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Theoretical investigation of hydroxytyrosol and its radicals

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Abstract

The structural and electronic properties of hydroxytyrosol, its three radical isomers, and its three analogues hydroxytyrosol-dihydroxyl isomers have been investigated theoretically by performing semi-empirical self-consistent field molecular orbital theory calculations. The geometry of the systems have been optimized and the electronic properties have been calculated at the level of AM1 method.

Keywords: Hydroxytyrosol; Hydroxytyrosol radical; Hydroxytyrosol-dihydroxyl; Antioxidant; Semi-empirical AM1 method; Structure activity relationship

1. Introduction

Antioxidants are considered as an important active ingredient of nutritionally functional foods. Reactive oxygen species (ROS) generated during the life of living cells are thought to play a major role in the pathology of degenerative chronic diseases such as aging, cardiovascular diseases and cancer. The oxidative damage by ROS to critical cellular macromolecules of biomembranes and DNA can be prevented by antioxidants where reactive free radical scavenging by the antioxidants quench the initiation and propogation steps of autooxidation chain reactions [1]. Examples of cytotoxic free radicals include peroxyl, alkoxyl, alkyl, superoxide and hydroxyl. α-Tocopherol in human plasma is an endogen antioxidant, regenerated by reaction with vitamin C. Mechanisms involved in radical scavenging by antioxidants are hydrogen atom transfer and single electron transfer [2,3].

Phenolic antioxidants are present in a wide variety of food products and have at least one hydroxy group attached to a benzene ring. Hydroxytyrosol (HTy) is a natural phenolic antioxidant found in olives and olive oil. It is a catechol derivative of oleuropein and belongs to the secoiridoid group [4]. HTy radical scavenging activity proceeds according to the following scheme, where ArOH represents HTy as phenolic antioxidant and $ArO^{\cdot}$ is the corresponding (relatively stable) HTy free radical:

$$RO_2^{\cdot} + ArOH \rightarrow ROOH + ArO^{\cdot}$$

HTy was able to scavenge hydroxy radical, the most reactive and hence toxic of the ROS known to

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present, along with lipid radicals in iron-loaded hepatocyte culture. However, only caffeic acid and oleuropein could scavenge superoxide anion [5].

Later, Viscoli et al. [6] have shown HTy and the parent compound oleuropein to be potent scavengers of superoxide radicals and inhibitors of neutrophil respiratory burst with low EC_{50}s. Along this line, several other biological and pharmacological activities of these molecules have been reported: protective effect against lipoperoxidative damage and lipoprotein oxidation; antimicrobial, vasodilator, hypotensive, hypoglycemic actions; protective effect against peroxynitrite-dependent DNA damage; potential anticancer activity; inhibitory effects on eicosanoid production and in platelet aggregation and interaction with nitric oxide [4,7,8].

Olives and olive oil are components in food commodities contributing to the well established beneficial health effects of the Mediterranean diet [6]. Disease protective effects range from chemoprotection against cancer (especially of the colorectum, prostate and breast) to heart disease [6,9]. Recently Bianco and Uccella [10] investigated the concentrations of different biophenolic compounds, including HTy, in olives from several Mediterranean countries. Olive oil, another Mediterranean food component carrying biophenolic antioxidants, can be consumed in the natural unrefined state (extra virgin oil quality) or as refined product. Both states have biophenols as minor components but the extra virgin oil quality has them in higher absolute amounts. Owen et al. [9] studied the composition and antioxidant/anticancer potentials of HTy and other phenolic compounds isolated from olive oil and compared them with seed oils. The positive outcome of the published health benefits of the Mediterranean diet is clearly seen in increasing preference of Western countries consumers in olive oil consumption to the Western high fat diets.

Recent trends are elucidation of structure activity relationships of natural and synthetic antioxidants using theoretical approaches to overcome discrepancies in experimental studies and clarify structural/-conformational aspects for the interpretation of ligand (in this case antioxidant) and target/receptor. Structural requirements for optimum scavenging activity can be predicted with molecular modeling and quantum chemical calculations. Another justification of theoretical investigations is the short half-lives of reactive free radicals leading to problems in experimentation. Finally, results of these studies will be used in the development of potent antioxidants and anticancer agents where data on structure activity relationships are needed.

