A theoretical study of the structure-radical scavenging activity of trans-resveratrol analogues and cis-resveratrol in gas phase and water environment

3 AUTHORS, INCLUDING:

Rafał Górniak
Adam Mickiewicz University
2 PUBLICATIONS   61 CITATIONS

SEE PROFILE

Marcin Molski
Adam Mickiewicz University
116 PUBLICATIONS   797 CITATIONS

SEE PROFILE

Original article

A theoretical study of the structure–radical scavenging activity of trans-resveratrol analogues and cis-resveratrol in gas phase and water environment

Damian Mikulski [a,*], Rafał Górniak [b], Marcin Molski [a]

[a] *Department of Theoretical Chemistry, Faculty of Chemistry, A. Mickiewicz University ul. Grunwaldzka 6, 60-780 Poznań, Poland*
[b] *Department of Physical Chemistry, Faculty of Chemistry, A. Mickiewicz University ul. Grunwaldzka 6, 60-780 Poznań, Poland*

ABSTRACT

Quantum calculations based on the density functional theory (DFT) have been employed to study the relation between the structure and antioxidant activity of trans-resveratrol (TR), cis-resveratrol (CR), trans-4.4′-dihydroxystilbene (trans-4,4′-DHS), trans-3,4-dihydroxystilbene (trans-3,4-DHS), trans-3,4,4′-trihydroxystilbene (trans-3,4,4′-THS), trans-3,4,5-trihydroxystilbene (trans-3,4,5-THS) and α,β-dihydro-3,4′,5-trihydroxystilbene (α,β-dihydro-3,4′,5-THS) in the gas phase and water environment. We have shown that the antioxidant activity of trans-stereoisomers of the compounds considered is related to their phenoxy radicals showing a planar and semiquinone conformation that allows delocalization of the unpaired electron through the whole trans-stilbene skeleton. The calculations have revealed that trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS and trans-4,4′-DHS exhibit higher antioxidant activity than TR, while α,β-dihydro-3,4′,5-THS and CR are less efficient antioxidants than TR. We have proved that all compounds have higher ability to donate hydrogen atom in the gas phase than in the presence of water medium in which they are very sensitive to electron donation. The results obtained demonstrate that the H-transfer mechanism for scavenging of the free radicals is more preferable than the single-electron transfer in both types of environment. They are consistent with the experiments confirming the specific biological activity of the compounds considered.

© 2009 Elsevier Masson SAS. All rights reserved.

1. Introduction

Over the past few years, several studies have been devoted to the therapeutic properties of TR (trans-3,4′,5-trihydroxystilbene, Fig. 1). The most important biological activity of this compound is related to the antioxidant [1–3] and cancer chemo-preventive activity [4,5]. It is a scavenger of free radicals against the peroxidation of low density proteins [6–8] and a potent inhibitor of cycloxygenase COX-1 and COX-2 in living cells [9]. These properties have been confirmed by Wright et al. [10] with the use of the quantum-mechanical computations in the gas phase and the locally dense basis sets (LDBS), employed in the investigation of the antioxidant activity of several classes of phenolic antioxidants including compounds related to vitamin *E*, flavonoids, hydroxylated stilbenes and sterically hindered phenols. In this work two pathways of oxidation in which phenolic antioxidants can react with free radicals have been studied. The results clearly indicate that in most cases H-atom transfer mechanism will be dominant in gas phase.

Theoretical study of the antioxidant properties of trans-resveratrol in the gas phase has been also performed by Cao et al. [11] by making use of B3LYP/6-31G(d,p) level of theory. It has been demonstrated that the 4′-hydroxyl group in this compound is more reactive than the other ones. On the basis of the analysis of the spin density they concluded that 4′-O-radical is more stable than 5-O-radical. Moreover, the authors proved that the antioxidant activity of trans-resveratrol is related to the spin density, unpaired electron distribution on the oxygen atoms and the semiquinone structure of its phenoxy radicals.

Recently, the common theoretical recognition of the anti-oxidative features of TR and its hydroxylated derivatives was reported by Quieroz et al. [12]. Employing the B3LYP hybrid functional and the 6-31G(d) basis set, they have determined the physical parameters characterizing the antioxidant activity of these compounds in the gas phase. Moreover, in this important study the authors have proved that the antioxidant activity of the polyphenols studied is associated with the stabilization energy of 4′-O

* Corresponding author. Tel.: +48 607608785; fax: +48 8291505.
E-mail address: dmkwant@amu.edu.pl (D. Mikulski).

radicals and that the hydroxyl bond in the para position strongly contributes to the decrease in the parameters characterizing the antioxidant behaviour of the polyphenolic compounds studied.

Parallel to theoretical calculations, some experimental studies of the antioxidant activity of TR and its derivatives have been performed. Recently, Fan et al. [13] synthesized a new antioxidant trans-4,4′-DHS (Fig. 1). In their work the antioxidant activity of this compounds has been analyzed in reactions with the glavinoxyl radical. Also, the geometric properties of trans-4,4′-DHS dimer have been confirmed by spectroscopic methods. The authors claimed that this dimer was an oxidative product of dimerization of TR in the presence of Cu(II). Finally, the experimental results lead to the conclusion that trans-4.4′-DHS possesses stronger antioxidant activity and cytoxicity against human promyelocitic leukemia cells than TR.

On the basis of the kinetic data Fang et al. [14], investigated the antioxidant effect of TR, trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS, trans-4,4′-DHS, trans-4-hydroxystilbene and trans-3,5-DHS, against the peroxidation of linoleic acid in sodium dodecyl sulfate (SDS) and cetyl trimethylammonium (CTAB) micelles. In order to investigate the reactions kinetic, the rate of propagation, chain initiation, peroxide formation, the inhibition period, kinetic chain lengths and inhibition time have been determined. Their results imply that except for trans-4-hydroxystilbene and trans-3,5-dihydroxystilbene, the compounds mentioned are significantly stronger antioxidants than TR. However, trans-3,4,4′-THS has

extremely high antioxidant activity, thus it completely inhibits peroxidation of linoleic acid. It can be seen from Table 9 that trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS, trans-4,4′-DHS are less effective antioxidants than TR since they are characterized by a lower values of the oxidation potential than TR. In addition, these authors show that the molecules with ortho-dihydroxyl and para-hydroxyl groups show high antioxidant activity.

The experimental study of the antioxidant activity of TR and its various derivatives was also presented by Stivala et al. [15]. In this work, the antioxidant activity of these molecules was evaluated measuring the inhibition of citronellal thermo-oxidation and the reduction of 2,2-diphenyl-1-picrylhydrazyl radical. These authors also compared the antioxidant activity of α,β-dihydro-3,4′,5-THS and TR and claimed that α,β-dihydro-3,4′,5-THS was significantly less active antioxidant than TR. Additionally, in their study based on the values of EC_{50} and EQ (Table 9) as measures of antioxidant activity it has been demonstrated that CR shows lower antioxidant activity than its trans-stereoisomer.

Considering the above remarks together with the importance of the antioxidative activity of TR, trans-4,4′-DHS, trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS and α,β-dihydro-3,4′,5-THS (Fig. 1) protection against free radicals, we demonstrate the structure–radical scavenging activity of these compounds in gas phase and aqueous environment by means of the DFT method. To elucidate the geometric stereoisomer–antioxidant activity relation, the computations were performed for trans-resveratrol and CR. Because of the
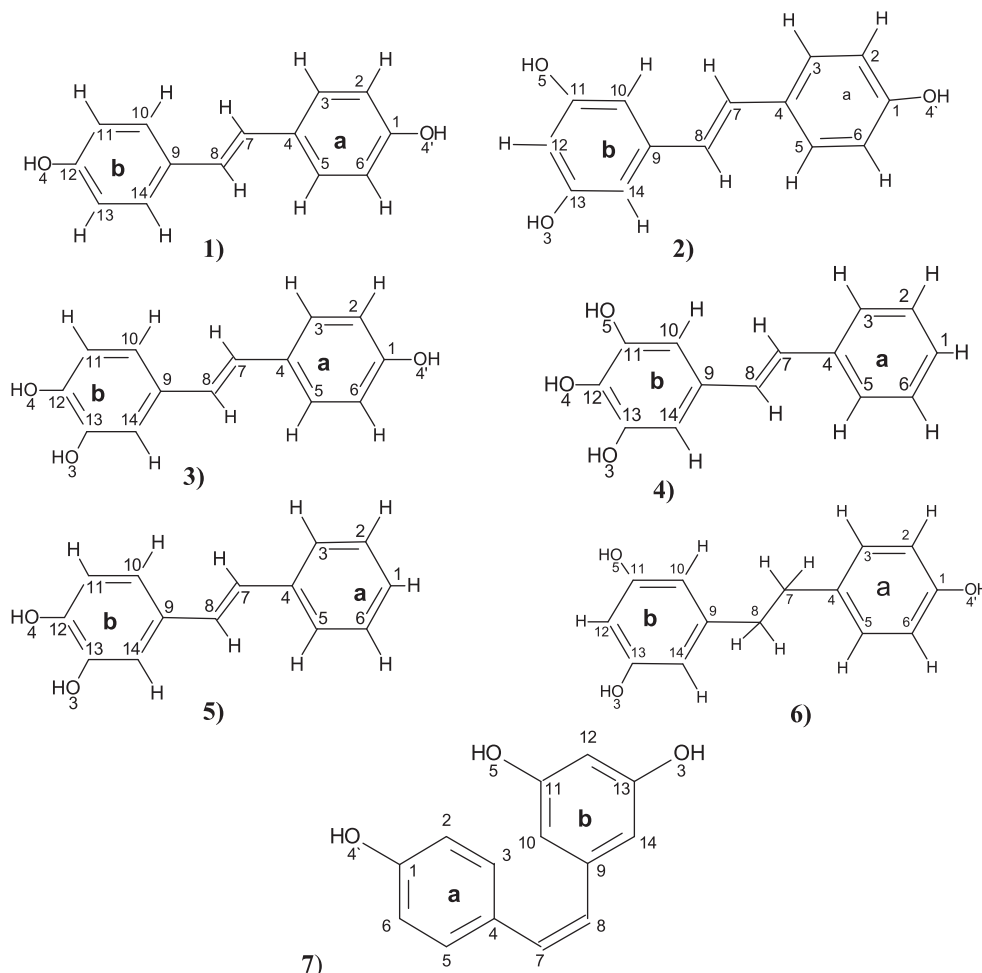

Fig. 1. Molecular structures of trans-4,4′-DHS (1), TR (2), trans-3,4,4′-THS (3), trans-3,4,5-THS (4), trans-3,4-DHS (5), α,β-dihydro-3,4′,5-THS (6) and CR (7).

