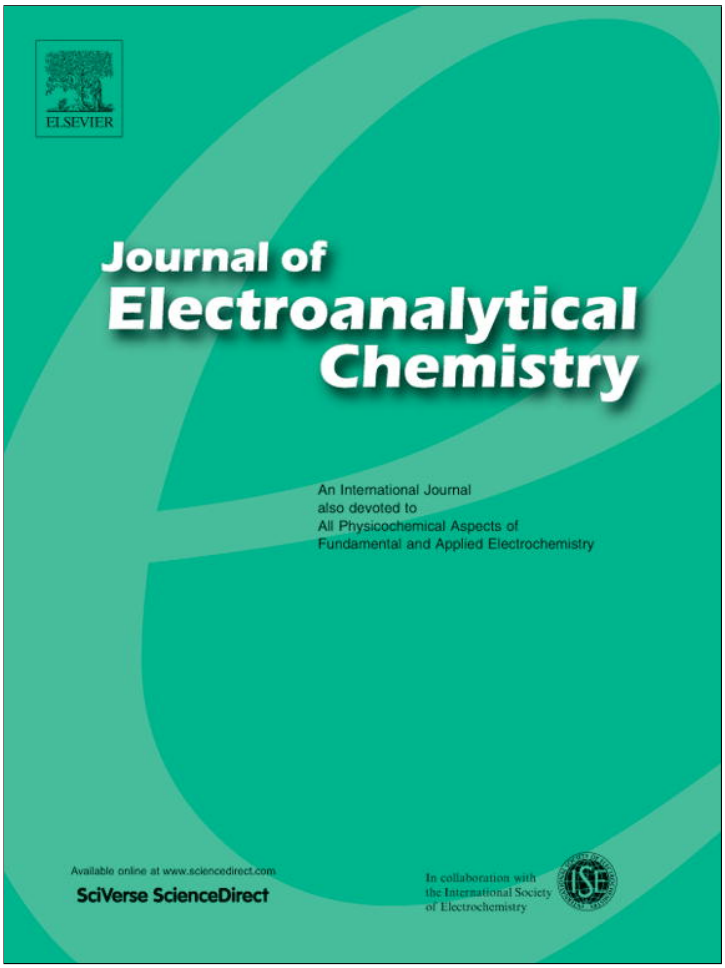


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# New hydroxylated 3-arylcoumarins, synthesis and electrochemical study

Patricia Janeiro [a,b], Maria J. Matos [b], Lourdes Santana [b], Eugenio Uriarte [b], Ana M. Oliveira-Brett [a,*]

[a] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal
[b] Departamento de Química Orgánica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

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ABSTRACT

A selected series of new different substituted 3-arylcoumarins have been designed, synthesized, and evaluated their electrochemical redox mechanisms. The relevant structural information about coumarins was considered in order to better understand the structure/electrochemical relationship. The influence of bromine, methyl or other hydroxyl groups in different positions of the coumarin scaffold, by cyclic, differential pulse and square wave voltammetry at a glassy carbon electrode at different pHs was investigated. A comparative study of differently substituted 8-hydroxy-3-arylcoumarins was performed.



## 1. Introduction

In recent years, phytochemical compounds have achieved 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.

Recent studies have shown that many dietary phenolic compound constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to the protective effects *in vivo*. These compounds are known to be the most common secondary metabolites in the vegetable kingdom [1–3]. Studies demonstrated the antioxidant properties of phenolic compound constituents of plants, and their help in the identification of the active constituents in beverages, vegetables and fruit that may help sustain antioxidant status and protect against free radical damage [1].

Phenolic compounds are a very wide group of compounds important in hormones, vitamins, and food antioxidants. Their mechanism of action as antioxidants seems to involve the ability of phenols to scavenge radicals by an electron transfer process in which phenol is converted into a phenoxyl radical. A simple method was developed for estimating flavonoid antioxidant activity involving the quantitative correlation between lipid peroxidation inhibition and the half-wave potential ($E_{1/2}$) [4].

Most phenolic compounds can be electrochemically oxidized due to the hydroxyl groups attached to the aromatic rings. It is known that pH is one of the most significant factor determining the antioxidant activity of phenolic compounds. The dependence of the phenol derivatives oxidation potential, $E_{1/2}$, on solution pH has been studied thoroughly for different classes of polyphenols using a glassy carbon electrode [5–11].

These parameters gave information not only for evaluating the antioxidant potentialities of polyphenols but also for understanding their reaction mechanisms. Cyclic voltammetry has also been used for the evaluation of the antioxidant capacity of several polyphenols and their mixtures [12].

The activity of phenols towards free radical protection is important due to their scavenging role, related to their ability to react with radicals much more rapidly than with other organic substrates. Interest in the potential health benefits associated with dietary consumption of phenolic compounds has increased significantly in the last decades.

A well know and extensively studied polyphenolic natural phytoalexin is resveratrol [13]. This 3,4′,5-trihydroxystilbene is produced by some spermatophytes species, such as vines, in response to damage. Resveratrol has already been studied for its antioxidant, anti-inflammatory, cardio-protective (vasodilator and platelet anti-aggregator), anticancer and enzymatic inhibitory properties, proving to be very efficient in a large group of *in vitro*, *ex vivo* and/or *in vivo* experiments [14,15].

The fusion of a pyrone with a benzene ring gives rise to a class of heterocyclic compounds known as benzopyrones or coumarins [16]. Coumarins are a wide group of compounds present in remarkable amounts in the nature [17]. Representatives of this group occur in the vegetable kingdom, either in free or combined state [18]. Due to their structural variability, they are an elite class of compounds which occupy an important role in synthetic

* Corresponding author. Tel.: +351 239 835295.
*E-mail address:* brett@ci.uc.pt (A.M. Oliveira-Brett).

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organic chemistry [19]. Coumarins have been attracting considerable interest due to their numerous biological activities, usually associated to low toxicity, depending on their substitution pattern [20]. 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 [21]. In the literature, coumarins are described as anticancer [22,23], antioxidant [24,25], antimicrobial [26], antiviral [27], vasorelaxant [28], anti-inflammatory [29] and enzymatic inhibitors [30–33]. Indeed, some coumarins are now commercially available as medicines.

Natural products have a special ability to interact with more than one target [29]. In medicinal chemistry, this represents a significant source of inspiration for the design and synthesis of properly functionalized analogues with the aim of improving the pharmacological profile. Limited data are available to demonstrate the antioxidant activities of coumarins [34,35].

In this study an efficient methodology of synthesis followed by the investigation of the electrochemical mechanism of oxidation of several coumarin-resveratrol hybrids was undertaken. The electrochemical mechanisms were studied for a wide range of solution conditions, using cyclic, differential and square wave voltammetry. The information on these mechanisms was obtained at different pH and is shown to play a crucial role in understanding its antioxidant activity.

