Electrochemistry and Reactivity Against Superoxide Anion Radicals of Hydroxycoumarins and Its Derivatives

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Electrochemistry and Reactivity Against Superoxide Anion Radicals of Hydroxycoumarins and Its Derivatives

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The present studies reveal superoxide radical anion reactivity against synthesized coumarins. Free radicals play an important role in many diseases and they have a protective function also. Therefore, we must keep a balance in their concentration, and this is where exogenous antioxidants such as coumarins become important. There are multiple methodologies to quantify the efficiency of an antioxidant due to vast amounts of mechanism with which a radical can act. Electrochemistry is a useful tool for this purpose. In this work, the cyclic voltammetry-based methodology was used to generate superoxide anion radical through oxygen one-electron reduction in a dimethyl sulfoxide solution. Plotting the remaining percentage of initial current against compound concentration we can establish a Reactivity Index (RI_{50}), for comparative purpose. This index means the value of concentration to reduce in a 50% the initial peak current. The most reactive coumarin against electrogenerated superoxide anion radical was 3-acetyl-7,8-dihydroxycoumarin (7,8-coum).

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Coumarins are heterocyclic compounds that were first isolated from Tonka beans in 1820 by Vogel.[1,2] They possess a great interest from a biological point of view due to their properties, such as anticoagulant,[3,4] antimicrobial,[5–7] estrogenic,[8] vasorelaxant,[9,10] anti-inflammatory,[11,12] antioxidant,[13–15] and analgesic activity.[16,17] Consequently, due to their great pharmacological potential, they have received vast amounts of attention, and their study still continues. There are two main direct routes to prepare coumarins: through Perkin's reaction[18] and through Pechmann's condensation.[19,20]

It's well known that coumarin nuclei can be reduced through several chemical ways, but one of the first articles about coumarin reduction was an electrochemical study performed by polarography at different pH values and was described as a pH-independent single wave.[21] Electroanalytical measurements for coumarin determination concerning essential oils and medicinal herbs with glassy carbon electrodes were presented by Wang. using cyclic voltammetry and differential pulse voltammetry, observing a reduction wave at approx. −1.4 V vs Ag/AgCl at a pH value between 8.07–8.96.[22]

Coumarin nuclei is founded in plants and also there are synthetic compounds and both are able to react against free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) for this reason its antioxidant capacity is studied.[23] Free radicals are highly reactive species; which can react with molecules of their surroundings, modifying them and therefore altering their primary function, converting them into secondary products or other reactive species.[23] In the organism, free radicals are produced by the aerobic metabolism; some of these radicals are the superoxide anion radical, hydroxyl radical, and peroxyl radical.[24] It is known that the increase of free radicals concentration, known as oxidative stress, is related to diverse pathologies such as heart disease or cancer.[25] These free radicals can cause cellular damage, therefore, we need molecules capable of reacting with radicals, i.e., the antioxidants.[26] Antioxidants can be endogenous, i.e., enzymatic mechanism of the organism (dismutase superoxide, catalase glutathione, peroxidase, coenzyme Q) and exogenous, which are incorporated through the diet (vitamin E, C, β-carotene and phenolic compounds).[27] Phenolic coumarins can react against free radicals.[28] Identifying the capacity and reactivity of antioxidants against free radicals is of great interest. There are many methodologies to evaluate the efficiency of antioxidants reaction against free radicals, some of these methodologies are, ORAC,[29] FRAP,[30] CUPRAC,[31] TEAC,[32] and redox-based methods.[33] An electrochemical methodology is selected because it is related to the redox behavior, which is very important since one action mechanism of antioxidants is oxidation. For this reason, studying the oxidation process is relevant and the electrochemical methodology gives us the tools to characterize the behavior of these phenolics coumarins. Besides, the redox behavior of phenolics compounds is very interesting due to its relationship with chemical and biological properties.

Experimental

Synthesis.—All reagents were of analytical grade and were obtained from Sigma Aldrich and used without further purification. Melting points were determined using a Kofler camera Bock monoscope. A 300-MHz spectrometer (Bruker, WM 300). Mass analysis for R-cin-6-coum derivatives was performed in MALDI-TOF Microflex (Bruker Daltonics Inc., MA-USA) and ESI-IT Esquire 4000 (Bruker Daltonik GmbH, Germany).

Synthesis of R-coum derivatives.—All hydroxylated coumarins (R-coum) were obtained by Knoevenagel reaction[34] following the next procedure: addition of an equimolar quantity of corresponding hydroxyl salicylaldehyde (1 mmol) and ethyl acetoacetate (1 mmol) using catalytic amounts of piperidine in ethanol (25 ml) under reflux and monitoring by thin-layer chromatography. Final products were purified through crystallization in ethanol and structure was confirmed by ^{1}HNMR. Figure 1 and Table I.

Synthesis of R-cincoum derivatives.—All hydroxylated cinnamoylcoumarins derivatives (R-cincoum) were obtained by addition of an equimolar quantity of previously synthetized 3-acetylcoumarin (1 mmol) and respective hydroxybenzaldehyde (1 mmol) in ethanol (25 ml) under reflux with catalytic amounts of piperidine. Final products were purified through chromatographic column and structure was confirmed by ^{1}HNMR. Results are shown in Fig. 1 and Table II.