Table 1
The B3LYP/6-311G(d,p) optimized in the gas phase bond lengths [Å], dihedral angles [deg], total energies [hartree] of TR, CR, trans-4,4′-DHS, trans-3,4,5-THS, trans-3,4,4′-THS, trans-3,4-DHS, 4′-O-radical of trans-TR, 4′-O-radical of trans-4,4′-DHS, 4-O-radical of 3,4-trans-DHS, 4-O-radical of trans-3,4,5-THS, 4′-O-radical of trans-3,4,4′-THS, 4-O-radical of 3,4,4′-THS, 4′-O-radical of α,β-dihydro-3,4′,5-THS and 4′-O–radical of CR.

Bond and Dihedral angles	C=C	O_4–H_4	O_3–H_3	$O_{4'}$–$H_{4'}$	$C_5C_4C_7C_8$	$C_7C_8C_9C_{10}$	Energy
TR	1.345	–	0.962	0.963	0.02	0.02	−766.571045
CR	1.345	–	0.962	0.963	27.43	39.22	−766.562776
trans-4′,4-DHS	1.346	0.962	–	0.962	0.00	0.00	−691.328145
trans-3,4,5-THS	1.345	0.965	0.962	–	6.98	6.47	−766.573012
trans-3,4,4′-THS	1.345	0.963	0.961	0.962	0.29	0.46	−766.571878
trans-3,4-DHS	1.345	0.962	0.960	–	0.00	0.00	−691.328528
α,β-dihydro-3,4′,5-THS	–	–	0.960	0.962	–	–	−767.793375
4′-O radical of TR	1.363	–	0.962	–	0.00	0.00	−765.934850
4′-O-radical of 4′,4-DHS	1.361	0.961	–	–	0.00	0.00	−690.693727
4-O-radical of 3,4,5-THS	1.362	–	0.961	–	0.00	0.00	−765.948469
4-O-radical of 3,4-DHS	1.362	–	0.962	–	0.00	0.00	−690.707321
4′-O-radical of α,β-dihydro-3,4′,5-THS	–	–	0.960	–	–	–	−767.151238
4′-O-radical of 3,4,4′-THS	1.362	0.963	0.980	–	0.00	0.00	−765.937949
4-O-radical of 3,4,4′-THS	1.362	–	0.980	0.963	0.04	0.00	−765.951976
4′-O-radical of CR	1.361	–	0.962	–	15.69	36.55	−765.924442

lack of experimental data for other cis-stereoisomers of trans-analogues of trans-resveratrol, the antioxidant properties of these cis-stereoisomers have been not investigated. It was our intention to employ the B3LYP/6-311G(d,p) level of theory to perform the most reliable optimization of the geometrical parameters of these compounds and to calculate physical descriptors characterizing their antioxidant ability. It particular, the homolytic bond dissociation enthalpy (BDE) of 4′-O–H and 4-O–H bonds, adiabatic ionization potential (AIP), HOMO orbital distribution and spin density in the 4′-O and 4-O-free radicals were calculated. Moreover, the purpose of this study was to show that trans-4,4′-DHS, trans-3,4-DHS, trans-3,4,4′-THS and trans-3,4,5-THS are more active antioxidants than TR, whereas α,β-dihydro-3,4′,5-THS and CR have significantly lower ability to scavenge free radicals than TR. The results were compared with the experimental conclusions reported by Fan [13], Fang, [14] and Stivala et al. [15]. It should be emphasized that we did not have the experimental values of AIP and BDE. Because of no experimental values of BDE and AIP, we could not express the errors in predictions of these calculated antioxidant factors. Note that in the experimental works the antioxidant properties of the compounds studied were estimated on the basis the EQ, EC_{50} and the kinetic parameters as well as oxidation potential reported by Fang et al. [14]. EC_{50} is the antioxidant concentration necessary to decrease the initial amount of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) by 50% while EQ is the concentration required for each compound to double the half-life with respect to control reaction. Unfortunately, these purely empirical parameters cannot be calculated with the use of the Gaussian 03W package. Hence, in this study we explain the differences in the antioxidant activity of the compounds studied and confirm the conclusions of the experimental studies.

In contrast to the works by Cao et al. [11] and Quieroz et al. [12], in our study new phenolic antioxidants are examined and detailed analysis of the geometric parameters of these compounds, their 4′-O, 4-O-radicals in gas phase and water medium is performed. We

present a detailed analysis of the geometrical parameters of these chemical systems. Our study carefully explains the influence of these optimized geometries and the presence of the vinyl bond on the antioxidant properties of the compounds studied. Note that the novelty of our study is investigation of the antioxidant activity of two stereoisomers of resveratrol in both environments. Hence, for the first time the stereochemical contribution to this activity of the stereoisomers of resveratrol is considered with the aid of ab initio quantum computations in polar medium. To investigate the influence of solvent on antioxidant properties of the compounds examined, the solvent effect was also included in computations. Therefore, our results contribute to explanation of the antioxidant activity of the compounds studied in living cells in which the environment with the participation of free radicals is polar. On the basis of this fact we conclude that the computations in the gas phase performed by Cao [11] et al. and Quieroz et al. [12] are very useful only for preliminary elucidation of the antioxidant properties of these compounds.

2. Methods

All computations were performed using the GAUSSIAN 03W software package [17]. The images presented in the figures were generated by using ChemSketch and GaussView visualization programs. Full optimization of molecular geometry and energy was carried out in the gas phase and water environment. Full optimizations were made without symmetry constraints. Geometries of the neutral compounds in their ground state were optimized employing the DFT method with restricted Becke's three-parameter hybrid functional and the nonlocal Lee, Yang and Parr gradient-corrected correlation functional B3LYP [18,19] combined with the 6-311G(d,p) functional basis set. However, for the geometry optimization of the

Table 2
The calculated at B3LYP/6-311G(d,p) level of theory total molecular energies [hartree] of the cis-stereoisomers and relative energies [kcal/mol] for the geometric stereoisomers in the gas phase.

Compounds	Energy	Relative energy
CR	−766.562776	5.189
cis-4,4′-DHS	−691.320744	4.644
cis-3,4,5-THS	−766.559248	8.637
cis-3,4,4′-THS	−766.564754	4.470
cis-3,4-DHS	−691.321182	4.609

Table 3
The total molecular energies [hartree] of the 4′-O and 4-O radicals of cis-stereoisomers and relative energies [kcal/mol] of these radicals of the geometric stereoisomers studied in gas the phase calculated at unrestricted B3LYP/6-311G(d,p) level of theory.

Radicals	Energy	Relative energy
4′-O-radical of CR	−765.924442	6.531
4′-O-radical of cis-4,4′-DHS	−690.683669	6.311
4-O-radical of cis-3,4,5-THS	−765.939325	5.738
4′-O-radical of cis-3,4,4′-THS	−765.927584	6.504
4-O-radical of cis-3,4-DHS	−690.697201	6.350
4-O-radical of cis-3,4,4′-THS	−765.942150	6.166

Table 4
The calculated at B3LYP/6-311G(d,p) level of theory total molecular energies [hartree] of the cis-stereoisomers and relative energies [kcal/mol] for the geometric stereoisomers in the water environment.

