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Electrochemical behaviour of new dimeric esters and amides derived from caffeic acid in dimethylsulfoxide†

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The electrochemical oxidation in DMSO of four new derivatives of caffeic acid (CA), two dimeric amides and two dimeric esters, is reported in this article. Although all of them contain two caffeoyl electroactive moieties in their structures, small differences in the connectors result in interesting changes in the electrochemical behaviour of this type of compound. Voltammograms of both esters do not show appreciable differences between them; however, an electrografting process occurs during the electrochemical oxidation of one of them, which suggests that the identity of the connector has an influence on the ability of the diesters to interact with the electrode surface. On the other hand, voltammograms of dimeric amides were more complex than those corresponding to dimeric esters. Electronic effects of diamine connectors seem to be related to the fact that caffeoyl moieties suffer from separate oxidation processes in both compounds. In contrast to their ferulic acid (FA) analogues, which have been studied by our group before, CA dimeric amides do not interact in an appreciable way with the electrode surface. In addition, a relationship between the oxidation potential and the inhibition percentage of the DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical was not observed for the symmetrical CA derivatives studied here. However, the molecular flexibility seems to play a very important role in the Free Radical Scavenging Activity (FRSA) of this type of compound.

1. Introduction

Caffeic acid (CA in Fig. 1) is a phenolic compound that belongs to the group of hydroxycinnamic acids. It is found naturally in plants and vegetables such as carrots, tomatoes, strawberries, blueberries, or potatoes.[1–4] The antioxidant properties and biological importance of CA and some of its natural esters, such as chlorogenic[5–10] and rosmarinic acids,[11–13] as well as the caffeic acid phenethyl ester (CAPE),[14–17] are well-documented in the literature. In addition, caffeic acid amide derivatives have also been identified in natural foods appreciated for their beneficial effect on the human health, such as oats[18] and amaranth.[19]

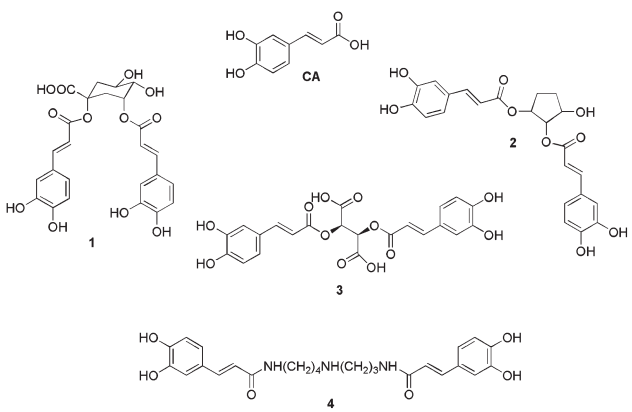

Fig. 1 Caffeic acid (CA) and natural dimeric derivatives containing two caffeoyl units.

Dimeric esters containing two caffeoyl units are also present in nature (Fig. 1). For example, 1,3-di-*O*-caffeoylquinic acid (**1**) was identified from yerba mate (*Ilex paraguariensis*),[20] whose benefits to health have been connected to its high content of polyphenolic secondary metabolites. 1,2-di-*O*-Caffeoylcyclopenta-3-ol (**2**) was isolated from *Acanthopanax*

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†Electronic supplementary information (ESI) available: Additional ESI corresponding to cyclic voltammetry of compounds **6** (Fig. S1) and **8** (Fig. S2) at different scan rates are provided. The dependence of the anodic (Fig. S1) and cathodic peak current (Fig. S2), respectively, on the square root of the potential scan rate (v) is also shown. ^{13}C NMR spectra of the new compounds **6–9** appear as Fig. S3–S6. See DOI: 10.1039/c4ob00823e

koreanum,[21] which is a plant used in traditional Korean medicine in the treatment of rheumatism, hepatitis, and diabetes. This compound has been found in *Daphne feddei* too,[22] a kind of shrub whose leaves and stems are used in some provinces of China to treat injuries from falls and bruises. On the other hand, chicoric acid (**3**), a natural diester derived from tartaric and caffeic acids, was reported as the main component in the leaves and roots of dandelion (*Taraxacum officinale*),[23] which has shown anti-inflammatory activity in animal studies. In addition, L-chicoric acid is a potent and selective inhibitor of type 1 HIV replication. This fact has motivated during the last few years the synthesis of compounds that employ L-chicoric acid as a template, and also several activity relationship (SAR) studies, in order to identify the structural features involved in selectivity and potency against integrase and HIV replication.[24–28] Less common are the reports about natural dimeric amides derived from CA. An example of this kind of compounds is *N*,*N*′-dicaffeoylspermidine (**4**), identified from eggplant (*Solanum melongena* L),[29] which is among the top 10 vegetables with oxygen radical trapping capacity.

