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Radical Scavenging Potential of Phenolic Compounds Encountered in *O. europaea* Products as Indicated by Calculation of Bond Dissociation Enthalpy and Ionization Potential Values

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The radical scavenging potential of phenolic compounds occurring in *Olea europaea* and of recently identified hydroxytyrosol metabolites was evaluated by means of quantum chemical calculations. The bond dissociation enthalpy (BDE) of phenolic hydroxyl groups and the ionization potential (IP) were calculated as descriptors to predict the H-atom-donating and electron-donating abilities of antioxidants, respectively. Catechol derivatives had the lowest BDE values (77.7−80.1 kcal/mol) whereas the lignans, pinoresinol and 1-acetoxypinoresinol, and other monophenols had much higher BDE values (85.1−88.0 kcal/mol), which suggested a lower potential for radical scavenging. Side chain characteristics were not found to affect the size of BDE values although differences in lipophilicity (on the basis of calculated Log *P* values) indicate variability in the activity in real systems. Conclusions for the antioxidant potential could not be drawn based on the IP values. Lack of experimental data for most of the studied compounds due to oxidative instability and difficulties in synthesis or isolation supports the usefulness of a computational approach for those interested in the antioxidant potential of phenolics encountered in *O. europaea* products.

KEYWORDS: Bond dissociation enthalpy; density functional theory; ionization potential; lignans; *O. europaea* products; oleuropein; hydroxytyrosol metabolites

INTRODUCTION

This work is a further step of our investigation on structure−activity relationships of phenolic antioxidants (*1*−*5*). The oxidative instability and difficulties in synthesis or isolation of individual compounds seem to restrict respective studies for a great number of phenolics. The aim of the present study was the elucidation of the radical scavenging potential of compounds naturally encountered in methanol or water/methanol extracts of *Olea europaea* products (leaves, unprocessed and processed olives, and virgin olive oil) (*6*−*15*) as there is a lack of published experimental data for most of them. To our knowledge, no bond dissociation enthalpy (BDE) or ionization potential (IP) values are also available for the compounds under investigation. In the most relevant studies, especially those using density functional theory (DFT) or ab initio calculations, the antioxidants examined are of a moderate molecular size (i.e., MW 94−224) (*1*, *4*). Given that the size of certain selected compounds did not allow the application of the above methodologies, a combined DFT/AM1 method was employed, which provides a good combination of economy and accuracy (*16*) despite some limitations. The lack of experimental data for the most of the compounds supports the usefulness of such a computational approach for those interested in the antioxidant potential of bioactive compounds such as oleuropein. The results may assist decisions on the use of antioxidants from *O. europaea* products in medical or food applications.

MATERIALS AND METHODS

Method of Calculation. By definition, (i) BDE = $H_r + H_h - H_p$, where H_r is the enthalpy for a radical generated after H-atom abstraction, H_h is the enthalpy for a hydrogen atom, and H_p is the enthalpy for a parent molecule, and (ii) IP = $E_c - E_P$, where E_c is the energy of a cation radical generated after electron transfer and E_p is the energy of parent phenol. Lower values for the two molecular descriptors indicate a higher radical scavenging activity.

For the compounds under investigation, the O−H BDEs and IPs were calculated by the combined DFT method proposed by Wright et al. (*16*), which takes advantages of economy and accuracy. The detailed procedures are as follows. The geometry optimization and the total

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determination of vibration frequencies were performed on the AM1 level (*17*). Electronic energies were obtained by the B3LYP functional on the 6-31G (*p*′) level, where *p* represents polarization functions only on hydrogen and *p*′ indicates that the normal *p* exponent has been modified to the value 1.0 (*18*). Using the electronic energies and the scaled zero point vibrational energies (the scaled factor is 0.947), O−H BDE and IP values were obtained (*16*). For certain compounds, in water IP values were also calculated. The solvent effect was considered by employing the self-consistent reaction field method with a polarized continuum model to do a single point calculation on the B3LYP/6-31G (*p*′) level. This method has been justified in a recent study (*19*). All of the quantum chemical calculations were accomplished by Gaussian 94 (*20*).

Calculation of Partition Coefficient (Log *P*). Calculation of the Log *P* values, simulating partitioning of phenols in an *n*-octanol/water (1:1, v/v) system, was based on Broto's fragmentation method (*21*) and was accomplished using the Chem Draw program (4.5 Ultra, Cambridge Soft Corp., 1985−1997).