## 2. Experimental

### 2.1. Materials and methods

The coumarin-resveratrol hybrid derivatives **1**–**10** [33,36–38] were efficiently synthesized according to the protocol outlined in Scheme 1. The general conditions and the compounds characterization are described below.

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-phenylcoumarins **1**–**5**. Compounds **6**–**10** were synthesized starting from the respective methoxy/ethoxy derivatives **1**–**5** by hydrolysis reaction, using hydriodic acid 57%.

Melting points were determined using a Reichert Kofler thermopan or in capillary tubes on a Büchi 510 apparatus. IR spectra were recorded on a Perkin-Elmer 1640FT spectrophotometer. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AMX spectrometer at 300 and 75.47 MHz, respectively, using TMS as internal standard (chemical shifts in $\delta$ values, $J$ in Hz).

Mass spectra were obtained using a Hewlett Packard 5988A spectrometer. Elemental analyses were performed using a Perkin-Elmer 240B microanalyser and were within ±0.4% of calculated values in all cases. Silica gel (Merck 60, 230–00 mesh) was used for flash chromatography (FC).

Analytical thin layer chromatography (TLC) was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

All supporting electrolyte solutions were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leqslant$ 0.1 μS cm$^{-1}$), Table 1. Experiments were carried out at room temperature (25 ± 1 °C) and in the presence of dissolved oxygen.

The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C) and microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA).

### 2.2. General procedure for the preparation of 3-phenylcoumarins (1–5)

To a solution of the (7.34 mmol) hydroxybenzaldehyde and (9.18 mmol) phenylacetic acid in (15 mL) dimethyl sulfoxide, (11.46 mmol) *N*,*N*′-dicyclohexylcarbodiimide was added and the mixture was heated in an oil-bath at 110 °C 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 × 25 mL). The organic layer was extracted with sodium bicarbonate solution (50 mL, 5%) and then water (20 mL). The solvent was dried with sodium sulfate and evaporated under vacuum. The residue was purified by FC (hexane/ethyl acetate 9:1).

#### 2.2.1. 8-Ethoxy-3-phenylcoumarin (**1**)

Yield 55%; mp 117–118 °C. $^1$H NMR ($CDCl_3$): 1.50 (t, 3H, –$CH_3$, $J$ = 7.0), 4.21 (dd, 2H, –$CH_2$, $J$ = 14.0, 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, 1.4), 7.79 (s, 1H, H-4). $^{13}$C NMR ($CDCl_3$): 14.8, 65.0, 114.5, 119.3, 120.4, 124.3, 128.3, 128.4, 128.5, 128.8, 134.8, 140.1, 143.4, 146.3, 160.2. MS *m*/*z* (%): 267 ([M+1]$^+$, 16), 266 (M$^+$, 83), 239 (16), 238 (20), 212 (14) 211 (20), 181 (15), 153 (25). Anal. Calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.66; H, 5.28.

#### 2.2.2. 8-Ethoxy-3-(4′-methoxyphenyl)coumarin (**2**)

Yield 61%; mp 99–100 °C. $^1$H NMR ($CDCl_3$): 1.50 (t, 3H, –$CH_3$, $J$ = 7.0), 3.84 (s, 3H, –$OCH_3$), 4.19 (dd, 2H, –$CH_2$, $J$ = 14.0, 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). $^{13}$C NMR ($CDCl_3$): 14.8, 55.4, 64.8, 113.8, 114.0, 119.0, 120.5, 124.2, 127.0, 127.8, 129.7, 129.8, 138.6, 138.7, 146.2, 160.0. MS *m*/*z* (%): 297 ([M+1]$^+$, 35), 296 (M$^+$, 100), 268 (54), 240 (47) 225 (45), 197 (13), 152 (11), 139 (15). Anal. Calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.91; H, 5.39.

#### 2.2.3. 8-Ethoxy-3-(4′-methylphenyl)coumarin (**3**)

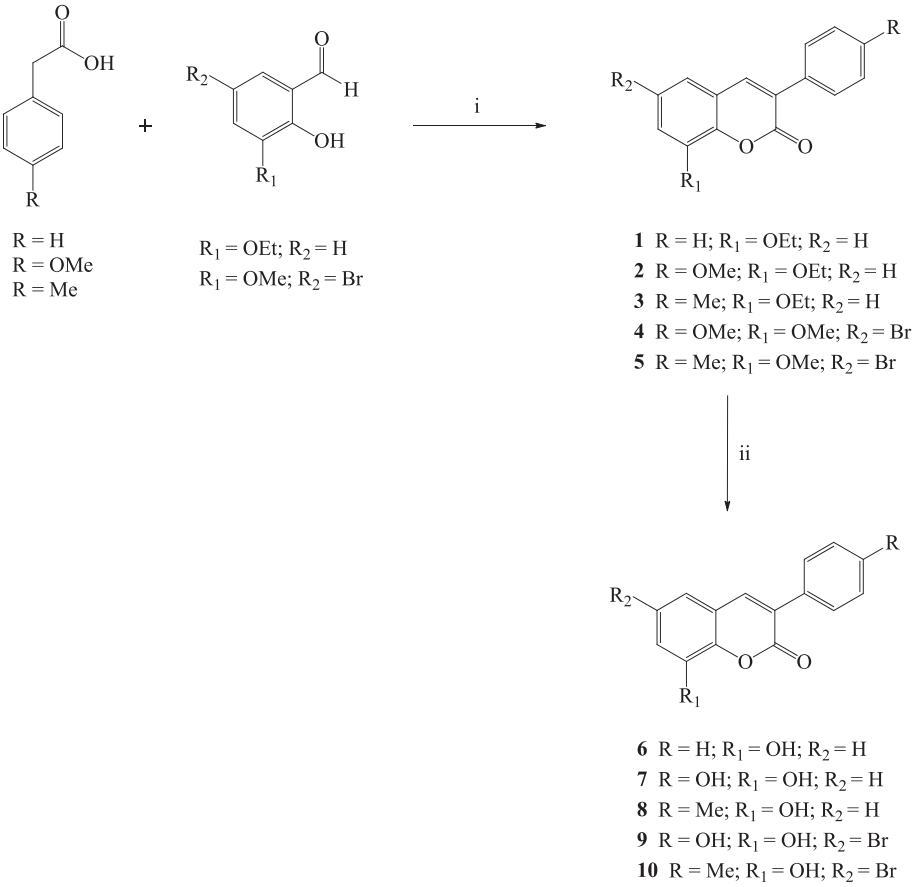
Yield 48%; mp: 109–110 °C. $^1$H NMR ($CDCl_3$): 1.51 (td, 3H, –$CH_3$, $J$ = 7.0, $J$ = 1.8), 2.39 (s, 3H, –$CH_3$), 4.20 (dd, 2H, –$CH_2$, $J$ = 7.0, $J$ = 1.8), 7.06–7.10 (m, 2H, H-5, H-6), 7.18 (dd, 1H, H-7, $J$ = 8.0, 1.9), 7.26 (d, 2H, H-3′, H-5′, $J$ = 1.6), 7.61 (dd, 2H, H-2′, H-6′, $J$ = 8.1, 1.8), 7.74 (d, 1H, H-4, $J$ = 1.9). $^{13}$C NMR ($CDCl_3$): 14.81, 21.30, 64.96, 114.37, 119.24, 120.51, 124.26, 128.34, 128.39, 129.15, 131.88, 138.83, 139.39, 143.31, 146.32, 160.26. MS *m*/*z* (%): 281 ([M+1]$^+$, 16), 280 (M$^+$, 80), 252 (78), 225 (16), 224 (56), 223 (20), 165 (30), 142 (45), 115 (17). Anal. Calcd for $C_{18}H_{16}O_3$: C, 77.12; H, 5.75. Found: C, 77.07; H, 5.80.

