There are strong evidences that phenolic antioxidants present in plants are at least in part responsible for these protective effects [3]. Hydroxycinnamic acids or phenylpropanoids are widely distributed in the plant kingdom and are important sources of antioxidants due to their free radical scavenging properties [4]. It has been shown that they protect LDL against oxidation induced by metmyoglobin [5], Cu<sup>2+</sup> ions and 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) [6]. They can also act as scavengers of hypochlorite [7], peroxyxynitrite [8]

and 2,2-diphenyl-1-picrylhydrazyl radical [9]. In particular, caffeic acid and chlorogenic acid have been proved to be highly active in most of these studies.

Both electrochemical [10–13] and chemical oxidation [10,14–16] of these compounds have been studied by many authors, especially in aqueous medium at different pH, but the mechanisms involved in their oxidative process are not yet well understood, above all because the radical species likely formed during their oxidation (phenoxyl radicals and radical anions) are highly reactive and unstable and may be involved in complicated chemical reactions [15,17,18]. Their possible pro-oxidant activity and their metabolic fate in human body are still scarcely known, too, limiting the possible employment of these natural compounds in the preventive medicine as well as in the recognized pathology.

In this context, we studied, in the present work, a series of mono- and di-hydroxycinnamic acids with the aim to investigate

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the mechanisms occurring in the antioxidant activity in a protons poor medium, under electron transfer (ET) conditions (anodic oxidation) and H-atom transfer (HT) conditions (chemical oxidation with a H-atom acceptor).

## 2. Materials and methods

### 2.1. Materials

Reagents and solvents were purchased from Sigma–Aldrich and used without further purification. Sodium perchlorate was purchased from BDH and dried under vacuum after crystallisation. Water for HPLC was deionized by Millipore Milli-Q Purification System. 3-(3,4-Dihydroxy-phenyl)-acrylic acid ethyl ester (CAE) was prepared according to the following procedure: 2 mmols of caffeic acid (360 mg) were dissolved in 10 mL of ethyl alcohol in the presence of a catalytic amount of *p*-toluenesulfonic acid. The solution was refluxed for 6 h, washed with NaHCO<sub>3</sub> 0.5 mol L<sup>-1</sup> and extracted with CHCl<sub>3</sub>. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated to dryness. <sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>): δ = 1.39 (t, 3H, *J* = 7.6 Hz); 4.26 (q, 2H, *J* = 7.6 Hz); 6.26 (d, 1H, *J* = 17.3 Hz); 6.81–6.89 (m, 1H); 6.90–7.10 (m, 1H) ppm.

### 2.2. Electrochemical experiments

Voltammetric measurements were performed with a three-electrode multipolarograph AMEL 472 coupled with a digital x/y recorder AMEL 863, with a static glassy-carbon (GC) working electrode and a glassy-carbon Radiometer rotating disk electrode (BM-EDI 101) coupled with a Radiometer speed control unit (CTV 101), Ag–AgClO<sub>4</sub> (0.1 mol L<sup>-1</sup>)/MeCN – fine porosity fritted glass disk – MeCN/NaClO<sub>4</sub> (0.1 mol L<sup>-1</sup>) – sintered glass disk [19] as reference and a platinum wire as counter electrode. All experiments were carried out at room temperature on nitrogen purged solution of anhydrous acetonitrile containing 0.1 mol L<sup>-1</sup> NaClO<sub>4</sub> as supporting electrolyte, 1 × 10<sup>-3</sup> mol L<sup>-1</sup> substrate and increasing amounts of 2,6-lutidine as deprotonating agent. Scan rates varied from 0.020 to 0.200 V s<sup>-1</sup>. The accuracy of the potentials reported in Tables 1 and 2 is ±5 mV.