This study was undertaken to investigate the structural and electronic properties of the HTy molecule for the first time theoretically by performing semi-empirical self-consistent field molecular orbital (SCF MO) calculations with special emphasis on the hydrogen-donating capacities of the hydroxyl groups. The results are discussed with relation to effectiveness of its antioxidant activity and lipophilicity and compared with the experimental results of other investigators.

2. Method of calculation

In the present study, isolated hydroxytyrosol molecule and its three radical isomers with three analogues hydroxytyrosol-dihydroxyl isomers have been considered theoretically by performing semi-empirical SCF MO theory calculations. Preoptimization has been performed by applying the molecular-mechanics method [11] using MM + force field [12]; this makes easier to perform full optimization by extended methods. The Austin Model 1 (AM1) semi-empirical molecular orbital (MO) method [13] within the Unrestricted Hartree–Fock (UHF) [14] formalism has been considered to optimize fully the geometry of the systems considered. Geometry optimization is carried out by using a conjugate gradient method (Polak–Ribiere algorithm [15]).

Table 1
Some molecular properties of the systems studied. Number of α electrons, N_α; number of β electrons, N_β; total number of molecular orbitals, N_o; multiplicity; and molecular point group, MPG

Molecule	N_α	N_β	N_o	Multiplicity	MPG
HTy	30	30	54	Singlet	C_1
HTyR1	30	29	53	Doublet	C_1
HTyR2	30	29	53	Doublet	C_1
HTyR3	30	29	53	Doublet	C_1
HTyD1	27	27	50	Singlet	C_1
HTyD2	27	27	50	Singlet	C_1
HTyD3	27	27	50	Singlet	C_1

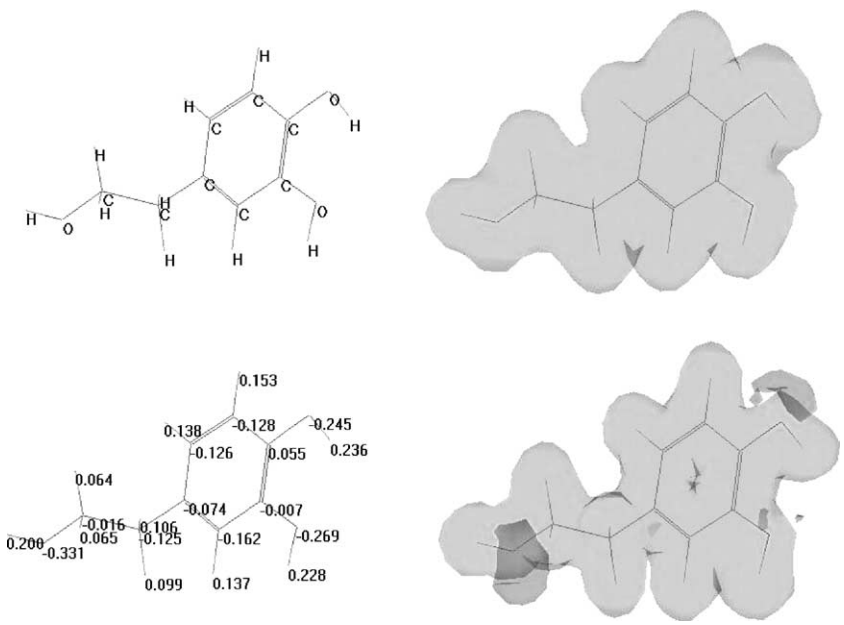

Fig. 1. The optimized structure of hydroxytyrosol molecule (HTy) (upper left), excess charge on the atoms (lower left), 3D charge density plot (upper right), and 3D electrostatic potential plot (lower right).

