Electrochemical Approach to the Radical Anion Formation from 2′-Hydroxy Chalcone Derivatives

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Abstract
Three 2′-hydroxy chalcone derivatives were electrochemically reduced to the radical anion by a reversible one-electron transfer followed by a chemical dimerization reaction. Under suitable conditions of the medium, the one-electron reduction produces very well resolved cyclic voltammograms due to the formation of the radical anion. By using appropriately the wide versatility of the cyclic voltammetric technique, was possible to study the generation of the radical anion and its stability.
We have found a direct relation between the A-ring substitution and the radical anion formation; consequently, it is possible to modulate the anion radical formation with different substituents on the A-ring.

Keywords: Chalcones, Cyclic voltammetry, Radical anion

Induction of phase 2 enzymes (e.g., glutathione transferases, NAD(P)H:quinone reductase, glucuronosyltransferases, epoxide hydrolase) is a major strategy for reducing the susceptibility of animal cells to neoplasia. Michael reaction acceptors (i.e., olefins or acetylenes that are conjugated to electron-withdrawing groups) are a major group of inducers of induction of phase 2 enzymes. The potencies of these compounds in inducing NAD(P)H:quinonereductase activity in murine hepatoma cells paralleled their Michael reaction acceptor activity [1]. Chalcones belong to this wide class of compounds. Chemically, chalcone is 1,3-diphenyl-2-propen-1-one and depending on the substitution pattern on the two aromatic rings, a wide variety of pharmacological properties have been identified. These include antioxidant [2, 3], anticancer [4, 5], antimutagenic [6] and antimalarial [7] activities. On the other hand a number of phenolic antioxidants have been shown to protect against carcinogenesis and mutagenesis [8 – 12].

The relationship between their antioxidant properties and their ability to serve as chemoprotective agents have been linked because free radical-mediated processes are believed to be associated with the occurrence of many human diseases, including cancer [13]. Moreover has been published that the free radical-scavenging capacity of several quinone reductase inducers show good correlation with their potencies as inducers of phase 2 enzymes suggesting that the regulation of phase 2 enzymes may involve both Michael reaction reactivity and radical quenching mechanism. It has also been described that the introduction of *o*-hydroxyl groups on the aromatic rings of these kind of compounds dramatically enhanced their potencies not only as inducers for quinone reductase but also as quenchers of superoxide [14]. On the other hand, It seems noteworthy that a phenolic aryl ketone grouping is a common feature of many biologically active compounds. In addition, we have demonstrated that compounds with this functionality are able to inhibit tumor cell respiration in the TA3 and multidrug-resistant TA3-MTX-R cell lines [15].

Considering that chalcones can act as electron transfer agents and as Michael acceptors it is feasible to think in a probable, but non-explored, enzymatic reduction by NAD(P)H: reductases as an alternative biological mechanism. Taking advantage of the similarity between enzymatic and electrochemical reductions we have been interested in checking the feasibility of reduction of some chalcones incorporating a phenolic hydroxyl in the *ortho* position with regard to the carbonyl group.

The electrochemistry of chalcones was initiated at the middle of the past century wherein some Russian authors [16, 17] proposed that the reduction of chalcones involved that the first electron attack takes place on the carbonyl group and the free radical formed undergoes isomerization into another free radical which is further reduced or can be converted into a dimer. In other work the formation of a radical anion and radical dianion by the electrolysis of chalcone in anhydrous dimethylformamide was reported

[18]. The interpretation of the reduction process of o-hydroxy chalcone and its heterocyclic analogues by Meunier et al. [19] proved that saturated ketone results from the acceptance of the first two electrons. Zuman et al. [20] informed the polarographic reduction of chalcone in aqueous medium finding a radical anion which is protonated with an approximate pK value of 10.2. Furthermore cyclic voltammetric studies [21] carried out in DMSO were consistent with a mechanism involving the reduction of the α,β-unsaturated ketone to its radical anion followed by irreversible dimerization. Recently [22, 23], it was found that electron affinities of a series of monosubstituted chalcones computed at the density functional level, were highly linearly correlated with voltammetric potentials measured in aprotic medium.