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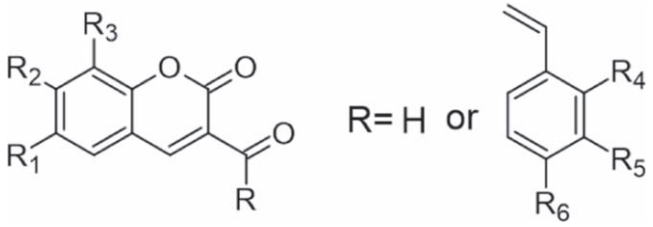

Figure 1. Chemical structure of the synthesized compounds.

Table I. Substituents of R-coum derivatives.

Compound[a)]	R_1	R_2	R_3
3-acetyl-6-hydroxycoumarin **(6-coum)**	–OH	–H	–H
3-acetyl-7-hydroxycoumarin **(7-coum)**	–H	–OH	–H
3-acetyl-8-hydroxycoumarin **(8-coum)**	–H	–H	–OH
3-acetyl-7,8-dihydroxycoumarin **(7,8-coum)**	–H	–OH	–H

a) R-coum abbreviature is used for hydroxycoumarin derivatives.

Table II. Substituents of hydroxylcinnamoylcoumarins derivatives.

Compound[a)]	R_1	R_2	R_3
3-(2-hydroxycinnamoyl)-coumarin **(2-cincoum)**	–OH	–H	–H
3-(3-hydroxycinnamoyl)-coumarin **(3-cincoum)**	–H	–OH	–H
3-(4-hydroxycinnamoyl)-coumarin **(4-cincoum)**	–H	–H	–OH

a) R-cincoum abbreviature is used for hydroxycinnamoylcoumarin derivatives.

Synthesis of R-cin-6-coum derivatives.—All derivatives hydroxycinnamoyl-6-hydroxycoumarin, R-cin-6-coum, were obtained with the same methodology of R-cincoum derivatives but using 3-acety-6-hydroxycoumarin (1 mmol) instead of 3-acetylcoumarin. Final products were purified through chromatographic column. Results are shown in Fig. 1 and Table III.

3-acetyl-6-hydroxycoumarin: **6-coum**. Physical characterization: m.p: 254 °C–256 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (2.57, s, 3 H), (7.16, dd, 1H), (7.23, d, 1H), (7.58, d, 1H), (8.56, s, H-chromene), (9.92, s, OH-Ar) ppm.

3-acetyl-7-hydroxycoumarin: **7-coum**. Physical characterization: m.p: 234 °C–236 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (2.49, s, 3H), (6.41, d, 1H), (6.60, dd, 1H), (7.58, d, 1H), (8.44, s, H-cromeno) ppm.

3-acetyl-8-hydroxycoumarin: **8-coum**. Physical characterization: m.p: Sublimates > 200 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (2.58, s, 3H), (7.21–7.17, m, 2H), (7.33, t, 1H), (8.57, s, H-chromene), (10.41, s, OH-Ar) ppm.

3-acetyl-7,8-dihydroxycoumarin: **7,8-coum**. Physical characterization: m.p: Sublimates > 180 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (2.5, s, 3H), (6.78, d, 1H), (7.30, d, 1H), (8.54, s, 1H), (9.6, s, O–H), (10.67, s, OH–Ar) ppm.

3-(2-hydroxycinnamoyl)-coumarin: **2-cincoum**. Physical characterization: m.p: 208 °C–210 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (m, 10H, 7.05–8.10, H-Ar), (s, 1H, 8.82, H-chromene), (s, 1H, 10.5, OH-Ar) ppm.

3-(3-hydroxycinnamoyl)-coumarin: **3-cincoum**. Physical characterization: m.p: 205 °C–207 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (m, 10 H, 6.85–7.95, H-Ar), (s, 1H, 8.64, H-chromene), (s, 1H, 9,7, OH–Ar), ppm.

3-(4-hydroxycinnamoyl)-coumarin: **4-cincoum**. Physical characterization: m.p: 235 °C–240 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): (m, 10H, 6.85–7.95, H-Ar), (s, 1H, 8.52, H-cromeno, (s, 1H, 10.2, OH-Ar) ppm.

3-(2-hydroxycinnamoyl)-6-hydroxycoumarin: **2-cin-6-coum**. Physical characterization: m.p: Sublimates > 200 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): 8.82, (s, 1 H, H-chromene), 7.05–8.10, (m, 10 H, H- aromatic), 9.91, (s, 1 H, OH-Ar) 10.5, (s, 1 H, OH-Ar) ppm. ^{13}CNMR (DMSO-d_6 δ (ppm)): 114.33, 114.61, 117.59, 118.71, 119.34, 120.02, 122.84, 125.01, 126.12, 129.36, 132.79, 147.25, 148.30, 150.42, 154.44, 157.88, 159.17, and 187.99. MALDI-TOF: $C_{18}H_{12}O_5$ $[M + H]^+$ 309.0744, (309.0757 theoric) and $[M–H]^-$ 307.0615, (307.0612 theoric). ESI-IT $[M–H]^-$ 307, $[M–H_2O–H]^-$ 289, $[M–CO–H]^-$ 279, $[M–CO_2–H]^-$ 263. Yield 11%.