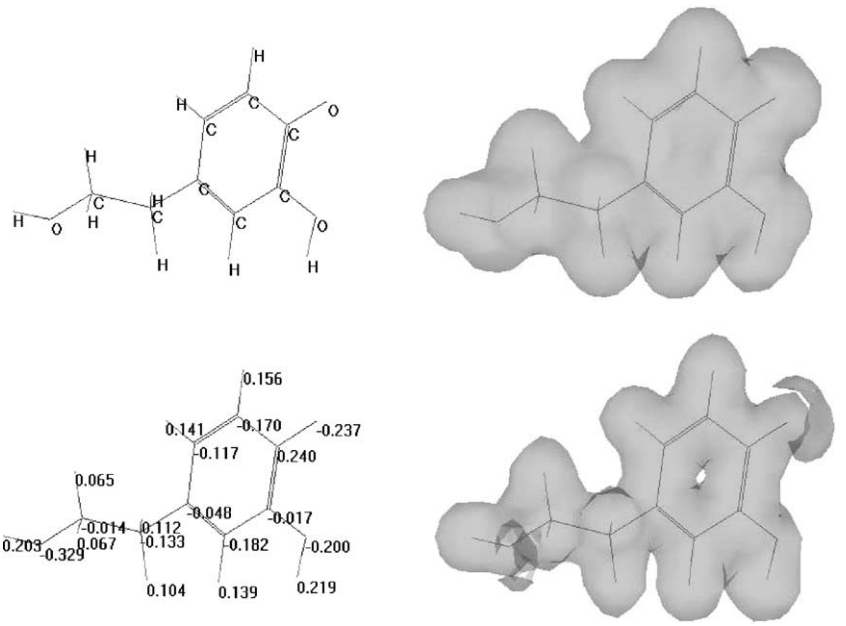

Fig. 2. Same as Fig. 1 but for HTyR1 molecule.

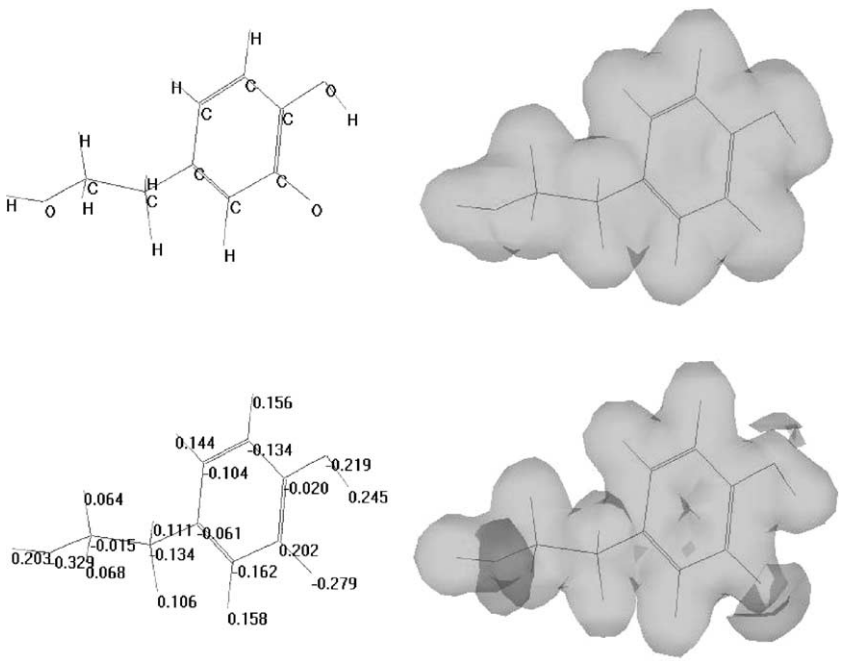

Fig. 3. Same as Fig. 1 but for HTyR2 molecule.