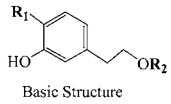
Evaluation of Antioxidant Activity in Bulk Oils. Purified triolein samples containing caffeic acid, hydroxytyrosol, or oleuropein at the level of 10 mg/kg were stored in an oven at 45 °C. Aliquots of triolein (2.5 g) were then distributed in a series of clear open transparent glass bottles of pharmacopeia quality (18 mm i.d.). The process of oxidation was followed by periodic measurement of the peroxide value (PV) in duplicate (CV % = ±2.6, $n = 7$ for PV = 10). Triolein was purified as reported elsewhere (*3*).

RESULTS AND DISCUSSION

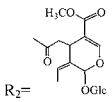
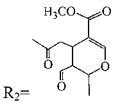
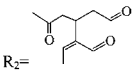
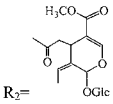
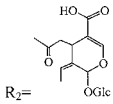
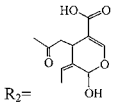
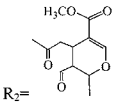
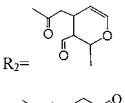
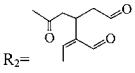
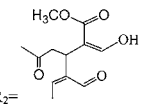
The examined components were the simple phenols tyrosol (**1**), hydroxytyrosol (**6**), secoiridoid oleuropein (**8**), verbascoside (**16**), and derivatives (**2**−**5**, **7**, and **9**−**14**) and also the lignans pinoresinol (**17**) and 1-acetoxypinoresinol (**18**) (Chart 1). The set included certain nonphenolic compounds reported to be present in the above extracts (**19**−**21**). The radical scavenging activity of tyrosol, hydroxytyrosol, and oleuropein has been studied extensively (*6*−*10*), but published works for the activities of other derivatives are rather limited. Indeed, limited is the experimental evidence for the acetate analogue of hydroxytyrosol (*11*), the dialdehydic form of elenolic acid linked to hydroxytyrosol or tyrosol (*12*, *13*), an aldehydic form of oleuropein aglycone (*11*, *12*), verbascoside (*14*) for the lignan 1-acetoxypinoresinol (*15*) and also for certain metabolites of hydroxytyrosol identified in human and rats urine (*22*). Because the study of the radical scavenging potential of all of the selected compounds was not feasible experimentally, a computational approach was employed. To this point, the values of molecular descriptors such as the O−H BDE and the IP were calculated as indices of expected differences in the radical scavenging activity of phenolic compounds through hydrogen atom or electron donation (*23*). The calculated BDE and IP values for the compounds of **Figure 1** are given in **Table 1**. In the same table, IP values in the gas phase, the molecular weights, and calculated Log *P* values are also given.

Grouping of Compounds of Figure 1 on the Basis of BDE Values. On the basis of the calculated BDE values (**Table 1**), the investigated compounds were sorted into three groups according to their hydrogen atom-donating ability. Compounds **1**−**5**, **17**, and **18**, which are all monophenols, comprised group A; compounds **6**−**16** possessing a catechol moiety comprised group B, whereas the nonphenolic compounds cornoside, helleridone, and elenolic acid (**19**−**21**) were group C. The catechol derivatives (i.e., hydroxytyrosol, oleuropein, and verbascoside) presented lower BDE values (77.7−80.1 kcal/mol) in comparison to those of monophenols (i.e., tyrosol, ligstroside, and pinoresinol) (85.1−88.0 kcal/mol). Caffeic acid (**15**), included in the study for comparison since both experimental and theoretical evidence can be found in the literature (*1*, *3*, *4*, *24*), had the lowest BDE value. The difference between the highest BDE value calculated for members of group A and the lowest one for members of group B was approximately 5 kcal/mol, whereas the differences among members of each group were much smaller. It can, therefore, be postulated that all of the compounds of group B will be more efficient antioxidants than the monophenols. Elenolic acid, cornoside, and halleridone (group C) are not expected to exhibit any antioxidant properties, and indeed, their corresponding BDE values were much higher (96.14−102.63 kcal/mol) even than that of unsubstituted phenol

Chart 1



Basic Structure

R_1=H	R_2=H	Tyrosol (**1**) 2-(4-hydroxyphenyl)ethanol
R_1=H	R_2=Glc	Tyrosol glucoside (**2**)
R_1=H	R_2= 	Ligstroside (**3**)
R_1=H	R_2= 	Aldehydic form of ligstroside aglycone (**4**)
R_1=H	R_2= 	Tyrosol-dialdehydic form of elenolic acid (**5**)
R_1=OH	R_2=OH	Hydroxytyrosol (**6**) 2-(3,4-dihydroxyphenyl)ethanol
R_1=OH	R_2=$COCH_3$	Hydroxytyrosol acetate (**7**)
R_1=OH	R_2= 	Oleuropein (**8**) (ester of 3,4-dihydroxyphenylethanol with elenolic acid glucoside)
R_1=OH	R_2= 	Demethyloleuropein (**9**)
R_1=OH	R_2= 	Oleuropein aglycone I (**10**)
R_1=OH	R_2= 	Aldehydic form of oleuropein aglycone (**11**)
R_1=OH	R_2= 	Oleuropein aglycone II (**12**)
R_1=OH	R_2= 	Hydroxytyrosol-dialdehydic form of elenolic acid (**13**)
R_1=OH	R_2= 	Oleuropein aglycone III (**14**)