3-(3-hydroxycinnamoyl)-6-hydroxycoumarin: **3-cin-6-coum**. Physical characterization: m.p: 237 °C–240 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): 6.87, (dd, 1 H, H-Ar), 7.13–7.35, (m, 7 H), 7.64, (d, 1 H, H-Ar), 8.59, (s, 1 H, H-chromene), 9.70, (s, 1 H, OH-Ar), 9.92, (s, 1 H, OH-Ar) ppm. ^{13}CNMR (DMSO-d_6 δ (ppm)): 114.35, 11.79, 117.60, 119.38, 120.00, 121.63, 122.84, 124.47, 126.18, 129.37, 132.76, 140.00, 147.24, 148.33, 154.51, 157.90, 159.18 and 188.00. MALDI-TOF: $C_{18}H_{12}O_5$ $[M + H]^+$ 309.0755, (309.0757 theoric)) and $[M-H]^-$ 307.0712, (307.0612 theoric). ESI-IT $[M–H]^-$ 307, $[M–H_2O–H]–$ 289, $[M–CO–H]–$ 279, $[M–CO_2–H]^-$ 263. Yield 15%.

3-(4-hydroxycinnamoyl)−6-hydroxycoumarin: **4-cin-6-coum**. Physical characterization: m.p: 290 °C–292 °C, ^{1}HNMR (DMSO-d_6 δ (ppm)): 6.85, (d, 2 H, H-Ar), 7.16, (dd, 1 H, H-Ar), 7.23, (d, 1 H, H-Ar), 7.34, (d, 1 H, H-Ar), 7.45, (d, 1 H, H-vinylic), 7.62, (d, 2 H, H-Ar), 7.68, (d, 1 H, H-vinylic), 8.53, (s, 1 H, H-chromene), 9.91, (s, 1 H, OH-Ar) and 10.18, (s, 1H, OH-Ar) ppm. ^{13}CNMR (DMSO-d_6 δ (ppm)): 114.40, 114.94, 117.62, 118.66, 119.35, 120.58, 122.95, 125.05, 125.87, 130.60, 136.16, 144.51, 147.49, 148.37, 154.49, 158.20, 159.25 and 187.81. MALDI-TOF: $C_{18}H_{12}O_5$ $[M + H]^+$ 309,0760 (309,0757 theoric), $[M–H]^-$ 307,0556 (307,0612 theoric). ESI-IT $[M–H]^-$ 307; $[M–H_2O–H]^-$ 289; $[M–CO–H]^-$ 279; $[M–CO_2–H]^-$ 263. Yield 17%.

Electrochemical characterization.—Differential pulse voltammetry (DPV) was performed with a CH Instrument 760 C assembly. A glassy carbon electrode (GCE) was used as the working electrode (CHI 104). The surface of the electrode was polished to a mirror finish with alumina powder (0.3 μm and 0.05 μm) and was washed with Milli-Q water before use and after measurement. Ag/AgCl (isolated with a filter medium) CHI 111 was used as a reference electrode and a platinum wire CHI 115 was used as an auxiliary electrode. Dimethylformamide (DMF) containing 0.1 M tetrabutylammonium hexafluorophosphate (Bu_4NPF_6) was used as support electrolyte. Concentration was 5 mM for all the compounds of this study.

Superoxide anion radical generation.—Cyclic voltammetry (CV) was performed with the same conditions of DPV experiments

Table III. Substituents of hydroxycinnamoyl-6-hydroxycoumarin derivatives.

Compound[a)]	R_1	R_2	R_3
3-(2-hydroxycinnamoyl)-6-hydroxycoumarin **(2-cin-6-coum)**	–OH	–H	–H
3-(3-hydroxycinnamoyl)-6-hydroxycoumarin **(3-cin-6-coum)**	–H	–OH	–H
3-(4-hydroxycinnamoyl)-6-hydroxycoumarin **(4-cin-6-coum)**	–H	–H	–OH

a) R-cin-6-coum abbreviature is used for hydroxycinnamoyl-6-hydroxycoumarin derivatives.

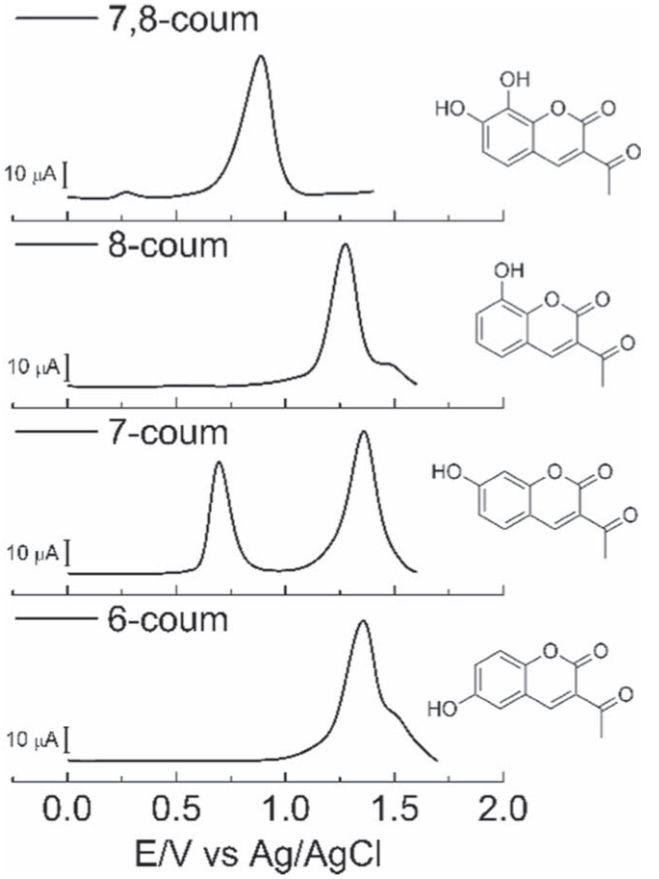

Figure 2. DPV of R-coum derivatives in DMF.

