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Evaluation of the Antioxidant Activity of New Coumarin Derivatives by Cyclic Voltammetry

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Introduction

Free radicals, species with one or more unpaired electrons are produced in normal or pathological cell metabolism, from xenobiotics, or trough ionizing radiation. Electron acceptors such as molecular oxygen react easily with free radicals, to become radicals themselves. Oxygen free radicals or reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxy radicals (·OH), and singlet oxygen ($^1O_2$) are continuously generated in cells exposed to an aerobic environment, and have been associated with the genesis of tumors as well as age-dependent diseases such atherosclerosis, arthritis, and neurodegenerative disorders. In this context, the chemopreventive role of antioxidants present in consumable fruits, vegetables, and beverages has received considerable attention and has been a growing interest in finding novel antioxidants in order to meet the requirements of pharmaceutical industries [1].

Several analytical methods have been used to evaluate the activity of antioxidant compounds, such as the cyclic voltammetry[2] and the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay[3].

In this work the antioxidant activity of two new synthetic coumarin derivatives, 3-bromo-6,7-dihydroxycoumarin and 6,7-dihydroxy-3-[(*E*)-2-phenylethenyl]coumarin, is evaluated by cyclic voltammetry. The anodic peak potentials were measured and compared to their antioxidant activities measured by the DPPH radical assay. The voltammetric experiments were carried out at pH 7.0 (phosphate buffer) in order to resemble the conditions of DPPH assays. Both voltammetric and DPPH assay data were compared with data obtained for 6,7-di-hydroxycoumarin and quercetin, with high antioxidant activity. The structure–activity relationships of the synthesized coumarins as potential antioxidants was investigated in order to understand how the different 3-substitutions affect the antioxidant activity of the 6,7-dihydroxycoumarin.

Results and Discussion

Coumarins with an ortho-catechol moiety showed low oxidation potentials and high scavenging activities for free radicals. These molecules are known to be oxidized to form orthosemiquinones, which are thought to contribute to their oxidizability, that is, antioxidant activity. This characteristic may explain why compounds with an ortho-catechol moiety show high antioxidant activities.

Radical-scavenging properties of the coumarins 1-3 were evaluated against the DPPH radical in a spectrometric assay. Quercetin and BHT were used as reference compounds. Activity of 6,7-dihydroxycoumarin (1) remained lower than that of quercetin but was higher than that of BHT (Table 1).

In this assay the new 3-substituted coumarin derivatives, 3-bromo-6,7-dihydroxycoumarin (2) and 6,7-dihydroxy-3-[(*E*)-2-phenyl-ethenyl]coumarin (3), showed higher activity than that 6,7-dihydroxycoumarin (1) (Table 1), suggesting that the higher delocalization of the π-electron system, induced by the 3-substituitions, increases the antioxidant activity of these molecules with relation to the 6,7-dihydroxy-coumarin (1).

The cyclic voltammograms of coumarins and quercetin are shown in Fig. 2. Within the investigated potential range, two anodic peaks were observed for coumarins (1) and (2), while for coumarin (3) only one peak was observed. Quercetin presents the typical voltammetric response already reported in the literature. The corresponding anodic peak potentials for these four compounds are summarized in Table 1. Comparing the values of the first anodic peak potential with each other, can be observed that susceptibility to the electrochemical oxidation increases in the following order: 6,7-dihydroxy-3-[(*E*)-2-phenylethenyl]coumarin > 3-bromo-6,7-dihydroxycoumarin ~ 6,7-dihydroxycoumarin > quercetin. This suggests that the antioxidant strength (reducing power) of these molecules increases in the same order, and that among the coumarins, the molecule with higher delocalization of the π-electron system (coumarin (3)), is less prone to lose electrons. The effect of substitution of 3-bromo also seems to diminish the ability of the molecule to donate electrons, but in a little insignificant way. As can be seen, the correlation of the oxidation potentials with their DPPH radical scavenging activities was not high, because of the discordant response obtained with coumarin (1). These findings are most probably related with several differences that exist between the chemical and electrochemical oxidation mechanisms of these molecules, with the oxidant DPPH and at the anode, respectively.

With respect to the counterpart cathodic response of the anodic peaks, it was found for all coumarins that the reduction peaks were very small or even non-observable (in particular, especially, at lower scan rates), indicating that the electrooxidation processes are followed by complex chemical reactions, such as a dimerization/polymerization reaction between the molecules of reactants and products of their oxidation. To verify this possibility other voltammograms were recorded at higher scan rates.