We are interested in the antioxidant properties of dimeric derivatives of hydroxycinnamic acids, since some of them have been suggested as therapeutic agents against human health disorders caused by oxidative stress.[24–28,30,31] Cyclic voltammetry is a promising tool in the study of antioxidant compounds, and numerous reports are found in the literature about the application of this electrochemical technique to characterize polyphenolic compounds.[32–37] We have reported recently the electrochemical behaviour of monomeric esters and amides derived from CA and ferulic acid (FA),[38] as well as mechanistic aspects of electrochemical oxidation of dimeric FA amides.[39] In those studies, we found that all compounds followed a similar oxidation mechanism; however, FA amides additionally undergo electrografting reactions. In addition, dimeric topology seems to favour this tendency. In contrast, caffeic acid benzyl amide (CABzA) showed an oxidation potential even lower than that of CAPE and CA. This amide was also the compound, of the complete series, with a higher capacity to scavenge the DPPH (2,2′-diphenyl-1-picrylhydrazyl) radical.

In this context, the aim of this study was to determine the electrochemical behaviour of new compounds with dimeric topology: two symmetrical esters (**6** and **7** in Fig. 2) and two symmetrical amides (**8** and **9**) derived from caffeic acid in an aprotic medium. The influence of the connectors on the oxidation mechanism is also discussed here. Since dimethylsulfoxide (DMSO) was the best solvent to dissolve all compounds studied in this work, we decided to carry out the analysis of the complete series in this medium. Cyclic voltammetry of CABzA (**10** in Fig. 2) in DMSO was also performed in order to contrast the effect of monomeric *versus* dimeric topologies on the electrochemical behaviour of compounds **8–10**. In addition, the radical scavenging capacity of the symmetrical compounds was evaluated using the DPPH assay. The obtained data were analyzed and compared with the oxidation potential values in order to investigate whether there is a relationship between them.

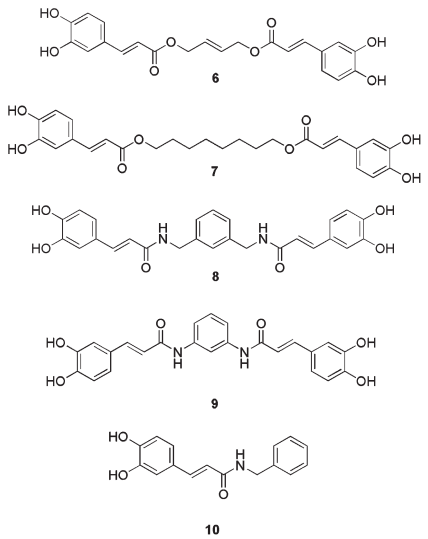


Fig. 2 Caffeic acid ester and amide derivatives studied in the present work.

2. Results and discussion

2.1 Electrochemical study of diesters 6 and 7

Fig. 3 shows the voltammograms of dimeric esters **6** and **7** (3a and 3b, respectively). It can be observed that there are no appreciable differences between them. Typical chemically irreversible oxidation waves (I) and the corresponding broadened reduction waves (II) appear in both cases. The values registered for these peaks are the following: 0.84 and 0.14 V *vs*. SCE in the case of **6**, and 0.89 and 0.08 V *vs*. SCE for compound **7**. A very similar electrochemical behaviour was observed before for CA and CAPE employing the same experimental conditions, which allows us to infer that these dimeric compounds follow

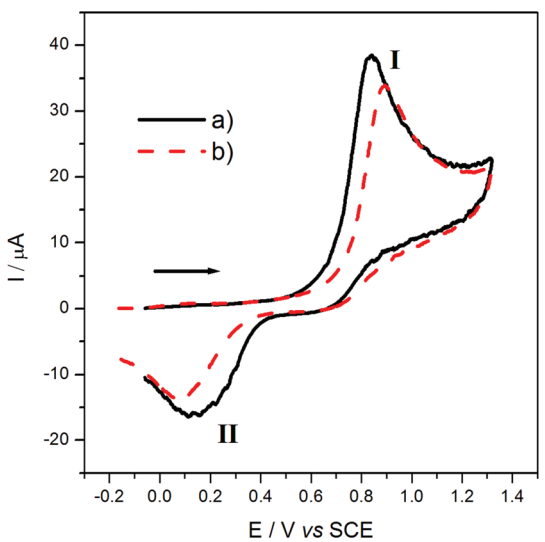


Fig. 3 Cyclic voltammetry of (a) compound **6** and (b) compound **7**, 2 mM in DMSO + 0.1 M *n*-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1}.

an ECE mechanism during their electrochemical oxidation. This mechanism involves the loss of one electron followed by a fast deprotonation and the subsequent loss of a second electron from each electroactive catechol moiety. A further deprotonation can be the final step in the oxidation of this kind of compounds, since *o*-quinone is the product suggested by spectroscopic studies after the oxidation of CA, which has been reported by other authors.[40] However, dimeric topology produces a slight increment in the oxidation potential, since the corresponding values reported for CA and CAPE were 0.78 and 0.73 V *vs.* SCE, respectively, under the same experimental conditions.[38]

In addition, in the case of compound **6**, the anodic peak current is linearly dependent on the square root of the scan rate, which indicates that transport of this compound toward the electrode surface is diffusion controlled (see Fig. S1 in the additional ESI†).[41] On the other hand, when the scan rate is increased, the peak potential is anodically shifted, presenting a linear variation with respect to the logarithm of the scan rate. The transition from a mechanism, the rate controlled by the follow-up chemical reaction, to a mechanism where the electron transfer is the rate determining step has been well characterized.[42] Thus, at 25 °C, this transition presents a variation of $\delta E_p/\delta \log \nu$ from 29.6 to 59.2 mV dec^{-1}. Our experimental results are in an intermediate situation (40 mV dec^{-1}), which suggests that the initial electron and proton transfer reactions are stepwise and they occur under a mixed kinetic control.