Table IV. DPV oxidation potentials for R-coum derivatives.

Compound	E_1/V	E_2/V
6-coum	1.09	—
7-coum	1.36	0.69[a)]
8-coum	1.27	—
7,8-coum	0.89	0.17[a)]
2-cincoum	1.25	1.55
3-cincoum	1.32	—
4-cincoum	1.14	1.44
2-cin-6-coum	1.16	—
3-cin-6-coum	1.38	—
4-cin-6-coum	1.03	—

a) corresponding to phenolate form oxidation.

with slight modification, dry dimethyl sulfoxide (DMSO) was used as a solvent in a thermoregulated 25 ml cell. Voltammograms were recorded at 0.1 V s^{-1} scan rate and potential work between −0.2 to −1 V. Working electrode was polished with alumina powder 0.3 μm and 0.05 μm. To ensure a constant oxygen concentration the cell is saturated with a flow of oxygen and when a $O_2^{\cdot-}$ constant value oxidation peak is reached the addition of coumarin start.

Results and Discussion

Electrochemistry (differential pulse voltammetry, DPV).— *R-coum.*—All the studied compounds are oxidizable due to the presence of phenolic hydroxyl and results are shown in Fig. 2 and Table IV. The lower oxidation potential for R-coum series is 7,8-coum which possess two hydroxyl group in its structure and the highest oxidation potential was seen in 7-coum derivative. 7,8-coum

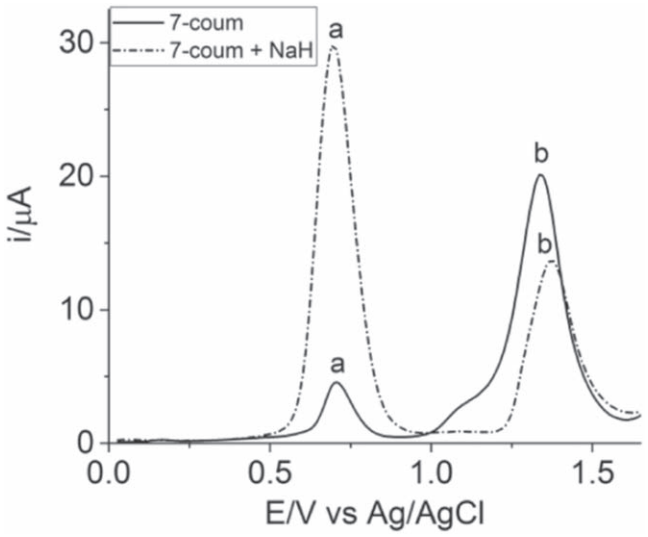

Figure 3. Effect of NaH addition on 7-coum.

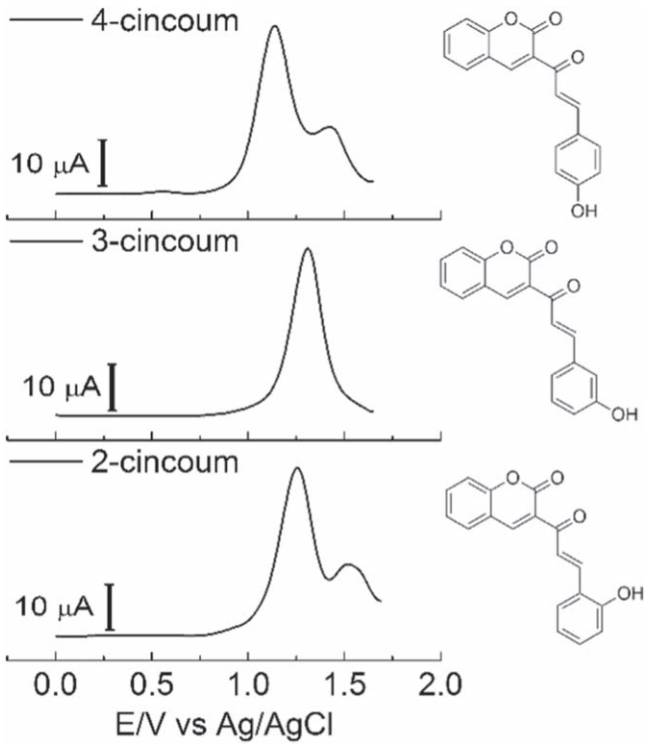

Figure 4. DPV of R-cincoum derivatives in DMF.

and 7-coum show an oxidation peak at lower potential and with low intensity, presumably due to an acidic equilibrium, thus, to verify this behavior, NaH is added to 7-coum for shift the equilibrium towards its phenolate form Ref. 35. Figure 3.

Considering the behavior of the 7-coum and 7,8-coum derivatives, it is possible to attribute the low-intensity peak—of approximately 0.69 V and 0.17 V, respectively—to phenolate anion oxidation corresponding to "peak a." Phenolate anion of 7-coum shows stronger acidity phenol character, which is directly related with its conjugation with electron-withdrawing acetyl group; thus, the first peak is at 0.69 V for 7-coum, corresponding to the oxidation of the phenolate anion (E phenolate) form Fig. 2. As we can see in Fig. 3, when base is added (NaH), peak b is increased by the displacement of equilibrium towards phenolate form.