All the previous electrochemical studies about the reduction of chalcones unanimously revealed that the anion radical generated as the consequence of the one electron acceptance is the obligate first step in its reduction mechanism. In spite of the above, not any electrochemical studies have been reported about the stability of this radical anion from chalcones. Our current research is focused to propose a possible nondescribed role between the radical anion and the pharmacological action of chalcones. Consequently in the first step we are looking for more information about the formation and stability of this anion.

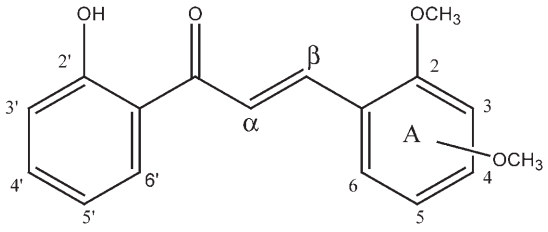
In the present study, we have used cyclic voltammetry in order to quantitatively characterize the formation and stability of the anion radical from three synthesized 2′-hydroxy chalcones.

All the three synthesized 2′-hydroxy chalcones (Fig. 1) were electrochemically reduced on Hg electrode in a non-aqueous medium containing DMSO and tetrabutylammonium hexafluorophosphate (TBFP) as supporting electrolyte. The reduction pattern of the three compounds was similar wherein a first reversible reduction couple was clearly identified from the voltammograms (Fig. 2). The observed 60 mV separation of the anodic – cathodic peaks strongly supports that the couple is due to a one electron reduction to the radical anion. Furthermore, ΔE_p remains constant (63 ± 4.3 mV) for sweep rates between 500 mV/s a 5 V/s. In this case, the reduction of the α,β-unsaturated ketone moiety to produce the carbanion radical, as shown in the following equation:

$$\mathrm{ArCOCH{=}CHAr + e^- \leftrightarrow ArC(O^-)CH{=}\dot{C}HAr} \quad (1)$$

In spite of recently [22, 23] the substituent effects on the reduction potentials of a large numbers of chalcone derivatives have been studied, none of the reduction potentials of the current synthesized 2′-hydroxy chalcones can be anticipated from that study.

By comparing the reduction potentials for the first electron transfer (Table 1) we can appreciate that the 2,4 dimethoxy substituted derivative required more energy to



Compound	A ring substitution
2,3-DIMECHA	3-OCH_3
2,4-DIMECHA	4-OCH_3
2,5-DIMECHA	5-OCH_3

Fig. 1. Molecular structures of the three 2′-hydroxy chalcone derivatives.

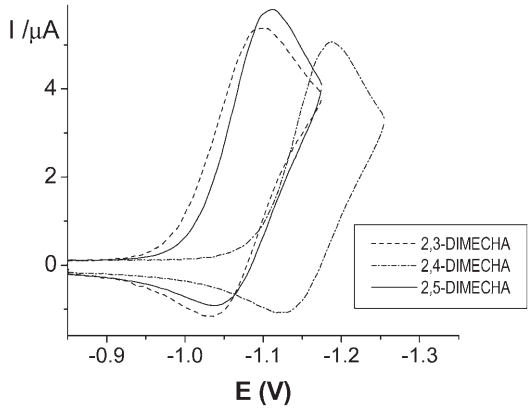


Fig. 2. Cyclic voltammogram of the first redox couple of 1 mM 2′-hydroxy chalcone derivatives in DMSO at 1 Vs^{-1}.

the one-electron transference than the two other compounds. This effect can be ascribed to the electron donor effect of the 4-methoxy group on the electron density of the β-carbon of the α,β insaturated ketone moiety. In fact, donor groups in ortho and/or para positions of the A ring will produce an enhanced electron density on the β-carbon thus hindering the reduction. Only in the case of the 2,4-dimethoxy derivative the effect of donor groups in ortho and para can be gathered, i.e. in the case of 2,4-DIMECHA the formation of the anion radical was more difficult than at the other two compounds.

