Food Chemistry 246 (2018) 481–489

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Theoretical study of the thermodynamics of the mechanisms underlying antiradical activity of cinnamic acid derivatives

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ARTICLE INFO

Chemical compounds studied in this article:
Dihydrocaffeic acid (PubChem CID: 348154)
Dihydroferulic acid (PubChem CID: 14340)
Caffeic acid (PubChem CID: 689043)
Ferullic acid (PubChem CID: 445858)

Keywords:
Cinnamic acids isomers
Radical scavenging
Guaiacyl, catechol and carboxyl moiety
Dienone lactone
Double HAT
Double SPLET

ABSTRACT

The role of antiradical moieties (catechol, guaiacyl and carboxyl group) and molecular conformation in antioxidative potency of dihydrocaffeic acid (DHCA) and dihydroferulic acid (DHFA) was investigated by density functional theory (DFT) method. The thermodynamic preference of different reaction paths of double ($2H^+/2e^-$) free radical scavenging mechanisms was estimated. Antiradical potency of DHCA and DHFA was compared with that exerted by their unsaturated analogs – caffeic acid (CA) and ferulic acid (FA). *Cis/trans* and *anti*-isomers of studied cinnamic acid derivatives may scavenge free radicals *via* double processes by involvement of catechol or guaiacyl moiety. Carboxyl group of *syn*-isomers may also participate in the inactivation of free radicals. Gibbs free energies of reactions with various free radicals indicate that *syn*-DHCA and *syn*-DHFA, colon catabolites that could be present in systemic circulation in low μM concentrations, have a potential to contribute to health benefits by direct free radical scavenging.

1. Introduction

Epidemiological studies indicate that regular consumption of foods and beverages of plant origin could be associated with better human health and longevity. The beneficial effects of fruits, vegetables, grains, olive oil, red wine and tea have been mostly ascribed to health protecting activities of (poly)phenols, ubiquitously present in plant kingdom. Recently, it has been suggested that active compounds are not intact plant (poly)phenolics such as polymeric proanthocyanidins, acylated flavonoid glycosides, tea catechins and esterified hydroxycinnamates, which usually possess very low bioavailability, but their highly bioavailable phenolic colonic catabolites (Dangles, 2012; Rodriguez-Mateos et al., 2014).

Free radicals are produced in low levels by any cell and are essential for life. However, in excessive amounts under oxidative stress conditions, they may cause damage to cell macromolecules, which leads to the onset of various diseases including diabetes, cardiovascular disorders, neurological diseases, different types of cancer, and aging (Quideau, Deffieux, Douat-Casassus, & Pouysegu, 2011). (Poly)phenolic colon catabolites could be important endogenous antioxidants capable to scavenge excess free radicals and suppress oxidative processes. Their free radical scavenging activity may be physiologically relevant *in situ* and perhaps in the blood, where they could be present in relatively high concentrations (Halliwell, Rafter, & Jenner, 2005; Rodriguez-Mateos et al., 2014). Low concentrations of circulating catabolites, usually observed after (poly)phenol-rich food consumption, do not exclude their accumulation at much higher local concentrations at specific sites of inflammation and oxidative damage (Dangles, Dufour, Tonnele, & Trouillas, 2017). Their spatial position and orientation in lipid bilayer membranes also considerably enhances their local concentration in this vital region, thus increasing their importance for *in vivo* biological activities including free radical scavenging (Košinova, Berka, Wykes, Otyepka, & Trouillas, 2012).

Dihydrocaffeic acid (DHCA) and dihydroferulic acid (DHFA) are colon catabolites produced by the gut microbiota, which are found in fecal water, plasma, and excreted urine after consumption of (poly) phenol-rich diet (Ludwig, Clifford, Lean, Ashihara, & Crozier, 2014; Poquet, Clifford, & Williamson, 2008; Rodriguez-Mateos et al., 2014). Ludwig et al. (2014) showed that coffee consumption in 2–3 h intervals may result in 1 μM DHFA concentration in plasma. This, coupled with DHCA and DHFA produced by colon catabolism of other (poly)phenolic compounds from diet, may result in sufficient concentration in

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https://doi.org/10.1016/j.foodchem.2017.11.100
Received 29 September 2017; Received in revised form 24 November 2017; Accepted 28 November 2017
Available online 02 December 2017
0308-8146/

circulatory system to exert biological activities (Feliciano et al., 2016). It has been shown that both DHCA and DHFA at low μM concentrations protect against oxidative stress induced in cultured human neuroblastoma SK-N-MC cells (Verzelloni et al., 2011), and that DHCA prevents lipid peroxidation in human plasma and erythrocytes (Lekse, Xia, Stark, Morrow, & May 2001).

DHCA and DHFA are weak acids: pK_{a1} (−COOH) = 3.84 (3.95) and pK_{a2} (4-OH) = 9.29 (9.95), respectively (Lopez-Munguia et al., 2011). They are slightly water soluble and at the physiological pH of 7.4 are in their deprotonated forms, i.e., as carboxylate anions. Free radical scavenging activity of DHCA, DHFA, and their structural analogs with unsaturated side chain, i.e., caffeic (CA) and ferulic (FA) acids has been extensively experimentally investigated *in vitro* by using $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ assays. Representative published results compiled in Table S1 (in Supplementary data) reveal that dihydrocinnamic acids (DHCA and DHFA) are better free radical scavengers than their structural counterparts possessing propenoic side chain at the phenolic ring (CA and FA). However, unambiguous underlying mechanism(s) has not been fully clarified yet (Dangles, 2012; Silva et al., 2000). It is noteworthy that kinetic results indicate that DHCA and DHFA react slower with $DPPH^{\cdot}$ radical than CA and FA, but are more efficient in terms of the number of radicals trapped per antioxidant molecule (Ordoudi, Tsimidou, Vafiadis, & Bakalbassis, 2006; Roche, Dufour, Mora, & Dangles, 2005).

Reaction mechanisms involved in free radical scavenging by (poly) phenols can be divided into two types of processes: H-atom abstraction and radical adduct formation (RAF). H-atom abstraction processes may occur *via* mechanisms such as hydrogen atom transfer (HAT), proton-coupled electron transfer (PCET), electron transfer followed by proton transfer (ET-PT), sequential proton loss electron transfer (SPLET), and sequential proton-loss hydrogen-atom transfer (SPLHAT) (Galano et al., 2016; Klein, Rimarčík, Senajová, Vagánek, & Lengyel, 2016). Previously published studies focused on the free radical scavenging have been mainly based on single, $1H^+/1e^-$ processes involving phenolic O—H group of a molecule (Galano et al., 2016; Leopoldini, Chiodo, Russo, & Toscano, 2011). However, depending on the number and positions of phenolic O—H groups and the presence of free radical scavenging moieties such as catechol moiety, guaiacyl moiety and carboxyl group, they may also proceed as double (multiple) sequential $1H^+/1e^-$ mechanisms (Anouar & Calliste et al., 2009; Cheng, Dai, Zhou, Yang, & Liu, 2007; Iwasaki, Cohen, & Witkop, 1963; Kozlowski et al., 2007). The catechol and guaiacyl moieties are potent H-atom/electron donors for the inactivation of free radicals. Recently, we have computationally investigated the role of catechol moiety (Amić et al., 2017) and carboxyl group (Amić, Lučić, Marković, & Amić, 2016) in free radical scavenging by colonic catabolites considering $2H^+/2e^-$ mechanisms.

