Insights into the antioxidant activity of phenolic compounds: Synthesis and electrochemical study of new series of hydroxycoumarins

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"Abstract." Phenolic compounds are bioactive substances widely distributed in the vegetable kingdom. They act as natural antioxidants, and their presence contributes to the color, flavor and aroma of food. This group of micronutrients is composed of one or more aromatic benzene rings with one or more hydroxyl groups and their redox properties are related with their chemical structure characteristics. The knowledge of their redox potentials may help the food industry, because when phenolic compounds are oxidized they could affect the quality of the wines, beers, grape juices, etc.

Coumarins are a large family of compounds, of natural and synthetic origin, that show important biological activities. Therefore, they occupy an important place in the study of natural products and synthetic organic chemistry. Recent studies pay special attention to their antioxidative, anticarcinogenic and enzymatic inhibition properties. Their preparation, and the versatility of the synthetic methodology, allowed us obtaining a wide family of compounds with substituent in different positions in the molecule. The election of these derivatives has considered the later pharmacological evaluation.

The investigation of the properties of these compounds, the study of the structural pattern and the elucidation of their biological role is of great interest for further development of coumarin-like antioxidant drugs. The electrochemical behaviour of a group of differently substituted hydroxycoumarins was investigated using cyclic, differential pulse and square wave voltammetry, in aqueous media at a glassy carbon electrode over the whole pH range. The antioxidant reactivity and capacity were also evaluated through a competition assay with hydroxyl radical (OH•) and ORAC-FL methodology. Number and positions of the hydroxyl groups were important factors in the antioxidant activities against peroxyl and hydroxyl radicals of the coumarin derivatives.

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1. Introduction

In recent years, phytochemical compounds have been showing a great interest due to their presence in bioactive dairy products. Fruits, vegetables, oil and wine have been studied as health protectors because of the antioxidant potential of their phenolic compounds. These compounds are known to be the most common secondary metabolites in the vegetable kingdom.[1,2,3]

The fusion of a pyrone with a benzene ring gives rise to a class of heterocyclic compounds known as benzopyrones or coumarins.[4] Coumarins are a wide group of compounds present in remarkable amounts in the nature.[5] Representatives of this group occur in the vegetable kingdom, either in free or combined state.[6] Due to their structural variability, they are an elite class of compounds which occupy an important role in synthetic organic chemistry.[7]

