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Quantum Mechanical and Experimental Oxidation Studies of Pentadecylresorcinol, Olivetol, Orcinol and Resorcinol

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Resorcinols (pentadecylresorcinol, olivetol, orcinol and resorcinol) exhibit antioxidant properties in liposomal systems. Antioxidant potency depends on the length of the alkyl chain. Pentadecylresorcinol has been demonstrated to be the most active antioxidant, indicating significance of its alkyl chain in a lipid bilayer. Quantum DFT computations demonstrated that hydroxyl group attached to the ring is the first target for the hydrogen abstraction after formation of the radical. However, the carbons of the side chain could also participate in the antioxidant properties of the alkylresorcinols. Formation of the radical at the hydroxyl oxygen initiates changes in the electron density which destabilise the whole system and subsequently leads to oxidation of the ring. The detailed study of lipophilicity and electrostatic properties of resorcinols is discussed.

Keywords: Antioxidants, resorcinols, liposomes, hydrogen abstraction, DFT computations, B3LYP, molecular modelling

INTRODUCTION

Peroxidation of lipids in biological membranes is a complex process in which rearrangement and destruction of double bonds in lipid molecules occurs through propagation of free radicals formed at the beginning of the process.[1,2] Lipid oxidation products and products of degradation of free radicals affect the structures of membranes and they are of great importance in cancer biogenesis, development and ageing.[3–6] However, some strong synthetic antioxidants while being very effective in scavenging free radicals are not beneficial in humans since they may also be carcinogenic.[4] The free radical chain reaction of lipid peroxidation[2] is also a major problem for food manufacturing. Lipid oxidation is responsible for the formation of cancerogens during food processing[7] and such processed

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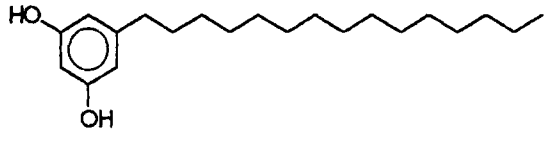
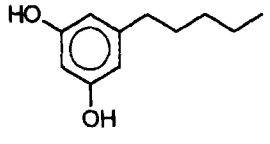
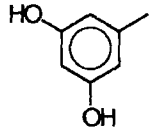
food is supposed to be responsible for colon and breast cancer. Lipid peroxidation is minimised by the use of antioxidants. Most natural and synthetic compounds acting as antioxidants, i.e., protecting lipids against nonenzymatic oxidation *in vitro* and *in vivo*, are of phenolic nature, e.g., tocopherol, ubiquinons, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), t-butylated hydroxyquinone (TBHQ) and flavonoids.[8,9] However, the use of some synthetic molecules is being increasingly limited by safety considerations and their replacement by "natural" antioxidants is being widely advocated. Alk(en)ylresorcinols (1,3-dihydroxy-5-n-alk(en)-ylbenzenes) are natural, amphiphilic, phenolic compounds, with an antioxidant activity.[10] Due to their amphiphilic nature, alk(en)ylresorcinols can be active in hydrophilic and hydrophobic media. Recently, cereal grain alk(en)ylresorcinols have been shown to be antimutagenic.[11] In this study we assume that the demonstrated antioxidant activity of alk(en)ylresorcinols occurs due to their oxidation. The mechanism of phenols' oxidation is highly variable and has been extensively investigated.[12] However the mechanism for diphenols with hydroxyl groups in the meta position in the benzene ring is not known, which is the case of alk(en)ylresorcinols. The mechanism(s) of this activity and reasons for observed differences with respect to the hydrocarbon chain length[10] are also not yet known. Demonstrated antioxidant and antimutagenic properties of alk(en)ylresorcinols motivated us to study this class of compounds to establish physico-chemical mechanisms of their activity against free-radical induced damage of lipid components of biomembranes. The purpose of this study is to find the structure–antioxidant activity relationships (both from experiment and quantum mechanics) and to suggest a possible mechanism in the antioxidant activity of alk(en)ylresorcinols. The studied structures are presented in Scheme 1. We have investigated the mechanisms of oxidation of 5-pentadecyl-1,3-benzenediol (pentadecylresorcinol) and three additional compounds with shorter hydrocarbon chain: 5-pentyl-1,3-benzenediol (olivetol), 5-methyl-1,3-benzenediol (orcinol), and without hydrocarbon chain, 1,3-benzenediol (resorcinol) as models of pentadecylresorcinol. The reference