The main goal of this research is to shed light on the possible processes underlying higher free radical scavenging activity of DHCA and DHFA in comparison to their unsaturated counterparts, CA and FA. To achieve this, we computationally investigated the role of catechol, guaiacyl and carboxyl moieties in the thermodynamics of the free radical scavenging proceeding *via* double, i.e., two sequential $1H^+/1e^-$ processes. The importance of catechol moiety to the free radical scavenging activity of (poly)phenols is well known (Amić et al., 2017; Dangles, 2012; Klein et al., 2016; Quideau et al., 2011), whereas the contribution of guaiacyl and carboxyl moieties remains to be fully elucidated.

2. Computational details

All calculations were performed using the Gaussian 09 program package (Frisch et al., 2013). Geometry optimizations and frequency calculations for studied acids and their radical cations, radicals, anions, radical anions, as well as for ten selected free radicals and their species involved in studied reactions were carried out using the M06-2X functional and the 6-311 + + G(d,p) basis set. The M06-2X has been chosen because it is one of the best performing functionals for modelling reaction energetics involving free radicals (Zhao & Truhlar, 2008). The influence of water and pentyl ethanoate as solvents was calculated using an implicit continuum solvation model – SMD (Marenich, Cramer, & Truhlar, 2009), which considers the full solute electron density in the estimation of energy of solvation. SMD is a universal solvation model and in conjunction with the M06-2X density functional has been successfully used for study of thermodynamics and kinetics of free radical scavenging mechanisms (Galano et al. 2016). Unrestricted calculations were used for open shell systems. No spin contamination was found for radical species, because before and after annihilation of the first spin contaminant the deviations from the correct value ($\langle S^2\rangle$ = 0.75 for singlet and $\langle S^2\rangle$ = 2.00 for triplet) were negligible. Therefore, the accuracy of energies of structures of open-shell systems are reliable. To analyze the electronic structures and the distribution of the unpaired electron in the radical species, natural bond orbital (NBO) analysis was performed using the NBO 5.9 software (Glendening et al., 2009). Enthalpies and free energies were calculated at 298.15 K. For the gas-phase total enthalpy of proton and electron, as well as for the solvation enthalpies of hydrogen atom, proton and electron, published values were employed (Tables S2a and S2b). Reaction enthalpies and free energies related to investigated free radical scavenging mechanisms were calculated using procedures described in our recently published reports (Amić et al., 2016, 2017).

3. Results and discussion

3.1. Validation of method applied

Over the past several decades, due to the lack of experimental data related to the reaction enthalpies involved in different free radical scavenging mechanisms of diverse (poly)phenolic compounds, antioxidant potency of these compounds has become the subject of intense theoretical research (Dangles et al., 2017; Galano et al., 2016; Klein et al., 2016). For this purpose, various methodologies have been used. To test the reliability of our approach, among scarce published experimental data related to (poly)phenolic compounds, we used BDE values of 18 substituted phenols determined in benzene by Lucarini, Pedrielli, Pedulli, Cabiddu, and Fattuoni (1996). As can be seen from Fig. S1, chosen level of theory (SMD/M06-2X/6-311 + + G(d,p)) may be considered reliable because the computed values of BDE in the same solvent are in very good agreement with experimental results (r = 0.993, s = 0.395).

3.2. Conformational analysis

Since the activity of different antiradical moieties of DHCA and DHFA could be greatly influenced by the geometry of the molecule, the conformation represents an important parameter affecting the antioxidant capacity of these molecules. Performed conformational analysis indicates that the energy differences between the most stable structures of *anti* and *syn* conformations of DHCA as well as of DHFA are small (< 0.5 kcal/mol) which indicate that corresponding structures interconvert easily at room temperature (Fig. 1). This indicates that both conformations could be nearly equally participating in the free radical scavenging. The analysis has been performed using water and pentyl ethanoate as solvents, representing biological liquids and membrane lipids, respectively, i.e., the natural environments for radical inactivation. In this report, particular attention was devoted to the antiradical potency of *syn* conformations of DHCA and DHFA, which has not been investigated so far. It should be noted that in the minimum energy structure both *trans*-CA and *trans*-FA are planar species, in accordance with published results (Leopoldini et al., 2011).

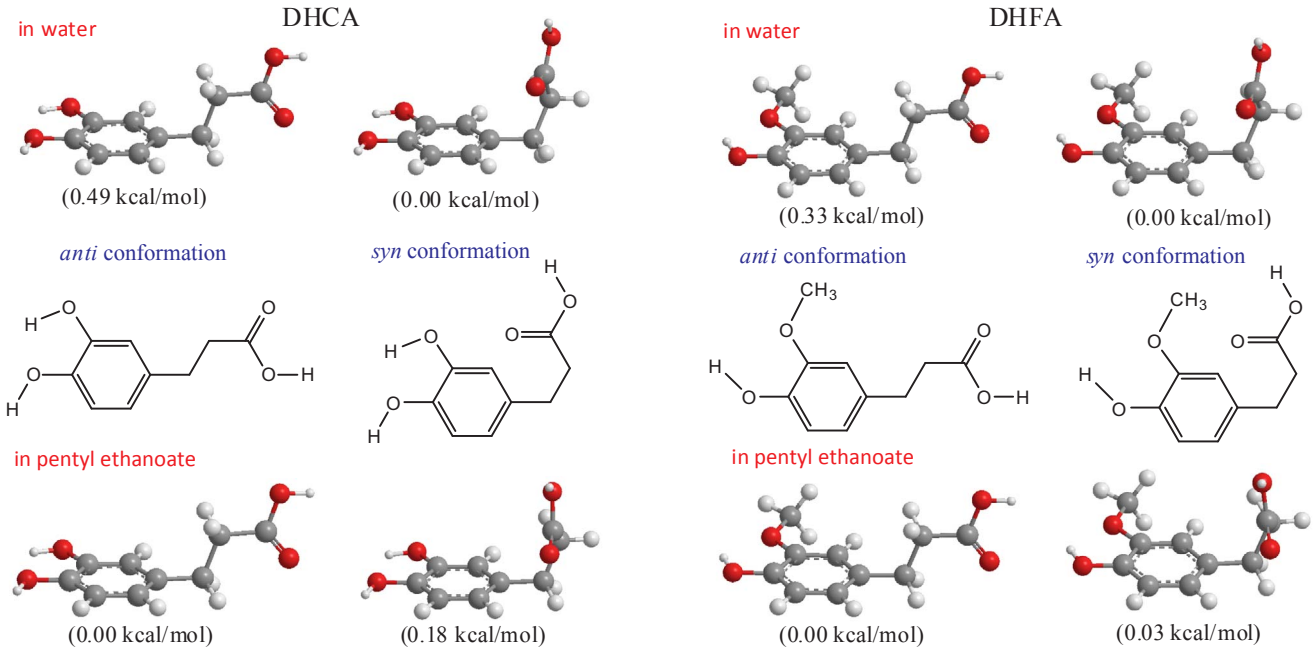

Fig. 1. Optimised structures of DHCA and DHFA calculated by using SMD/M06-2X/6-311 + + G(d,p) level of theory.