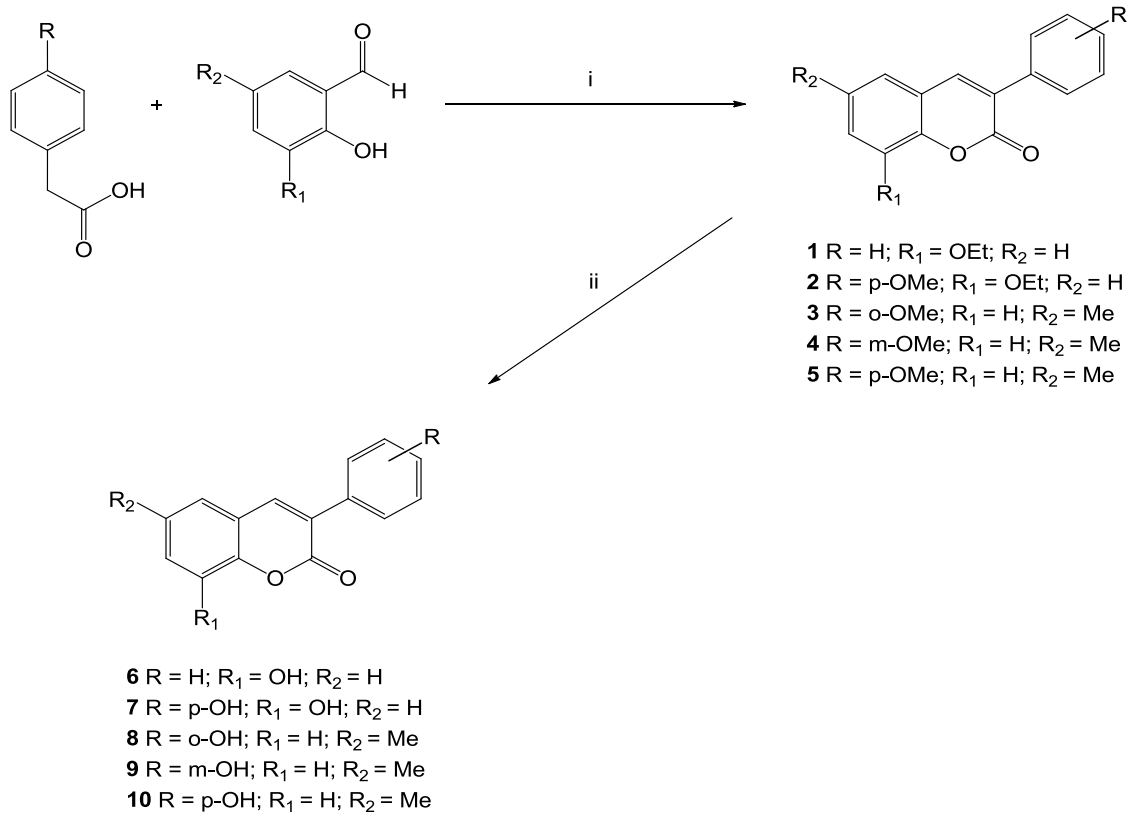
Coumarins have been attracting considerable interest due to their numerous biological activities, usually associated to low toxicity, depending on their substitution pattern.[8] There are many possible permutations offered by substitution and conjugation, and this readily explains why so many synthetic analogues featuring coumarin structural motif are investigated due to their wide range of biological properties.[9] In the literature, coumarins have been described as anticancer,[10,11] antioxidant,[12,13] antimicrobial,[14] antiviral,[15] vasorelaxant,[16] anti-inflammatory[17] and enzymatic inhibitors.[18,19,20,21] Indeed, some coumarins are now commercially available as medicines.

2. Chemistry

The coumarin derivatives **1-10**[15,22,23,24] were efficiently synthesized according to the protocol outlined in Scheme 1. The general conditions and the compounds characterization were described in the experimental section.

Perkin condensation of differently substituted *ortho*-hydroxybenzaldehydes with the corresponding arylacetic acids, using *N,N'*-dicyclohexylcarbodiimide (DCC) as dehydrating agent, in DMSO, afforded the 3-arylcoumarins **1-5**. Compounds **6-10** were synthesized starting from the respective methoxy/ethoxy derivatives **1-5** by hydrolysis reaction using hydriodic acid 57 %.

Scheme 1. Synthetic strategy



1 R = H; R_1 = OEt; R_2 = H
2 R = p-OMe; R_1 = OEt; R_2 = H
3 R = o-OMe; R_1 = H; R_2 = Me
4 R = m-OMe; R_1 = H; R_2 = Me
5 R = p-OMe; R_1 = H; R_2 = Me

6 R = H; R_1 = OH; R_2 = H
7 R = p-OH; R_1 = OH; R_2 = H
8 R = o-OH; R_1 = H; R_2 = Me
9 R = m-OH; R_1 = H; R_2 = Me
10 R = p-OH; R_1 = H; R_2 = Me

[a] Reagents and conditions: (i) DCC, DMSO, 110 ºC, 24 h; (ii) HI, AcOH, Ac_2O, 110 ºC, 3 h.

3. Results and discussion

All the described 3-arylcoumarins (compounds **1-10**) were efficiently synthesized, characterized and evaluated for their antioxidant functionality. Relevant aspects concerning the electrochemistry of this new series of synthesised coumarins can be obtained. Considering that the electrochemical behaviour of these compounds depends on their structural features, useful information on their antioxidant functionality can be drawn. For this purpose cyclic (CV), differential pulse (DPV), at different pH values, and ORAC-FL experiments for some of the hydroxyl derivatives were performed.