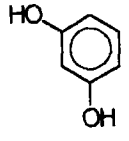
5-pentadecyl-l,3-benzenediol
Pentadecylresorcinol



5-pentyl-l,3-benzenediol
Olivetol



5-methyl-l,3-benzenediol
Orcinol



1,3-benzenediol
Resorcinol

SCHEME 1 The studied structures.

model compounds of pentadecylresorcinol were studied to find the reasons for the changes in the antioxidant activity caused by the presence of the alkyl chain and its length. We were comparing highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies, lipophilicity, electrostatic potential and dipole moments of the closed shell molecules. The hydrogen abstraction as the first step of oxidation of alkylresorcinols was considered. The formation of tautomeric radicals of olivetol, orcinol and resorcinol was analysed with Density Functional Theory (DFT) and the detailed total energy analysis of all the tautomers and closed shell molecules has been performed. The experimental study of antioxidation properties of alkylresorcinols was performed in FAT-PC liposome model. The comparison of the theoretical analysis with experimental results is briefly discussed.

MATERIALS AND METHODS

Egg-yolk lecithin was from Lipid Products, Nutfield, UK. Pentadecylresorcinol was obtained from Aldrich, USA and purified as described in.[13] Olivetol and orcinol were obtained from Sigma, USA; resorcinol was obtained from POCh, Poland. All reagents were of highest available purity and were used as stock chloroform solutions. All the preparations, solutions and buffers were prepared using nanopure water.

Experimental Methods

All homologues of alkylresorcinols were used as methanolic solutions. Membrane oxidation was studied on FAT-PC liposome model. The liposomes were prepared as follows: lipid solutions (10 mg of lipid) were mixed in round bottom flask and the solvent was evaporated using rotary vacuum evaporator. The lipid film was additionally kept overnight in vacuum desiccator over the calcium chloride. The lipids were hydrated at 40°C until multilammellar vesicles were formed. Liposomal suspensions were further subjected to seven freezing–thawing cycles involving freezing in liquid nitrogen and thawing in warm (50°C) water.[14] Peroxidation of lipids was induced by Fe^{2+} ions. The incubation mixtures contained 1 mg/ml liposomes, resorcinolic compound (pentadecylresorcinol or olivetol or orcinol or resorcinol) in concentration range 0.5–200.0 μM and oxidation inducer (0.03 mM Fe^{2+}). The extent of oxidation after 15 min reaction was estimated by determination of thiobarbituric acid reacting substances (TBARS).[15] The level of TBARS was detected by absorbance measurements at 535 nm using UV-VIS spectrometer Cary2E. Inhibition of peroxidation in the sample was calculated in a standard way, $I = (A_{max} - A)/(A_{max} - A_{nat}) \times 100\%$, where A_{max}, A_{nat} and A are absorbances registered for maximally oxidised, native and studied (oxidised in the presence of a resorcinolic compound) samples, respectively. Concentrations of a resorcinolic compound, at which I is equal to 50% or 100%, are denoted as IC_{50} and IC_{100}, respectively.

Computational Methods

To study the problem by molecular modelling methods and methods of quantum chemistry, the molecules of interest: pentadecylresorcinol, olivetol, orcinol, resorcinol were built with a molecular modelling software package Sybyl v.6.2.[16] The geometry of each closed shell molecule and its radical was optimised using Gaussian 94 Rev. D.4[17] with the Hartree–Fock and B3LYP density functional methods with the 6-31G** functional basis set. The optimised geometries of the molecules and their radicals, as well as electronic structures at these geometries, were analysed with Cerius2 v.2.1[18] and

Sybyl v.6.3[19] software package on a Silicon Graphics workstation. HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) and dipole moments were determined at the Hartree–Fock level. The Connolly surfaces for resorcinolic compounds were created in Sybyl v.6.3 with the standard probe sphere radius 1.4 Å. Sybyl was used to visualise the molecular geometry, partial net atomic charges, dipole moments, lipophilic and electrostatic potentials. The total energies E_{tot} were determined at the B3LYP level to include electron correlation and exchange effects. The total energy increase $\Delta E_{tot} = (E^{R}_{tot} + E^{H}_{tot}) - E^{RH}_{tot}$ was calculated for the reaction: R–H→R + H as the difference of total energy of products and the total energy of the substrate. R–H denotes the studied resorcinolic compound, R denotes resorcinolic compound radical, and H denotes atomic hydrogen; E^{RH}_{tot} denotes the total energy of resorcinolic compound, E^{R}_{tot} denotes the total energy of its radical and E^{H}_{tot} is the total energy of hydrogen atom (−0.5 Hartree).