3.3. Free radical scavenging potency of cinnamic acid isomers and conformers

Published theoretical reports on the antiradical potency of cinnamic acids are dealing almost exclusively with *trans* isomers by considering single $1H^+/1e^-$ mechanisms *via* 4-OH phenolic group (Leopoldini et al., 2011; Lopez-Munguia et al., 2011; Nenadis, Zhang, & Tsimidou, 2003; Ordoudi et al., 2006). In the case of *trans*-CA, the $2H^+/2e^-$ mechanisms *via* catechol moiety were also investigated (Amić et al., 2017; Cheng et al., 2007; Hotta et al., 2002). CA and FA exist in the *cis* or *trans* form because of the presence of a vinyl group in the side chain. Absorption of energy can unpair the π-electrons in the π-bond of a vinyl group. After rotation around C—C single bond of the resulting diradical by 180°, the unpaired electrons can pair up again. In this way, the other geometric isomer is formed. Both isomers were detected in the gastrointestinal tract after ingestion of radiolabelled *trans*-CA (Omar et al., 2012).

As mentioned in the *Introduction* section, experimental results revealed DHCA and DHFA as more potent free radical scavengers than CA and FA (Table S1). Using classical approach (single H-atom donation from phenolic OH group), these differences could be explained by means of the BDE, a primary descriptor of antioxidant action thermodynamics. A lower BDE value indicates more potent antioxidant molecule. In Table 1 calculated BDE1 values (in kcal/mol) for phenolic 4-OH group of *syn* and *anti* conformations of DHCA and DHFA, as well as of *cis* and *trans* isomers of CA and FA in polar (water) and non-polar (pentyl ethanoate) medium are presented.

In water, calculations were performed for both neutral and anionic (carboxylate) form of acids, while in pentyl ethanoate only for neutral molecules. Results presented in Table 1 enable estimation of the contribution of electron donating (EDG) and electron-withdrawing groups (EWG) to the O—H BDE1 values. As expected, EWG causes a decrease in the antioxidant activity due to the increase in BDE. EDG shows the opposite effect (Bakalbassis, Lithoxoidou, & Vafiadis, 2006). Saturated alkyl group (EDG) in the side chain of *syn/anti* conformations induces the decrease in the O—H BDE1 up to 1.62 kcal/mol in comparison to propenyl group (EWG) of *cis/trans* isomers. Easier hydrogen atom abstraction (hence, more efficient free radical scavenging) in the case of species with saturated side chain is in accordance with experimental results (Table S1). Similarly, in water at physiological pH of 7.4, carboxylate anion (EDG) of studied cinnamic acids causes decrease in the O − H BDE1 by up to 2.15 kcal/mol, in comparison with carboxyl group (EWG) of non-dissociated acids.

On average, *syn* and *anti* conformations of dihydrocinnamic acids have ~1 kcal/mol lower BDE1 values than *cis* and *trans* isomers of corresponding unsaturated analogs. Although these slight differences in 4-OH BDE1 are in line with observed experimental results, it seems that

Table 1
Bond dissociation enthalpy (BDE) values (in kcal/mol) of studied cinnamic acid derivatives in water (neutral molecule and carboxylate ion) and pentyl ethanoate (neutral molecule).

	Water					Pentyl ethanoate		
	−COOH			$-COO^-$		−COOH		
a)	BDE1[a]	BDE2[b]	BDE2[c]	BDE1[a]	BDE2[b]	BDE1[a]	BDE2[b]	BDE2[c]
syn-DHCA	80.81	70.60	66.36	80.13	70.16	79.71	71.06	66.36
anti-DHCA	80.87	69.63	–	80.13	69.44	79.98	70.64	–
cis-CA	82.06	71.73	–	79.93	71.76	81.29	72.30	–
trans-CA	82.47	73.26	–	80.46	72.61	81.09	74.05	–
Average values								
syn/anti-DHCA	80.84	70.11	66.36	80.13	69.80	79.84	70.85	66.36
cis/*trans*-CA	82.26	72.49	–	80.19	72.18	81.19	73.17	–
ΔBDE	1.42	2.38	–	0.06	2.38	1.35	2.32	–
b)	BDE1[a]	BDE2[d]	BDE2[c]	BDE1[a]	BDE2[d]	BDE1[a]	BDE2[d]	BDE22[c]
syn-DHFA	82.46	45.35	64.85	81.75	45.63	85.46	43.76	64.09
anti-DHFA	82.31	45.54	–	81.62	45.95	85.41	43.60	–
cis-FA	83.85	44.50	–	81.70	46.50	86.52	43.91	–
trans-FA	84.15	44.29	–	82.13	46.10	86.20	43.48	–
Average values								
syn/anti-DHFA	82.38	45.44	64.85	81.68	45.79	85.43	43.68	64.09
cis/*trans*-FA	84.00	44.39	–	81.91	46.30	86.36	43.69	–
ΔBDE	1.62	−1.05	–	0.23	0.51	0.93	0.01	–

a) ΔBDE = [BDE(*cis*-CA) + BDE(*trans*-CA)] − [BDE(*syn*-DHCA) + BDE(*anti*-DHCA)].
b) ΔBDE = [BDE(*cis*-FA) + BDE(*trans*-FA)] − [BDE(*syn*-DHFA) + BDE(*anti*-DHFA)].
[a] 4-OH group.
[b] Catechol moiety.
[c] Carboxyl group.
[d] Guaiacyl moiety.

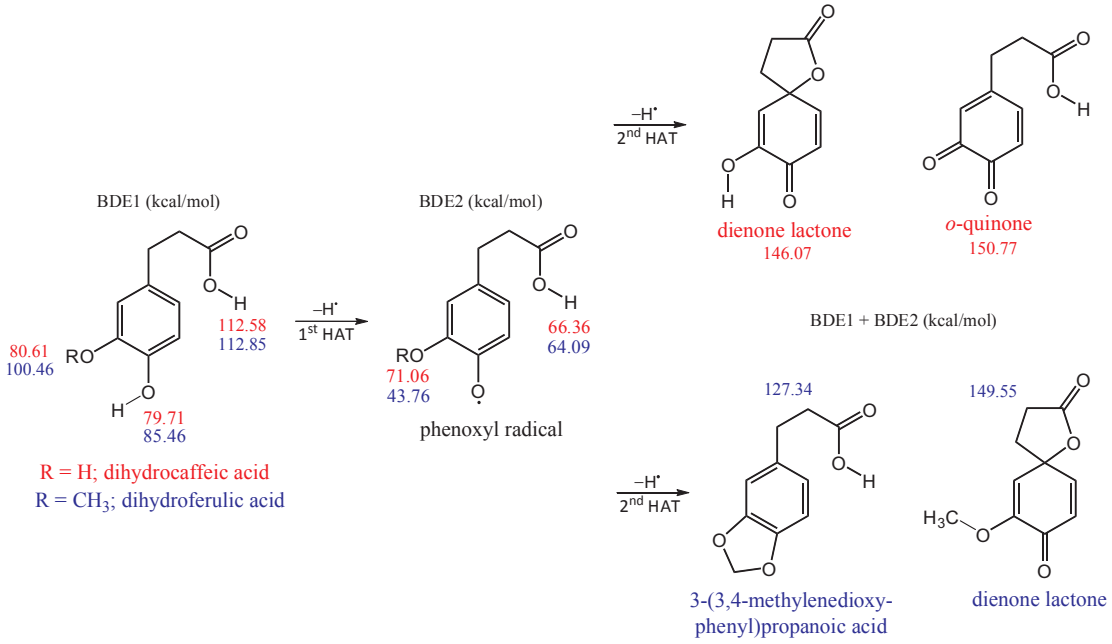

Fig. 2. dHAT mechanism of *syn*-DHCA and *syn*-DHFA in pentyl ethanoate.