Voltammetric study of compound **6** at pH 7.0, figure 1, shows on the first scan an oxidation peak at E_{p1}= + 0.650 V corresponding with an irreversible reaction. A value of E_p-$E_{p/2}$= + 0.050 V indicates that one electron is involved in the first oxidation process. A reduction peak, on the inverse sense, at E_{p2c}= + 0.044 V could also be seen, corresponding to the reduction of the oxidation products formed during oxidation of peak P_1. The reversibility of peak 2 was confirmed in the second scan where a new oxidation peak appears at lower potentials, E_{p2a}= + 0.076 V. This reversible oxidation process corresponding with peak P_2 occurs with transference of two electrons.

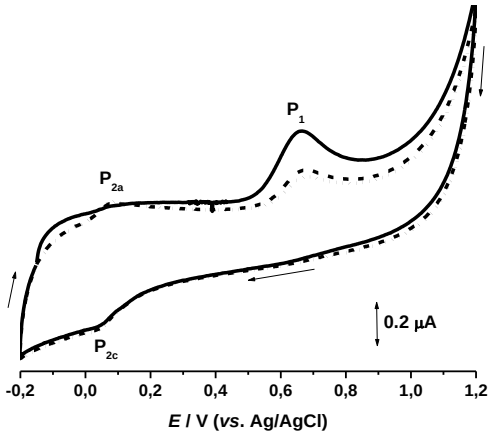


Figure 1. Cyclic voltammograms of compound **6**, 0.5 mM, pH 7.0, 0.2 M phosphate buffer: (—) 1st, (— —) 2nd and (●●) 3rd scan. Scan rate 50 mVs^{-1}.

When two hydroxyls groups are present in the molecule, as compound **7**, the differential pulse voltammogram should show two oxidation peaks due to both oxidizable groups. Figure 2A presents the first and second scan of compound **7**. The first one shows an oxidation peak with a $W_{1/2}$ ~200 mV suggesting that could be two oxidation peaks. This is confirmed on figure 2B, on the second scan, when the baseline is subtracted. First scan shows also another oxidation peak at lower potentials, called P_2 at E_{p2}= + 0.167 V. This potential value can be compared with that one for a catecol group. On the second scan an oxidation product peak, P_3, appears at E_{p3}= + 0.047 V. As was seen before though CV, P_3 corresponds to the oxidation product formed after P_1 oxidation. The strong adsorption of the oxidation products, which blocked the electrode surface, makes possible to observe more easily on the second scan both oxidation peaks, P_1 and $P_{1'}$. Oxidation processes due to P_1 and $P_{1'}$ occur with one electron transference in each case. A value of $W_{1/2}^{P3}$= + 0.057 V indicates that one electron is involved on the oxidation process. The value of $W_{1/2}$ obtained for P_2 was + 0.054 V indicating that two electron are involved in the oxidation process. This would confirm that P_2 is an oxidation product of P_1 corresponding with a catecol group.

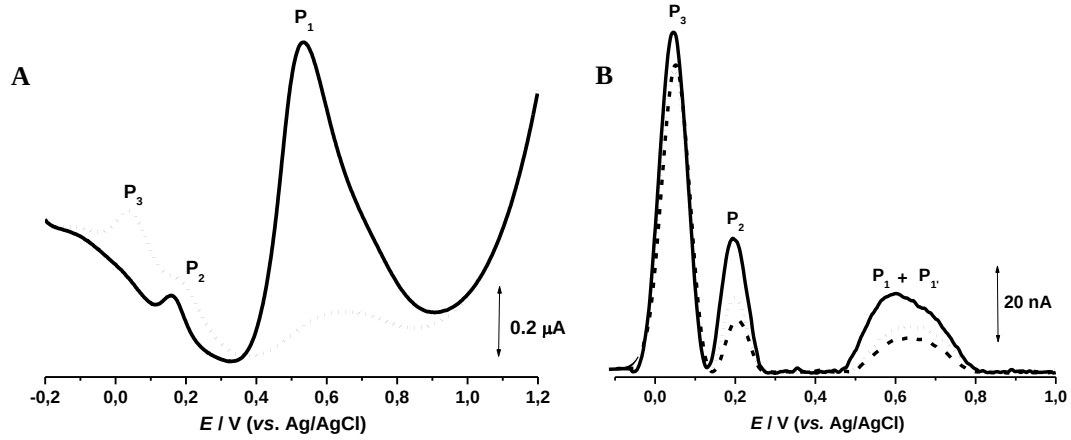


Figure 2. Differential pulse voltammograms of compound **7**, 0.5 mM at pH 7.0, 0.2M phosphate buffer. A) First (—) and second (●●) scans. Scan rate 5 mV s^{-1}. B) 2nd (—), 3rd (●●) and 4th (— —) scans with baseline subtraction.