RESULTS

Experimental Results

The antioxidant activity of resorcinol, orcinol, olivetol and pentadecylresorcinol is shown by their IC_{50} and IC_{100} values in Table I. Resorcinol, i.e., the simplest of the studied molecules was able to protect liposomes against Fe^{2+} peroxidation completely at concentration of 150 μM.

TABLE I The antioxidant activity of resorcinol, orcinol, olivetol and pentadecylresorcinol shown by their IC_{50} and IC_{100} values

Phenolic compound	IC_{50} [μM]	IC_{100} [μM]
Resorcinol	40 ± 1.4	150 ± 1.7
Orcinol	35 ± 1.6	140 ± 1.6
Olivetol	19 ± 1.1	85 ± 1.4
Pentadecylresorcinol	14 ± 0.8	75 ± 1.2

The characteristic feature of the results is the dependence of IC_{50} and IC_{100} values on the presence and length of the alkyl chain in the resorcinolic compound structure. It is evident that pentadecylresorcinol is the most active, but only about 11% more active than olivetol. The most significant increase of the antioxidant properties is observed with the increase of the alkyl chain up to 5 carbons. This fact proves that the chain plays an important but unrecognised role in the process of protection of the lipid membrane against free radical damage. The results suggest the importance, in relation to biological system, of both the resorcinolic ring and side attachments.

Computational Results

The optimised geometries for pentadecylresorcinol, olivetol, orcinol and resorcinol are presented in Figure 1 (part A, B, C and D, respectively). One can see the similarity in the optimised geometries, which are almost identical in the common fragment of their structure. Also, the geometry of the chains differs only by the length. The net atomic charges resulting from the B3LYP/6-31G** computations were assigned to atoms in Sybyl and used in the analysis of the electrostatic and the lipophilic potentials mapped on the Connolly surfaces surrounding the studied structures of resorcinolic compounds. The lipophilic potential (LP) on the Connolly surfaces for pentadecylresorcinol, olivetol, orcinol and resorcinol is presented in Figure 2 (part A, B, C and D, respectively). In Figure 3 the electrostatic potential (EP) on Connolly surfaces for pentadecylresorcinol, olivetol, orcinol and resorcinol is visualised (part A, B, C and D, respectively). The results of computations of total energy for the studied structures are summarised in Tables II–IV and classified in Schemes 2–4 for resorcinol, orcinol and olivetol, respectively. HOMO and LUMO energies and dipole moments for resorcinol, orcinol, olivetol and pentadedylresorcinol are presented in Table V.