antioxidative activity of studied cinnamic acid derivatives is not governed only by phenolic OH group(s). Namely, 4-OH group is not the only structural motif which may affect the free radical scavenging by investigated cinnamic acids. By visual inspection of studied acids structures, it could be expected that due to the presence of catechol, guaiacyl and carboxyl moieties, phenoxyl radical formed after the first H-atom transfer (from 4-OH group) may scavenge another free radical by the second H-atom donation from 3-OH, 3-OCH_3 or −COOH group (Amić et al., 2017; Anouar & Calliste et al., 2009; Iwasaki et al., 1963; Kozlowski et al., 2007). BDE2 values of the second H-atom transfer from those groups are also included in Table 1. It is obvious that BDE2 values are sufficiently less energy demanding to provide a driving force for the second H-atom abstraction. In the case of catechol moiety of DHCA and CA isomers, BDE2 values in both, polar and non-polar, media are in average by ~10 kcal/mol lower. These differences are more pronounced for guaiacyl moiety of DHFA and FA isomers: on average they amount ~39 kcal/mol. Moreover, involvement of carboxyl group of *syn*-DHCA in second H-atom donation reduces energy cost by additional ~5 kcal/mol (in comparison with catechol moiety). On the contrary, involvement of carboxyl group of *syn*-DHFA increases energy cost by ~20 kcal/mol (in comparison with guaiacyl moiety). Obviously, double H-atom processes should be considered to ascertain the free radical scavenging potency of investigated cinnamic acid derivatives.

3.4. dHAT mechanisms of syn-DHCA and syn-DHFA

Fig. 2 presents the results of calculations for double HAT mechanisms by which *syn*-DHCA and *syn*-DHFA may scavenge free radicals in non-polar medium. Calculated BDE1 values in both acids indicate as the most abstractable H-atom that one from phenolic 4-OH group and as the less abstractable H-atom that from −COOH group. It should be emphasized that energy cost of second H-atom abstraction is considerably reduced, particularly from phenoxyl radical of *syn*-DHFA.

Hydrogenation of the C=C double bond of cinnamic acids by gut microbiota enables participation of carboxyl group in free radical scavenging. Abstraction of the H-atom from carboxyl group of phenoxyl radical results in the formation of dienone lactone (Iwasaki et al., 1963). This is operative in the case of *syn*-DHCA and *syn*-DHFA in contrast to *cis*-CA and *cis*-FA. Our calculations indicate that a completely planar conformation of *cis*-CA has by 1.02 kcal/mol higher energy than the two low-energy conformations in which propenoic side chain lies out of plane of benzene ring by ± 34.5° in water and ± 6.1° in pentyl ethanoate, respectively. Deviation from planarity is similar in *cis*-FA where C_3 side chain lies out of plane of benzene ring by ± 32.5° and ± 6.2° in water and pentyl ethanoate, respectively (Fig. S2). Consequently, the rigid C_6-C_3 sp^2-hybridized backbone of *cis*-CA and of *cis*-FA with moderate deviation from planarity, stabilized through π-electron delocalization, does not allow cyclization contrary to flexible sp^3-hybridized side chain of *syn*-DHCA and *syn*-DHFA which by folding of −$COO^{\cdot}$ group (formed after second H-atom abstraction) towards the phenyl ring enables the formation of dienone lactone (Iwasaki et al., 1963). Such kind of double H-atom transfer from *syn*-DHCA and *syn*-DHFA may contribute to free radical scavenging activity. This is in line with results of Silva et al. (2000) who stated that molecular conformation of the (poly)phenolic compounds could be one of the factors affecting their antiradical activity and found that blocking of −COOH group of DHCA by esterification markedly led to a huge decrease in its scavenging activity.

For *syn*-DHCA in pentyl ethanoate, the second BDE (H-atom abstraction from 3-OH group of phenoxyl radical, BDE2 = 71.06 kcal/mol) is by 8.65 kcal/mol less energy demanding than the first BDE (H-atom abstraction form 4-OH group of *syn*-DHCA, BDE1 = 79.71 kcal/mol) (Fig. 2). Lower BDE2 value indicates that second HAT mechanism should be even more rapid than the first one (Hussain et al., 2003). On average, *syn* and *anti* conformations of DHCA have BDE2 values lower by 2.36 kcal/mol than *cis* and *trans* isomers of CA (Table 1), in accordance with experimentally observed higher antiradical activity of DHCA. The high free radical scavenging capacity of phenoxyl radical of *syn*-DHCA arises from the fact that product formed after the second HAT is stabilized in a singlet state and not in a triplet state. As earlier emphasized by Anouar and Calliste et al. (2009), such stabilization in a singlet state favours double HAT mechanism. Our calculations indicate that singlet state of produced *o*-quinone is by 33.06 kcal/mol more stable than the triplet state (Fig. S3a).

H-atom abstraction from −COOH group is by 71.06–66.36 = 4.70 kcal/mol less energy demanding in comparison to 3-OH group (resulting in formation of *o*-quinone) and singlet state of formed dienone lactone is by 47.46 kcal/mol more stable than the triplet state (Fig. S3b). All those facts undoubtedly indicate that

thermodynamically preferred product of second H-atom abstraction from phenoxyl radical of *syn*-DHCA is dienone lactone. In this way, free radical scavenging by *syn*-DHCA may avoid production of potentially harmful electrophilic *o*-quinone which may contribute to DNA damage by forming covalent adducts with DNA and thereby exerting mutagenic effects (Quideau et al., 2011). It arises that higher *in vitro* antiradical activity of DHCA in comparison with CA is governed by lower BDE2 value of reaction path by which *syn*-DHCA produces dienone lactone. Poquet et al. (2008) found that during the phase II metabolism of DHCA conjugation reactions to form methylated, sulfated, and glucuronidated derivatives are regioselective, preferably on the 3-OH group. Consequently, all conjugates retain free 4-OH group which in the case of *syn*-DHCA enables free radical scavenging *via* $2H^+/2e^-$ mechanisms resulting in dienone lactone. The methyl conjugate, DHFA, may be oxidized to FA, and the reverse reaction, i.e. reduction of FA to DHFA, may also occur. Thus, the main form of DHCA circulating in plasma is a mixture of different conjugates and catabolites.