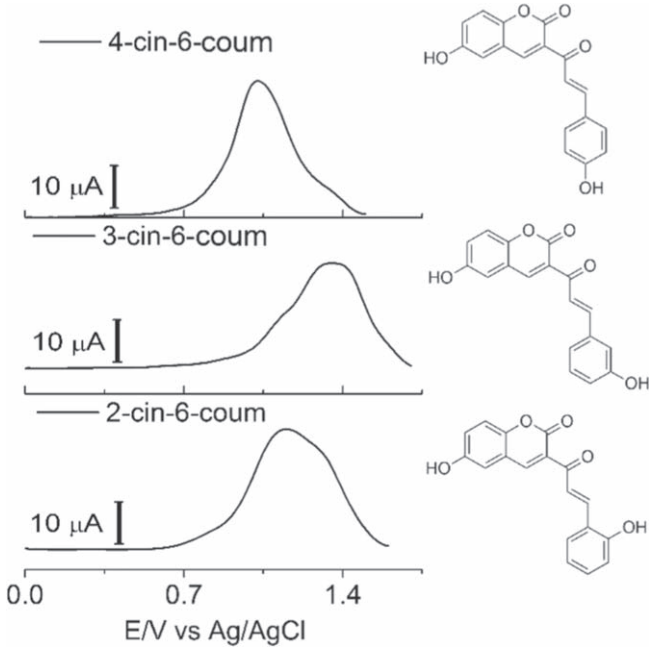

Figure 5. DPV of R-cin-6-coum derivatives in DMF.

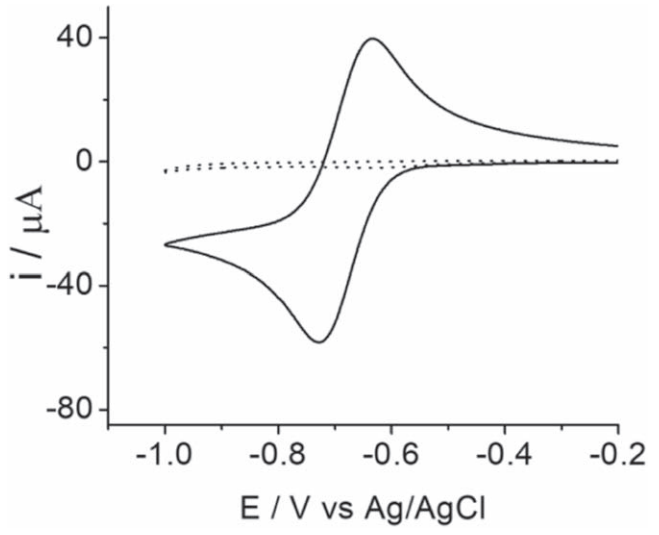

Figure 6. CV for (···) Degassed solution and (–) molecular oxygen in DMSO.

R-cincoum.—All compounds are monophenolic and oxidizable, results are shown in Fig. 4 and Table IV. Only 3-cincoum, has a hydroxyl not conjugated to carbonyl of the cinnamoyl fragment and showed one oxidation peak. The 2-cincoum and 4-cincoum have conjugation between hydroxyl group and carbonyl of the cinnamoyl fragment and we observe an intense oxidation peak and a second oxidation peak at higher oxidation potential.

R-cin-6-coum.—DPV for all synthesized compounds were made and results are shown in Fig. 5 and Table IV As shown in Table IV, the 4-cin-6-coum presents a lower potential value of 1.03 V. This compound has a hydroxyl, the cinnamoyl fragment with a hydroxyl conjugated to a keto group carbonyl. These compounds show lower potential when the hydroxyl of cinnamoyl fragment has conjugation with a chalcone group.

The oxidation potentials are influenced by the substituent, 7,8-coum has lower potential due to donor effect of hydroxyl in ortho

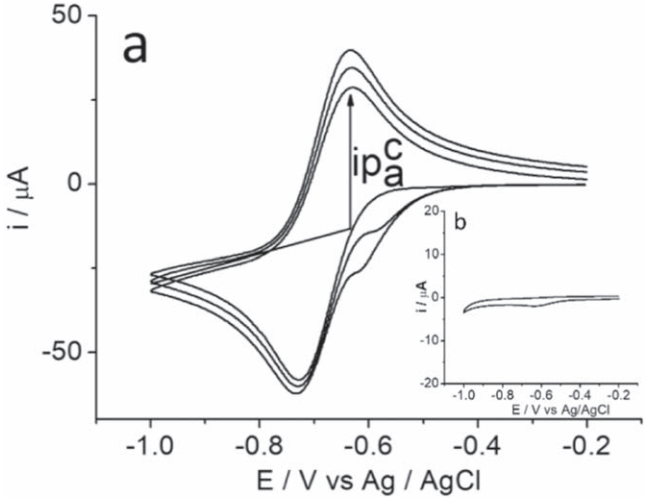

Figure 7. CV of molecular oxygen in presence of different concentrations 8-coum (a). CV of 8-coum 5 mM in a degassed solution (b).