Accordingly, we observed: a) the development of a cathodic peak, which gradually grows with the scan rate, b) the peak current ratio ($i_{p,c}/i_{p,a}$), which is less than unit, increases slightly with increasing scan rate, until it reaches a fixed value. In addition, proportionally to the increase of scan rate, the scan rate normalized current ($i_{p,a}/\nu^{1/2}$) diminishes gradually. Meantime, the oxidation peak potential is positively shifted (see Fig 3), indicating that the charge transfer of coumarins to the electrode was impaired (the $\partial E_{p,a}/\partial \log \nu$ increases from the 6,7-dihydroxycoumarin to the coumarin with higher delocalization of the π-electron system, coumarin (3)). Besides that, good linear relationships were obtained for the plot of $\log i_{p,a}$ vs. $\log \nu$ (see Fig 3). The slope $\partial i_{p,a}/\partial \log \nu$ increase from the 6,7-dihydroxy-coumarin to the 6,7-dihydroxy-3-[(*E*)-2-phenylethenyl]coumarin, suggesting that the charge transfer is diffusion controlled with the former coumarin and with other two, some surface effects have an increasing influence.

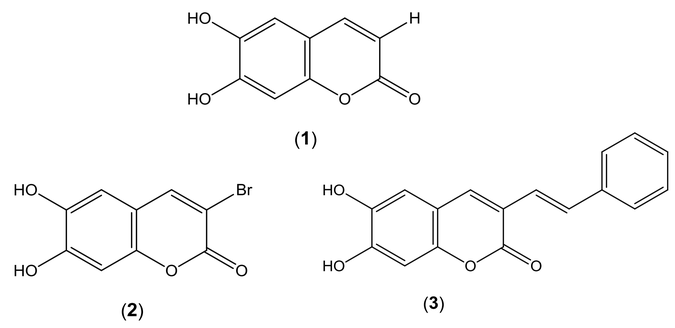

Fig.1. Chemical structure of coumarins: **6,7-dihydroxycoumarin** (esculetin) (**1**), **3-bromo-6,7-dihydroxycoumarin** (**2**), and **6,7-dihydroxy-3-[(*E*)-2-phenyl-ethenyl]coumarin** (**3**) .

Table 1. DPPH spectrophotometric assay results and voltammetric data of coumarins, quercetin and BHT .

	IC_{50} (µg/mL)	E_{pa} [a] (mV)	E_{pa} [b] (mV)
6,7-dihydroxycoumarin (1)	8,8	321	855
3-bromo-6,7-dihydroxycoumarin (2)	4,8	326	816
3-[(*E*)-2-phenylethenyl]-6,7-dihydroxycoumarin (3)	5,2	574	--
Quercetin	2,1	195	430, 935
BHT	13,8	--	--

[a] First anodic peak and [b] Other anodic peaks (*vs.* Ag/AgCl, KCl 3 M).

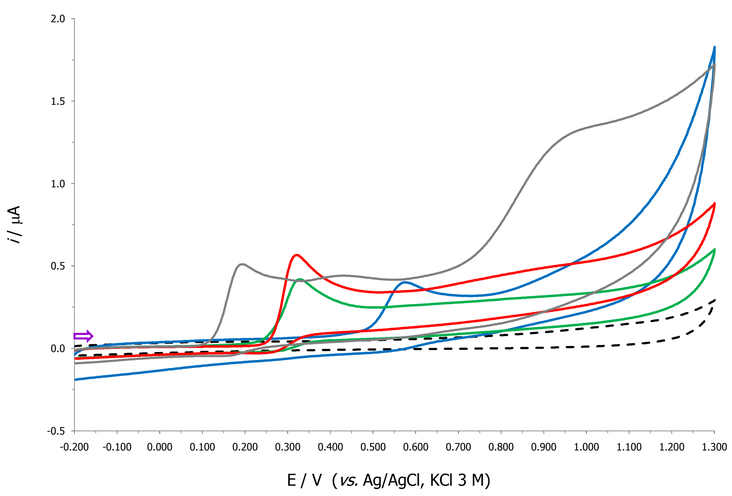

Fig. 2. Cyclic voltammograms of **6,7-dihydroxycoumarin**; **3-bromo-6,7-dihydroxycoumarin**, **6,7-dihydroxy-3-[(*E*)-2-phenylethenyl]coumarin**, and **quercetin (50 µM)**. Initial potential (E_i): -0.200 V; scan rate (ν): 25 mV s^{-1}. The dotted line corresponds to the blank response.