In the case of *syn*-DHFA, abstraction of the second H-atom from 3-OCH_3 group (BDE2 = 43.76 kcal/mol) of the phenoxyl radical is significantly less energy demanding than from carboxyl group (BDE2 = 64.09 kcal/mol). Accordingly, thermodynamically favoured product is 3-(3,4-methylenedioxyphenyl)propanoic acid, i.e., product obtained *via* double HAT mechanism which embraces guaiacyl moiety. Such cyclization naturally occurs in plants, confirming that this reaction path is chemically feasible (Kozlowski et al., 2007). Singlet state of produced 3-(3,4-methylenedioxyphenyl)propanoic acid is by 59.40 kcal/mol more stable than the triplet state (Fig. S4a). Thermodynamically less probable product is corresponding dienone lactone because of additional energy cost of 149.55 − 127.34 = 22.21 kcal/mol (Fig. 2). Singlet state of the resulting dienone lactone is by 49.59 kcal/mol more stable than the triplet state (Fig. S4b).

The distribution of spin density was considered to determine potential for delocalization and consequently the stability of investigated *syn*-DHCA and *syn*-DHFA phenoxyl radicals. The spin density values, obtained by the NBO analysis are depicted in Fig. S5. Obtained values of the spin density clearly reveal that the unpaired electron is similarly delocalized in both radicals. Actually, the oxygen atom is the most probable radical center (spin density 0.34 and 0.35 for *syn*-DHCA and *syn*-DHFA in pentyl ethanoate), the rest of the spin density is delocalized over the *para* and *ortho* carbons of aromatic ring and slightly over oxygen of hydroxyl and methoxy group. High delocalization of the unpaired electron indicates low probability of radical inactivation by dimerization and justifies the study of double processes.

Feasibility of involvement of guaiacyl moiety in double H-atom donation can be supported by the fact that simple guaiacol molecule scavenge 2 molecules of $DPPH^{\cdot}$ as has been experimentally determined by Brand-Williams, Cuvelier, and Berset (1995). Possible underlying mechanism has been proposed by Nenadis and Sigalas (2008), based on calculations performed using IEF-PCM/B3LYP/6-311 + + G(2d,2p) level of theory. After the first H-atom transfer from phenolic OH group (BDE1 = 83.5 kcal/mol), the methoxy group of guaiacyl moiety donates the second H-atom forming cyclic compound 1,3-benzodioxole with the energetic cost of second process of only 44.0 kcal/mol (BDE2). Our corresponding values are 86.81 kcal/mol and 42.47 kcal/mol (for BDE1 and BDE2) respectively, calculated in pentyl ethanoate as a solvent.

3.5. dSPLET mechanisms of syn-DHCA and syn-DHFA

Examination of SPLET mechanism, which is generally feasible in polar ionization supporting media, indicates that *syn*-DHCA may scavenge free radicals by involvement of phenolic 4-OH group and carboxyl group *via* sequential double proton loss double electron transfer (SdPLdET) and by involvement of catechol moiety *via* sequential triple proton loss double electron transfer (StPLdET) pathway. Former mechanism results in the formation of dienone lactone and the latter in the formation of *o*-quinone. Corresponding steps of these reaction paths can be characterized by proton affinity (PA) and electron transfer enthalpy (ETE). Obtained results are summarized in Fig. 3.

Both SdPLdET and StPLdET mechanisms start with deprotonation of the most acidic, i.e., carboxyl group which has the lowest PA value (16.47 kcal/mol). Then second deprotonation occurs from 4-OH group of dihydrocaffeate which is energetically more favourable (PA2 = 24.07 kcal/mol) than electron transfer from $-COO^-$ group (ETE = 101.19 kcal/mol). Third step is electron transfer from the dianion leading to the formation of radical anion. Electron transfer from $-COO^-$ group of radical anion terminates SdPLdET mechanism by production of dienone lactone (Fig. 3, upper part). Overall energetic cost of this reaction path amounts 182.38 kcal/mol (PA1 + PA2 + ETE1 + ETE2 = 16.47 + 24.07 + 73.66 + 68.18 = -182.38). Another possible reaction path, StPLdET, which proceeds *via* catechol moiety, continues by deprotonation of 3-OH group of formed radical anion and terminates by the electron transfer from radical dianion (Fig. 3, lower part). The final product of this mechanism is *o*-quinone. Total energetic cost for StPLdET mechanism amounts: PA1 + PA2 + ETE1 + PA3 + ETE2 = 16.47 + 24.07 + 73.66 + -16.47 + 71.30 = 201.97 kcal/mol.

In an analogous way *syn*-DHFA may scavenge free radicals by involvement of carboxyl group *via* SdPLdET and guaiacyl moiety *via* StPLdET processes (Fig. 4).

As it can be deduced from the upper part of Fig. 4, the overall energetic cost of SdPLdET mechanism by which *syn*-DHFA may inactivate free radicals amounts 182.51 kcal/mol. This is a very close value to the corresponding value for *syn*-DHCA (182.38 kcal/mol). StPLdET mechanism which proceeds *via* guaiacyl moiety of *syn*-DHFA continues by reorientation of the $-OCH_3$ group of the radical anion (Fig. 4, lower part). Such reorientation, at the cost of 4.45 kcal/mol, favours the formation of the cyclic compound, i.e., 3-(3,4-methylenedioxyphenyl) propanoate as the final product. This occurs *via* proton transfer from the twisted methoxy group followed by the electron transfer from radical dianion as the ultimate step. Examination of the lower part of the Fig. 4 shows total energetic cost of StPLdET mechanism amounts: PA1 + PA2 + ETE1 + RE + PA3 + ETE2 = 16.45 + 27.47 + 71.89 + 4.45 + 39.48 + 19.30 = 179.04 kcal/mol.

3.6. dET-PT mechanisms of syn-DHCA and syn-DHFA

We have also considered double electron transfer followed by proton transfer (dET-PT) mechanism, for both *syn*-DHCA and *syn*-DHFA, in water as a solvent. Corresponding steps of this mechanism can be characterized by ionization potential (IP) and proton dissociation enthalpy (PDE). Obtained results for *syn*-DHCA are presented in Fig. S6. The first step is the transfer of an electron from *syn*-DHCA by which the radical cation is formed. The second step is deprotonation of radical cation (i.e. proton loss from 4-OH group with the lowest PDE1 value) which results in the formation of phenoxyl radical. The second electron transfer results in the formation of cationic intermediate. In the final step, two competitive proton transfers from cationic intermediate may occur: deprotonation of $-COOH$ group gives dienone lactone (Fig. S6, upper part), while deprotonation of 3-OH group gives *o*-quinone (Fig. S6, lower part). Total energy requirement for dET-PT mechanism *via* carboxyl group amounts 182.38 kcal/mol (IP1 + PDE1 + IP2 + PDE2 = 103.19 − 4.77 + 98.83 − 14.87). As expected, because the reactants and products are the same, this is equal energetic cost as in the case of SdPLdET mechanism which embraces 4-OH group and carboxyl group (Fig. 3). Total energy requirement for dET-PT mechanism *via* catechol moiety (IP1 + PDE1 + IP2 + PDE2 = 103.19 − 4.77 + 98.83 − 10.63) amounts 186.62 kcal/mol. Addition of the energetic cost of deprotonation of *o*-quinone (15.36 kcal/mol) to that value results in the same sum as in the case of StPTdET mechanism (201.97 kcal/mol). The first step of studied dET-PT mechanisms is much more energetically demanding (IP1 = 103.19 kcal/mol) than the first step of SdPLdET mechanisms (PA1 = 16.47 kcal/mol),