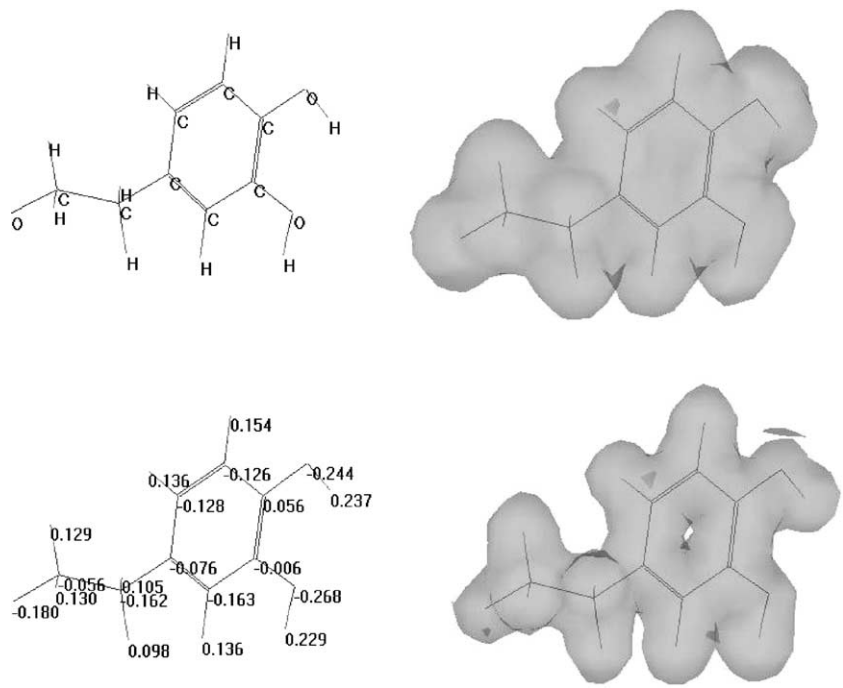

Fig. 4. Same as Fig. 1 but for HTyR3 molecule.

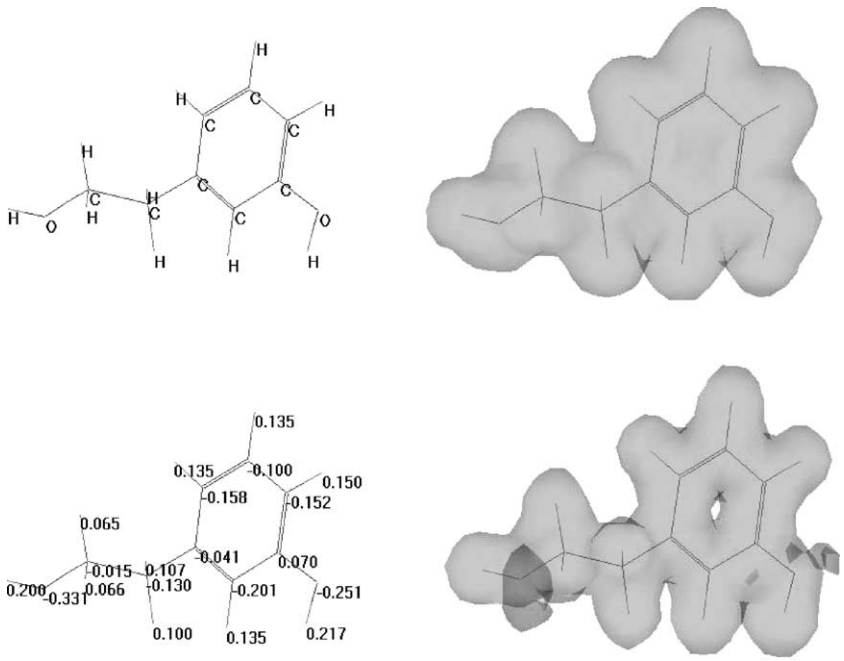

Fig. 5. Same as Fig. 1 but for HTyD1 molecule.