Spectroelectrochemical experiments were carried out with a diode array spectrophotometer HP 8452A and a potentiostat AMEL 552 coupled with an integrator AMEL 731 and a x/y recorder LINSEIS L250E for controlled potential electrolyses, using a three-electrode modified UV cell, a platinum wire as working electrode, an Ag–AgClO<sub>4</sub> (0.1 mol L<sup>-1</sup>)/MeCN – fine porosity fritted glass disk – MeCN/NaClO<sub>4</sub> (0.1 mol L<sup>-1</sup>) – sintered glass disk as reference and a platinum wire (placed on the inner wall of a glass tube containing MeCN/NaClO<sub>4</sub> 0.1 mol L<sup>-1</sup> and connected to the test solution via a sintered glass-disk) as auxiliary electrode. The solution was stirred by purging with a continuous nitrogen flux. All experiments were carried out at room temperature on solution of anhydrous acetonitrile containing NaClO<sub>4</sub> 0.1 mol L<sup>-1</sup> as supporting electrolyte and 1 × 10<sup>-4</sup>–5 × 10<sup>-4</sup> mol L<sup>-1</sup> substrate.

Table 1

Electrochemical data of HCAs, C and TBC in anhydrous acetonitrile 0.1 mol L<sup>-1</sup> NaClO<sub>4</sub>, at a static GC electrode, scan rate 0.200 V s<sup>-1</sup>, vs. Ag/AgClO<sub>4</sub> in the absence and in the presence (\*) of 2,6-lutidine

[S] (1 × 10 <sup>-3</sup> mol L <sup>-1</sup> )	<i>E</i> <sub>ap</sub> (V)	<i>E</i> <sub>ap</sub> (*) (V)	<i>E</i> <sub>cp</sub> (V)	<i>E</i> <sub>cp</sub> (*) (V)
C	+0.85	+0.28	+0.18	–0.30
TBC	+0.77	+0.26	+0.13	–0.34
CA	+0.79	+0.26	+0.36	–0.30
CAE	+0.83	+0.26	+0.12	–0.31
CGA <sup>a</sup>	+0.80	–	+0.25	–
RA	+0.86	+0.25	+0.13	–0.15/–0.25
FA	+0.91	+0.40	+0.32	–0.27
SA	+0.78	+0.25	+0.49	–0.15/–0.30
CUA <sup>b</sup>	+1.20	–	–	–

<sup>a</sup> Some data undetectable because of the presence of an abundant precipitate of lutidinium salt.

<sup>b</sup> Some data undetectable because of adsorption phenomena at the electrode surface.

### 2.3. Chemical oxidation

Chemical oxidation was carried out by adding directly in the UV cell small amounts of freshly prepared PbO<sub>2</sub> [20] to solutions of anhydrous acetonitrile containing 1 × 10<sup>-4</sup>–5 × 10<sup>-4</sup> mol L<sup>-1</sup> substrate, purged with nitrogen.

### 2.4. HPLC–MS instrumentation and conditions

HPLC–MS analyses were carried out on an HPLC separation module 1525 μ Waters, linked to a Quattro Micro Tandem MS–MS with an electro-spray interface Waters (Micromass, Manchester, UK). Data were processed by MassLynx software (Data Handling System for Windows, Version 4.0, Micromass, UK).

The solution obtained from the oxidation with PbO<sub>2</sub> of each HCA dissolved in acetonitrile was filtered through PTFE 0.45 μm filter disk and injected (10 μL) into a C18 reversed phase column (Waters Spherisorb 5 μm ODS2, 2.1 mm × 150 mm). Solvent A consisted of deionized water 5 mM formic acid and solvent B consisted of methanol. At a flow rate of 200 μL min<sup>-1</sup>, the following binary gradient with linear interpolation was used: 0 min, 20% B; 2 min, 20% B; 12 min, 40% B; 15 min, 40% B; 25 min, 60% B; 40 min, 60% B. Before starting and at the end of each gradient, the column was equilibrated to initial condition for 30 min. The effluent was analysed by an electrospray source (source temperature, 100 °C; desolvation temperature, 250 °C; capillary voltage, 2.7 kV; cone voltage, 25 V). Nitrogen was used as nebulizing (550 L h<sup>-1</sup>) and as drying gas (50 L h<sup>-1</sup>). Electrospray ionization-mass spectrometry (ESI–MS) data were acquired in negative mode.

### 2.5. ESR experiments

ESR spectra were recorded on a Bruker EMX EPR spectrometer (Bruker, Karlsruhe, Germany) equipped with an XL Microwave frequency counter, Model 3120 for the determination of the *g*-factors. Computer simulation of EPR spectra were

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