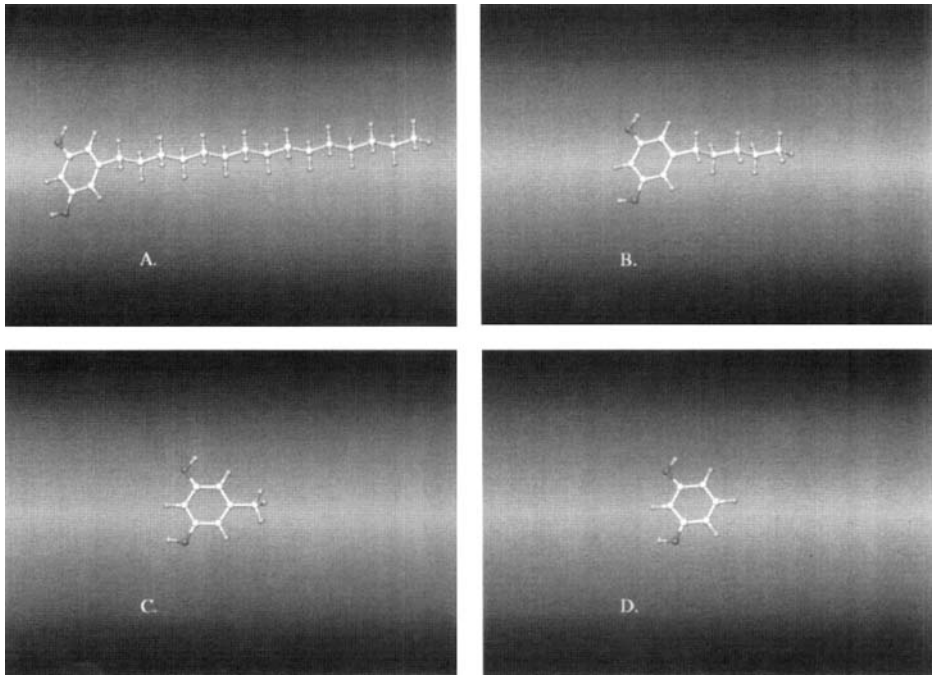


FIGURE 1 The B3LYP optimised geometries of: pentadecylresorcinol (A), olivetol (B), orcinol (C) and resorcinol (D). Oxygen is denoted in red, carbon atoms are in antique white and light cyan balls denote hydrogen atoms. (See Colour Plate I at the end of this issue.)

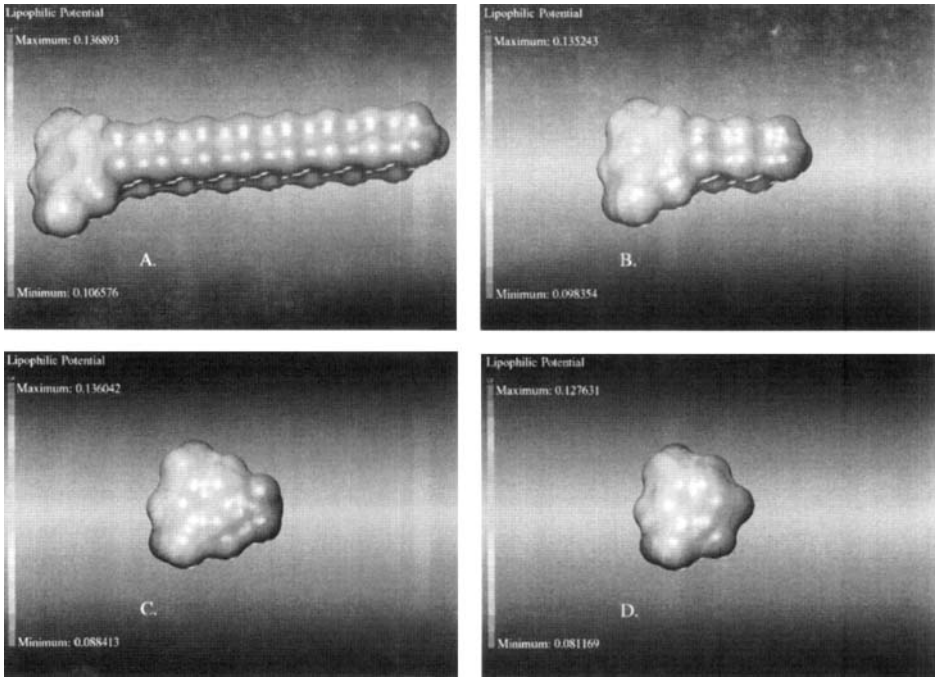


FIGURE 2 The lipophilic potential (LP) mapped on the Connolly surface of: pentadecylresorcinol (A), olivetol (B), orcinol (C) and resorcinol (D). The blue colour corresponds to hydrophilic and the brown to lipophilic areas on the surface. (See Colour Plate II at the end of this issue.)