The peak P_1 potential of each compound is displaced to more negative values with increasing pH. A slope of proximately + 0.059 V suggests that the oxidation processes involve the same number of electrons and protons. P_1 and $P_{1'}$ occurs by transference of one electron and one proton whereas, P_2 and P_3, both oxidation products, occur by transference of two electrons and two protons.

Oxidation product peaks of each compound involved two electrons and two protons. Oxidation products showed a very low oxidation potential. This behaviour, which is related to their molecular structure, clearly shows their good antioxidant properties. Also, ORAC-FL indexes were calculated by fluorescence measurements, comparing with the reference compound Trolox. The values obtained in ORAC-FL experiment are showed in table 1. Positions of the hydroxyl groups were important factors in the antioxidant activities against peroxyl and hydroxyl radicals of the coumarins derivatives.

Table 1. ORAC-FL results of some of the described compounds

Compounds	ORAC-FL
6	No evaluated
7	13,5
8	8,4
9	6,7
10	6,1
Trolox	1

4. Conclusions

We synthesized a new series of compounds using an efficient and versatile synthetic route. The experimental results demonstrated that using electrochemical methods as CV and DPV, it can be clarified the mechanism of electron transfer of a new series of 3-arylcoumarins. Compound **7** is the best antioxidant candidate since it has the lowest oxidative potential and also the highest ORAC-FL value of this new series.

5. Experimental section

General Procedure for the Preparation of 3-phenylcoumarins (1-5). A solution of hydroxybenzaldehyde (7.34 mmol) and the corresponding phenylacetic acid (9.18 mmol) in dimethyl sulfoxide (15 mL) was prepared. *N,N'*-dicyclohexylcarbodiimide

(11.46 mmol) was added and the mixture was heated in an oil-bath at 110 ºC for 24 h. Triturate ice (100 mL) and acetic acid (10 mL) were added to the reaction mixture. After keeping it at room temperature for 2 h, the mixture was extracted with ether (3 x 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5 %) and then water (20 mL). The solvent was evaporated under vacuum and the dry residue was purified by FC (hexane/ethyl acetate 9:1).

8-Ethoxy-3-phenylcoumarin (1). Yield 55 %; mp 117-118 ºC. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 1.50 (t, 3H, -CH_3, *J*=7.0), 4.21 (dd, 2H, -CH_2, *J*=14.0, *J*=7.0), 7.09 (t, 2H, H-6, H-7, *J*=6.9), 7.21 (t, 1H, H-5 *J*=7.8), 7.42-7.48 (m, 3H, H-3', H-4', H-5'), 7.72 (dd, 2H, H-2', H-6', *J*=7.7 and *J*=1.4), 7.79 (s, 1H, H-4).

8-Ethoxy-3-(4'-methoxyphenyl)coumarin (2). Yield 61 %; mp 99-100 ºC. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 1.50 (t, 3H, -CH_3, *J*=7.0), 3.84 (s, 3H, -OCH_3), 4.19 (dd, 2H, -CH_2, *J*=14.0 and *J*=7.0), 6.84-7.26 (m, 5H, H-3', H-5', H-5, H-6, H-7), 7.69 (t, 2H, H-2', H-6', *J*=7.7), 7.73 (s, 1H, H-4).

3-(2'-methoxyphenyl)-6-methylcoumarin (3). Yield 59 %; mp 177-178 ºC. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.41 (s, 3H, -CH_3), 3.82 (s, 1H, -OCH_3), 7.02 (m, 2H, H-3', H-4'), 7.24-7.41 (m, 5H, H-5, H-7, H-8, H-5', H-6') 7.69 (s, 1H, H-4).

3-(3'-methoxyphenyl)-6-methylcoumarin (4). Yield 53 %; mp 84-85 ºC. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.44 (s, 3H, -CH_3), 3.88 (s, 1H, -OCH_3), 6.97 (m, 1H, H-4'), 7.26-7.42 (m, 6H, H-5, H-7, H-8, H-2', H-5', H-6') 7.78 (s, 1H, H-4).