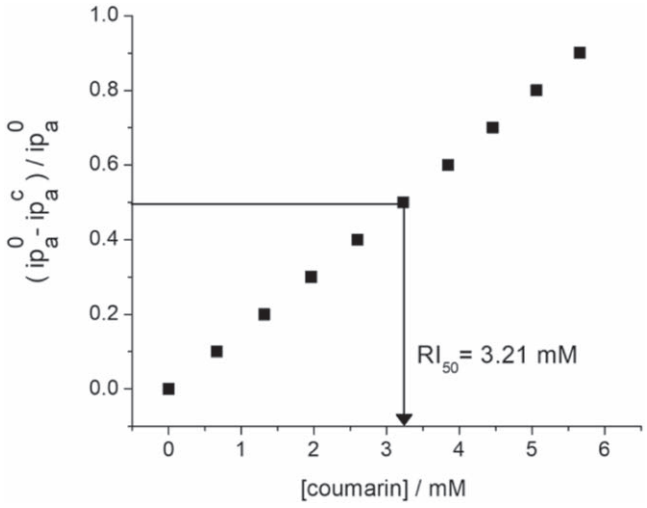

Figure 8. Determination of RI_{50} for 8-coum of $(ip_a^0 - ip_a^C)/ip_a^0$ vs coumarin concentration curve.

position and 7-coum has a hydroxyl conjugated with electron withdrawing carbonyl keto and ester, showing a higher oxidation potential for phenol form 1.36 V and 6-coum and 8-coum conjugated with donor electron group and oxygen of pyrone ring show the lower potential for monophenolic coumarins. Not only oxidation potential is affected by hydroxyl position, also we can see an ionic equilibrium of 7-coum, which was confirmed by base addition and subsequently shifting the equilibrium to ionic form and that was expected due to electron withdrawing conjugation of phenol. For 7,8-coum showed a similar behavior that 7-coum where we can see an oxidation peak with low intensity at 0.17 V for 7,8-coum attributable to phenolate form. 7-coum and 7,8-coum present a lower-potential oxidation process, corresponding to phenolate oxidation.

Reactivity against radical anion superoxide.—Electrochemical reduction of oxygen, to generate a superoxide anion radical, $O_2^{\cdot-}$, in situ, through one electron using cyclic voltammetry (CV) is shown in Fig. 6. All studied compounds are not electroactive in the range of work. For this cyclic voltammogram, the ip_a^0 value is taken and compared with the addition of all antioxidant. As we can see in Fig. 7, the oxidation peak of superoxide anion radical decreases with the addition of coumarin, due to the reaction between radicals and

Table V. Summary of reactivity against superoxide radical anion electrogenerated.

Compound[a]	Lineal range	RI_{50}/mM	r^2
6-coum	0.38–3.35	1.90	0.995
7-coum	0.33–5.10	3.51	0.997
8-coum	0.66–5.70	3.21	0.983
7,8-coum	0.32–2.21	1.67	0.985
2-cincoum	0.32–5.66	3.93	0.976
3-cincoum	0.32–3.14	2.24	0.978
4-cincoum	0.32–3.39	2.48	0.985
2-cin-6-coum	0.32–2.59	1.88	0.994
3-cin-6-coum	0.33–3.85	2.04	0.999
4-cin-6-coum	0.6–3.85	1.99	0.991
Trolox ®	0.67–2.94	1.93	0.995

phenol groups of coumarin, decreasing radical concentration, and subsequently, causing the fall of intensity of the ip_a.

Plotting dimensionless parameter $(ip_a^0 - ip_a^C)/ip_a^0$ vs coumarin concentration[36] Fig. 8, a linear tendency is shown and represents the consumption of $O_2^{\cdot-}$. Where ip_a^0 is the peak current of oxygen before of coumarin addition, ip_a^C is the peak current in presence of coumarin, when coumarin concentration increase, the $O_2^{\cdot-}$ anodic peak current decreases. The compound concentration value that corresponds to $(ip_a^0 - ip_a^C)/ip_a^0 = 0.5$, is the compound concentration value needed to decrease the value of current by 50%, which is called Reactivity Index, RI_{50}, Fig. 8. We can estimate a comparative value—reactivity index—(RI_{50}) to compare all coumarin derivatives. Table V.

The RI_{50} of Trolox ®, an analog of the vitamin E used for comparative purposes, is 1.93 mM, in agreement with the value of 1.97 mM previously reported.[36] As we can see, for R-coum derivatives, 7-coum shows the lowest reactivity (RI_{50} 3.51). For R-coum derivatives we can observe oxidation potential and RI_{50}, both increase in the sequence 7,8-coum > 6-coum > 8-coum > 7-coum; thus, for R-coum series studied, the lower potential, the lower RI_{50} (most reactive compound). If we consider that one of the possible reaction mechanism for $O_2^{\cdot-}$ is the hydrogen atom transfer (HAT), which is the remotion of one hydrogen atom,[37] phenolic hydrogen, is expected that 7-coum, the most acidic derivative,[38] shows the lowest reactivity, highest RI_{50} value, due to its deprotonation in aprotic medium. For 8-coum, phenolic hydroxyl is in ortho position to the oxygen of pyrone ring and, thus, hydrogen of phenol could present a chelation with the oxygen of pyrone reducing the reactivity. All monohydroxylated R-cincoum derivatives, show a lower reactivity than Trolox being the lowest the ortho position, 2-cincoum (RI_{50} 3.93). The dihydroxylated cinnamoyl derivatives, R-cin-6-coum, show a similar reactivity and close to 2 mM. The most reactive compound was 7,8-coum, RI_{50} 1.67 mM, possibly due to the capacity of radical stabilization of phenolic hydroxyl in the ortho position.