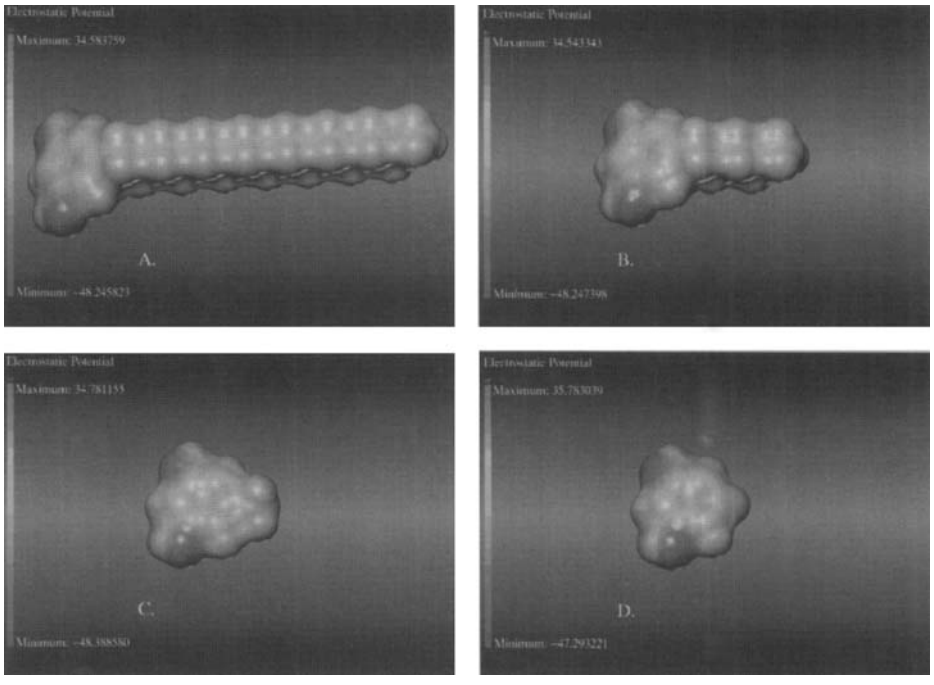


FIGURE 3 The electrostatic potential (EP) in e/Å units mapped on the Connolly surface of: pentadecylresorcinol (A), olivetol (B), orcinol (C) and resorcinol (D). The highest values are denoted in red while the lowest are in violet. (See Colour Plate III at the end of this issue.)

TABLE II The results of computation of the total energy of resorcinol and its radicals at the B3LYP level. Notation for radicals and their classes is given in Scheme 2

Resorcinol and its radicals	Total energy E_{tot} [Hartree]	ΔE_{tot} [kcal/mol]
Resorcinol	−382.6992496	—
R10/a	−382.0542932	91.0
R4/a	−382.0566476	89.5
R7/b	−382.0057192	121.4
R9/b	−382.0051298	121.5
R11/b	−382.0077954	120.1
R12/b	−382.0103485	118.5

TABLE III The results of computation of the total energy of orcinol and its radicals at the B3LYP level. Notation for radicals and their classes is given in Scheme 3

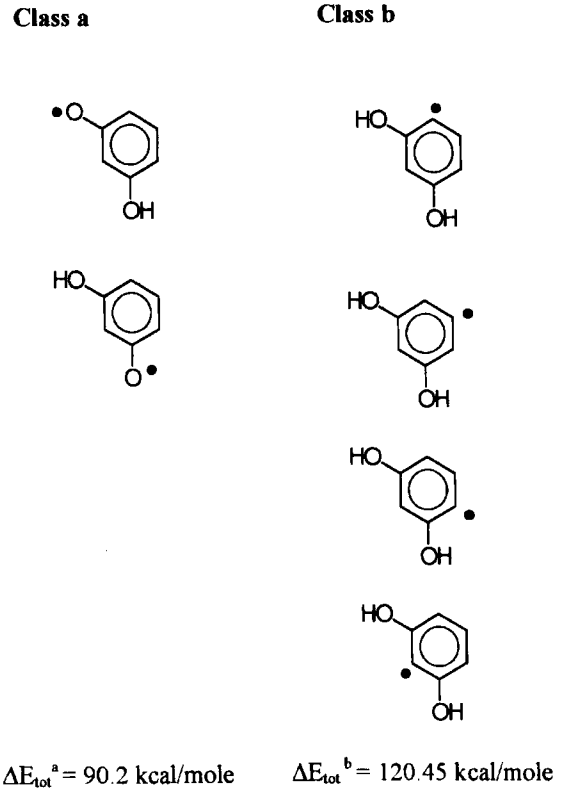
Orcinol and its radicals	Total energy E_{tot} [Hartree]	ΔE_{tot} [kcal/mol]
Orcinol	−422.019984	—
R7/c	−421.326090	121. 7
R9/c	−421.326323	121.5
R10/c	−421.329005	119.8
R14/a	−421.377922	89.1
R17/a	−421.375628	90.6
R12/b	−421.366447	96.3
R15/b	−421.366447	96.3
R16/b	−421.366447	96.3