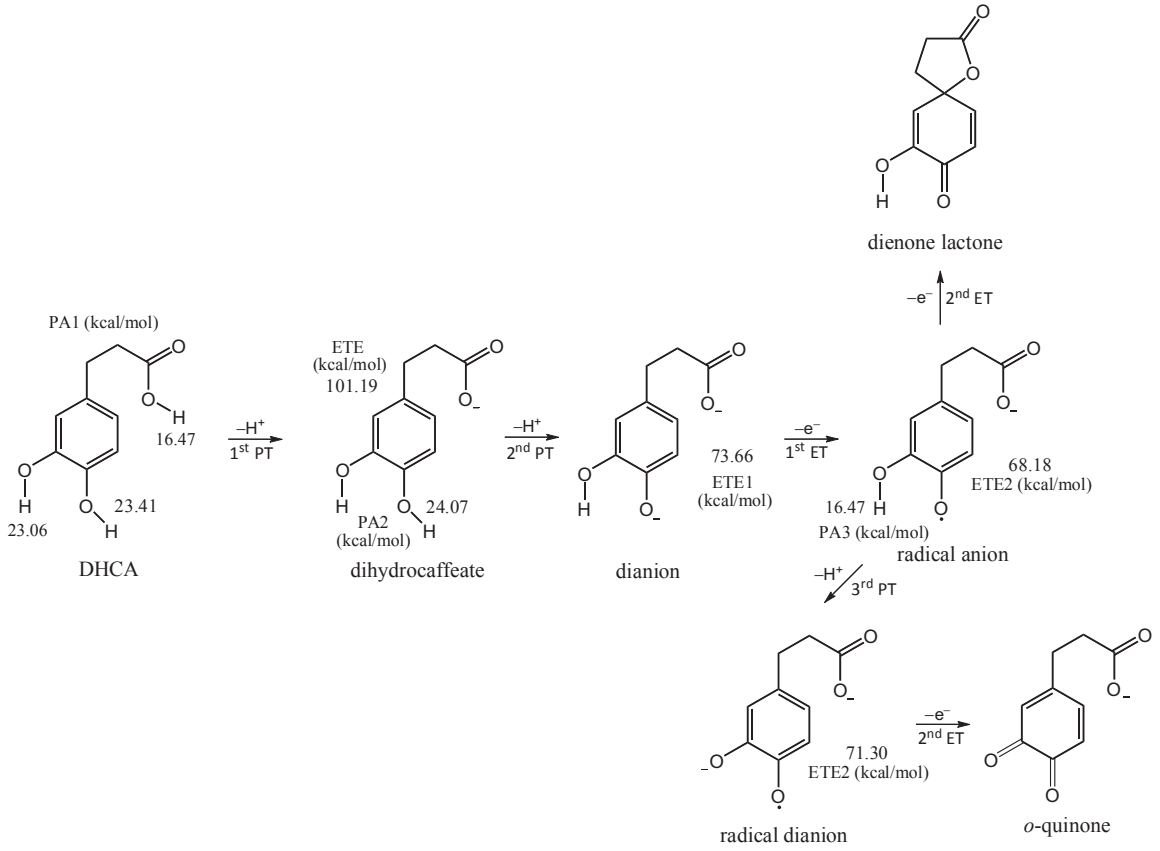

Fig. 3. Reaction paths of SdPLdET mechanism of *syn*-DHCA which proceed by involving carboxyl group (upper part) and catechol moiety (bottom part) in water.

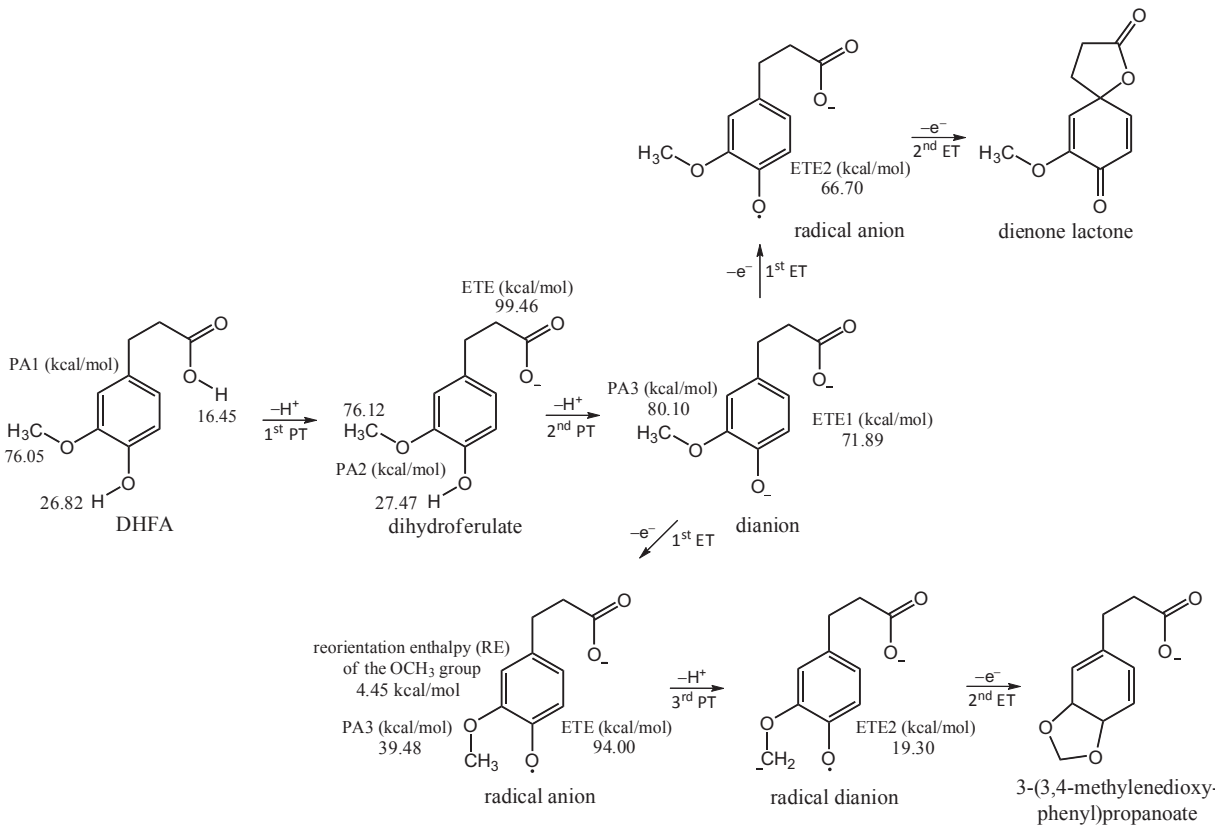

Fig. 4. Reaction paths of SdPLdET mechanism of *syn*-DHFA which proceed by involving carboxyl group (upper part) and guaiacyl moiety (bottom part) in water.

indicating SdPLdET mechanism as thermodynamically favoured.