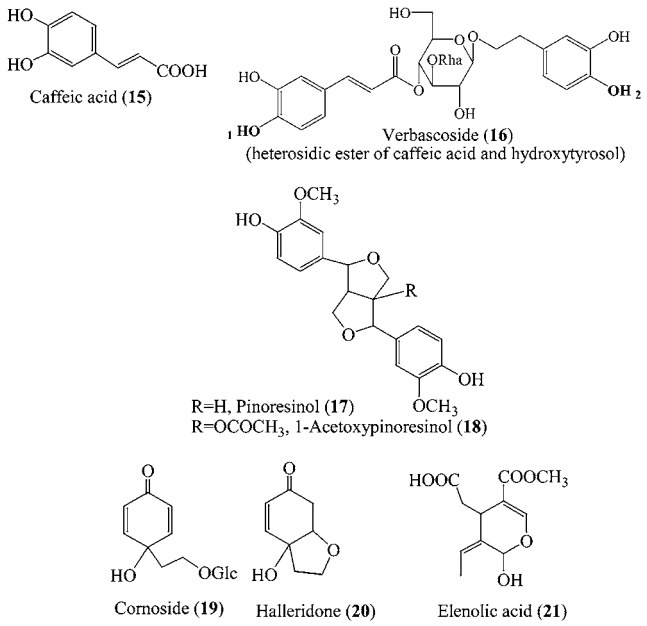


Figure 1. Phenolic compounds encountered in *O. europaea* products.

Table 1. B3LYP/6-31G (*p*′)//AM1-Calculated O–H BDEs, IPs, and Other Physicochemical Parameters for Phenolic Compounds Encountered in *O. europaea* Products

AH	O–H BDE (kcal/mol)[a]	IP (kcal/mol)[b]	MW	Log *P*
1	85.4	177.4	138.16	1.18
2	85.1	173.7	300.30	–0.39
3	85.9	176.6	529.51	–0.58
4	85.9	171.4	362.14	1.04
5	86.2	175.7	304.34	1.23
6	78.4	171.8	154.16	0.79
7	78.9	177.2	196.2	1.39
8	79.9	176.0	540.19	–0.97
9	80.1	169.4	526.17	–1.24
10	78.6	170.9	378.37	1.64
11	78.7	169.9	378.37	0.65
12	78.6	169.4	320.34	1.14
13	79.3	168.2	320.34	0.84
14	78.8	171.5	378.37	0.94
15	77.7	176.6	180.16	1.15
16		167.0	624.12	–0.13
16–H(1)	78.8			
16–H(2)	77.9			
17	86.0	156.2	358.39	1.93
18	88.0	161.2	416.5	1.54
19	100.4	195.8	316.3	–2.50
20	102.6	202.7	154.16	–1.06
21	96.1	178.4	242.23	0.14
phenol	86.4	187.8		

[a] O–H bond disociation enthalpy. [b] IP.

(86.42 kcal/mol). The predicted superiority for the radical scavenging activity by the catechol derivatives is in line with experimental data for phenolic antioxidants (*25*).

IP values did not exhibit any regularity as this descriptor depends on the whole structure of the molecule and not on the effects introduced to the phenolic ring by substituents, so that no similar grouping could be derived. It may be argued that electron donation is not the prevalent mechanism of action for all phenolic antioxidants (*23*).