Such as in the case of monomeric and dimeric FA amides studied before by our group, the current intensity of the oxidation peak of compound **7** decreased until it disappeared completely after 25 cycles (see Fig. 4b). Nevertheless, the electrochemical response is recovered after the electrode surface is polished. This behaviour suggests that parallel to the oxidation mechanism described before, an electrografting process also takes place during the experiment. Interestingly, the electrochemical response of compound **6** remains almost the same after several cycles, which suggests that the connector could exert an influence on the ability of these dimeric esters to interact with the surface of the glassy carbon electrode (GCE). In order to know more details about the electrochemical processes occurring during the oxidation and the electrografting process of compound **7**, we carried out a series of additional experiments, which are described below.

In the first place, after the complete blocking of the electrode surface, the electrochemical reduction of the solution of compound **7** was performed immediately. As is shown in Fig. 4c, a cathodic peak at −1.92 V *vs.* SCE was registered, which corresponds to the reduction of the carbonyl of the ester group present in molecules of the sample in solution.[43] In addition, a further oxidation cycle was also carried out in the same cell and the electrochemical response is shown in Fig. 4d. The recovery of peak I is clear, which implies that, at least in part, a desorption phenomenon occurred at cathodic potentials as a consequence of an oxidation–reduction process between the modified GCE surface and molecules of compound **7** in solution.

In a new experiment and after the electrografting process (Fig. 5a and b), the electrode was rinsed several times with acetone, followed by sonication in pure DMSO and then transferred to a cell containing only the solution of the supporting electrolyte. No peak was registered during the electrochemical reduction of the modified carbon electrode (Fig. 5c), which allows us to infer that the pending chain is not electroactive.

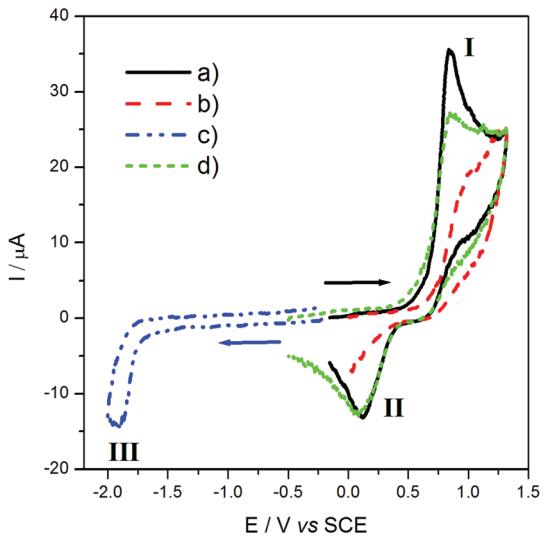


Fig. 4 Cyclic voltammetry in DMSO + 0.1 M *n*-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1} of: (a) compound **7**, 2 mM; (b) cycle 25; (c) cyclic voltammetry to the cathodic direction of blocked GCE into a solution of **7**; and (d) further cyclic voltammetry to the anodic direction after the procedure described in c.

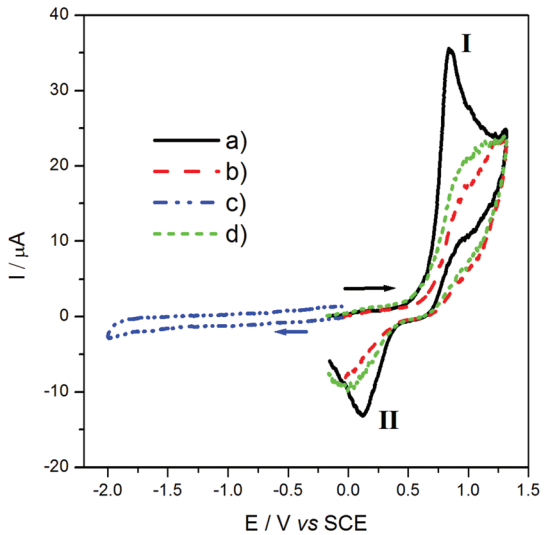
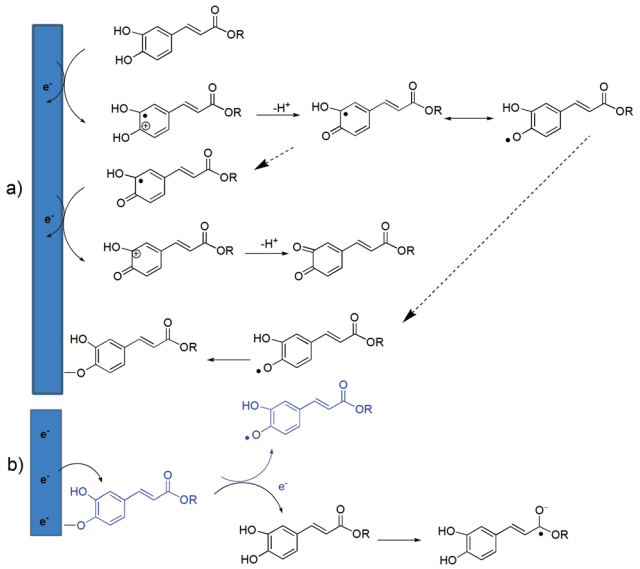