DISCUSSION

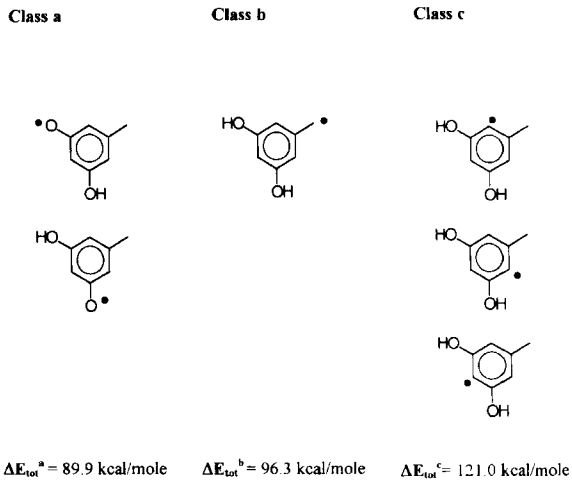
As it was mentioned by us earlier the antioxidant activity of the resorcinols is assumed to occur by their oxidation. On the basis of HOMO (the highest occupied molecular orbital) and LUMO (the lowest unoccupied molecular orbital) energy values we may try to establish if the differences in antioxidant efficiency of resorcinols (which is manifested in experiments) can be interpreted in terms of electron transfer. HOMO and LUMO energies, which are listed in Table V, show small trend for increase of the highest occupied energy levels with the alkyl chain length increase. The highest difference in HOMO value corresponds to the appearance of the methyl group attached

TABLE IV The results of computation of the total energy of olivetol and its radicals at the B3LYP level. Notation for radicals and their classes is given in Scheme 4

Olivetol and its radicals	E_{tot} [Hartree]	ΔE_{tot} [kcal/mole]
Olivetol	−579.2860883	—
R7/e	−578.5922171	121.7
R9/e	−578.5926412	121.4
R11/e	−578.5952816	119.7
R10/a	−578.6416466	90.6
R17/d	−578.6121268	109.2
R28/d	−578.6122297	109.1
R22/c	−578.6186276	105.1
R23/c	−578.6183270	105.3
R25/c	−578.6185598	105.1
R26/c	−578.6187602	105.0
R20/b	−578.6368262	93.7
R19/a	−578.6439720	89.2



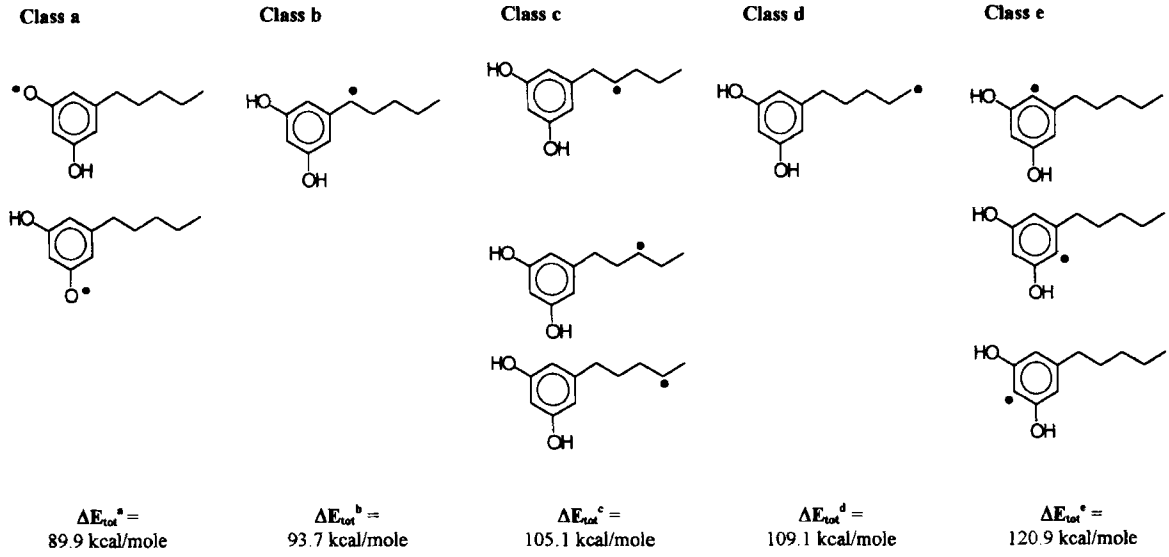
SCHEME 2 Classification of radicals of resorcinol. Class a denotes radicals on a hydroxyl oxygen (radicals R4 and R10 in Table II); class b denotes radicals on a ring carbon (R7, R9, R11 and R12 in Table II).