Compounds	Energy	Relative energy
CR	−766.604433	5.743
cis-4,4′-DHS	−691.349062	7.314
cis-3,4,5-THS	−766.594239	6.642
cis-3,4,4′-THS	−766.599655	5.900
cis-3,4-DHS	−691.346727	5.966

free radicals and cation radicals the unrestricted uB3LYP/6-311G(d,p) level of theory was applied. We employed unrestricted B3LYP exchange-correlation functional for the open-shell molecular systems since it provides the most reliable results. The computations performed for the radicals were made for the optimized most stable structure of the neutral molecules, after H atom abstracted from 4′-OH or 4-OH groups. Since the 4′-OH and 4-OH groups are the most effective in scavenging of free radicals [16], the radicals without 4′- and 4-hydrogen were investigated. In the process of optimization of the structure of radicals and cation radicals the spin contamination was monitored. The mean value of the square of the total spin of 0.750 was maintained. In order to determine the starting geometry of the compounds studied and the approximate location of the minimum energy structures, the potential energy surface scans were made. At first step, the potential energy surfaces, at restricted B3LYP/6-31G(d) level were built by changing the dihedral angles α (C_5–C_4–C_7–C_8) and θ (C_7–C_8–C_9–C_{10}) in the compounds studied. The conformational energy maps were obtained through the discrete rotation of these selected angles in 10° increments, ranging from 0° to 180° with constraint on all other geometrical parameters. Afterwards, the most stable structures obtained from the scan were fully optimized without any constraint around each potential minimum. For all optimized structures, we calculated the harmonic vibrational frequencies of the corresponding bonds to determine stationary points on the potential energy surface. Since the relative hydrogen orientation in OH groups is also the most important geometrical parameter, for the most stable geometries obtained from previous full optimizations, the potential energy surfaces describing the relation between these orientations and total energy were built. These surfaces were generated at B3LYP/6-31G(d) level of theory by changing the dihedral angles β (C_6–C_1–$O_{4′}$–$H_{4′}$), γ (C_{10}–C_{11}–O_5–H_5), φ (C_{14}–C_{13}–O_3–H_3) and δ (C_{11}–C_{12}–O_4–H_4) in 60° increments with constraint on all other geometrical parameters. In the calculations we used the angle increment by 60° in order to find out whether in

the most stable conformations the hydrogen atoms from the OH groups are in the plane of the molecule or out of the plane. Finally, the most stable conformations obtained from these scans were fully optimized at 6-311G(d,p) level of theory and vibrational characterized to check for the presence of imaginary frequencies in the minima. The total molecular energies of the compounds studied assumed to have the optimized structures were almost the same. In this way, the most reliable structures of the antioxidants investigated in the global energy minimum were determined, which seem to be the best theoretical result generated by the methods used in the calculations. In order to explain the influence of the resonance stabilization on the antioxidant activity of trans-4,4′-DHS, the resonance forms of its 4′-O-radical were determined. The homolytic BDE values of the O–H bond ware calculated as the differences in the enthalpy of the reactants at 298.15 K and 1.00 atm with the use of the following equation: $BDE = H_{radical} + H_H − H_{molecule}$ where $H_{radical}$ is the enthalpy of the free radical, H_H is the enthalpy of the H-atom (−0.49764 [hartree]) with the vibrational correction [10] whereas $H_{molecule}$ indicates the enthalpy of the parent molecule. The AIP values were obtained according to the formula $AIP = E_{CR} − E_P$ in which E_P is the total energy of the parent molecule whereas E_{CR} denotes the corresponding total energy of the cation radical generated after the electron transfer. In all computations, cation radicals from the optimized neutral compounds in the global energy minimum were generated and further fully optimized. The HOMO orbital distribution and spin density distribution were determined by using the B3LYP/6-311G(d,p) level of theory for the fully optimized structure of the compounds and their 4′-O and 4-O-radicals. Additionally, the spin density for each atom of the 4′-O and 4-O-radicals studied was computed using the unrestricted B3LYP/6-311G(d,p) level of theory in gas phase and water environment.

Since the free radical-scavenging action strongly depends on polar solution in the real biological systems, solvent effect was taken into account in this study. The solvent effects were computed by using the conductor polarizable solvation model (CPCM) [20] as implemented in the Gaussian 03W program. In this model water is assumed as a macroscopic continuum characterized a dielectric constant $\varepsilon = 78.39$. The UAO solvent cavity model was used in the computations. The UAO cavity is built-up using the united atom topological model UATM [21] applied on atomic radii of the universal force field UFF [22]. The CPCM computations were performed with tesserae of 0.2 Å^2 average size. We investigated the influence of water environment on the stability of O–H bonds, the values of AIP and spin density. The solvent effect in the conformational equilibrium was obtained for the fully optimized most stable trans-stereoisomers, CR, α,β-dihydro-

Table 5
The B3LYP/6-311G(d,p) optimized in the water medium bond lengths [Å], dihedral angles [deg], total energies [hartree] of TR, CR, trans-4,4′-DHS, trans-3,4,5-THS, trans-3,4,4′-THS, trans-3,4-DHS, 4′-O-radical of trans-TR, 4′-O-radical of trans-4,4′-DHS, 4-O- radical of 3,4-trans-DHS, 4-O-radical of trans-3,4,5-THS, 4′-O-radical of trans-3,4,4′-THS, 4-O-radical of 3,4,4′-THS, 4′-O-radical of α,β-dihydro-3,4′,5-THS and 4′-O-radical of CR.

Bond and Dihedral angles	C=C	O_4–H_4	O_3–H_3	$O_{4′}$–$H_{4′}$	$C_5C_4C_7C_8$	$C_7C_8C_9C_{10}$	Energy
TR	1.347	–	0.982	0.983	0.10	0.00	−766.613485
CR	1.347	–	0.982	0.983	26.51	38.62	−766.604333
trans-4′,4-DHS	1.347	0.982	–	0.982	0.01	0.00	−691.360718
trans-3,4,5-THS	1.347	0.977	0.975	–	4.58	4.04	−766.604823
trans-3,4,4′-THS	1.347	0.983	0.976	0.982	0.33	0.46	−766.609058
trans-3,4-DHS	1.347	0.983	0.976	–	0.00	0.00	−691.356234
α,β-dihydro-3,4′,5-THS	–	–	0.981	0.982	–	–	−767.832870
4′-O radical of TR	1.368	–	0.983	–	0.11	0.13	−765.975119
4′-O-radical of 4′,4-DHS	1.372	0.985	–	–	0.00	0.00	−690.725411
4-O-radical of 3,4,5-THS	1.366	–	0.985	–	0.00	0.00	−765.980779
4-O-radical of 3,4-DHS	1.363	–	0.984	–	0.00	0.00	−690.728335
4′-O-radical of α,β-dihydro-3,4′,5-THS	–	–	0.982	–	–	–	−767.187787
4′-O-radical of 3,4,4′-THS	1.369	0.985	0.978	–	0.00	0.00	−765.973781
4-O-radical of 3,4,4′-THS	1.367	–	0.983	0.984	0.04	0.00	−765.983506
4′-O-radical of CR	1.366	–	0.982	–	19.43	36.55	−765.962693

3,4′,5-THS, 4′-O, 4-O radicals and cation radicals in the gas phase, at the B3LYP/6-311G(d,p) level of theory.

3. Results and discussion

3.1. The optimized geometries of TR, CR, trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS, trans-4,4′-DHS, α,β-dihydro-3,4′,5-THS, their free radicals and cation radicals

The O_4–H_4, $O_{4'}$–$H_{4'}$, O_3–H_3 and C=C bond lengths and the torsion angles between two phenyl rings, which are the most significant structural parameters of the equilibrium optimized neutral structure of all the compounds investigated and their 4′-O and 4-O-radicals in

their ground states, are summarized in Table 1. The optimization of all compound structures using 6-311G(d,p) basis set leads to the absolute energy minimum on the potential energy surface, whose presence is confirmed by the lack of imaginary frequencies. The energetic analysis performed reveals that α,β-dihydro-3,4′,5-THS is the most stable compound in the mediums studied. Probably it is due to the absence of the vinyl bond. Additionally, the all cis-stereoisomers and their 4-O and 4′-O-radicals are energetically less stable than their trans-stereoisomers in the environment studied (Tables 2–5). This result is confirmed by the values of relative energies calculated as the difference between total energies of the geometric stereoisomers studied (Table 2). The small values of these relative energies reveal the high torsional flexibility of the compounds
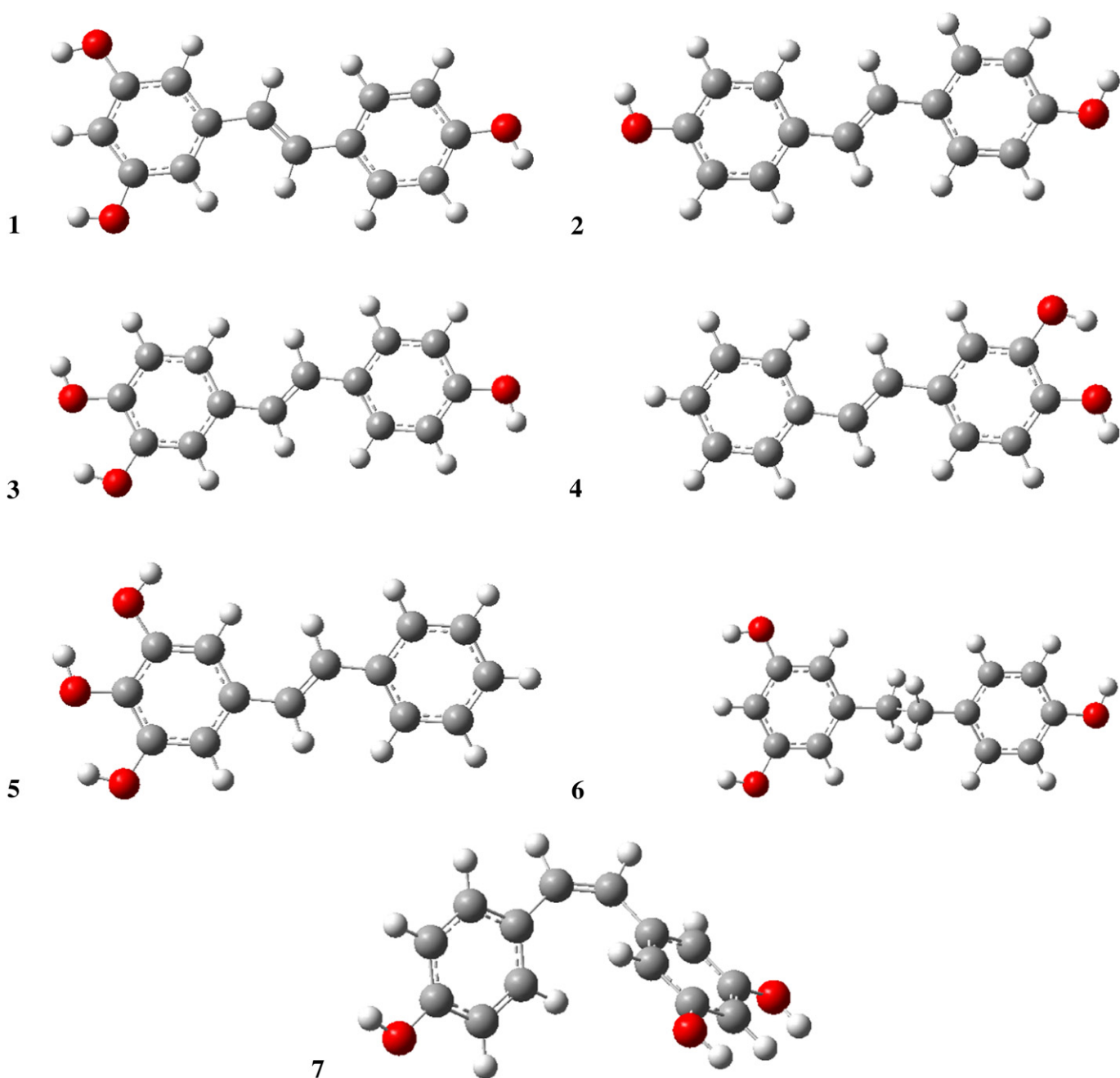