Fig. 5 Cyclic voltammetry in DMSO + 0.1 M *n*-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1} of: (a) compound **7**, 2 mM; (b) cycle 25; (c) cyclic voltammetry to the cathodic direction of blocked GCE into a solution of the supporting electrolyte; and (d) cyclic voltammetry to the anodic direction of GCE after the procedure described in c, employing a fresh solution of compound **7**.



Scheme 1 (a) Electrografting process taking place during oxidation of compound **7**. (b) Desorption phenomenon of compound **7** at cathodic potentials.

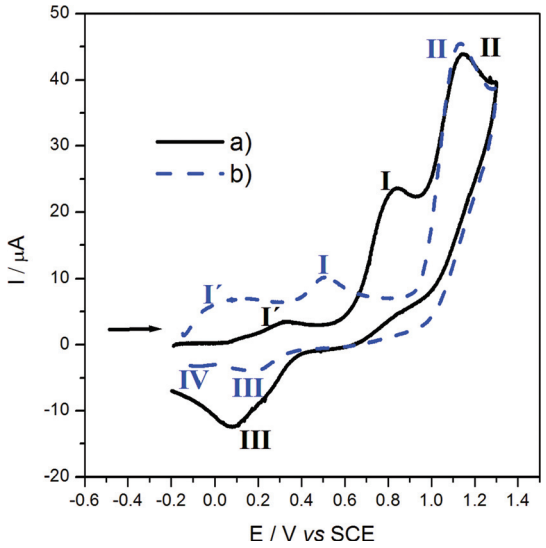


Fig. 6 Cyclic voltammetry of compounds **8** (a) and **9** (b), 2 mM in DMSO + 0.1 M n-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1}.

Immediately after that, the electrode was transferred to a cell containing a fresh solution of compound **7**, and a further oxidation cycle was carried out. Interestingly, peak I was not recovered, which means that the surface of the electrode was still blocked (Fig. 5d), and that the desorption phenomenon described above is only possible at cathodic potentials and in the presence of molecules of compound **7** in the solution. Scheme 1 summarizes the mechanism that could explain the electrochemical behaviour of compound **7**.

2.2 Electrochemical study of dimeric amides 8 and 9

The electrochemical response of CA dimeric amides differs from the data registered for both esters described above. Fig. 6a and b show the voltammograms of compounds **8** and **9**, respectively. As can be observed, a prewave and two peaks, labeled as I′, I, and II respectively, appear in the anodic direction in both compounds at 0.32, 0.84, and 1.14 V *vs*. SCE for the dimeric amide **8**, whereas 0.08, 0.51, and 1.13 V *vs*. SCE were the corresponding values registered during the electrochemical oxidation of compound **9**. In addition, a broad wave III was observed at cathodic potentials for compound **8** at 0.08 V *vs*. SCE, whereas two waves at 0.17 and −0.08 V *vs*. SCE appear in the voltammogram of **9**, which were labelled as III and IV, respectively. From these data, it is clear that at least two main oxidation phenomena occur during the experiment in both cases, and also that the identity of the connector exerts a type of influence on the electrochemical behaviour of this kind of compounds, since prewave I′ and peak I in compound **8** are shifted to more anodic potentials with respect to the corresponding values registered in the voltammogram of compound **9**.

Since compounds **6–9** share dimeric topology with the same electroactive caffeoyl moieties but their electrochemical behaviour is clearly different, we suspected that two structural features could be responsible for the oxidation peak II, observed only in the case of **8** and **9**: (1) the presence of an additional electroactive functional group, such as an amide group, since its oxidation is well documented in the literature,[44] or (2) an effect of the diamine spacer, which provokes that caffeoyl units give rise to separate oxidation processes, similar to those described for bis-ferrocenyl compounds.[45] In order to rule out any of these hypotheses, we decided to carry out cyclic voltammetry of CABzA (compound **10**) in DMSO (electrochemical behaviour of this monomeric amide in acetonitrile was reported before by our group[38]). We rationalized that two peaks must be observed if, in addition to the caffeoyl moieties, the amide group undergoes an oxidation process during the electrochemical experiment on compound **10**. On the other hand, if only one peak is observed in the voltammogram of **10** that would mean that caffeoyl moieties undergo separate oxidation processes, in the case of dimeric amides.