#### 2.2.4. 6-Bromo-8-methoxy-3-(4′-methoxyphenyl)coumarin (**4**)

Yield 53%; mp 144–145 °C. $^1$H NMR ($CDCl_3$): 3.85 (s, 3H, –$OCH_3$), 3.96 (s, 3H, –$OCH_3$), 6.93–6.96 (m, 2H, H-3′, H-5′), 7.12 (d, 1H, H-7, $J$ = 1.8), 7.23 (d, 1H, H-5, $J$ = 2.0), 7.63–7.67 (m, 2H, H-2′, H-6′), 7.69 (s, 1H, H-4). $^{13}$C NMR ($CDCl_3$): 55.7, 56.8, 114.2, 116.2, 116.8, 121.5, 126.8, 129.4, 130.2, 130.8, 137.3, 142.2, 147.8, 159.8, 160.6. MS *m*/*z* (%): 363 ([M+1]$^+$, 19), 362 (M$^+$, 100), 361 (19), 360 (59), 334 (24), 332 (23), 319 (33), 317 (34), 291 (11), 289 (11), 182 (18), 167 (17), 139 (21). Anal. Calcd for $C_{17}H_{13}BrO_4$: C, 56.53; H, 3.63. Found: C, 56.55; H, 3.68.

#### 2.2.5. 6-Bromo-8-methoxy-3-(4′-methylphenyl)coumarin (**5**)

Yield 56%; mp 164–165 °C. $^1$H NMR ($CDCl_3$): 2.40 (s, 3H, –$CH_3$), 3.98 (s, 3H, –$OCH_3$), 7.15 (s, 1H, H-7), 7.22–7.28 (m, 3H, H-3′, H-5′, H-5), 7.61 (d, 2H, H-2′, H-6′, $J$ = 8.2), 7.67 (s, 1H, H-4). $^{13}$C NMR ($CDCl_3$): 21.3, 56.5, 116.0, 116.1, 116.6, 121.3, 128.4, 129.2, 129.6, 131.3, 137.6, 137.8, 139.3, 147.6, 159.4. MS *m*/*z* (%): 347 (25), 346 (98), 345 ([M+1]$^+$, 26), 344 (M$^+$, 100), 318 (47), 316 (46), 275




**Scheme 1.** Synthesis experimental conditions: (i) DCC, DMSO, 110 °C, 24 h; (ii) HI, AcOH, $Ac_2O$, reflux, 3 h.

**Table 1**
Supporting electrolytes.

| pH | Composition |
|---|---|
| 4.5 | HAcO + NaAcO |
| 7.0 | $NaH_2PO_4 + Na_2HPO_4$ |
| 8.5 | $NaH_2PO_4 + Na_2HPO_4$ |

(18), 273 (18), 208 (11), 166 (52), 165 (43), 115 (15), 83 (14). Anal. Calcd for $C_{17}H_{13}BrO_3$: C, 59.15; H, 3.80. Found: C, 59.11; H, 3.87.

### 2.3. General procedure for the preparation of hydroxy-3-phenylcoumarins (6–10)

To a solution of (0.50 mmol) substituted methoxy/ethoxy-3-phenylcoumarin in (5 mL) acetic acid and (5 mL) acetic anhydride at 0 °C, (10 mL) hydriodic acid 57% was added dropwise. The mixture was stirred, under reflux, for 3 h. The solvent was evaporated under vacuum and the dry residue was purified by $CH_3CN$ crystallization.

#### 2.3.1. 8-Hydroxy-3-phenylcoumarin (**6**)

Yield 64%; mp 199–200 °C [33].

#### 2.3.2. 8-Hydroxy-3-(4′-hydroxyphenyl)coumarin (**7**)

Yield 41%; mp 237–238 °C [33].

#### 2.3.3. 8-Hydroxy-3-(4′-methylphenyl)coumarin (**8**)

Yield 56%; mp 199–200 °C. $^1$H NMR ($CDCl_3$): 2.33 (s, 3H, $-CH_3$), 7.06 (dd, 2H, H-5, H-6, $J$ = 6.8, 2.9), 7.10–7.21 (m, 3H, H-3′, H-5′, H-7), 7.25 (d, 2H, H-2′, H-6′, $J$ = 8.0), 8.15 (s, 1H, H-4), 10.22 (s, 1H, –OH). $^{13}$C NMR ($CDCl_3$): 20.8, 117.8, 118.5, 120.4, 124.5, 126.6, 128.3, 128.8, 131.8, 138.0, 140.3, 141.5, 144.2, 159.7. MS $m/z$ (%): 253 ($[M+1]^+$, 38), 252 ($M^+$, 100), 225 (26), 223 (23), 165 (15), 153 (12), 152 (25), 115 (11). Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.08; H, 4.79. Found: C, 76.02; H, 4.83.

#### 2.3.4. 6-Bromo-8-hydroxy-3-(4′-hydroxyphenyl)coumarin (**9**)

Yield 56%; mp 249–259 °C. $^1$H NMR ($CDCl_3$): 6.83 (d, 2H, H-3′, H-5′, $J$ = 8.8), 7.14 (d, 1H, H-7, $J$ = 1.9), 7.36 (d, 1H, H-5, $J$ = 2.0), 7.56 (d, 2H, H-2′, H-6′, $J$ = 8.5), 8.00 (s, 1H, H-4). $^{13}$C NMR ($CDCl_3$): 115.6, 116.0, 119.9, 120.6, 122.5, 125.4, 128.2, 130.4, 138.0, 141.2, 146.0, 158.6, 159.8; MS $m/z$ (%): 335 (35), 334 (99), 333 ($[M+1]^+$, 45), 332 ($M^+$, 100), 307 (31), 305 (35), 225 (26), 197 (29), 169 (35), 168 (46), 153 (24), 141 (21), 140 (16), 139 (51), 118 (17), 115 (28), 84 (18), 84 (46). Anal. Calcd for $C_{15}H_9BrO_4$: C, 54.08; H, 2.72. Found: C, 54.05; H, 2.69.