Fig. 2. The B3LYP/6-311G(d,p) optimized geometries of TR (1), trans-4,4′-DHS (2), trans-3,4,4′-THS (3), trans-3,4-DHS (4), trans-3,4,5-THS (5), α,β-dihydro-3,4′,5-THS (6) and CR (7) in the gas phase.

studied. The significant value of the relative energy for the 3,4,5-THS stereoisomers in the gas phase can be related to the high energy of cis-stereoisomer whose geometry is strongly deviated from planarity (α (C_5–C_4–C_7–C_8) = 38.89°, θ (C_7–C_8–C_9–C_{10}) = 31.14°). Note that the energy differences between the lowest energy conformations of trans and cis-stereoisomers (except for stereoisomers of 3,4,5-THS) are lower than that of their 4′-O and 4-O radicals. The calculated C=C bond lengths of the central moiety of the molecules studied have similar values in the gas phase. However, in the radical forms the C=C bonds are longer than those in the neutral molecules. We observe that all O_3–H_3 bond lengths in the compounds studied are slightly shorter than the O_4–H_4 and $O_{4'}$–$H_{4'}$ bonds. The optimized values of the β (C_6–C1–$O_{4'}$–$H_{4'}$), γ (C_{10}–C_{11}–O_5–H_5), φ (C_{14}–C_{13}–O_3–H_3) and δ (C_{11}–C_{12}–O_4–H_4) dihedral angles

indicate that hydrogen atoms from OH groups lie in the surface of the all compounds, their radical forms and cation radicals in both environments. According to the calculation results, these dihedral angles are approximately 0° and 180° in the mediums studied. On the basis of the optimized values of the α (C_5–C_4–C_7–C_8) and θ (C_7–C_8–C_9–C_{10}) dihedral angles, it is evident that the geometries of the trans-stereoisomers studied (Fig. 2) and their radicals (Fig. 3) as well as cation radicals (Fig. 4) are strictly planar in the gas phase. In comparison with the gas phase, the water environment causes an increase in the lengths of the C=C bonds and a significant increase in the O–H bond lengths in all compounds and their 4′-O-radicals (Table 5). This finding shows that water environment evidently facilitates the heterolytic dissociation of the hydroxyl bonds. Also, in water environment all trans-stilbene derivatives studied (except for
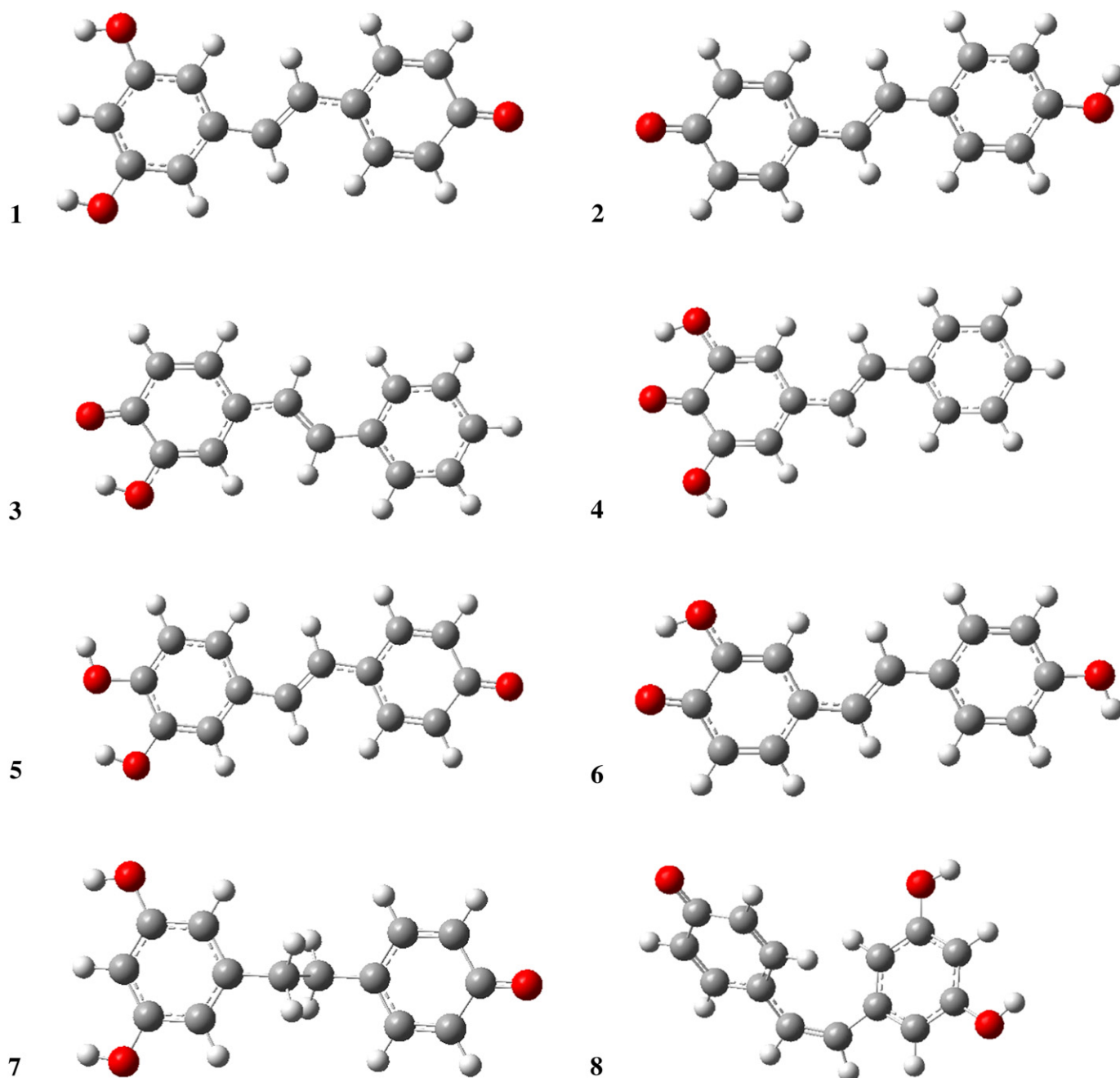

Fig. 3. The uB3LYP/6-311G(d,p) optimized geometries of the 4′-O-radical of TR (1), 4′-O-radical of trans-4,4′-DHS (2), 4-O-radical of trans-3,4-DHS (3), 4-O-radical of trans-3,4,5-THS (4), 4-O-radical of trans-3,4,4′-THS (5), 4-O-radical of trans-3,4,4′-THS (6), 4-O-radical of α,β-dihydro-3,4′,5-THS (7) and 4′-O-radical of CR (8) in the gas phase.

trans-3,4,5-THS), their 4′-O, 4-O-radicals and cation radicals have planar equilibrated geometries. Besides, the all chemical systems studied in water environment are more energetically stable than in the gas phase (Table 5). The slight deviation from the planarity found for trans-3,4,5-THS in both environments is probably due to the unfavourable location of hydroxyl groups. In our opinion the strong interaction between closely localized hydroxyl groups is the main contribution to this distortion of the planar geometry. It is interesting to note that also CR (Fig. 9), its 4′-O-radical and cation radical have geometry significantly deviating from the planarity in the environments studied. The values of the α (C_5–C_4–C_7–C_8) and θ (C_7–C_8–C_9–C_{10}) dihedral angles in CR and its 4′-O-radical explicitly confirm this finding (Table 1). Thus, this deviation can be the main reason for the higher energy of CR than TR in the environments studied. It is well