Results for dET-PT mechanisms by which *syn*-DHFA may scavenge free radicals are summarized in Fig. S7: the upper part shows production of dienone lactone *via* 4-OH group and carboxyl group, and the lower part shows production of 3-(3,4-methylenedioxyphenyl)propanoic acid *via* guaiacyl moiety. Phenoxyl radical of *syn*-DHFA produced after the first $1e^-/1H^+$ process may undergo the second $1e^-/1H^+$ process. Cationic intermediate formed by second ET may produce dienone lactone by deprotonation of −COOH group (Fig. S7, upper part). If second electron transfer occurs from phenoxyl radical with twisted methoxy group, then $-OCH_3$ group of cationic intermediate deprotonates yielding 3-(3,4-methylenedioxyphenyl)propanoic acid (Fig. S7, lower part). Total energy requirements of the dET-PT mechanism which terminates *via* carboxyl group amounts 182.51 kcal/mol, as in the case of SdPLdET mechanism (IP1 + PDE1 + IP2 + PDE2 = PA1 + PA2 + ETE1 + ETE 2). As for *syn*-DHCA, thermodynamically feasible mechanisms in water is SdPLdET because PA1 (16.45 kcal/mol) is much less energy demanding than IP1 (101.62 kcal/mol). dET-PT and StPLdET processes *via* guaiacyl moiety are also isoenergetic proviso that final product of both mechanisms is the same. Again, StPLdET is thermodynamically preferred mechanism. Obtained results unequivocally indicate that electron transfer from neutral molecule of *syn*-DHCA and *syn*-DHFA is highly thermodynamically unfavorable and that proposed hypothetical dET-PT mechanism is not relevant in water, where the two acids under physiological conditions exist as carboxylate anions.

3.7. Estimation of the reliability of calculated reaction enthalpies

To compare our calculated reaction enthalpies describing studied free radical scavenging mechanisms with the published ones, we have first considered some of the theoretical approaches which were tested on simple phenols for which experimental results are available. Among reaction enthalpies, mostly BDE is experimentally determined. BDE (related to HAT) can serve as a general parameter of free radical scavenging potency because it is entirely correlated with the energy cost for SET-PT (*via* sum of IP and PDE) as well as for SPLET (*via* sum of PA and ETE) mechanism (Amić et al., 2016). In extensive study, Trouillas, Marsal, Siri, Lazzaroni, and Duroux (2006), by comparing 3 different methods, 4 different functionals and 7 different basis sets found that (U)B3P86/6-311 + G(d,p) is the most relevant method for reproduction of experimentally determined BDE values for phenol and catechol. By using this method at the + G(d,p) level, Anouar et al. (2009b), for *trans*-FA in water, estimated BDE1 value of 83.3 kcal/mol. Our value of 84.15 kcal/mol obtained using SMD/M06-2X/6-311 + + G(d,p) level of theory nicely matches this value (Table 1). Galano et al. (2016) compared 14 different levels of theory by using 6-311 + G(d,p) basis set in benzene to reproduce BDE value of phenol. Among the different functionals tested, M05-2X and M06-2X gave the best agreement with experimental data. Using SMD/M05-2X/6-311 + G(d,p) level of theory in benzene, for *trans*-CA they obtained BDE value of 78.8 kcal/mol which is in good agreement with the value of 80.65 kcal/mol that we obtained in the same solvent. Recently, our SMD/M05-2X/6-311 + + G(d,p) calculations in benzene, nicely reproduced experimental BDE value of phenol determined in the same solvent (88.62 kcal/mol *vs* 88.3 kcal/mol, respectively) (Amić et al., 2017). For carboxylate anions of *trans*-CA and *anti*-DHCA in water using the same method we obtained BDE values of 80.34 kcal/mol and 79.61 kcal/mol. These are very close to the values obtained in the present work, i.e., 80.46 kcal/mol and 80.13 kcal/mol, respectively (Table 1). Secondly, we compared our results with some gas-phase ones. Borpuzari, Rohman, and Kar (2015) published BDE, IP, PDE, PA and ETE values for *trans*-CA in the gas-phase at LC-BLYP/6-311 + + G(3df,2p) level of theory. Additionally, they calculated BDE at nine other levels of theory. M06-2X functional provided BDE of 78.77 kcal/mol. For *trans*-FA, Nenadis et al. (2003) using B3LYP/6-311 + G(2d,2p) approach, obtained gas-phase value of 84.32 kcal/mol. Corresponding BDE values obtained by our approach amount to 81.09 kcal/mol and 86.20 kcal/mol for *trans*-CA and *trans*-FA in pentyl ethanoate, respectively. This is in line with the published results that the gas-phase BDEs of polyphenolics are close but usually lower than solution ones (Klein et al., 2016).

3.8. Influence of the free radicals nature on scavenging potency of syn-DHCA and syn-DHFA

Mechanisms of free radical scavenging depend on many factors among which the characteristics of the free radical play a significant role. We have selected the set of ten free radicals of different nature: $HO^{\cdot}$ (hydroxyl radical), $HOO^{\cdot}$ (hydroperoxyl radical), $CH_3O^{\cdot}$ (methoxyl radical), $(CH_3)_3CO^{\cdot}$ (*t*-butoxyl radical), $PhO^{\cdot}$ (phenoxyl radical), $O_2^{\cdot -}$ (superoxide radical anion), $CH_3-OO^{\cdot}$ (methyl peroxyl radical), $CH_2{=}CH-OO^{\cdot}$ (vinyl peroxyl radical), $CH_2{=}CH-CH_2-OO^{\cdot}$ (allyl peroxyl radical), and $Cl_3C-OO^{\cdot}$ (trichloromethyl peroxyl radical). Some of them can be involved in *in vivo* processes (Galano et al., 2016). Among others, we are dealing with the most biologically damaging $HO^{\cdot}$, although some authors discourage that as meaningless because of its exceptionally high reactivity and low selectivity, i.e. $HO^{\cdot}$ can abstract an electron or H-atom from almost any compound in its vicinity.

The calculated free energy of reactions (Δ_rG^o) of *syn*-DHCA and *syn*-DHFA with studied free radicals considering catechol, guaiacyl and carboxyl group as reaction centers for dHAT, dET-PT and SdPLdET mechanisms in water and pentyl ethanoate are compiled in the Tables S3–S10. All studied mechanisms have equal energy requirements ($\Delta_rG^o_{dHAT} = \Delta_rG^o_{dET\text{-}PT} = \Delta_rG^o_{SdPLdET}$, i.e., $\Delta_rG^o_{dHAT1} + \Delta_rG^o_{dHAT2} = \Delta_rG^o_{dET\text{-}PT1} + \Delta_rG^o_{dET\text{-}PT2} = \Delta_rG^o_{SdPLdET1} + \Delta_rG^o_{SdPLdET2}$) and consequently may occur simultaneously. Thermodynamically preferred mechanism could be deduced from the energetic cost of the first step in radical inactivation: $\Delta_rG^o_{BDE1}$, $\Delta_rG^o_{IP1}$ and $\Delta_rG^o_{PA1}$. The most exergonic process is the preferred one. It should be also noted that the sum of $\Delta_rG^o_{dBDE1}$ and $\Delta_rG^o_{dBDE2}$ related to the first and second HAT mechanisms, respectively, assigned as $\Delta_rG^o_{dBDE}$, given in the last column of the Tables S3–S10, represents total energy cost of all studied mechanisms. Closer look at Tables S3–S10, where studied free radicals are tabulated in descending order of $\Delta_rG^o_{dBDE1}$ and $\Delta_rG^o_{dBDE2}$ values, clearly shows that the second $1H^+/1e^-$ processes are more exergonic.