3-(4'-methoxyphenyl)-6-methylcoumarin (5). Yield 61 %; mp 144-145ºC (biblio. 143ºC). ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.40 (s, 3H, -CH_3), 3.84 (s, 3H, -OCH_3), 6.96 (dd, 2H, H-3', H-5', *J*=6.8 and *J*=2.1), 7.26 (m, 3H, H-4, H-7, H-8), 7.66 (m, 3H, H-3, H-2', H-6').

General Procedure for the Preparation of hydroxy-3-phenylcoumarins (6-10). A solution of substituted methoxy/ethoxy-3-phenylcoumarin (0.50 mmol) in acetic acid (5 mL) and acetic anhydride (5 mL), at 0 ºC, was prepared. Hydriodic acid 57 % (10 mL) was added dropwise. The mixture was stirred, under reflux temperature, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by CH_3CN crystallization.

8-Hydroxy-3-phenylcoumarin (6). Yield 64 %; mp 199-200 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm), *J* (Hz): 7.10-7.19 (m, 3H, H-5, H-6, H-7), 7.38-7.45 (m, 3H, H-3', H-4', H-5'), 7.71 (d, 2H, H-2', H-6', *J*=6.7), 8.19 (s, 1H, H-4), 10.25 (s, 1H, -OH).

8-Hydroxy-3-(4'-hydroxyphenyl)coumarin (7) Yield 41 %; mp 237-238 ºC. ^{1}H NMR (DMSO-d_6) δ (ppm), *J* (Hz): 6.84-6.97 (m, 2H, H-3', H-5'), 7.00-7.12 (m, 3H, H-5, H-6, H-7), 7.53-7.61 (m, 2H, H-2', H-6'), 8.05 (s, 1H, H-4), 9.80 (s, 1H, -OH), 10.15 (s, 1H, -OH).

3-(2'-hydroxyphenyl)-6-methylcoumarin (8). Yield 56 %; mp 167-168 ºC. ^{1}H NMR (DMSO-d_6) 2.38 (s, 3H, -CH_3), 6.30-6.35 (m, 2H, H-3', H-5'), 7.22-7.24 (d, 1H, H-5, *J*=1.5), 7.29-7.31 (m, 2H, H-4', H-6'), 7.34 (d, 1H, H-8, *J*=8.4), 7.44 (dd, 1H, H-7, *J*=8.4 and *J*=1.5), 7.97 (s, 1H, H-4), 9.60 (s, 1H, -OH).

3-(3'-hydroxyphenyl)-6-methylcoumarin (9). Yield 53 %; mp 160-161 ºC. ^{1}H NMR (DMSO-d_6) 2.32 (s, 3H, -CH_3), 6.77 (dd, 1H, H-4', *J*=7.1 and *J*=2.4), 7.05-7.07 (m, 2H, H-5, H-8), 7.21-7.25 (m, 2H, H-5', H-6'), 7.38 (dd, 1H, H-7, *J*=8.5 and *J*=1.8), 7.50 (s,

1H, H-2'), 8.08 (s, 1H, H-4), 9.52 (s, 1H, -OH).

3-(4'-hydroxyphenyl)-6-methylcoumarin (10). Yield of 63 %; mp 217-218 ^{o}C. ^{1}H NMR ($CDCl_3$) δ (ppm), *J* (Hz): 2.37 (s, 3H, $-CH_3$), 6.84 (d, 2H, H-3', H-5', *J*=8.8), 7.31 (d, 1H, H-8, *J*=8.4), 7.40 (dd, 1H, H-7, *J*=8.4 and 1.9), 7.56 (m, 3H, H-2', H-6', H-5), 8.06 (s, 1H, H-4).

ACKNOWLEDGEMENT

We are grateful to the Xunta de Galicia (PGIDIT09CSA030203PR) and Ministerio de Sanidad y Consumo (FIS PS09/00501) for the partial financial support. M.J.M. and A.G. also thank Fundação de Ciência e Tecnologia for the fellowships.

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