#### 2.3.5. 6-Bromo-8-hydroxy-3-(4′-methylphenyl)coumarin (**10**)

Yield 43%; mp 192–193 °C. $^1$H NMR ($CDCl_3$): 2.35 (s, 3H, $-CH_3$), 7.19 (d, 1H, H-7, $J$ = 2.3), 7.27 (d, 2H, H-3′, H-5′, $J$ = 8.0), 7.40 (d, 1H, H-5, $J$ = 2.2), 7.61 (d, 2H, H-2′, H-6′, $J$ = 8.1), 8.10 (s, 1H, H-4), 10.80 (s, 1H, –OH). $^{13}$C NMR ($CDCl_3$): 20.9, 115.52, 119.8, 120.3, 121.8, 127.8, 128.4, 128.8, 131.5, 138.4, 139.0, 141.0, 145.6, 159.2. MS $m/z$ (%): 333 (17), 332 (98), 331 ($[M+1]^+$, 18), 330 ($M^+$, 100), 305 (13), 304 (77), 303 (22), 302 (79), 223 (12), 166 (14), 165 (30),

152 (30), 115 (14). Anal. Calcd for $C_{16}H_{11}BrO_3$: C, 58.03; H, 3.35. Found: C, 58.06; H, 3.37.

### 2.4. Voltammetric measurements

Voltammetric experiments were carried out using an Autolab PGstat 10 running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a three-electrode system in a 0.5 mL one-compartment electrochemical cell (Cypress System Inc., USA). Glassy carbon electrode (GCE, $d$ = 1.5 mm) was the working electrode, Pt wire the counter electrode and the Ag/AgCl (3 M KCl) reference electrode. The experimental conditions for cyclic voltammetry were scan rate 50 mV $s^{-1}$. For differential pulse (DP) voltammetry were: pulse amplitude 50 mV, pulse width 70 ms and scan rate 5 mV $s^{-1}$. For square wave (SW) voltammetry were: pulse of 50 mV, frequency of 10 Hz and a potential increment of 2 mV, corresponding to an effective scan rate of 20 mV $s^{-1}$.

The GCE was polished using diamond particles of 3 μm (Kemet, UK) before each electrochemical experiment. After polishing, it was rinsed thoroughly with Milli-Q water. Following this mechanical treatment, the GCE was placed in buffer supporting electrolyte and voltammograms were recorded until a steady state baseline voltammograms were obtained. This procedure ensured very reproducible experimental results.

## 3. Results

Relevant aspects concerning the electrochemistry of the hydroxylated 3-arylcoumarins were investigated using cyclic voltammetry (CV), differential pulse (DP) voltammetry and square wave (SW) voltammetry.

The effect on the oxidation potential caused by the presence of a halogen on the coumarin structure was investigated. Halogens, substituents that are more electronegative than carbon, will inductively pull the electron density out of the ring. Due to the lone electron pairs this compounds are able to donate electronic density stabilizing the reaction intermediates by resonance in the *ortho* and *para* positions.

The effect of an alkyl group, in this specific case a methyl group, in the oxidation potential of a compound was also studied. The alkyl groups are electron donating, activating the ring enabling faster reaction rates.

### 3.1. Cyclic voltammetry

The compound **6** was investigated by CV in pH 7.0, Fig. 1, showing on the first scan oxidation peak $P_1$, at $E_{p1}$ = +0.65 V, corresponding with an irreversible reaction. The value of $|E_p - E_{p/2}|$ = +50 mV indicates that one electron is involved in the first oxidation process [39]. A reduction peak $P_{2c}$, on the reverse scan, appears at $E_{p2c}$ = +0.05 V corresponding to the reduction of the oxidation products formed during the oxidation at peak $P_1$.

In the second scan at lower potentials a new oxidation peak $P_{2a}$, at $E_{p2a}$ = +0.08 V, confirmed the reversibility of peak $P_2$, that occurs with the transfer of two electrons [40]. The same behavior was found for compounds **8** and **10**, both with only one oxidizable hydroxyl group on their structure.

CVs of compound **8** showed an identical behavior to compound **6**. That means that the presence of a weak electron donating substituent, in a different ring where the hydroxyl group is, does not influence the oxidation potential. The strong adsorption of the oxidation products, which blocked the electrode surface, was also observed for compounds **6**, **8** and **10**, and oxidation peak $P_1$ current always decreased in the second scan.

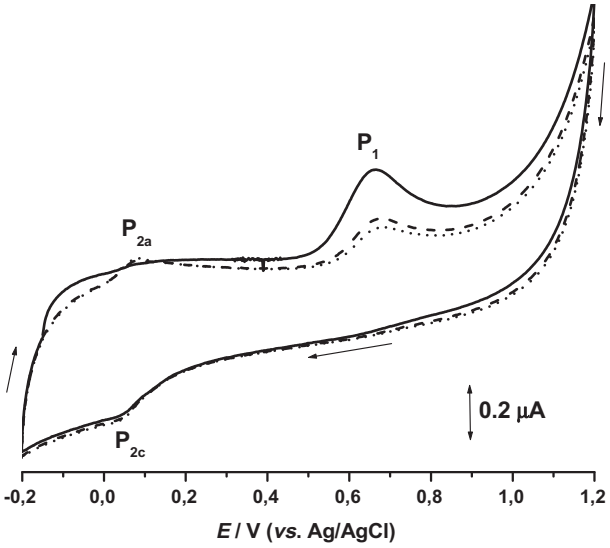



**Fig. 1.** CVs in 0.5 mM compound **6**, in 0.2 M phosphate buffer pH = 7.0: (—) first, (- - -) second and (•••) third scans. Scan rate 50 mV $s^{-1}$.

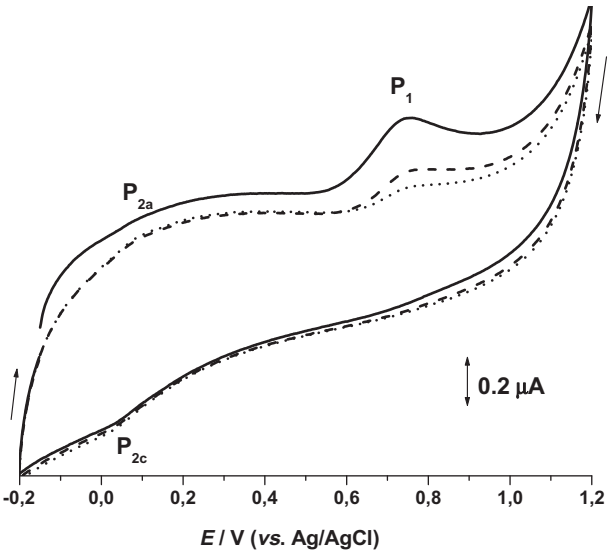



**Fig. 2.** CVs in 0.5 mM compound **10**, in 0.2 M phosphate buffer pH = 7.0: (—) first, (- - -) second and (•••) third scans. Scan rate 50 mV $s^{-1}$.