Radical Scavenging Potential of Compounds Belonging to Group A. The most common members of the group (**1**−**5**) are not expected to show differences in radical scavenging potential on the basis of BDE values. Differences could be expected in real systems due to differences in molecular weight

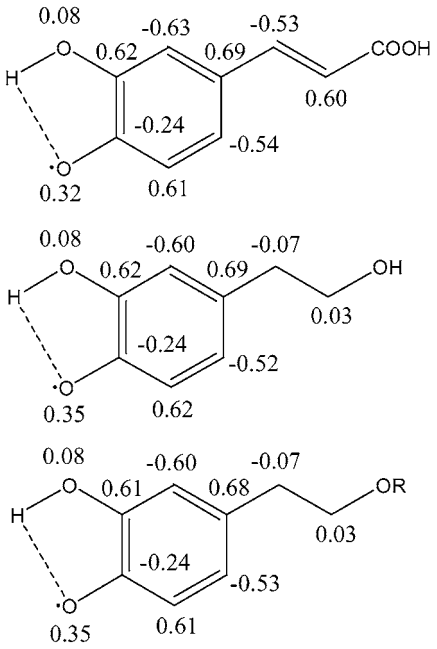


Figure 2. Spin density of the phenoxy radical of caffeic acid; hydroxytyrosol and oleuropein calculated by the AM1 method.

and consequently in lipophilicity of phenolic antioxidants. However, no such data are available for all of the above-mentioned compounds. Data found for tyrosol activity were mainly for comparison with that of olive product polyphenols proving that the former is rather inactive (e.g., *6*, *15*).

The results for the radical scavenging potential of the two lignans, pinoresinol (**17**) and 1-acetoxypinoresinol (**18**), which were recently identified in some virgin olive oils in significant amounts (*26*), were not in agreement with those reported by Owen et al. (*15*). These investigators, using a hypoxanthine/xanthine oxidase system to produce hydroxyl radicals, reported that 1-acetoxypinoresinol was more efficient than the catechol derivatives caffeic acid (6.6×), hydroxytyrosol (1.5×), and oleuropein (8.6×). However, such efficiency is not expected on the basis of the calculated BDE values for the two lignans and it also cannot be attributed to the structural characteristics of the lignans. If the exceptionally high activity of **18** is not related to experimental conditions, i.e., inhibitory effect on xanthine oxidase, then the low IP value that was calculated for it in the gas phase (161.2 kcal/mol) could suggest electron donation ability.

Radical Scavenging Potential of Compounds Belonging to Group B. Within this group of compounds, the type or the size of the carbon side chain is not expected to affect significantly the radical scavenging potential because the differences in BDE values vary within ∼2 kcal/mol. An exception could be verbascoside (**16**), which possesses two catechol moieties with similar hydrogen-donating activity **16**−H(1) and −H(2). The high activity predicted for verbascoside was not supported by the limited experimental data (*14*).

Extended conjugation and the stereochemistry of the molecule contribute to the further stabilization of phenoxy radicals as shown in **Figure 2** for spin distribution in the cases of **6**, **8**, and **15** resulting in lower BDE values (**Table 1**). Although the relative order for the radical scavenging ability toward DPPH$^{\bullet}$ (*24*) was in line with the respective BDE values, the antioxidant performance in triolein autoxidation (**Figure 3**) was obviously influenced by other factors (e.g., kinetics).

Other factors that may contribute to the overall performance of the compounds in real systems are the size and the

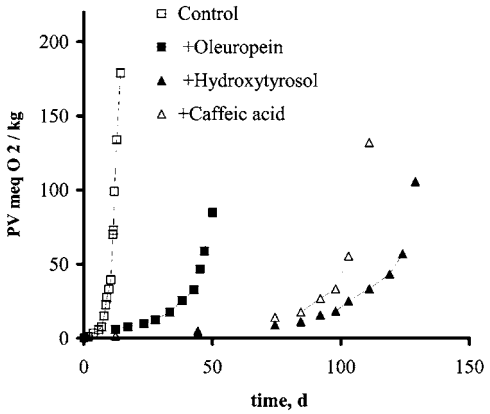


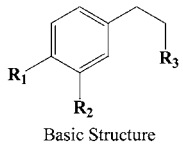
Figure 3. Antioxidant activity of caffeic acid, oleuropein, and hydroxytyrosol under triolein autoxidation (45 °C, 10 ppm).

lipophilicity of the molecules. Compounds **6**−**16**, which seem to be equally active as hydrogen atom donors, differ in size (1−4×). The three-dimensional configuration of the compounds (caffeic acid being an exception) is expected to moderate penetration into membranes and thus affect antioxidant performance in biological systems (*27*, *28*). Paiva-Martins et al. (*28*) reported that **6**, its acetate analogue (**7**), the dialdlehydic form of elenolic acid (**13**), and the aldehydic form of oleuropein aglycone (**11**) cannot penetrate the membranes, due to association with the surface of the phospholipid bilayer.