known that the molecular structure of TR and other antioxidant can contribute to the electron transfer during scavenging of free radicals [15]. The stacking interactions between the planar investigated molecules can permit energetically favourable hydrogen atom transfer in the interaction and facilitate the interaction with free radicals. The energetic analysis reveals that all 4′-O, 4-O-radicals are less stable than the neutral molecules in the examined mediums, therefore they can be more reactive in the biochemical reactions. The optimized structures of the 4′-O and 4-O radicals are planar and have semiquinone form; the unpaired electron is localized in these forms mainly on the O atom in the para position leading to the stabilization of the radicals. Also, the high delocalization of the unpaired electron on the vinyl bond is strongly emphasized. Note that the presence of the vinyl bond and the electron donation hydroxyl groups in these
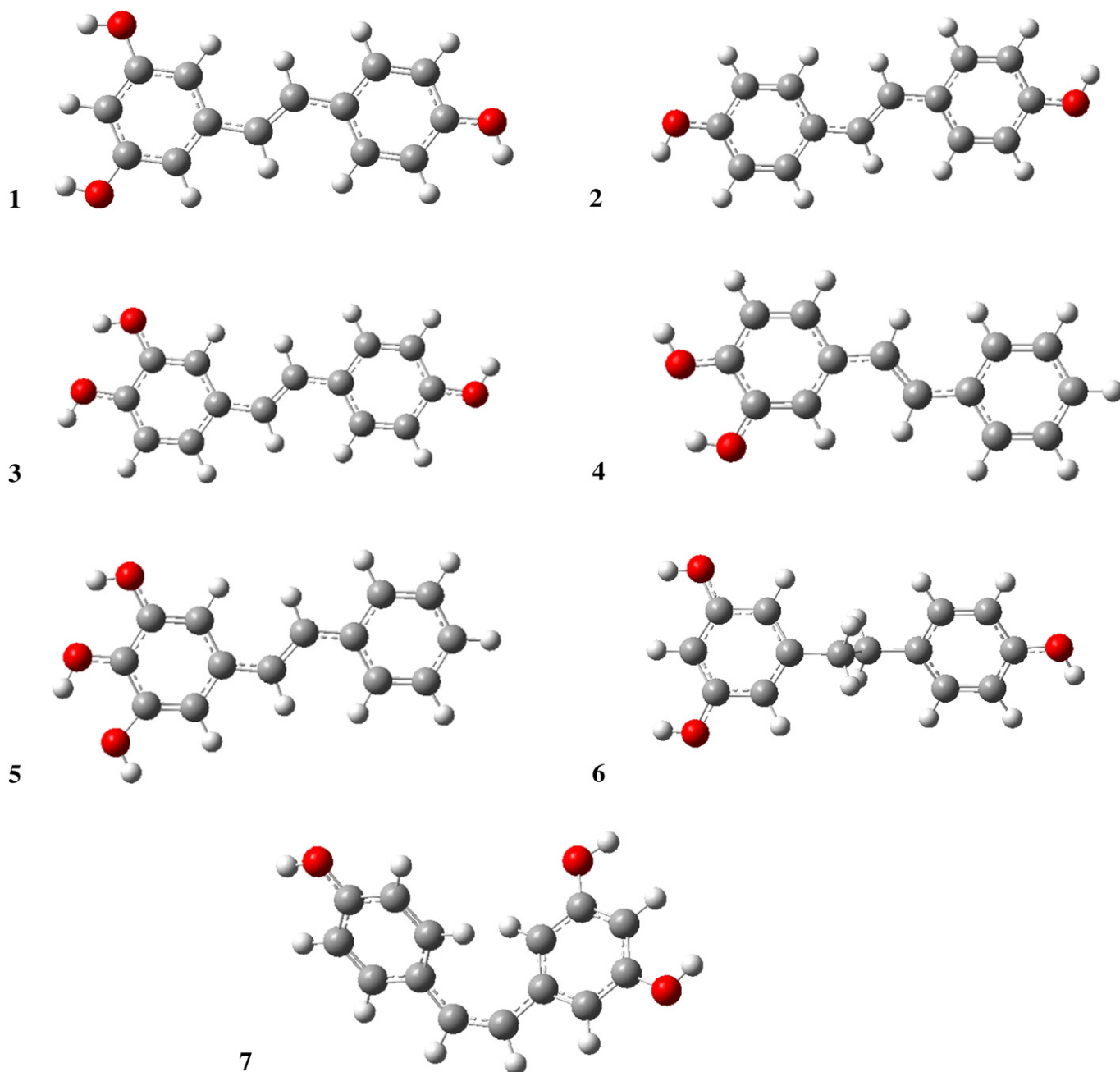

Fig. 4. The uB3LYP/6-311G(d,p) optimized geometries of the cation radicals of TR (1), trans-4,4′-DHS (2), trans-3,4,4′-THS (3), trans-3,4-DHS (4), trans-3,4,5-THS (5), α,β-dihydro-3,4′,5-THS (6) and CR (7) in the gas phase.
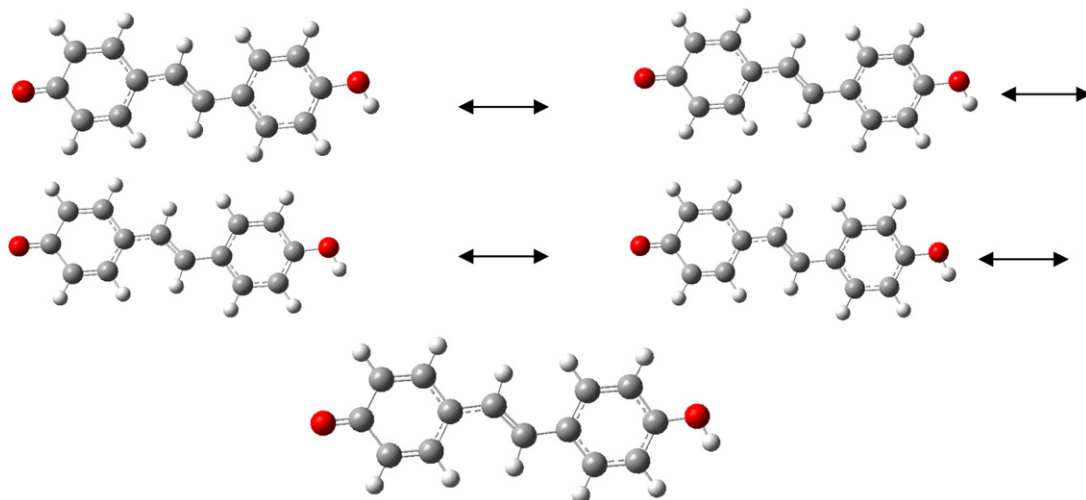
Fig. 5. The uB3LYP/6-311G(d,p) optimized geometries of the resonance forms of trans-4,4′-DHS in the gas phase.

radicals may also stabilize their semiquinone geometry. The corresponding resonance forms of the 4′-O-radicals of trans-4.4′-DHS (Fig. 5) are almost isoenergetic ($E = -690.69372721$ [hartree]), thus, they are characterized by similar stability and are strongly stabilized by the resonance. All geometric parameters in these forms take the comparable values and the unpaired electron is distributed mainly on the vinyl bond in their planar conformation.

3.2. BDE, AIP, HOMO orbital distribution and spin density distribution. The influence of water environment

The hydrogen donating ability of the wide class of polyphenols and the ability of these compounds to form the radical forms are characterized by BDE, whereas AIP is an important physical factor indicating the range of electron donation. The BDE corresponds to the O–H bond breaking (H abstraction), thus this parameter describes the stability of the hydroxyl bonds. The molecules with lower values of BDE and AIP are endowed with higher antioxidant activity. The calculated values of the BDE and AIP for the molecules studied in the gas phase are present in Table 6. The results indicate that AIP and BDE for trans-4.4′-DHS, trans-3,4-DHS, trans-3,4,4′-THS and trans-3,4,5-THS are lower than those for TR while the smallest value of AIP of trans-3,4,4′-THS. It proves that these compounds are more efficient free radical scavengers than TR and that these molecules seem to donate the single-electron during the interaction with free radicals. In particular, trans-3,4,4′-THS is mainly susceptible to electron transfer. Analyzing the values of BDE of the 4-O-H bond for all compounds one can find that for trans-3,4,4′-THS and trans-3,4-DHS this antioxidant factor takes the smallest value in the gas phase. In our opinion, this result for trans-3,4-DHS can be interpreted as a consequence of the presence of all hydroxyl groups in one phenyl ring. In such an arrangement the hydroxyl groups at 3 and 5 positions reduce the stability of the 4′-O–H and 4-O–H bonds by intermolecular hydrogen interaction. This result confirms the results reported by Fang et al. [14]. Additionally, 3,4,4′-trans-THS has a higher value of BDE of 4′-O–H bond than 4-O–H. Hence, in this molecule 4-O–H bond is more sensitive to interaction with free radicals. Similarly, the high antioxidant potency of trans-4.4′-DHS is related to the favourable location of phenolic hydroxyl groups. The presence of two strong donation hydroxyl groups in para positions contributes to the ease of the electron donation. It is well known that electron-donating groups increase the stability of polyphenolic free radicals and decrease the

stability of parent molecules. Thus, we conclude that the decrease in BDE for all polyphenols can arise from the number of the strong electron-donating hydroxyl groups and their favourable location in the para positions of the studied molecules. Moreover, the conjunction of the phenyl rings by double bond ensures the most energetically favourable resonance stabilization, which facilitates the high electron transfer after the hydrogen abstraction in para or orto positions. In contrast to the previous results, BDE of 4′-OH group and the AIP for α,β-dihydro-3,4′,5-THS are significantly higher than for TR and other antioxidants studied. This result shows that the double bond between the phenyl rings is a very important structural element which determines the antioxidant potency of the phenolic compounds.

The BDE and AIP values of CR are higher than those of its trans-stereoisomer in both types of environment. It proves that cis-stereoisomer is less efficient antioxidant than trans-resveratrol. This result confirms the experimental observation by Stivala et al. [15]. It should be pointed out that planar conformation of free radical and cation radical ensures favourable spin and unpaired electron distribution, which is an important parameter determining the stability of the radicals and antioxidant power. Therefore, the deviation from planarity of CR, its 4′-O-radical and cation radical is the main factor determining the decrease in the reduction of the antioxidant activity of CR. We can conclude that the non-planar geometry of the 4′-O-radical of CR can effectively prevent the delocalization of the unpaired electron in this radical.