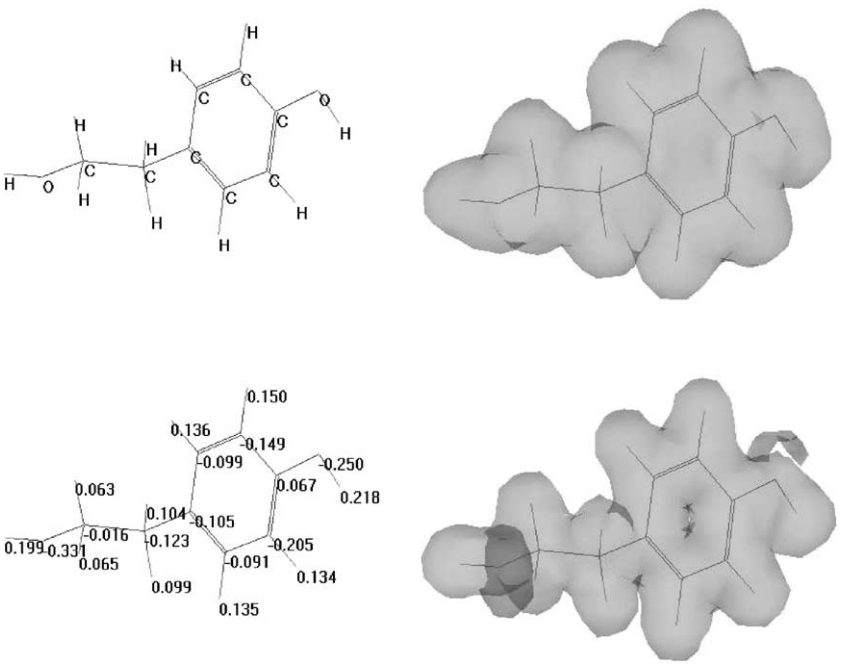

Fig. 6. Same as Fig. 1 but for HTyD2 molecule.

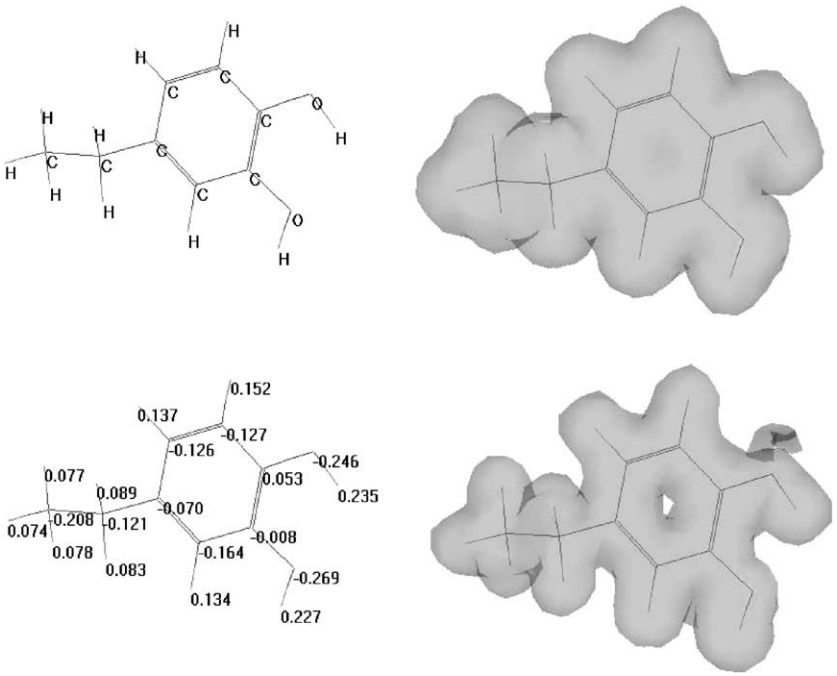

Fig. 7. Same as Fig. 1 but for HTyD3 molecule.

The electronic structure of the systems has been calculated in their ground state. The SCF convergency is set to 0.0001 kcal/mol and the RMS gradient is set to 0.001 kcal/mol in the geometry optimizations. We have performed all the calculations by using the HyperChem-5.1 packet [16].

3. Results and discussion

The closed formula of the hydroxytyrosol molecule (HTy) is in the form $C_8O_3H_{10}$, its radical isomers (hereafter referred to as HTyRx; $x = 1–3$) are in the form $C_8O_3H_9$, and its analogues hydroxytyrosol-dihydroxyl isomers (hereafter referred to as HTyDx; $x = 1–3$) are in the form $C_8O_2H_{10}$. The AM1 geometry optimization yields a nonplanar structure as the stable form of molecules studied. Some of the molecular properties of the systems studied are given in Table 1. The optimized structures (by the AM1 method), calculated excess charge on the atoms of the molecules, 3D charge density plots, and 3D electrostatic potential plots of the systems studied are shown in Figs. 1–7.