SCHEME 3 Classification of radicals of orcinol. Class a denotes radicals on hydroxyl oxygen (radicals R14 and R17 in Table III), class b denotes radicals on sp^3 carbon atom (R12, R15 and R16 in Table III), class c denotes radicals on a ring carbon (R7, R9 and R10 in Table III).

to the ring. The higher the HOMO level, the easier an electron can be donated by a molecule, and the easier the molecule can be oxidised by electron transfer mechanism. LUMO energies of resorcinolic compounds also increase with the presence and the length of the alkyl chain in the studied structures. In general, the increase of the LUMO energy also denotes that the reduction of a molecule is more difficult. However, it is easy to observe that the differences in HOMO energies are insignificant and less than $3kT$ (where k is the Boltzmann constant and T is the absolute room temperature). Also differences in LUMO energies are insignificant.

The electronic structure of olivetol or orcinol mimics well the electronic structure of pentadecylresorcinol (which is much more tedious for quantum DFT or *ab initio* computations). However, for the same reason, we find practically no rationale for the electron transfer mechanism of oxidation of resorcinolic compounds and cannot differentiate the antioxidant potency of different compounds by differences of HOMO and LUMO energies. While considering the possible alternative molecular mechanism of alkylresorcinols' oxidation we concentrated on the simplest



SCHEME 4 Classification of radicals of olivetol. Class a denotes radicals on hydroxyl oxygen (radicals R10 and R19 in Table IV). Class b denotes radicals on the first carbon atom in the chain (R20 in Table IV). Class c denotes radicals on the second, third and fourth carbon in the chain (R22, R25 and R26 in Table IV). Class d denotes radicals on the last carbon in the chain (R17, R28 in Table IV). Class e denotes radicals on a ring carbon (R7, R9 and R11 in Table IV).

TABLE V HOMO and LUMO energies and dipole moments determined for closed shell molecules of resorcinolic compounds by the Hartree-Fock method using the 6-31G** functional basis set

Resorcinolic compound	HOMO energy [eV]	LUMO energy [eV]	Dipole moment [Debye]
Resorcinol	−8. 293	4.077	1.43
Orcinol	−8.249	4.149	1.49
Olivetol	−8.223	4.155	1.46
Pentadecylresorcinol	−8.217	4.162	1.46

possibility, i.e., on the possibility of oxidation by dehydrogenation of alkylresorcinols by free radicals. Considering hydrogen abstraction as a possible first step of oxidation of alkylresorcinols, the systematic elaboration by the B3LYP/6-31G** density functional method was performed on the radical structures that can be obtained from resorcinol, orcinol and olivetol by abstraction of the hydrogen atom. The radical formation process occurs with the increase of the total energy of the studied molecular systems. The reason for the elaboration was to find such radical structures, for which the increase of total energy ΔE_{tot} is minimal. The results of the computation are presented in Tables II–IV (and classified in Schemes 2–4) for resorcinol, orcinol and olivetol, respectively. In all the compounds studied, the hydrogen bonded to the ring carbon atoms are most unlikely to be abstracted, since the increase of the total energy ΔE_{tot} in such cases is the highest. On the basis of the performed computation one cannot, however, distinguish between the radicals formed by hydrogen abstraction from the hydroxyl groups and by the abstraction from the first carbon of the alkyl chains. Both processes are of approximately the same ΔE_{tot}. ΔE_{tot} difference for the two classes of radicals is less than 5 kcal/mole for olivetol; in the case of orcinol, however, the difference is ca. 7 kcal/mole. Such values are of

the order of magnitude of a hydrogen bonding energy. ΔE_{tot} for the two classes of radicals cannot be distinguished by quantum computations of isolated molecules. In spite of a very high accuracy of computation (10^{-8} Hartree), the limitation comes from the quantum chemistry model. Also for these reasons we cannot interpret the higher or lower antioxidant activity of the compounds by differences of the total energy in hydrogen abstraction. However, the values of ΔE_{tot} can suggest to us that if the abstraction occurs (at least as the first step of the complex molecular oxidation process), the formation of radicals on hydroxyl oxygen or the first carbon after the ring is much more probable from an energetic point of view, than formation of other radicals by abstraction of hydrogen. The released hydrogen can easily be captured and strongly bonded to some reactive molecular species such as the hydroxyl radical. From the energetic point of view, the formation of the alkylresorcinol radical by hydrogen abstraction can lead to the lower energy level of the whole system (if chemical bonding of the released hydrogen leads to greater decrease of total energy of the newly formed molecule than the increase caused by breaking the bonding to the alkylresorcinol).