Finally, this study in non-aqueous media serves as an approximation to the behavior that they could be have in a bilipid membrane where reactive oxygen species can be found.[39]

Conclusions

In this work we have synthesized and described hydroxycinnamoyl coumarin derivatives that have antioxidant activities comparable to the Trolox® standard.

All compounds in this study consume superoxide anionic radicals as evidenced by their reactivity against the superoxide radical anion generated by cyclic voltammetry. Electronic effects are not only related to acid-base properties but also oxidation potential. For example, 7-coum and 7,8-coum were the most acidic compounds and showed an ionic equilibrium in an aprotic solvent (i.e. dimethyl formamide).

For R-coum derivatives, both the oxidation potentials and the reactivity against $O_2^{\cdot-}$ increase in the sequence 7,8-coum > 6-coum>8-coum > 7-coum; thus, for R coum series studied, the lower potential, the lower RI_{50} (more reactive compound).

This work could be useful as a preliminary rapid screening study for reactivity against superoxide radical anion of compounds and get a first approximation of antioxidant properties of them.

Electrochemistry is a tool that can be used to detect antioxidant activity expressed as quenching capacity to superoxide anion electrochemically generated.

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References

1. A. Al-Amiery, Y. Al-Majedy, A. Kadhum, and A. Mohamad, "Novel macromolecules derived from coumarin: synthesis and antioxidant activity." *Sci. Rep.*, **5**, 11825 (2015).
2. A. Stefanachi, F. Leonetti, L. Pisani, M. Catto, and A. Carotti, "Coumarin: a natural, privileged and versatile scaffold for bioactive compounds." *Molecules*, **23**, 250 (2018).
3. L. Lei et al., "Coumarin derivatives from Ainsliaea fragrans and their anticoagulant activity." *Sci. Rep.*, **5**, 13544 (2015).
4. R. A. O'Reilly, "Studies on the coumarin anticoagulant drugs: interaction of human plasma albumin and warfarin sodium." *J. Clin. Invest.*, **46**, 829 (1967).
5. S. Medimagh-Saidana, A. Romdhane, M. Daami-Remadi, H. Jabnoun-Khiareddine, D. Touboul, H. Ben Jannet, and M. A. Hamza, "Synthesis and antimicrobial activity of novel coumarin derivatives from 4-methylumbelliferone." *Med. Chem. Res.*, **24**, 3247 (2015).
6. J. Sahoo and S. K. Paidesetty, "Antimicrobial activity of novel synthesized coumarin based transitional metal complexes." *J. Taibah Univ. Med. Sci.*, **12**, 115 (2017).
7. D. Dastan, P. Salehi, A. Aliahmadi, A. R. Gohari, H. Maroofi, and A. Ardalan, "New coumarin derivatives from Ferula pseudalliacea with antibacterial activity." *Nat. Prod. Res.*, **30**, 2747 (2016).
8. M. A. Musa, J. S. Cooperwood, and M. O. F. Khan, "A review of coumarin derivatives in pharmacotherapy of breast cancer." *Curr. Med. Chem.*, **15**, 2664 (2008).
9. M. Campos-Toimil, F. Orallo, L. Santana, and E. Uriarte, "Synthesis and vasorelaxant activity of new coumarin and furocoumarin derivatives." *Bioorganic Med. Chem. Lett.*, **12**, 783 (2002).
10. E. Quezada, G. Delogu, C. Picciau, L. Santana, G. Podda, F. Borges, V. García-Morales, D. Viña, and F. Orallo, "Synthesis and vasorelaxant and platelet antiaggregatory activities of a new series of 6-halo-3-phenylcoumarins." *Molecules*, **15**, 270 (2010).
11. C. A. Kontogiorgis and D. J. Hadjipavlou-Litina, "Synthesis and antiinflammatory activity of coumarin derivatives." *J. Med. Chem.*, **48**, 6400 (2005).
12. D. J. Hadjipavlou-Litina, K. E. Litinas, and C. Kontogiorgis, "The anti-inflammatory effect of coumarin and its derivatives." *Antiinflamm. Antiallergy. Agents Med. Chem.*, **6**, 293 (2007).
13. R. Torres, F. Faini, B. Modak, F. Urbina, C. Labbé, and J. Guerrero, "Antioxidant activity of coumarins and flavonols from the resinous exudate of Haplopappus multifolius." *Phytochemistry*, **67**, 984 (2006).
14. D. Farmanzadeh and M. Najafi, "Antioxidant activity of aminothiazol hydroxycoumarin derivatives." *J. Theor. Comput. Chem.*, **12**, 1350058 (2013).
15. D. Hadjipavlou-Litina, C. Kontogiorgis, E. Pontiki, M. Dakanali, A. Akoumianaki, and H. E. Katerinopoulos, "Anti-inflammatory and antioxidant activity of coumarins designed as potential fluorescent zinc sensors." *J. Enzyme Inhib. Med. Chem.*, **22**, 287 (2007).
16. C. Lino, L. Taveira, B. Viana, and F. Matos, "Analgesic and antiinflammatory activities of Justicia pectoralis Jacq and its main constituents: coumarin and umbelliferone." *Phyther. Res.*, **11**, 211 (1998).
17. K. Koriem, G. Asaad, H. Megahed, H. Zahran, and M. Arbid, "Evaluation of the antihyperlipidemic, anti-inflammatory, analgesic, and antipyretic activities of ethanolic extract of ammi majus seeds in albino rats and mice." *Int. J. Toxicol.*, **31**, 294 (2012).
18. J. R. Johnson, "The perkin reaction and related reactions." *Org. React.*, **210**, 265 (2011).
19. M. S. Manhas, S. N. Ganguly, S. Mukherjee, A. K. Jain, and A. K. Bose, "Microwave initiated reactions: pechmann coumarin synthesis, Biginelli reaction, and acylation." *Tetrahedron Lett.*, **47**, 2423 (2006).
20. P. Kalita and R. Kumar, "Solvent-free coumarin synthesis via Pechmann reaction using solid catalysts." *Microporous Mesoporous Mater.*, **149**, 1 (2012).