Table 6
The B3LYP/6-311G(d,p) in the gas phase calculated values of the AIP [kcal/mol] and BDE [kcal/mol] of 4′-O–H bond of TR, 4′-O–H bond of trans-4,4′-DHS, 4-O–H bond of trans-3,4,5-THS, 4-O–H bond of trans-3,4,4′-THS, 4′-O–H bond of trans-3,4,4′-THS, 4-O–H bond of trans-3,4-DHS, 4′-O–H bond of α,β-dihydro-3,4′,5-THS and 4′-O–H bond of CR.

Antioxidant	BDE	AIP
TR	78.683	157.061
trans-4,4′-DHS	77.663	152.212
trans-3,4,5-THS	71.816	151.342
trans-3,4,4′-THS (4-OH)	68.986	150.227
trans-3,4,4′-THS (4′-OH)	77.845	150.227
trans-3,4-DHS	69.734	156.691
α,β-dihydro-3,4′,5-THS	82.401	168.183
CR	80.026	159.349

When the solvent effect was taken into account, a significant influence of water medium on AIP values was observed (Table 8). The values of this antioxidant factor in water medium are dramatically lower than in gas phase. It is well known that cation radicals are charged and quite sensitive to the polarity of various solvents. As expected in water environment the lower values of AIP than in gas phase were obtained. From this result it is evident that this polar medium can facilitate electron donation which is one of the most important process of free radical scavenging. Moreover, the values of AIP indicate that trans-3,4,4′-THS is the most efficient the electron donor from among the compounds studied and that the all trans-stereoisomers studied are more sensitive to electron donation than TR, while α,β-dihydro-3,4′,5-THS is less sensitive to the electron donation than TR.

Comparing the values of BDE (Tables 6, 8) in the environments studied, we observe that in water medium these values are higher than in gas phase. Hence, on the basis of the values of BDE in water environment it can be concluded that this medium decreases the ability of the hydroxyl groups to homolytic dissociation. This finding reveals that in water the antioxidants studied have a lower ability to hydrogen transfer than in gas phase. Similarly as in the gas phase the computations performed in water environment reveal that trans-3,4,4′-THS has highest the hydrogen atom donating ability whereas the ability of α,β-dihydro-3,4′,5-THS to this process is smallest. In comparison with TR, the all trans-stilbene derivatives in water medium are expected to show higher hydrogen atom donating activity since the corresponding BDE values are lower
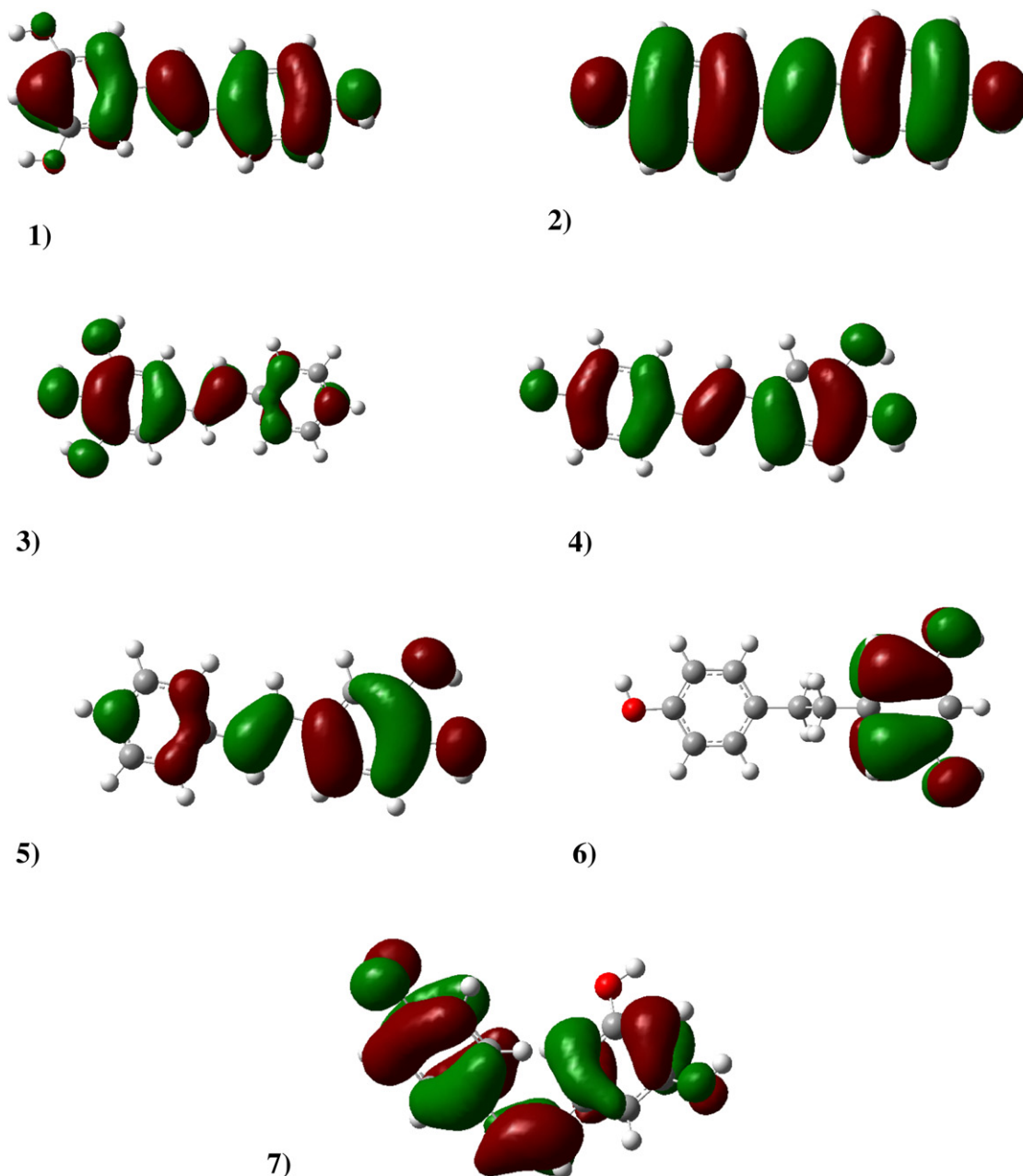

Fig. 6. The HOMO orbital distribution in TR (1), trans-4,4′-DHS (2), trans-3,4,5-THS (3), trans-3,4,4′-THS (4), trans-3,4-DHS (5), α,β-dihydro-3,4′,5-THS (6) and CR (7) in the gas phase.
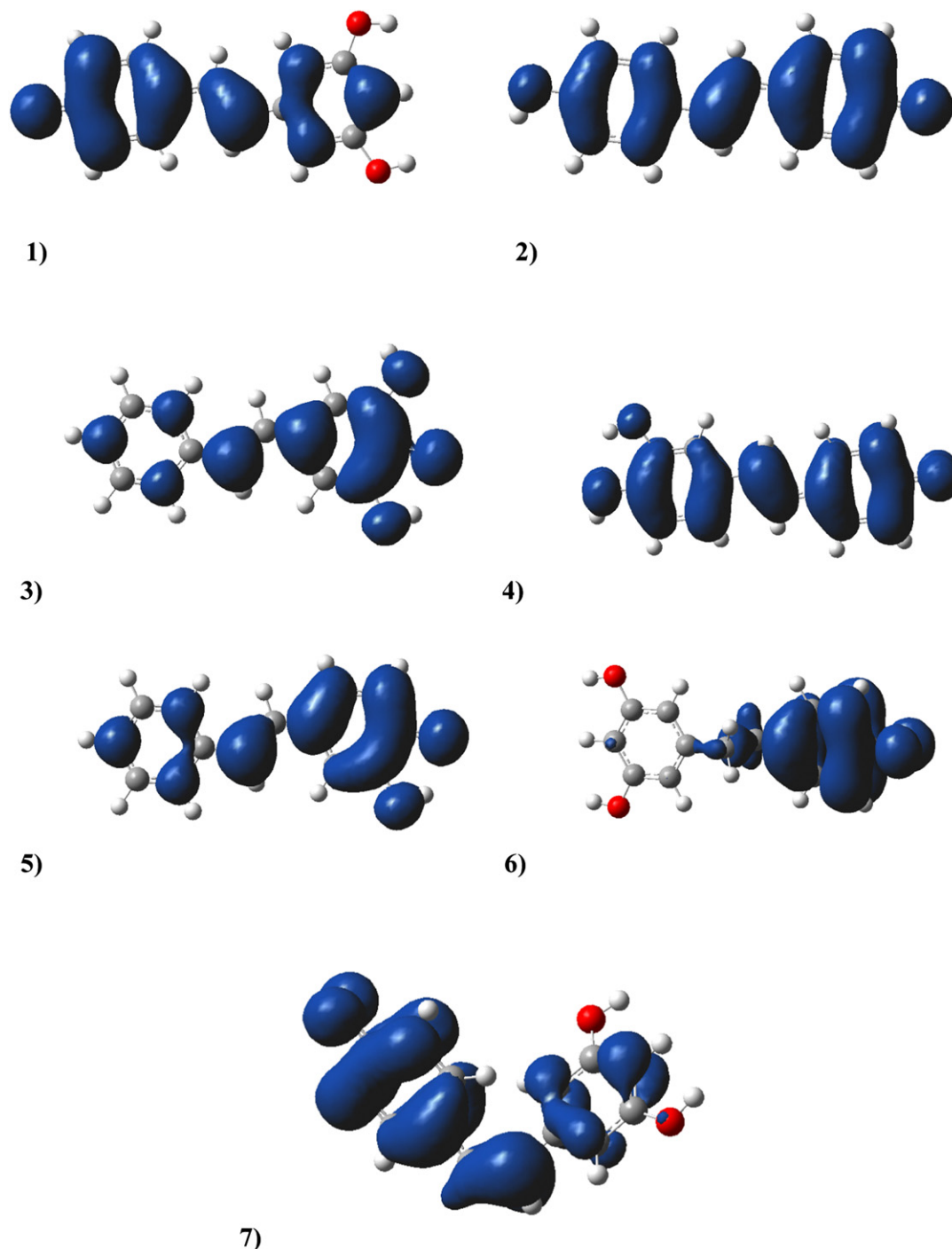
Fig. 7. The spin density distribution in 4′-O-radical of TR (1), 4′-O-radical of trans-4,4′-DHS (2), 4-O-radical of trans-3,4,5-THS (3), 4′-O-radical trans-3,4,4′-THS (4), 4-O-radical of trans-3,4-DHS (5), 4′-O-radical of α,β-dihydro-3,4′,5-THS (6) and 4′-O-radical of CR (7) in the gas phase.