In the case of compound **10**, Fig. 2, the oxidation peak $P_1$ appears at a slightly higher potential, at $E_{p1}$ = +0.71 V. This can be due to the presence of bromine, an electron withdrawing substituent which causes an acidity increment on the phenol moiety. The oxidation process corresponding to the peak $P_1$ is irreversible for compounds **6**, **8** and **10** and occurs with the transfer of one electron. It has been proved in this work that the oxidation of the hydroxyl group at the position C8 occurs irreversibly and it is formed an oxidation product which is oxidized at a lower potential in a reversible process.

The compounds **6**, **8** and **10** oxidation products are electroactive and reversibly oxidized at peak $P_{2a}$–$P_{2c}$ with the transfer of two electrons. The compound **10** peak $P_1$ oxidation product, occurs at reversible peak $P_{2a}$–$P_{2c}$, $E_{p2a}$ = +0.08 V and $E_{p2c}$ = +0.05 V, and presented a very similar behavior to compound **6**, Fig. 1, showing that bromine does not interfere on the redox reaction of compound **10** oxidation product, with the oxidisable hydroxyl group and the

halogen in the *meta* position in the same ring. It can be remarked that compound **10** peak $P_2$ current was lower than compound **6** peak $P_2$ current, which could indicate that the different substituents do not affect the peak $P_1$ current, but affect the peak $P_2$ current.

The presence of electron withdrawing substituents, that deactivate the ring, causes an increase in acidity of the phenol. This effect involves charge, when the substituents are situated in the *ortho*- and *para*-positions, in respect to the hydroxyl group, due to conjugation of these positions with the group. This explains that the oxidation potential of compound **10** peak $P_1$ occurs at a slightly higher oxidation potential than the compound **6** peak $P_1$ that presents a not so acidic hydroxyl group. The CV study was done in the pH range between 4.5 and 8.5. The oxidation process is complex and pH-dependent, indicating that the oxidation of all compounds occurs associated with a proton transfer.

### 3.2. Differential pulse voltammetry

To clarify the mechanism of oxidation of this series of compounds DP voltammetry was used. Successive DP voltammograms in 0.5 mM compound **8**, in pH 4.5, acetate buffer showed the occurrence of one oxidation peak $P_I$, at $E_{p1}$ = +0.80 V, and after the second scan peak $P_2$, at $E_{p2}$ = +0.20 V, which corresponds to the oxidation of the peak $P_I$ oxidation product, Fig. 3. The peak $P_I$ width at half height was $W_{1/2}$ = 100 mV indicating an oxidation process with one electron involved [39].

The peak $P_1$ current decreased and peak $P_2$ current increased with increasing number of scans. This confirms that a strong adsorption of the oxidation product of compound **8** on the electrode blocks the electrode surface.

The same DP voltammetric behavior found for compound **8** was observed for compounds **6** and **10**, Fig. 4, but the compound **10** peak $P_1$ oxidation was for a higher potential than at compounds **6** and **8**, while compound **10** peak $P_2$ had the same peak potential as in compounds **6** and **8**, but compound **10** peak $P_2$ current was lower.

The DP voltammogram in compound **7**, that has two hydroxyl groups, presented two oxidation peaks due to two oxidizable hydroxyl groups, Fig. 5. In the first DP voltammogram the oxidation peak $P_I$ width at half height was $W_{1/2} \sim 200$ mV corresponding to two oxidation peaks overlapped, that was confirmed after the baseline was subtracted in the DP voltammogram. The peaks overlap is due to the two similar hydroxyl groups in the compound **7**, the hydroxyl groups in positions C4′ and C8 of the 3-arylcoumarin, and the oxidation occurs at similar potentials, as they are both phenol groups.

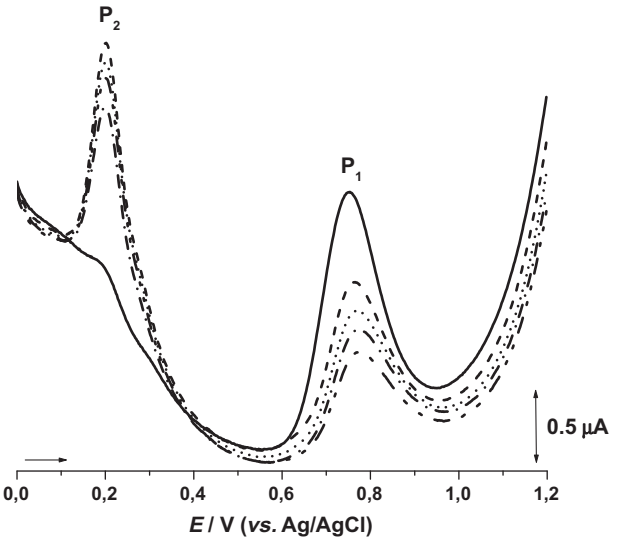



**Fig. 3.** Successive DP voltammograms (1 → 5) in 0.5 mM compound **8**, in 0.2 M acetate buffer pH = 4.5. Scan rate 5 mV $s^{-1}$.

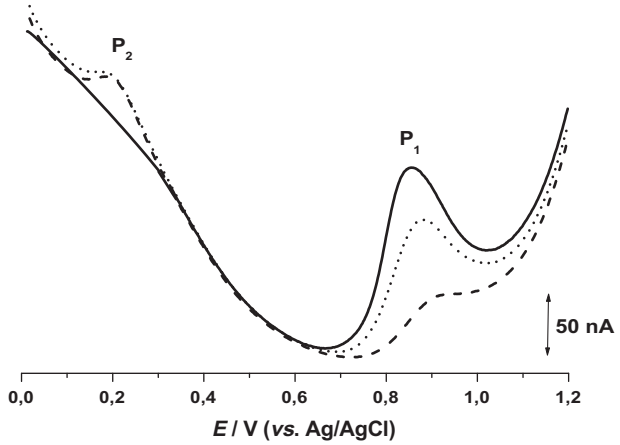



**Fig. 4.** DP voltammograms in 0.5 mM compound **10**, in 0.2 M acetate buffer pH = 4.5: (—) first, (•••) second and (- - -) third scans. Scan rate 5 mV $s^{-1}$.