21. A. J. Harle and L. E. Lyons, "The polarographic reduction of some heterocyclic molecules. Part II." *Coumarin and certain derivatives thereof, J. Chem. Soc.*, **1575**, 1575 (1950).
22. L. H. Wang and H. H. Liu, "Electrochemical reduction of coumarins at a film-modified electrode and determination of their levels in essential oils and traditional Chinese herbal medicines." *Molecules*, **14**, 3538 (2009).
23. K. Mehdi, E. Saeed, D. Gholamreza, F. Alireza, R. Ali, and S. Abbas, "Synthesis and free radical scavenging activity of coumarin derivatives containing a 2-methylbenzothiazoline motif." *Arch. Pharm.*, **344**, 588 (2011).
24. I. Gülçin, "Comparison of *in vitro* antioxidant and antiradical activities of L-tyrosine and L-Dopa." *Amino Acids*, **32**, 431 (2007).
25. M. B. Reddy and L. K. Clark, "Iron, oxidative stress, and disease risk." *Nutr. Rev.*, **62**, 120 (2004).
26. D. Huang, B. Ou, M. Hampsch-Woodill, J. A. Flanagan, and R. L. Prior, "High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format." *J. Agric. Food Chem.*, **50**, 4437 (2002).
27. A. C. Payne, A. Mazzer, G. J. J. Clarkson, and G. Taylor, "Antioxidant assays - consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves." *Food Sci. Nutr.*, **1**, 439 (2013).
28. M. J. Matos, F. Mura, S. Vazquez-Rodriguez, F. Borges, L. Santana, E. Uriarte, and C. Olea-Azar, "Study of coumarin-resveratrol hybrids as potent antioxidant compounds." *Molecules*, **20**, 3290 (2015).
29. M. Arts, G. Haenen, H.-P. Voss, and A. Bast, "Antioxidant capacity of reaction products limits the applicability of the trolox equivalent antioxidant capacity (TEAC) assay." *Food Chem. Toxicol.*, **42**, 45 (2004).
30. K. Thaipong, U. Boonprakob, K. Crosby, L. Cisneros-Zevallos, and D. H. Byrne, "Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts." *J. Food Compos. Anal.*, **19**, 669 (2006).
31. M. Olszowy and A. L. Dawidowicz, "Essential oils as antioxidants: their evaluation by DPPH, ABTS, FRAP, CUPRAC, and β-carotene bleaching methods." *Monatsh. Chem.*, **147**, 2083 (2016).
32. J. Malyszko and M. Karbarz, "Electrochemical oxidation of trolox and α-tocopherol in acetic acid: a comparative study." *J. Electroanal. Chem.*, **595**, 136 (2006).
33. E. I. Korotkova, Y. A. Karbainov, and A. V. Shevchuk, "Study of antioxidant properties by voltammetry." *J. Electroanal. Chem.*, **518**, 56 (2002).
34. H. Valizadeh, H. Gholipur, and A. Schockravi, ", Microwave assisted synthesis of coumarins via potassium carbonate catalyzed knoevenagel condensation in 1-n-butyl-3-methylimidazolium bromide ionic liquid." *J. Heterocycl. Chem.*, **44**, 867 (2009).
35. V. Pardo-Jiménez, C. Barrientos, J. A. Squella, P. A. Navarrete-Encina, and L. J. Nuñez-Vergara, "Electrochemical oxidation of 7-, 8- and 9-Hydroxy-3-ethoxycarbonyl-2,4-dimethyl coumarin[4,3-b]pyridine isomers at glassy carbon in dimethylformamide." *J. Electrochem. Soc.*, **158**, F166 (2011).
36. C. Le Bourvellec, D. Hauchard, A. Darchen, J. L. Burgot, and M. L. Abasq, "Validation of a new method using the reactivity of electrogenerated superoxide radical in the antioxidant capacity determination of flavonoids." *Talanta*, **75**, 1098 (2008).
37. S. J. L. Lauw, J. Y. H. Yeo, Z. Chiang, and R. D. Webster, "Comparing the relative reactivities of food and vitamin molecules toward electrochemically generated superoxide in dimethylformamide." *Chem. Electro. Chem.*, **4**, 1190 (2017).
38. C. Barrientos, P. Navarrete-Encina, J. Carbajo, and J. A. Squella, "New voltammetric method useful for water insoluble or weakly soluble compounds : application to pKa determination of hydroxyl coumarin derivatives." *J. Solid State Electrochem.*, **22**, 1423 (2018).
39. K. Pérez-Cruz, M. Moncada-Basualto, J. Morales-Valenzuela, G. Barriga-González, P. Navarrete-Encina, L. Núñez-Vergara, J. A. Squella, and C. Olea-Azar, "Synthesis and antioxidant study of new polyphenolic hybrid-coumarins." *Arab. J. Chem.*, **11**, 525 (2018).