that those obtained for TR. This result is in agreement with the calculations performed in the gas phase. The findings obtained lead to the conclusion that the trans-stereoisomers studied are stronger antioxidants than TR in the mediums considered. However, the calculated smaller values of BDE than AIP prove that the H-atom transfer mechanism for scavenging of free radicals is most favourable in the mediums studied. Hence, in physiological conditions, a scavenging of harmful free radicals by the antioxidants investigated may be achieved mainly *via* hydrogen donation.

Another important molecular parameter correlated with the free radical scavenging activity is the energy of the HOMO orbital. The molecules with a lower energy of the HOMO orbital have weaker electron donating ability. Besides, the electronic density distribution in these orbitals permits prediction of the most probable sites in the molecules investigated which can be easily attacked by free radicals and other reactive agents. More active redox sites of these molecules are characterized by high density of the HOMO orbital. Analysis of the distribution of HOMO orbital (Fig. 6) shows that for all trans-stereoisomers and CR it is delocalized on the whole
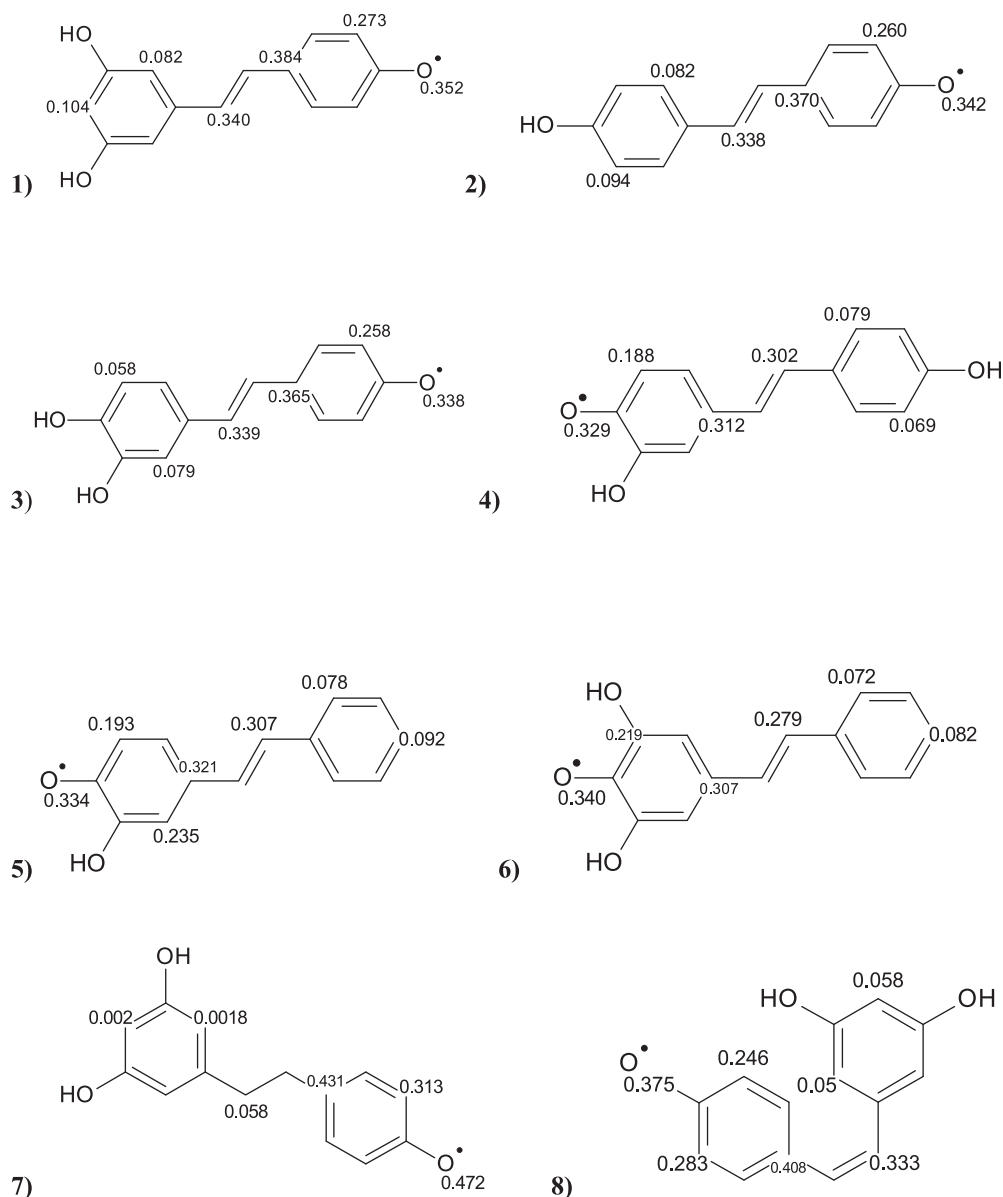
Fig. 8. The computed at uB3LYP/6-311G(d,p) level of theory spin density in the 4′-O-radical of TR (1), 4′-O-radical of trans-4,4′-DHS (2), 4′-O-radical of trans-3,4,4′-THS (3), 4-O-radical trans-3,4,4′-THS (4), 4-O-radical of trans-3,4-DHS (5), 4-O-radical of trans-3,4,5-THS (6), 4′-O-radical of α,β-dihydro-3,4′,5-THS (7) and 4′-O-radical of CR (8) in the gas phase.

molecule and particularly on the 4- and 4′-positions and double bond which connects the phenyl rings. Hence, the 4′-OH and 4-OH groups in both compounds can be easily attacked by the free radicals in the real biological systems. The high range of delocalization of this orbital indicates that the compounds investigated have many active redox sites. In contrast, the OH groups in meta position are insensitive to the interactions with free radicals. This observation for TR is in an excellent agreement with the results obtained by Cao et al. [11]. Furthermore, in all trans-stilbene derivatives studied the HOMO orbital is distributed along both phenyl rings. This finding is strongly correlated with the presence of the vinyl bond, which facilitates the HOMO orbital distribution between two phenyl rings.
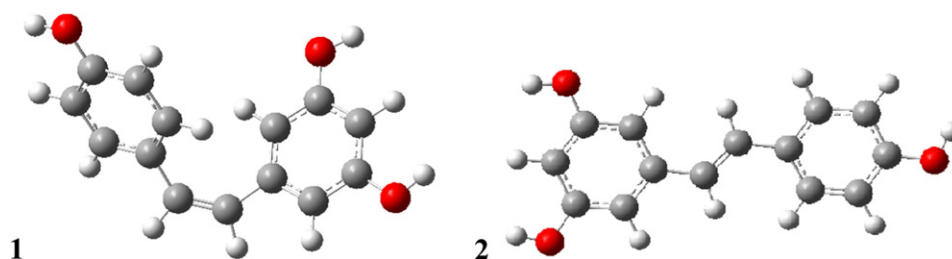

Fig. 9. The B3LYP/6-311G(d,p) fully optimized geometries of CR (1) and TR (2) in the water medium.

Table 7
The uB3LYP/6-311G(d,p) spin density calculated on the 4′-O and 4-O atoms in the 4′-O and 4-O-radicals of the compounds studied in the water environment.

Radical	Spin density
4′-O-radical of TR	0.315
4′-O-radical of trans-4,4′-DHS	0.314
4-O-radical of trans-3,4,5-THS	0.312
4-O-radical of trans-3,4,4′-THS	0.303
4′-O-radical of trans-3,4,4′-THS	0.311
4-O-radical of trans-3,4-DHS	0.309
4′-O-radical of α,β-dihydro-3,4′,5-THS	0.443
4′-O-radical of CR	0.334

Table 9
The experimental values of the oxidation potential E [14] and EQ [15] for the antioxidants studied.

Antioxidant	E [V]	EQ [μM]
TR	0.62	135 ± 8.81
trans-4,4′-DHS	0.40	–
trans-3,4,5-THS	0.24	–
trans-3,4,4′-THS	0.32	–
trans-3,4-DHS	0.34	–
α,β-dihydro-3,4′,5-THS	–	260 ± 11.2
CR	–	241 ± 38.0

Comparing the HOMO orbital distribution in CR and TR we observe that CR shows significant reduction of the density of the HOMO orbital at 3-OH and 5-OH group. However, α,β-dihydro-3,4′,5-THS is characterized by total reduction of the HOMO orbital distribution at a-phenyl ring.