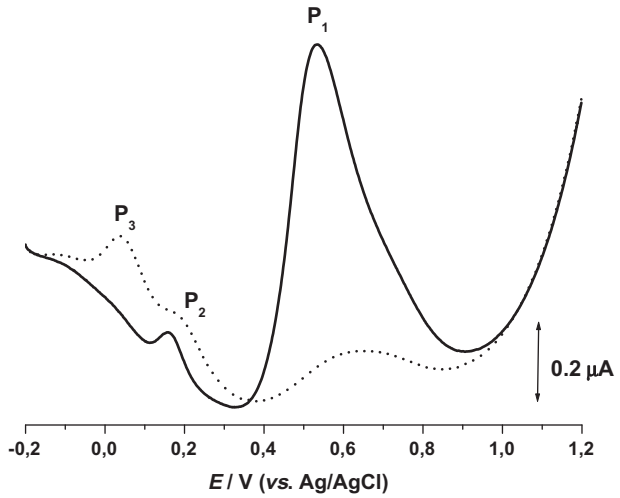



**Fig. 5.** DP voltammograms in 0.5 mM compound **7**, in 0.2 M phosphate buffer pH = 7.0: (—) first and (•••) second scans. Scan rate 5 mV $s^{-1}$.

The first scan DP voltammogram in compound **7** showed also another oxidation peak, peak $P_2$, at $E_{p2}$ = +0.17 V, at a lower potential, corresponding to the oxidation of a catechol group. Although peak $P_2$ appears already on the first scan, peak $P_2$ is due to the oxidation product of peak $P_1$. This denotes that compound **7** was oxidized during its synthesis before the electrochemical experiences. The peak $P_2$ width at half height was $W_{1/2} \sim 60$ mV indicating that two electron are involved in the oxidation process, confirming that is a catechol moiety oxidation product of peak $P_1$ [39,40].

On the second scan, the oxidation product formed after $P_1$ oxidation, peak $P_3$, at $E_{p3}$ = +0.05 V, occurs, as observed by CV, and is due to the oxidation of the hydroxyl group at position C8 in the $P_1$ oxidation product. This is confirmed by the appearance of peak $P_3$ only in the second scan and also at lower oxidation potential. The peak $P_3$ width at half height was $W_{1/2} \sim 60$ mV indicates that two electrons are involved on the oxidation of the hydroxyl group at position C8 oxidation product of compound **7**. The strong adsorption of the oxidation products, which blocked the electrode

surface, enabled to observe more easily on the second scan the overlap that occurs in $P_1$ corresponding to a transfer of one electron in each peak.

The DP voltammetric study was undertaken for compounds **6**, **8** and **10**, for a wide pH range and the potentials of peaks $P_1$, $P_2$ and $P_3$ were shifted to more negative values with increasing pH. The dependence of $E_p$ vs. pH was linear the whole pH range studied and followed the relationships presented in Table 1. The $E_p$ vs. pH, relationship enables the determination of the number of protons involved in the oxidation process. In all cases the oxidation potential was pH-dependent following a linear relationship with the slope of −0.059 V, which corresponds to a electron transfer reaction involving the same number of electrons and protons.

From Table 2, the transfer of one electron and one proton occurs for peak $P_1$, attributed to the hydroxyl group correspondent to a phenol group, whereas, oxidation product peaks $P_2$ and $P_3$, both occur with the transfer of two electrons and two protons.

Compound **9** shows a DP voltammogram very similar to that of compound **7**, Fig. 6. The oxidation peak $P_1$ observed is broad, indicating the presence of two hydroxyl groups that oxidize at similar potential. It also appears in the first scan a peak $P_2$ at lower potential. The peak $P_3$, a product of oxidation of $P_1$, appears in the second scan. A great decrease in peak $P_1$ current on the second scan indicates a strong adsorption of oxidation products formed on the electrode surface. The halogen group attached to the aromatic ring, with the hydroxyl group, has an electron withdrawing effect that makes the ring poorer in electron density. This causes a deactivating effect and a decreasing in the reactivity of the ring.

### 3.3. Square wave voltammetry

The advantages of SW voltammetry are greater speed of analysis, lower consumption of electro-active species in relation to DP voltammetry, and reduced problems with blocking of the electrode surface. A great advantage of the square-wave method is the possibility to see during one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both the positive and the negative-going pulses, peaks corresponding to the oxidation or reduction of the electroactive species at the electrode surface are obtained in the same experiment.

The SW voltammetry conditions chosen led to well-defined SW voltammograms, and showed similar results to CV and DP voltammetry, i.e. the same oxidation peaks and oxidation products peaks, a strong adsorption on the second scan and the decrease of the oxidation potential with increasing pH.

Therefore, the irreversibility of $P_1$ and the reversibility of their oxidation product $P_2$, in compounds **6**, **8** and **10**, was confirmed by SW voltammetry, Fig. 7. Compounds **7** and **9** with two hydroxyl groups in their structure, presented the irreversible peak $P_1$ with overlapping, at $E_{p1}$ = +0.85 V, and two reversible peaks, peak $P_2$, at $E_{p2}$ = +0.39 V, due to oxidation during synthesis, and peak $P_3$, at $E_{p3}$ = +0.21 V, Fig. 8, confirmed in the second scan after reversing the potential scan just before peak $P_1$ and obtaining $P_3$.

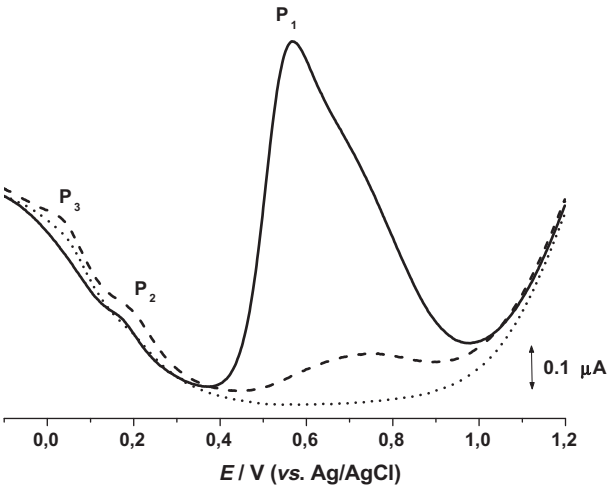



**Fig. 6.** DP voltammograms in 0.5 mM compound **9**, in 0.1 M phosphate buffer pH = 7.0: (—) first, (- - -) second and (•••) third scans. Scan rate 5 mV $s^{-1}$.