Besides exergonic processes, processes with low endergonicity (< 10 kcal/mol) could also be thermodynamically feasible proviso that they occur at significant rate (Perez-Gonzalez, Alvarez-Idaboy, & Galano, 2015). Obtained results indicate *syn*-DHCA and *syn*-DHFA as potent free radical scavengers. Their reactivity toward free radicals was predicted to decrease as follows: hydroxyl > > alkoxyls > phenoxyl ≈ peroxyls > > superoxide (Figs. 5 and S8, Tables S3–S10). Generally, because the first step of the dET-PT mechanism ($\Delta_rG^o_{IP1}$) was found to be endergonic in both solvents, this mechanism can be discarded as inoperative. It may be probable only in scavenging of highly electrophilic $HO^{\cdot}$ and $Cl_3C-OO^{\cdot}$ in water environment. dHAT and SdPLdET mechanisms are thermodynamically feasible because of negative or low positive values of $\Delta_rG^o_{BDE}$, $\Delta_rG^o_{PA}$ and $\Delta_rG^o_{ETE}$. In the case of relatively low reacting $O_2^{\cdot -}$ (conjugated base of $HOO^{\cdot}$), the first $1H^+/1e^-$ processes are highly endergonic ($\Delta_rG^o_{HAT1} \geq 10$ kcal/mol) in both polar and non-polar environments (Tables S3–S10). Accordingly, it could be predicted that *syn*-DHCA and *syn*-DHFA are unable to effectively scavenge this free radical *via* any of studied mechanisms.

Regardless of reaction path (*via* catechol moiety or carboxyl group), the first step of dHAT mechanism by which *syn*-DHCA scavenges free radicals takes place on 4-OH group, and consequently has equal energy requirement (equal $\Delta_rG^o_{BDE1}$). The $\Delta_rG^o_{BDE2}$ value, related to second step, differentiates total energy cost. As can be estimated from Fig. S8 and from Tables S5 and S6, reaction path *via* carboxyl group (final product dienone lactone) is on average by 2 kcal/mol less energy demanding than reaction path *via* catechol moiety (final product *o*-quinone), Tables S3 and S4. This difference is more pronounced in the case of *syn*-DHFA.

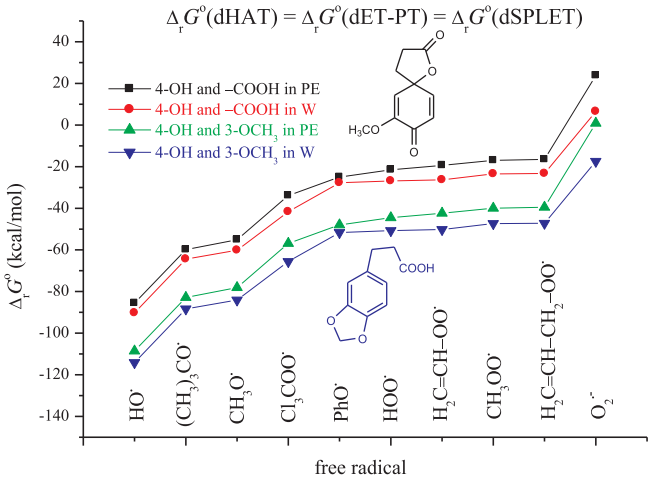

Fig. 5. Overall energy requirements for scavenging of selected free radicals *via* dHAT, dET-PT and SdPLdET by *syn*-DHFA.

In the first step the 4-OH group of *syn*-DHFA scavenges free radicals, and the second step makes differences. Reaction path which terminates *via* guaiacyl moiety (final product 3-(3,4-methyledioxyphenyl)propanoic acid) is on average by 23 kcal/mol more exergonic than reaction path *via* carboxyl group (final product dienone lactone) (Fig. 5 and Tables S7–S10). Because of equal total energy requirements for all studied mechanisms for particular reaction path (equal $\Delta_r G^o_{dBDE}$), the results described above are also identical for dET-PT and SdPLdET mechanisms (regardless of initial deprotonation site, −COOH or 4-OH). Obtained results also indicate that double $2H^+/2e^-$ mechanisms are less energy demanding in polar (water) environment, in accordance with published results (Amić et al., 2017).

As our recent reports emphasize (Amić et al., 2016, 2017), thermodynamic approach is valuable, but more complete picture of antiradical potency requires additional kinetic analysis. Understanding kinetics of the reactions involved in free radical scavenging is of particular significance because free radicals are very short-lived species, what implies that the impact of an antioxidant depends on its high reactivity towards free radicals. The higher the rate of the scavenging reaction the lower the extent of the damage of biological macromolecules. As was previously noted, slightly endergonic free radical scavenging reaction should not be *a priori* neglected. It could be operative if it takes place at a significant rate. Moreover, product generated *via* highly endergonic reaction may not be experimentally determined, even if it takes place at a significant rate (Perez-Gonzalez et al., 2015). Because it has been shown that a very good linear correlation may exist between log *k* and the BDE of phenolic antioxidants (Hussain et al., 2003), it could be expected that kinetic results will be in line with the thermodynamic analysis presented here.

4. Conclusions

In this paper, the natural antioxidants present in the body (DHCA, DHFA, CA, and FA) were computationally studied in terms of the thermodynamics of their primary antioxidant activity and their reactivity towards different free radicals. Theoretical background for experimentally observed better antioxidative activity of DHCA and DHFA in comparison with CA and FA was investigated. The role of molecular conformation in antiradical activity was investigated and discussed. Obtained results indicate that the presence of structural motifs such as the catechol or guaiacyl moiety and carboxyl group in *syn*-DHCA and *syn*-DHFA is beneficial for the high scavenging efficiency of investigated phenolic compounds toward free radicals. These antiradical moieties enable inactivation of free radicals *via* different paths of double $(2H^+/2e^-)$ free radical scavenging mechanisms. Gibbs free energies of reactions of inactivation of various free radicals indicate that *syn*-DHCA and *syn*-DHFA, colon catabolites found in systemic circulation in very low μM concentrations, have potential to contribute to health benefits by direct free radical inactivation.

Acknowledgements

This work was supported by The Foundation of the Croatian Academy of Science and Arts, under the project No. 10-102/244-1-2016, by the Ministry of Science of the Republic of Serbia (Projects No. 172015 and 174028), and the Slovak Grant Agency (VEGA 1/0594/16 and 1/0416/17).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.11.100.

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