According to the above we have found a direct relation between the A-ring substitution and the radical anion formation. Consequently, it is possible to modulate the radical formation with different substituents on the A-ring.

On investigating the substituent effects on the radical anion formation and stability, we have focused our attention only on the first reversible reduction couple. As can be seen from all previous evidences and Equation (1) the mono-electronic transfer of 2′-hydroxy chalcones to produce the corresponding anion radical is the first obligatory step in its

Table 1. Characteristic parameters of different compounds.

	$-E_{p,c}$ (mV)	$k_2 \times 10^{-3}$ (M^{-1} s^{-1})	$t_{1/2}$ (s)
2,3-DIMECHA	1096	5.6 ± 0.30	0.18
2,5-DIMECHA	1110	30.5 ± 0.30	0.03
2,4-DIMECHA	1187	40.5 ± 0.27	0.03
Metronidazole [29]	1090	0.22 ± 0.03	4.50
Nitrofurazone [30]	886	0.750 ± 0.04	1.33
Nifurtimox [30]	930	0.486 ± 0.04	2.05

reduction pathway. Under suitable conditions of the medium, the one electron reduction produces very well resolved cyclic voltammograms due to the formation of the radical anion. By using appropriately the wide versatility of the cyclic voltammetric technique, it is possible to study the generation of the radical anion and its stability. The stability of the radical anion depends fundamentally on the reaction media and is reflected in the decay of the corresponding oxidation current, $i_{p,a}$, subsequent to its electrochemical generation. In fact the stability of the radical anion is better expressed by the current ratio parameter, $i_{p,a}/i_{p,c}$, which reveals the tendency of an electrochemical generated species, i.e. radical anion, to undergo chemical following reactions [24]. Thus, the current ratio equals to unity in the absence of further reactions of radical anion but decreases if the radical reacts subsequently. Therefore the cyclic voltammetric experiments can be used to prove the stability of the formed radical anion from the chalcone derivatives by changing electrochemical or chemical conditions, and then by measuring the $i_{p,a}/i_{p,c}$ values of the radical anion couple. Consequently, the $i_{p,a}/i_{p,c}$ ratio will be the fundamental parameter in the characterization of the radical anion from the studied chalcones. According to the tendency of the curve between current ratio and log of sweep rate (Fig. 3A) the radical anion obeys an EC type mechanism wherein a first electrochemical step is followed by a coupled chemical reaction according to the following equations:

$$\mathrm{Ar-CH=CO-Ar} + \mathrm{e} \rightleftharpoons [\mathrm{Ar-CH=CO-Ar}]^{\bullet -} \qquad (2)$$

$$m\ [\mathrm{Ar-CH=CO-Ar}]^{\bullet -} \rightarrow \mathrm{Products} \qquad (3)$$

The chemical coupled reaction could be of first ($m = 1$) or higher ($m \neq 1$) order. A diagnostic criterion suitable for discriminating EC process involving chemical coupled reaction of first order from those of second order is given by the dependence of current ratio values on the concentration of the electroreducible compound which is observed only in the latter case [25]. Consequently, in order to investigate the order of the coupled chemical reaction we have tested cyclic voltammograms at different 2′-hydroxy chalcone derivative concentrations. According to the dependence obtained between $i_{p,a}/i_{p,c}$ and the 2′-hydroxy chalcone derivative concentration (Fig. 3B) we can affirm that the order of the coupled chemical reaction is different from order 1 and from the slope of the plot of the cathodic