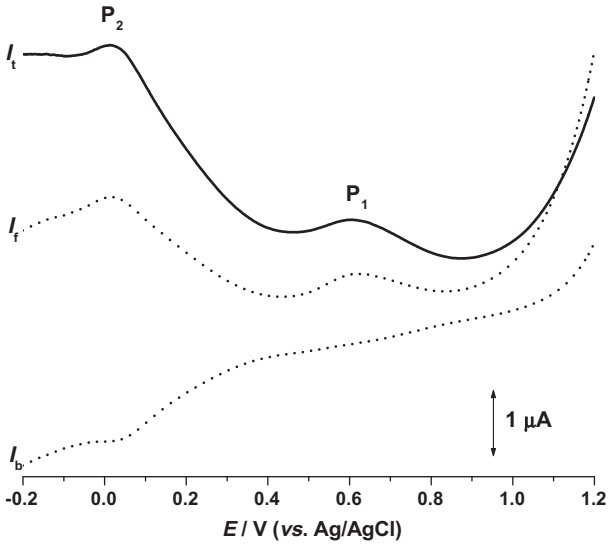



**Fig. 7.** Second SW voltammogram in 0.5 mM of compound **6**, in pH = 8.5, third scan. $I_t$ – total current, $I_f$ – direct current and $I_b$ – forward current; $f$ = 25 Hz, $\Delta E$ = 2 mV, $v_{eff}$ = 50 mV $s^{-1}$.

## 4. Discussion

All described 3-arylcoumarins (compounds **1–10**) were efficiently synthesized, characterized and evaluated for their antioxidant functionality. Relevant aspects concerning the electrochemistry (compounds **6–10**) of this selected series of synthesised coumarin-resveratrol hybrids were obtained. It was found that the different substituents have only a slightly effect upon

**Table 2**
DP voltammetric data for compounds **6–10**.

| | Peak $P_1$ | | | Peak $P_2$ | | | Peak $P_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $E_p$ vs. pH | $e^-$ | $H^+$ | $E_p$ vs. pH | $e^-$ | $H^+$ | $E_p$ vs. pH | $e^-$ | $H^+$ |
| 6 | $E_p$ = 0.98–0.057 pH pH | 1 | 1 | $E_p$ = 0.47–0.056 pH pH | 2 | 2 | – | – | – |
| 7 | $E_p$ = 0.96–0.061 pH | 1 | 1 | $E_p$ = 0.61–0.059 pH | 2 | 2 | $E_p$ = 0.48–0.059 pH | 2 | 2 |
| 8 | $E_p$ = 1.02–0.059 pH | 1 | 1 | $E_p$ = 0.46–0.060 pH | 2 | 2 | – | – | – |
| 9 | $E_p$ = 1.03–0.060 pH | 1 | 1 | $E_p$ = 0.62–0.058 pH | 2 | 2 | $E_p$ = 0.46–0.059 pH | 2 | 2 |
| 10 | $E_p$ = 1.08–0.060 pH | 1 | 1 | $E_p$ = 0.43–0.057 pH | 2 | 2 | – | – | – |

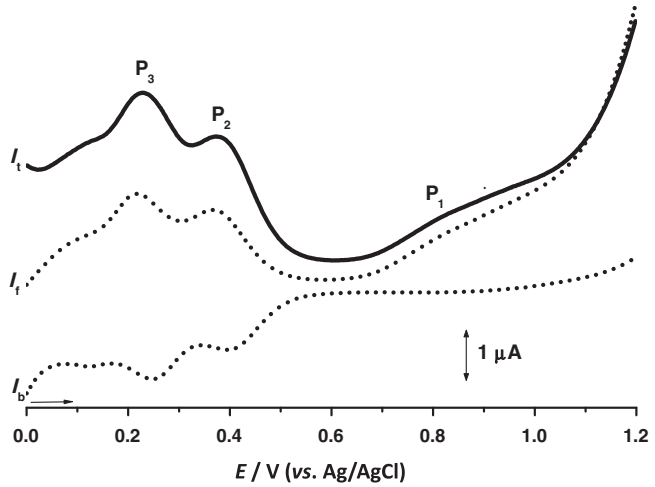



**Fig. 8.** Second SW voltammogram in 0.5 mM compound **9**, in 0.1 M acetate pH = 4.5 buffer, fourth scan. $I_t$ – total current, $I_f$ – forward current and $I_b$ – backward current; $f$ = 25 Hz, $\Delta E$ = 2 mV, $v_{eff}$ = 50 mV $s^{-1}$.

reactivity of the selected coumarins. Also differences in the electroactive substituents on similar structures lead to characteristic differences in their voltammetric behavior.

Considering that the electrochemical behavior of these compounds depends on their structural characteristics, useful information on their antioxidant functionality can be drawn from the CV, DP and SW voltammograms for each hydroxylized compound performed at different pH values.

All synthetized compounds possess in common a hydroxyl group on position C8 and differ by the presence or absence of bromine, an electron withdrawing atom, in the benzene ring and the presence or absence of a hydroxyl strong electron donating group or a methyl weak electron donating group in the benzene ring linked to the position C3 of the coumarin structure. Electrochemical studies of this series of compounds allowed to investigate the influence on the oxidation potential of hydroxycoumarins of different substituents.

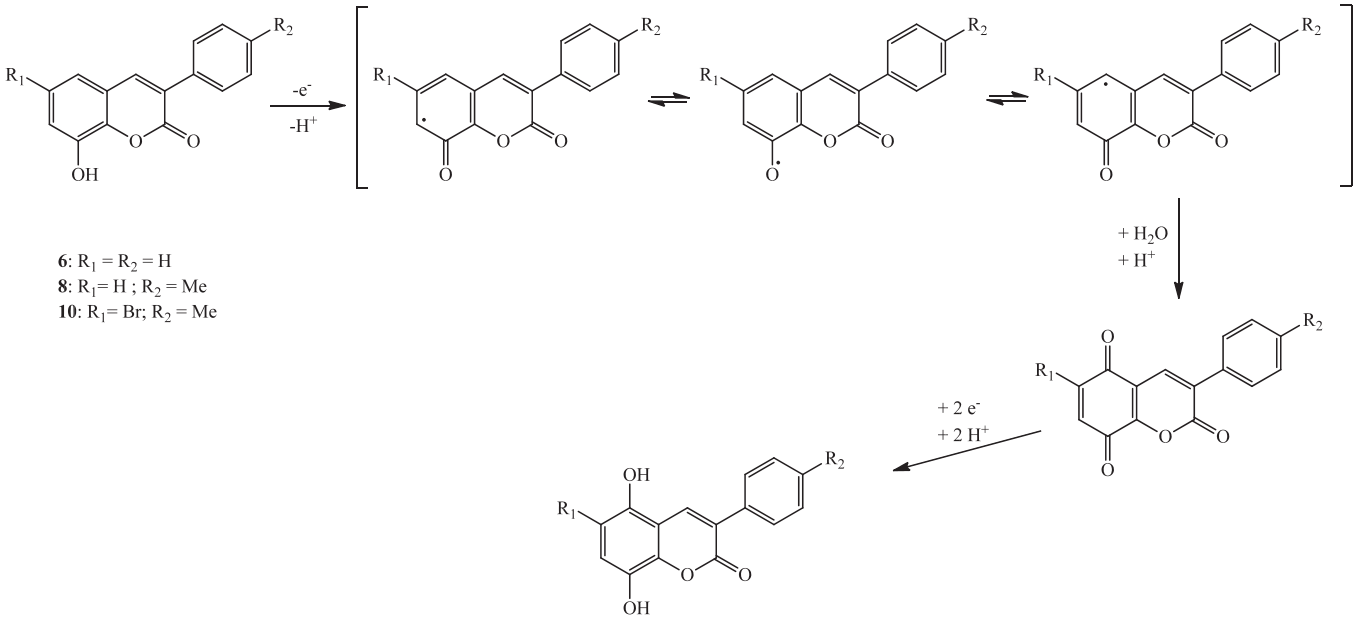
The results indicated that compounds **6**, **8** and **10** oxidation occurs in one step whereas compounds **7** and **9** oxidation occurs in two consecutive steps in for 4.5 < pH < 8.5. The irreversibility of all these steps was established by CV and SW voltammetry. The involvement of one electron and one proton in the oxidation steps was evaluated from the peak width at half height of the DP voltammograms and the slope of $E_p$ vs. pH plot for each compound is in Table 1. The oxidation products oxidation peaks of each compound involved always two electrons and two protons.