One of the most important parameters which can be used to characterize the antioxidant activity is also the spin density distribution in free radicals. The stability of free radicals and the antioxidant potency are mainly determined by this factor. The calculations performed reveal that in all compounds which are derivatives of trans-stilbene, the spin density is delocalized through the whole molecules but comes mainly from the oxygen atoms in para position and the vinyl bond. Moreover, Fig. 7 shows that the spin density is distributed over the whole molecules with high concentrations on the phenyl rings and vinyl bond. These results can be related to better stabilization of 4′-O and 4-O free radicals and support the results obtained for the HOMO orbital distribution. The high localization of the spin density on the hydroxyl bonds in para and ortho positions prove that TR, trans-4.4′-DHS, trans-3,4-DHS, trans-3,4,4′-THS and trans-3,4,5-THS are excellent antioxidants. High differences in the distribution of the spin density in α,β-dihydro-3,4′,5-THS were obtained. In the 4′-O- radical of this compound, the contribution of spin density from the all atoms of b-phenyl ring and C=C bond linking the phenyl rings has decreased. In our opinion it is mainly related to the lower ability of this radical to the resonance stabilization caused by the absence of the vinyl bond. Comparing the spin distribution in 4′-O-radical of CR and TR we find that 4′-O-radical of CR is characterized by significant reduction of the spin density at 3- and 5- position. However, this distribution is mainly concentrated around the atoms in the a-phenyl ring. This reduction observed is probably a result of the steric hindrance in the cis-4′-O radical of CR.

The computed spin density in 4′-O and 4-O radicals studied in gas phase (Fig. 8) indicates that the main contribution to this density comes from the oxygen at 4′- and 4-position. The contribution from this oxygen in (2), (3), (4), (5) and (6) radicals presented in the Fig. 8 is smaller than from those in TR. The smallest values of the spin density

on 4′ and 4-oxygen in 4′-O and 4-O-radical of trans-3,4,4′-THS indicate that this compound is most effective antioxidant from among the compounds studied. This finding is in accordance with the results of the computations of BDE and AIP. In the global spin density calculated for 4′-O-radicals of TR, trans-4,4′-DHS, trans-3,4,4′-THS, CR and α,β-dihydro-3,4′,5-THS the main contribution comes from a-ring, whereas in that calculated for 4-O-radicals of trans-3,4-DHS, trans-3,4,5-THS and trans-3,4,4′-THS the main contribution comes from b-ring. It should be emphasized that 4′-O-radical of α,β-dihydro-3,4′,5-THS shows a significant increase in the contribution of the spin density from 4′-O atom and atoms from the a-phenyl ring while very small values of spin density from b-phenyl rings including oxygen's at meta positions were obtained. The absence of vinyl bond in this radical decreases the spin density in b-phenyl ring. It is interesting to note that in cis-4′-O-radical of CR and all trans-4′-O-radicals the spin density takes large values on the carbon of vinyl bond and phenyl ring including 4′- or 4-oxygen. This result confirms the generated spin distribution (Fig. 7) and proves that 4′-OH and 4-OH groups are especially susceptible to free radicals attack. However the high contribution to global spin density from vinyl bond leads to the conclusion that this fragment of radicals can be also sensitive to attack of reactive radical forms. We also observe that the value of the spin density on the 4′-O atom is higher for CR than for TR. Hence, the antioxidant power of CR is lower than that the trans-stereoisomer. In our opinion, this result is related to the distorted geometry of CR. The calculated values of the spin density on the 4′-O and 4-O atoms of the corresponding radicals in aqueous medium are lower than in the gas phase (Table 7). Therefore, in water environment the 4′-OH and 4-OH groups in the all compounds can be more sensitive to the interaction with free radicals. From this fact we can conclude that in water environment the compounds studied show higher antioxidant activity than in the gas phase. Moreover, the lower values of the spin density in water medium are related to strong stabilization of the radicals studied by this medium. The computed values of the spin density confirm that all TR trans-derivatives are better antioxidants than TR in aqueous medium while α,β-dihydro-3,4′,5-THS and CR have weaker antioxidant potency than TR.

4. Conclusions

The results of DFT computations indicate that trans-4,4′-DHS, trans-3,4-DHS, trans-3,4,4′-THS, trans-3,4,5-THS are more active antioxidants than TR, whereas α,β-dihydro-3,4′,5-THS and CR are less able to scavenge free radicals than TR in the environments considered. It has been proved that the most favorable mechanism for radical scavenging is through hydrogen atom donation from the antioxidants studied and their activity can be related to the planar and semiquinone structure of the phenoxy free radicals stabilized by the resonance and the presence of the vinyl bond. The calculations demonstrate that the antioxidant potency strongly depends on the geometry of the neutral compounds and their phenoxy radicals, the number and position of the hydroxyl groups. Since

Table 8
The B3LYP/6-311G(d,p) in the water medium calculated values of the AIP [kcal/mol] and BDE [kcal/mol] of 4′-O–H bond of TR, 4′-O–H bond of trans-4,4′-DHS, 4-O–H bond of trans-3,4,5-THS, 4-O–H bond of trans-3,4,4′-THS, 4′-O–H bond of trans-3,4,4′-THS, 4-O–H bond of trans-3,4-DHS, 4′-O–H bond of α,β-dihydro-3,4′,5-THS and 4′-O–H bond of CR.

Antioxidant	BDE	AIP
TR	80.728	118.940
trans-4,4′-DHS	79.103	113.380
trans-3,4,5-THS	73.914	116.339
trans-3,4,4′-THS (4-OH)	70.075	112.398
trans-3,4,4′-THS (4′-OH)	79.977	112.398
trans-3,4-DHS	71.338	117.414
α,β-dihydro-3,4′,5-THS	85.545	129.106
CR	82.731	120.837

solvent effect is important for investigation of biological processes in living organisms, the influence of the water medium on an antioxidant activity should be taken into calculations. The findings obtained contribute to the understanding of the pharmacological activity of the compounds studied which thus can be exploited in the food chemistry, medicine and pharmacy.

Acknowledgements

References

[1] M.F. Wang, J.G. Li, M. Rangarajan, Y. Shao, E.J. Lavoie, T.C. Huang, C.T. Ho, J. Agric. Food Chem. 46 (1998) 4869.
[2] O. Brede, S. Stajanovic, H. Sprinz, Free Radic. Res. 36 (2002) 76.
[3] P. Brito, L.M. Almeida, T.C. Dinis, Free Radic. Res 36 (2002) 176.
[4] D. Pietraforte, L. Turco, E. Azzini, M. Minetti, Biochim. Biophys. Acta 1583 (2002) 176.
[5] B. Tadolini, C. Juliano, L. Piu, F. Franconi, L. Cabrini, Free Radic. Res 33 (2000) 105.
[6] L.M. Szewczuk, L. Forti, L.A. Stivala, T.M. Penning, J. Biol. Chem. 279 (2000) 22727.
[7] L. Belguendoz, L. Fremont, A. Linard, Biochem. Pharm. 33 (1997) 1347.
[8] L. Fremont, L. Belguendouz, S. Delpal, Life Sci. 64 (1999) 2511.
[9] M. Murias, N. Handler, T. Erker, K. Pleban, G. Ecker, P. Saiko, T. Szekeres, W. Jäger, Bioorg. Med. Chem. 12 (2004) 5571.
[10] J.S. Wright, R.J. Erin, G.A. DiLlabio, J. Am, Chem. Soc. 123 (6) (2001) 1173.
[11] H. Cao, X. Pan, C. Li, C. Zhou, F. Deng, T. Li, Bioorg. Med. Chem. Lett. 13 (2003) 1869.
[12] A.N. Queiroz, B.A.Q. Gomes, W.M. Moraes, R.S. Borges, Eur. J. Med. Chem. 44 (2009) 1644.
[13] J. G-Fan, X.-D. Liu, Y.-P. Qian, Y.-J. Shang, X.-Z. Li, F. Dai, X-L Jin J-G. Fang, B. Zhou, Bioorg. Med. Chem. 17 (2009) 2360.
[14] G. J-Fang, M. Lu, Z.-H. Chen, H.-H. Zhu, Y. Li, L.-M. Li YangWu, Z-Li. Liu, Chem. Eur. J. 8 (2002) 4191.
[15] L.A. Stivala, M. Savio, F. Carafoli, P. Perucca, L. Bianchi, G. Maga, L. Forti, U.M. Pagnoni, A. Albini, E. Prosperi, V. Vannini, Biolog. Chem. 276 (2001) 222586.
[16] A. Matsuoka, K. Takeshita, A. Furuta, M. Ozaki, K. Fukuhara, N. Miyata, Mutat. Res. Genet. Toxicol. Environ. Mutagen. 521 (2002) 29.
[17] M.J. Frisch, G.W. Trucks, H.B. Schlegel, G.E. Scuseria, M.A. Roob, J.R. Cheeseman, J.A. Montgomery, T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Menucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Ciosłowski, B.B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanayakkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople, Gaussian 03. Gaussian Inc., Pittsburgh, PA, 2003.
[18] A.D. Becke, J. Chem. Phys. 98 (1993) 5648.
[19] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785.
[20] A. Klamt, G. Schürmann, J. Chem. Soc. Perkin Trans. 2 2 (1993) 799.
[21] A. Ben-Naim, Y. Marcus, J. Chem. Phys. 81 (1984) 2016.
[22] A.D. Becke, Phys. Rev. A 38 (1988) 3098.