Fig. 7 shows the electrochemical behaviour of amide **10**, which is the monomeric analogue of compound **8**. Only a sharp oxidation peak I at 0.70 V *vs*. SCE and a broad reduction wave II at 0.18 V *vs*. SCE are observed. Such as in the case of diesters **6** and **7**, an ECE mechanism with a further deprotonation must be involved in the oxidation of this compound, since its voltammogram is very similar to those reported before for CA and CAPE in the same solvent.[38] More interesting is the effect of topology on the electrochemical response of monomeric *versus* dimeric amides. This result confirms that caffeoyl moieties in **8** and **9** undergo separate oxidation processes, as a consequence of the effect of diamine connectors. Looking at Fig. 6a and b, it is evident that insertion of two methylene units in the spacer (*m*-xylylenediamine *vs*. 1,3-phenylenediamine) makes the oxidation of the first caffeoyl moiety in compound **8** more difficult with respect to

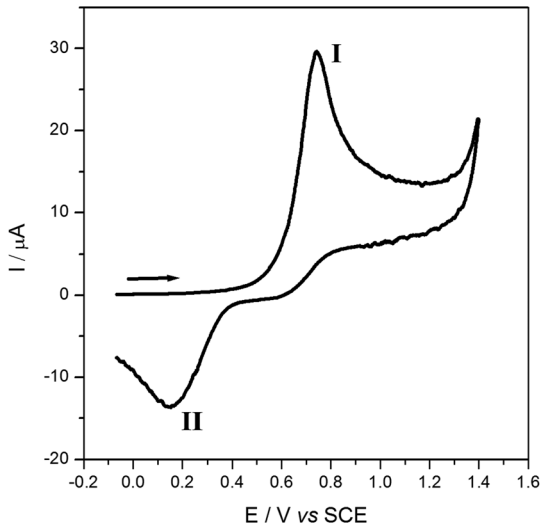


Fig. 7 Cyclic voltammetry of compound **10**, 2 mM in DMSO + 0.1 M n-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1}.

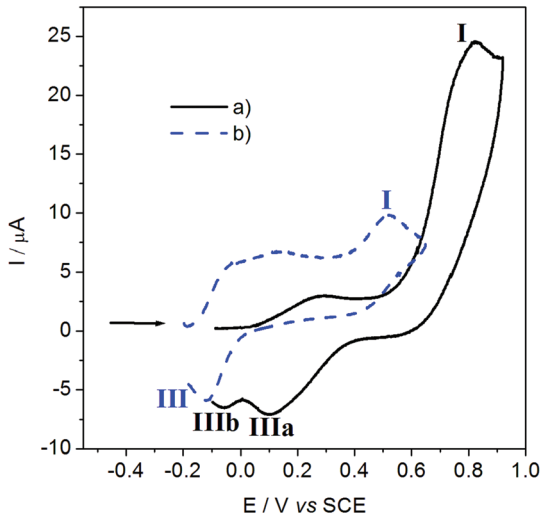


Fig. 8 Cyclic voltammetry of compounds **8** (a) and **9** (b), 2 mM employing E_λ = 0.90 and 0.65 V *vs.* SCE respectively, in DMSO + 0.1 M n-Bu_4NPF_6, on a glassy carbon electrode (3 mm ϕ) at 0.1 V s^{-1}.

compound **9**, and that electronic effects could account for this phenomenon. In addition, the difference between anodic potentials of peaks I and II (ΔE_p) decreases in a very important way, also as a consequence of this structural change (620 mV and 300 mV in the case of **9** and **8**, respectively).

On the other hand, very modest changes in the intensities of current peaks or in anodic potential values were observed after several cycles for both dimeric amides. Nevertheless, prewave I′ of both compounds decreases very slightly after cycling the electrode potential between the initial potential and the foot of peak I, which is consistent with the occurrence of a modest adsorption phenomenon during the experiments with solutions of compounds **8** and **9**.[46–48] In order to know more about the relationship between the anodic peaks I and II and the cathodic peak III observed in the voltammograms of symmetrical amides, we carried out additional electrochemical experiments, which are shown in Fig. 8. Inversion potentials (E_λ) at 0.90 and 0.65 V *vs.* SCE were employed during the acquisition of voltammograms of **8** (Fig. 8a) and **9** (Fig. 8b), respectively. As can be observed, peak III remains in both cases, which is evidence that it is coupled to the oxidation peak I. In addition, in the case of compound **8**, peak III becomes two peaks, labelled as IIIa and IIIb in Fig. 8a. It should be mentioned that linear relationships were obtained between current peaks of both IIIa and IIIb and the square root of the scan rate. From this analysis, we can deduce that these peaks correspond to diffusion waves (see Fig. S2 in additional ESI†).[41]

The behaviour of dimeric amides studied here contrasts with their analogues derived from FA reported before by our group, which after two or three cycles block, in a very efficient way, the electrode surface when the experiment is performed in acetonitrile. From these results it seems that electroactive guaiacol moieties, present in FA amides, contribute in a decisive manner to their ability to react with the electrode, whereas catechol units, present in compounds **8** and **9**, are less efficient. The difference in the behaviour of the two types of hydroxycinnamic derivatives could be related to the fact that after the first steps (EC) involved in the oxidation of both of them, a further delocalization of the unpaired electron of the radical toward the lateral chain occurs in a favourable way in the case of FA amides, which reacts with the electrode surface, as proposed by us in previous work,[39] whereas formation of *o*-quinone is preferred in the case of the analogous CA derivatives, through the loss of a second electron and a subsequent deprotonation.