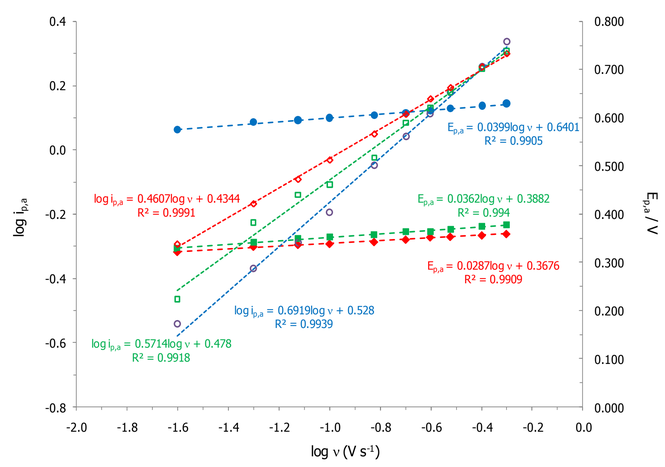

Fig. 3. Variation of the first anodic peak potential ($E_{p,a}$) and peak current ($i_{p,a}$) as a function of the scan rate ν (semi-log and log-log relationships, respectively).

Experimental

DPPH spectrophotometric assay: Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 250, 125, 50, 25, 10, 5, 3 and 1 µg/ml, in methanol. One mL of a 0.3 mM DPPH methanolic solution was added to 2,5 mL of sample solutions of different concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 518 nm and converted into percentage of antioxidant activity (AA) using the following formula: $AA\% = 100 - ((Abs_{sample} - Abs_{blank}) \times 100) / Abs_{control}$). Methanol (1,0 mL) plus compound solution (2.5 mL) was used for blank. DPPH solution (1.0 mL; 0.3mM) plus methanol (2,5 mL) was used for negative control. The positive controls were those using the standard solutions. The IC_{50} values are calculated by linear regression of plots where the abscissa represented the concentration of tested compounds and the ordinate the average percent of antioxidant activity from three separate tests. Activity criteria: IC_{50} < 200 µg/ml.

Cyclic voltammetric experiments: These were carried out using an Potentiostat/Galvanostat *AUTOLAB* (from Ecochemie B.V. - Holland , model PGSTAT 20, computer controlled (software GPES, version 4.9)) and a 663 VA Stand with a Multi Mode Electrode (Metrohm). All the measurements were carried out in a three-electrode measuring cell (all components from Metrohm): Working electrode – Glassy carbon electrode (ϕ = 2 mm); Reference electrode – Ag/AgCl, KCl 3 M; Auxiliary electrode – Platinum rod electrode (ϕ = 2 mm, l = 65 mm). The working electrode surface was hand-polished using 0.3 mm alumina powder on a polishing cloth, followed by successively rinsing with acetone and deionized water, before each experiment. Before each measurement this electrode surface was electrochemically activated in the coumarin-free (or quercetin-free) electrolyte solution, by performing 2 cycles between -1.000 and 1.000 V (vs. Ag/AgCl, KCl 3 M), at a scan rate of 10 mV s^{-1}.

Coumarins and quercetin stock solutions with 2.5 mM in each, were prepared in methanol. A phosphate buffer stock solution (Na_2HPO_4/KH_2PO_4, with pH 7.00 and 0.2 M) was used to prepare the supporting electrolyte solutions. The final concentration of coumarins and quercetin added to cell were of 50 µM. Solutions in the cell were purged with oxygen-free nitrogen for 6 min prior the measurement. All voltammograms were recorded at a constant temperature of 25ºC. Scan rates used: 25 to 500 mVs^{-1}.

Conclusions

No strong correlation was found between the oxidation potencial in voltammetry and the radical scavenging capacities exhibited by the coumarin derivatives, but trends were similar. This may indicate the dominant influence of structural effects on antiradical effects. The importance of structural effects and use of different assays and their similarities is a subject of continued debate among scientists in the field. The different mechanisms involved and the assay conditions in different media might also contribute to the existing differences in the results.

References

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