Compounds **9** and **10** with bromine at position C6 on the coumarin moiety have the highest oxidation potentials on the series studied. This is due to the presence of an electron withdrawing substituent which causes an acidity increment on the phenol. Therefore, the reactivity of these compounds is different from the others. An oxidation mechanism was proposed for compounds **6**, **8** and **10**, Scheme 2.

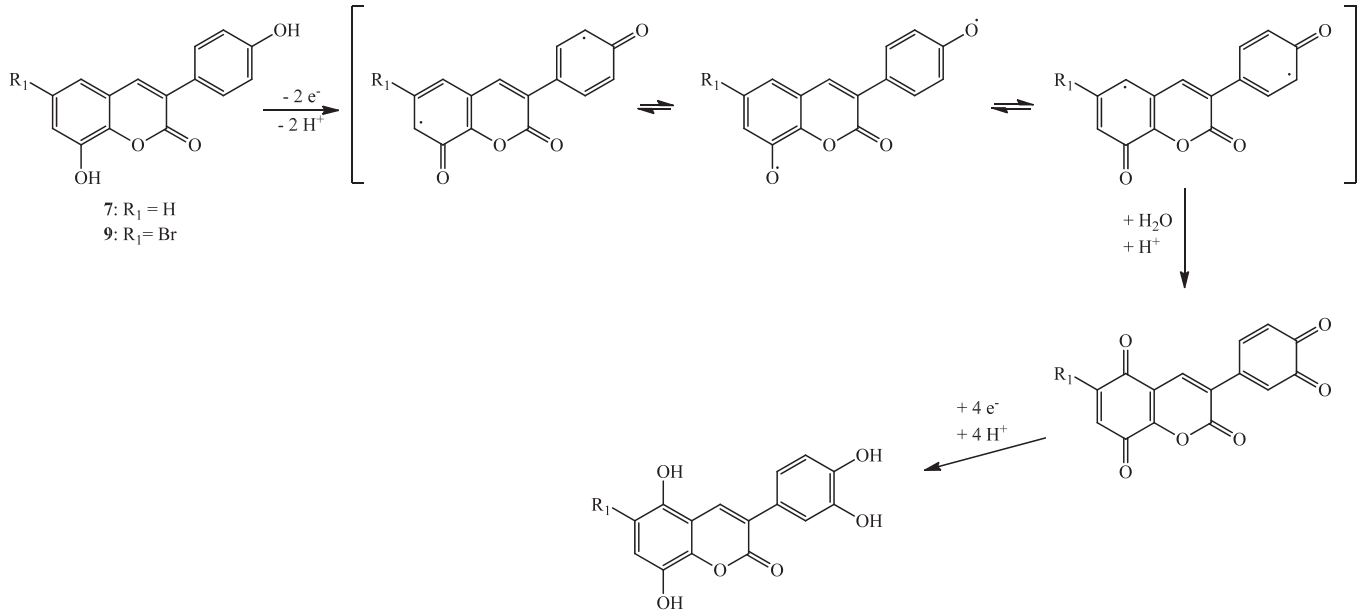
Cyclic voltammograms of compound **8** showed an identical behavior to compound **6**. That means that the presence of a methyl group weak electron donating substituent, in a different ring from the hydroxyl group, does not influence the reactivity of the compounds and the oxidation potential.

Compounds **7** and **9** besides the hydroxyl group on the position C8 have another hydroxyl group in position C4′, and this two oxidations potentials are very near and their peaks overlap. The oxidation mechanism occurs in two consecutive irreversible reactions with the transfer of an electron and a proton for the overlapped peaks in $P_1$.

The oxidation products formed after peak $P_1$, identified as peak $P_3$, corresponds to the oxidation of the hydroxyl group on position C8, present in compounds **6**, **8** and **10**. The oxidation products formed after peak $P_1$, identified as peak $P_2$, had a reversible oxidation process with transfer of two protons and two electrons with oxidation potential values with similar characteristics to a catechol group, corresponding to the oxidation of the hydroxyl group on position C8, present in compounds **7** and **9**. An oxidation mechanism was proposed for compounds **7** and **9**, Scheme 3.




**Scheme 2.** Proposed oxidation mechanism for compounds **6**, **8** and **10**.




**Scheme 3.** Proposed oxidation mechanism for compounds **7** and **9**.

## 5. Conclusions

The synthesis of ten new 3-arylcoumarins was carried out in an efficient, direct and versatile way using a Perkin reaction as key step. The ether derivatives had been hydroxylated, with good yields, giving the corresponding hydroxyl derivatives that were studied. All the new 3-arylcoumarins were electrochemically oxidized. The electrochemical data showed that all these novel coumarins can be oxidized at relatively low potentials, and the electrochemical results demonstrated that using electrochemical methods the electron transfer mechanisms of this new series of 3-arylcoumarins can be clarified. In compounds **6**, **8** and **10**, with only one oxidizable hydroxyl group on their molecule, the electrochemical behavior showed that one electron is involved in the oxidation process that occurs in one step. In compounds **7** and **9** the oxidation occurred in two consecutive steps. It was demonstrated than it was easier to oxidize the hydroxyl group at position C8 of the coumarin structure than the hydroxyl group at position C4′. As expected, the radical phenoxyl in C4′ is more stable. The experiments showed an irreversible reaction corresponding to the oxidation of the hydroxyl groups at positions C8 and C4′. This behavior, which is related with their molecular structure, clearly showed their good antioxidant properties.

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