2.3 Oxidation potentials *versus* free radical scavenging activity (FRSA)

Electrochemical parameters such as the oxidation potential, the number of electrons, and the electron transfer rate have been used to evaluate the oxidant capabilities of organic compounds.[49,50] Particularly, oxidation potentials have been used to study the electron-donating capacity of molecules and as an indicator of their radical scavenging ability, since it has been shown that compounds with a less positive oxidation potential possess higher radical scavenging activities.[32,38] The DPPH assay is an easy test commonly employed to evaluate FRSA of phenolic compounds, hydroxycinnamic acids and related systems. In order to see whether there is a relationship between the oxidation potential and the FRSA of symmetrical CA derivatives reported here, we carried out a DPPH assay with four new compounds.

The oxidation potentials of compounds **6–9** are shown in Table 1. CAPE was also included in the last entry as a reference, due to the fact that this compound has been recognized by its high antioxidant capacity and it is structurally related to the new dimeric CA derivatives. Since two waves (I and II) were observed for compounds **8** and **9** in the anodic direction, two values appear in Table 1 for each symmetrical amide. However, we take into account only the lower oxidation potentials of

Table 1 Oxidation potential of CA derivatives and their corresponding inhibition percentage of 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical

Compound	E_p/V *vs.* SCE[a]	Inhibition (%) (±SD) DPPH[b]
6	0.84	70.14 (±0.17)
7	0.89	94.07 (±0.06)
8	0.84	65.80 (±0.55)
	1.14	
9	0.51	40.57 (±0.92)
	1.13	
CAPE	0.73[c]	55.72 (±0.94)[d]

[a] Oxidation potentials of caffeic acid derivatives were obtained at the same concentration (2 mmol L^{-1}) and at 0.1 V s^{-1} in DMSO. [b] The values represent means ± S.D. from three experiments and indicate the percentage of inhibition for solutions 35 μmol L^{-1} of CA derivatives. [c] Oxidation potential of CAPE reported before by us.[38] This value was observed under the same experimental conditions employed to study **6–9**. [d] Inhibition percentage evaluated at 35 μmol L^{-1}.

both compounds to make a comparison with the inhibition percentage of the DPPH radical.

As can be observed, compound **9** has the lower oxidation potential of the complete series (0.51 V *vs.* SCE), whereas the symmetrical ester **7** showed the higher value (0.89 V *vs.* SCE). On the other hand, the anodic peaks of compounds **6** and **8** were registered at the same potential (0.84 V *vs.* SCE). Nevertheless three of the four compounds exhibited higher oxidation potentials with respect to CAPE (0.73 V *vs.* SCE); oxidation of compound **9** occurred at less anodic values. In a different way from the behaviour observed before by our group in the case of monomeric FA and CA derivatives,[38] there is no clear relationship between the oxidation potential of the new symmetrical compounds reported here and the corresponding inhibition percentages of the DPPH radical.

Compound **7** showed the higher inhibition percentage (94.07%), whereas the symmetrical amide **9** showed the lower one (40.57%). In addition, compounds **6** and **8** exhibited similar inhibition percentages (70.14 and 65.80%, respectively). From the data shown in Table 1, it is clear that the presence of two caffeoyl moieties in the dimeric compounds does not have a twofold effect on their capacity to react with the DPPH radical. Nevertheless, it seems that molecular flexibility could play the main role in the FRSA of this type of compound, since the compound with the aliphatic longer connector showed a higher inhibition percentage of the DPPH radical (**7**), whereas the compound with the more rigid aromatic connector showed a lower value (**9**).

3. Experimental

3.1 Instrumentation

Melting points were determined with Melt-Temp apparatus and are not corrected. ^{1}H and ^{13}C spectra were recorded on Varian Mercury Plus (300 MHz) and Agilent Technologies 400/54 Premium Shielded (400 MHz) spectrometers, using deuterated methanol (CD_3OD) and d_6-DMSO as solvents. Chemical shifts were recorded in δ (ppm) values from CD_3OD or d_6-DMSO (δ 3.31 and 2.50 ppm for ^{1}H, and δ 49.0 and 39.4 ppm for ^{13}C respectively, based on the middle peak of the solvents). Signal patterns are indicated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet and br, broad signal. Coupling constants (J) are given in hertz. HR-ESI-TOF MS spectra were measured on a Gi969A Agilent spectrometer. Cyclic voltammetry experiments were performed on a potentiostat PGZ-301 (Radiometer Copenhagen) with positive feedback resistance compensation. A spectrophotometer Multiskan Spectrum (Thermo Electron Corporation) was employed to carry out the DPPH assay.