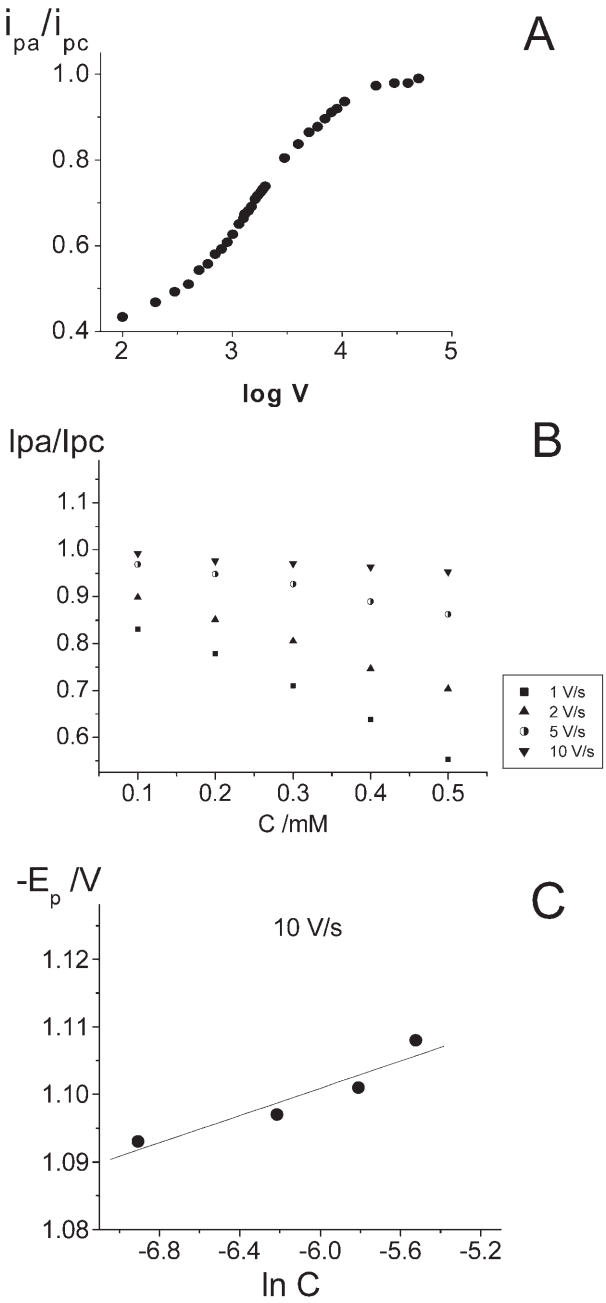


Fig. 3. A) Current ratio dependence on sweep rate for redox couple to the one electron of 2,5-DIMECHA in DMSO. B) Current ratio dependence on the 2,3-DIMECHA concentration at different sweep rates: (■) 1.0; (▲) 2.0; (◑) 5.0; (▼) 10.0 Vs^{-1}. (C) Peak potential dependence with the natural logarithm of 2,3-DIMECHA concentration.

peak potential (E_p) versus the natural logarithm of chalcone derivative concentration (Fig. 3C) we obtained an order 2 for the coupled chemical reaction. In fact, the slope of the E_p vs ln C plot shows a value of 10.06 mV. According to the previously described equation [25]:

$$\mathrm{d}E_p/\mathrm{d}\ \ln\ C_0 = (m-1)/(m+1)\ (RT/nF)$$

it is possible to obtain a "*m*" value of 2.28 which is indicative of order 2 for the coupled chemical reaction.

From the above experimental evidence we postulate that the coupled chemical reaction corresponds to dimerization. This postulation is in accordance with previous studies from related compounds wherein a dimerization reaction for radical anions of α,β-unsaturated ketones was described [21]. In order to confirm the proposed mechanism we applied the cyclic voltammetry theory for the dimerization reaction described by Olmstead et al. [26]. In fact, we obtained straight lines in the plots of the kinetic parameter ω versus τ in perfect accordance with that anticipated by the theory. Furthermore, according to the theory, for values $a\tau = 4$, it should be noted that $\omega = k_2 C^0 \tau$; consequently, we can obtain the dimerization rate constant (k_2) from the slope of the straight lines between ω and τ. We have calculated the $k_{2,\mathrm{dim}}$ values and the corresponding half-life times ($t_{1/2}$) for the radical anions of all the 2′-hydroxy chalcone derivatives (Table 1). Based on the $k_{2,\mathrm{dim}}$ obtained values, we can affirm that 2,4-DIMECHA generates a less stable radical species than the other two chalcones. Also, from Table 1, we can affirm that radical anion more easily formed (lower cathodic potentials) involved more stable radical anions i.e. the radical anion formation from 2,4-DIMECHA required more energy but it was the most unstable. In the previous paper of Evans et al. [21] a dimerization rate constant of 1.4×10^5 M s^{-1} for the radical anion formed from an α,β-unsaturated derivative was calculated. The enhanced stability of the radical anion from the 2′-hydroxy chalcone derivatives compared with the α,β-unsaturated derivative can be explained by the stabilizer effect of the phenyl substituted group neighbor to the carbonyl moiety which is failing in the previous studied α,β-unsaturated derivative.