Some of the calculated energy values such as total energy, binding energy, and heat of formation (by the AM1 method) of the systems studied are given in Table 2. The highest occupied and the lowest unoccupied molecular orbital energies (HOMO and LUMO, respectively) and the frontier molecular orbital energy gap (LUMO–HOMO

Table 2
Some of the calculated energies of the systems studied. Total energy, E_t; binding energy, E_b; and heat of formation, HoF. Energies are in kcal/mol

Molecule	E_t	E_b	HoF
Hty	−48978.311	−2192.431	−125.614
HTyR1	−48637.486	−2114.409	−99.694
HTyR2	−48640.718	−2117.641	−102.926
HTyR3	−48616.940	−2093.863	−79.148
HTyD1	−41584.748	−2088.450	−81.192
HTyD2	−41584.824	−2088.526	−81.268
HTyD3	−41583.912	−2087.614	−80.356

Table 3
Calculated highest occupied molecular orbital eigenvalue (HOMO); lowest unoccupied molecular orbital eigenvalue (LUMO); frontier molecular orbital energy gap (HOMO–LUMO difference, ΔE); and dipole moment (μ) of the systems studied. Energies are in eV, and dipole moment is in Debye

Molecule	HOMO (α)	LUMO (α)	HOMO(β)	LUMO(β)	ΔE (α)	ΔE (β)	μ
HTy	−8.974	0.386	−8.932	0.363	9.360	9.295	1.740
HTyR1	−9.119	0.200	−10.274	−1.263	9.319	9.011	3.659
HTyR2	−9.281	0.074	−10.027	−1.402	9.355	8.625	2.404
HTyR3	−9.020	0.257	−9.052	−0.619	9.277	8.433	1.455
HTyD1	−9.271	0.371	−9.095	0.364	9.642	9.459	1.249
HTyD2	−9.026	0.363	−9.143	0.456	9.389	9.599	1.547
HTyD3	−8.842	0.485	−8.877	0.477	9.327	9.354	2.328

energy difference, ΔE), and the calculated dipole moment values of the systems considered are given in Table 3.

According to AM1 calculation all the molecules considered are stable and exothermic. The radicals of hydroxytyrosol molecule are ordered with respect to the total energy, binding energy, and heat of formation values as the following: HTyR2 > HTyR1 > HTyR3; HTyR2 is the most stable radical. On the other hand, hydroxytyrosol analogues hydroxytyrosol-dihydroxyl isomers are comparable from stability point of view, their energy values are close to each other; considering the small differences in energies the similar order in stability appears, namely HTyD2 > HTyD1 > HTyD3; HTyD2 is slightly more stable.

According to dipole moment values, HTyR1 has the largest dipole moment (about 3.7 D) among the radical isomers, whereas HTyD3 has the largest dipole moment (about 2.3 D) among the hydroxytyrosol-dihydroxyl isomers. It is abvious that HTyR1 radical interacts with its aqeous environment, especially other polar molecules in the cell, more strongly. Saija et al. [4] have shown HTy to be hydrophilic (high polarity) and therefore have the ability of scavenging aqueous peroxyl radicals near the membrane surface, acting as a powerful antioxidant against lipid peroxidation induced by aqueous oxygen radicals. The present results support this experimental finding; calculated dipole moment of HTy (1.74 D) and its radicals HTyR1 (3.66 D), HTyR2 (2.40 D) show that these are highly hydrophilic molecules.

Excess charge accumulation on the atoms of HTy shows an interesting feature. All carbon atoms have negative excess charge except the one bonded to upper oxygen on the ring (see Fig. 1). Hydrogen atom bonded to the same oxygen (upper on the ring) has the largest positive excess charge. It is most probable that this hydrogen atom (having largest positive excess charge) is donated firstly and primarily during the radical scavenging reaction.

HTy was experimentally shown to be active as scavengers of reactive oxygen and nitrogen species that are implicated in human pathologies. Tyrosol was less active than HTy, higher activity was attributed to the presence of the catechol functionality in the HTy [8].

Although the physiological level of HTy in humans is not known [7], HTy derived from the diet due to consumption of olive oil, can be developed as a potential preventive therapeutic agent for use as a potent scavenger of peroxynitrite based on the results of quantum chemical calculations.

The results obtained in the present study may be used for the experimental works in the analysis of the structure activity relationship of the molecules studied as well as aiding in drug design against human diseases accompanied by free radical injury.

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