3.2 Chemicals

All chemical reagents and tetrabutylammonium hexafluorophosphate 99% (n-Bu_4NP_6) were purchased from Sigma-Aldrich and were used without purification. Dimethylformamide (DMF) and CH_2Cl_2 were of reagent grade and were distilled before use. DMSO (content of H_2O < 0.1%) spectra grade (Merck Uvasol) was used as the solvent during the electrochemical experiments, whereas ethanol chromasolv® was employed to perform the DPPH assay. Products were purified by flash column chromatography on silica gel 230–400 mesh using mixtures of EtOAc–hexanes as eluents.

3.3 Synthesis of esters 6 and 7

The dimeric esters were prepared following the procedure reported before by Son[51] with some modifications. In a 250 mL flask fitted with a dropping funnel and a stir bar, 8.88 mmol (1.600 g) of caffeic acid, 15 mL of DMF, and 1.4 mL of 25% NaOH solution (8.88 mmol) were placed. After that, 2.77 mmol of either *trans*-1,4-dibromo-2-butene or 1,8-dibromooctane previously dissolved in 6 mL DMF were added dropwise through the dropping funnel and the resulting solution was stirred for seven days. The crude from the reaction was washed with 30 mL of water at 0 °C and the product was extracted several times with EtOAc, dried over sodium sulfate and evaporated under reduced pressure. Purification of the crude products was accomplished through flash chromatography.

3.4 Synthesis of amides 8 and 9

In a 250 mL flask with a stir bar, 5.25 mmol (0.945 g) of caffeic acid, 20 mL of DMF and 5.25 mmol (0.73 mL) of Et_3N were placed. The solution was allowed to stir for 10 minutes at 0 °C, after that 2.5 mmol of either *m*-xylylenediamine or 1,3-phenylenediamine was added directly to the solution. About 5.25 mmol (2.32 g) of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) dissolved in 20 mL of CH_2Cl_2 were added *via* a syringe and the mixture was stirred at 0 °C for another 30 minutes. The reactions proceeded at room temperature for 48 h, after which CH_2Cl_2 and DMF were removed, and the crude from the reaction was diluted in 50 mL of EtOAc. The product was filtered, evaporated to dryness, and then washed with 1 M HCl , water, and an aqueous solution of 1 M $NaHCO_3$. Finally, it was diluted again

in EtOAc and dried over sodium sulfate. The solvent was removed in a rotary evaporator and the residue was chromatographed on silica gel employing mixtures of hexanes–EtOAc as eluents with a gradient from 80 : 20 to 50 : 50. Yields are given for isolated products.

3.5 Synthesis of monomeric amide 10

Compound **10** was prepared by us previously[38] following the procedure reported by Rajan.[52]

Compound **6**: 1,4-di-*O*-caffeoyl-*trans*-2-butenediol (0.23 g, 20.5%). m.p. 227–230 °C. δ_H(400 MHz; d_6-DMSO) 4.64 (4H, m, $-OCH_2$), 5.91 (2H, m, –CH═CH–), 6.25 (2H, d, J = 15.9 Hz, –CHCO), 6.73 (2H, d, J = 8.1 Hz, –CHAr), 6.97 (2H, dd, J = 8.2 Hz, J = 2.0 Hz, ArH), 7.02 (2H, d, J = 2.0 Hz, ArH), 7.47 (2H, d, J = 15.9 Hz, ArH), 9.24 (4H, br, OH). δ_C(100.5 MHz; d_6-DMSO) 63.6, 114.1, 115.3, 116.1, 121.8, 125.9, 128.4, 145.9, 146.0, 148.9, 166.6. HRMS (ESI-TOF) calculated for $[C_{22}H_{20}O_8Na]^+$ 435.1056, found $[M + Na]^+$ 435.1046.

Compound **7**: 1,8-di-*O*-caffeoyloctanediol (0.06 g, 5.0%). m.p. (decomposes before melting). δ_H(400 MHz; d_6-DMSO) 1.29 (8H, m, $-CH_2$), 1.59 (4H, m, $-CH_2$), 4.07 (4H, t, J = 6.6 Hz, $-CH_2O$), 6.21 (1H, d, J = 15.9 Hz, –CHCO), 6.72 (1H, d, J = 8.1 Hz, –CHAr), 6.95 (1H, dd, J = 2.0 Hz, J = 8.1 Hz, ArH), 7.00 (1H, d, J = 2.0 Hz, ArH), 7.42 (1H, d, J = 15.9 Hz, ArH), 9.00 (2H, s, –OH), 9.44 (2H, s, –OH). δ_C(100.5 MHz; d_6-DMSO) 25.7, 28.7, 28.9, 64.1, 114.4, 115.2, 116.1, 121.6, 126.0, 145.4, 146.0, 148.7, 166.9. HRMS (ESI-TOF) calculated for $[C_{26}H_{31}O_8]^+$ 471.2019, found $[M + H]^+$ 471.2018.