Furthermore, in Table 1 we can observe a comparison of both the cathodic peak potentials, $E_{\mathrm{p,c}}$ and the dimerization rate constants, $k_{2,\mathrm{dim}}$ of the radical anion obtained from the 2′-hydroxy chalcone derivatives with other radical anion (nitro radical anion) obtained from compounds with biological significance such as nitromidazol, nifurtimox and nitrofurazone. From the comparison we can deduce that the energy of formation of the nitro radicals are comparable, meaning that there is no electrochemical basis to discard a possible enzymatic reduction of the 2′-hydroxy chalcones . On the other hand the radical anion from the 2′-hydroxy chalcones are considerably lower stable than the nitro radical anion providing half-life times more shortened.

The electrochemical approach is very useful as a tool for investigating formation and stability of radical anions from chalcone derivatives.

We have found a direct relation between the A-ring substitution and the radical anion formation; consequently, it is possible to modulate the anion radical formation with different substituents on the A-ring.

All the studied 2′-hydroxy chalcones were easily reduced to the corresponding radical anion at similar potentials than enzymatically reduced compounds, making non discardable an hypothetic new reduction route for these compounds.

Experimental

2′-Hydroxy chalcones were prepared by adding dropwise a solution of the corresponding dimethoxy substituted benzaldehyde, (7.34 mmol in ethanol, 20 mL) to a stirred mixture of 2-hydroxyacetophenone solution (7.34 mmol, in ethanol, 20 mL) and potassium hydroxide solution (2g in 10 mL distilled water). The mixture was allowed to react overnight, an then was diluted with distilled water (200 mL) and neutralized with hydrochloric acid, and then extracted four times with ethyl acetate (50 mL). The combined organic phases were concentrated in vacuum and redissolved in ethanol and allowed to crystallize.

All these compounds were previously described in the literature:

2,3-dimethoxy-2′-hydroxy chalcone 2,3-DIMECHA [27] was obtained as yellow crystals (59 %); mp.102 – 103 °C;

2,4-dimethoxy-2′-hydroxy chalcone 2,4-DIMECHA [28] was obtained as yellow crystals (58%); mp. 107 – 109 °C.;

2,5-dimethoxy-2′-hydroxy chalcone 2,5-DIMECHA [29] was obtained as yellow crystals (72%); mp. 95 – 96 °C.

Electrochemical experiments were carried out using a totally automated BAS-100 voltammetric analyzer attached to a PC computer with proper BAS 100-W version 2.3 software for total control of the experiments and data acquisition and treatment.

The Cyclic voltammetry (CV) experiments were carried out in DMSO with tetrabutylammonium hexafluorophosphate (TBFP) as supporting electrolyte. A hanging mercury drop electrode (HMDE) was used as working electrode (area = 2.27 mm^2). A platinum wire counter electrode and Ag/AgCl as reference electrode were used for the measurements.

Stock solutions of each compound were prepared at a constant concentration of 20 mM in DMSO. The cyclic voltammetric working solutions were prepared by diluting the stock solution until final concentrations of 1 mM were obtained. The dilution solutions were DMSO containing 0.1 M tetrabutylammonium hexafluorophosphate (TBFP), as supporting electrolyte.

All the electrochemical experiments were obtained after purge with N_2 for ten minutes in the cell before each run.

All the experiments were carried out at 25 ± 1 °C.

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