Still looking for the answer to the question, what makes pentadecylresorcinol or olivetol more active than resorcinol or orcinol in lipid membrane reaction medium, we performed the comparative analysis of physical properties of resorcinolic compounds. The properties were mapped on the Conolly surfaces. The space inside the Connolly surface forms the molecule's body and the properties mapped on this surface are manifested by the molecule in its surrounding. We used the B3LYP optimised geometry and net atomic charges and performed a molecular modelling study of lipophilicity (hydrophobicity) and electrostatic potential of resorcinol, orcinol, olivetol and pentadecylresorcinol. The hydrophobicity of the molecules increases in obvious sequence: resorcinol, orcinol, olivetol and pentadecylresorcinol, which can be observed with the larger areas on Connolly surfaces coloured brown in the Figure 2. Also the minimal values of lipophilic potential (LP) can be ordered the same way, showing that the hydrophilicity of hydroxyl groups neighbourhood depends on the presence of the alkyl chain in the molecule and the length of the chain changes the area of the lowest values of LP. In the case of resorcinol, this region is not continuous. In the case of all other molecules studied, the area of the lowest LP is continuous and increases with the chain length. For resorcinol, one can see that its highest LP area is surrounding the three neighbouring nonhydroxyl hydrogens. For orcinol, the highest LP area is the area around the methyl group and the neighbouring nonhydroxyl hydrogens. The maximal value of LP for orcinol is nearly the same as it is for pentadecylresorcinol. For olivetol, the highest LP area is the area around the hydrogens of the alkyl chain and the nonhydroxyl hydrogen atoms while for pentadecylresorcinol, the highest LP area is around the terminal methyl group and hydrogens of the alkyl chain. The LP colour map of olivetol fits more closely the map of orcinol in its ring part, while it is more similar to the LP colour map of pentadecylresorcinol in the last 5 carbons ending the chain. In Figure 3, the electrostatic potential (EP) colour maps show that for all the molecules studied the regions of lowest and highest values of EP are located in their ring part. The neighbourhoods of oxygen are of the lowest EP values, while the highest values of EP are situated mainly on hydroxyl hydrogens and their hydrogen neighbours. For resorcinol and orcinol, the region of the highest EP extends also around other hydrogens. EP colour maps of pentadecylresorcinol and olivetol are nearly identical also in absolute values; EP map for olivetol fits well the map for pentadecylresorcinol in its ring part and first 5 carbons after the ring. The corresponding values of EP for resorcinol are about 1 e/Å greater than for the other molecules studied.

The comparison of physical properties of the resorcinolic compounds studied shows that the properties of their ring part depend on the presence and the length of the alkyl chain. The

presence of the chain increases the hydrophobicity of the molecule and decreases the values of electrostatic potential on the Connolly surfaces. One can expect that the more hydrophobic a resorcinolic compound is, the deeper it incorporates and the better it can anchor into the hydrophobic core of the lipid membrane. For these reasons, the antioxidant activity of different alkylresorcinols can be exhibited in different locations with respect to the phosphorus plane of the phosphatidylcholine liposome membrane. Also, the angular orientation of the less hydrophobic molecules is probably more random than it occurs for alkylresorcinols with longer chains. For the latter, the hydroxyl groups or first carbons after the ring can be oriented more efficiently to the water medium, where the radical attack comes from.

In conclusion, on the basis of results of the *quantum* mechanical computations and the molecular modelling study, we may consider the first step of oxidation of alkylresorcinols by hydrogen abstraction from hydroxyl group. Some involvement of the first sp^3 carbon after the ring should also be considered. We suggest that further oxidation of the studied structures can occur in the ring, but the alkyl chain can play some subsidiary role not only by the increase of the hydrophobicity of the molecule with its length.

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