Compound **8**: *N*,*N*′-dicaffeoylxylylenediamine (0.64 g, 25.5%). m.p. 119 °C. δ_H(300 MHz; CD_3OD) 4.46 (4H, s, $-CH_2Ar$), 6.39 (2H, d, J = 15.6 Hz, –CHCO), 6.74 (2H, d, J = 8.1 Hz, ArH), 6.87 (2H, d, J = 8.1 Hz, ArH), 7.00 (2H, s, ArH), 7.24 (2H, m, ArH), 7.41 (2H, d, J = 15.6 Hz, –CHAr), 7.46–7.55 (1H, m, ArH), 7.72–7.87 (1H, m, ArH). δ_C(75.5 MHz; CD_3OD) 44.3, 114.9, 116.3, 118.1, 122.1, 127.4, 127.7, 128.2, 129.8, 140.2, 142.4, 146.6, 148.5, 169.0. HRMS (ESI-TOF) calculated for $[C_{26}H_{25}N_2O_6]^+$ 461.1713, found $[M + H]^+$ 461.1707.

Compound **9**: *N*,*N*′-dicaffeoylphenylenediamine (0.14 g, 5.8%). m.p. (decomposes before melting). δ_H(300 MHz; CD_3OD) 6.57 (2H, d, J = 15.5 Hz, –CHCO), 6.75 (2H, d, J = 8.1 Hz, ArH), 6.96 (2H, d, J = 8.1 Hz, ArH), 7.08 (2H, s, ArH), 7.26 (2H, m, ArH), 7.37 (1H, m, ArH), 7.45 (2H, d, J = 15.5 Hz, –CHAr), 7.69 (1H, m, ArH). δ_C(75.5 MHz; CD_3OD) 112.4, 115.2, 116.5, 118.6, 122.5, 125.2, 128.1, 130.1, 140.4, 143.6, 146.9, 149.1, 167.3. HRMS (ESI-TOF) calculated for $[C_{24}H_{21}N_2O_6]^+$ 433.1400, found $[M + H]^+$ 433.1388.

3.6 Voltammetry experiments

A Pyrex glass three-electrode cell was used in the electrochemical experiments. The working electrode was a 3 mm diameter glassy carbon disk (Sigradur G from HTW, Germany). This electrode was polished with 0.4 μm alumina powder and rinsed in an ultrasound bath with distilled water and ethanol before each run. A platinum mesh was used as a counter electrode and a saturated calomel electrode (SCE) as a reference electrode. The reference electrode was connected to the cell through a salt bridge containing the same supporting electrolyte concentration as the working solution. All electrochemical experiments were performed at 25 °C under a high purity nitrogen atmosphere.

3.7 Free radical scavenging activity (DPPH assay)

The free radical scavenging activity (FRSA) was measured according to the following procedure: 100 μL of a DPPH ethanolic solution (300 μmol L^{-1}) was mixed with the same volume of the test compound solution (35 μmol L^{-1}). The reaction mixtures were vigorously vortexed for 10 s. The absorbance of the resulting solutions was read at 520 nm, after 30 min of incubation at room temperature in darkness. The scavenging activity was determined by comparing the absorbance with that of the blank containing only the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical and ethanol. Results were expressed as inhibition percentage with respect to control values.[53] CAPE was used as a standard antioxidant.

4. Conclusions

New dimeric derivatives of CA, two diesters and two diamides were prepared and their electrochemical behaviour was investigated in DMSO. Although all of them contain two caffeoyl electroactive moieties in their structures, small differences between the connectors provoke interesting changes in the electrochemical responses of this type of compound. Although appreciable differences were not observed from voltammograms of both diesters, only one of them (compound **7**) undergoes an electrografting process during the electrochemical experiment. Nevertheless, a desorption phenomenon occurs through a redox process at cathodic potentials between the molecules of compound **7** in solution and the modified electrode.

Voltammograms of the dimeric amides derived from CA, studied here, resulted in being more complex with respect to those obtained from dimeric esters. Electronic effects of the diamine connector seem to be related to the fact that caffeoyl moieties suffer from separated oxidation processes in this type of dimeric amide. In contrast to their FA analogues, which have been studied by our group before, CA dimeric amides do not interact in an appreciable way with the electrode surface. The difference in the behaviour of FA and CA diamides could be related to the fact that formation of *o*-quinone is mainly favoured during electrochemical oxidation of CA derivatives, whereas delocalization of the unpaired electron of the resulting radical toward the lateral chain could occur in the case of FA amides, which reacts with the electrode surface. Taking all these into account, we can conclude that although the representative electrochemical oxidation mechanism of hydroxycinnamic acids CA and FA and their derivatives involves ECE processes, small changes in the structure of dimeric compounds exert interesting effects on their electrochemical behaviour.

On the other hand, a clear relationship between the oxidation potential and the inhibition percentage of DPPH was not observed for the symmetrical CA derivatives studied here. However, the molecular flexibility seems to play an important role in the FRSA of this type of compound, since the compound with the aliphatic longer connector showed the highest inhibition percentage of the DPPH radical (**7**), whereas the compound with the more rigid aromatic connector showed the lowest value (**9**). Considering that in the last few years symmetrical derivatives of hydroxycinnamic acids have attracted the attention of research groups as potential therapeutic agents, it should be kept in mind that the type of connector is an important key to take into account in the molecular design.

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