Additionally, on the basis of the calculated Log *P* values (**Table 1**) and some literature data (*2*, *11*, *27*, *28*), it is obvious that the size of the side chain affects the lipophilicity of the compounds and, consequently, the performance in bulk oils and dispersed systems. Thus, although hydroxytyrosol was found to scavenge DPPH• 1.3 times more than its acetate counterpart and was 1.1 times more effective than it in bulk oil oxidation, the order of activity was reversed in an oil/water emulsion (*11*). This was due to the higher polarity of hydroxytyrosol, which resulted in the smaller concentration of the phenol in the lipid phase of the dispersed system. It should also be stressed that even if the size of the chain is the same (compounds **10**, **11**, **14** or **12**, and **13**) the presence of different groups affects the lipophilicity of the compounds (Log *P* values) as well. Therefore, even in this case, differences in the activity of the phenols can be expected experimentally. The latter is supported by the findings reported for coniferylaldehyde and coniferyl alcohol (*2*). The two compounds having the same molecular weight but also different lipophilicity due to the characteristic group at the end of the chain had significantly different performances in models imitating real systems.

In conclusion, all of the compounds of group B should be expected to have an appreciable radical scavenging potential. However, the efficiency in real systems (food or biological) is expected to be controlled by other factors as well. This fact does not hamper the importance of BDE, which can be currently calculated for a great number of compounds (natural or artificial) within a reasonable working time.

Radical Scavenging Potential of Hydroxytyrosol Metabolites Detected in Biological Fluids. Besides the interest in the potential of natural antioxidants, scientists are also interested in that exhibited by the bioavailable forms detected in various biological fluids. Therefore, in a subsequent step, the antioxidant potential of hydroxytyrosol and metabolites such as methoxylated derivatives, a sulfate, and a glucuronide conjugate (**Figure 4**) were also investigated theoretically. The formation of similar



Basic Structure

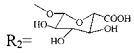
R_1=OH	R_2=OH	R_3=OH	Hydroxytyrosol (**5**)
R_1=OH	R_2=OH	R_3=CHO	3,4-dihydroxyphenylacetaldehyde ($\mathbf{5_a}$)
R_1=OH	R_2=OH	R_3=COOH	3,4-dihydroxyphenylacetic acid ($\mathbf{5_b}$)
R_1=OH	R_2=OCH_3	R_3=OH	Homovanillic alcohol ($\mathbf{5_c}$)
R_1=OH	R_2=OCH_3	R_3=COOH	Homovanillic acid ($\mathbf{5_d}$)
R_1=OH	R_2=OCH_3	R_3=CHO	Homovanillic aldehyde ($\mathbf{5_e}$)
R_1=OH	R_2= 	R_3=OH	Monoglucuronide conjugate ($\mathbf{5_f}$)
R_1=OH	R_2=OSO_3^-	R_3=OH	Monosulfate conjugate ($\mathbf{5_g}$)
R_1=OCH_3	R_2=OH	R_3=COOH	3-hydroxy-4-methoxyphenylacetic acid ($\mathbf{5_h}$)
R_1= OCH_3	R_2=OCH_3	R_3=OH	3,4-dimethoxyphenylethanol ($\mathbf{5_i}$)

Figure 4. Hydroxytyrosol metabolites found in biological fluids ($\mathbf{5_{a-i}}$).

Table 2. B3LYP/6-31G (*p*′)//AM1-Calculated O–H BDEs and IPs for Hydroxytyrosol Metabolites Found in Biological Fluids

AH	O–H BDE (kcal/mol)[a]	IP (kcal/mol)[b]
5	78.7	171.8 (127.9)
$\mathbf{5_a}$	79.3	176.4 (128.8)
$\mathbf{5_b}$	79.0	175.6 (125.0)[c]
$\mathbf{5_c}$	87.2	166.6 (125.5)
$\mathbf{5_d}$	87.7	170.2 (127.6)[c]
$\mathbf{5_e}$	87.9	170.2 (127.2)
$\mathbf{5_f}$	91.8	167.9 (136.9)
$\mathbf{5_g}$	91.3	93.3 (130.4)
$\mathbf{5_h}$	87.9	172.1 (126.0)[c]
$\mathbf{5_i}$	103.4	160.6 (124.5)

[a] O–H bond disociation enthalpy. [b] IP. In solvent values are in parentheses. [c] The proton of the –COOH group is dissociated.

conjugates is common in kidneys and liver to enhance excretion from the body. Calculated BDE and IP values are presented in **Table 2**.

The aldehyde and the acid metabolite of hydroxytyrosol ($\mathbf{5_a}$ and $\mathbf{5_b}$) had similar BDE values, which were lower than those calculated for the more complex ones. This finding was attributed to the presence of a catechol moiety in the two compounds rather than to other structural characteristics. For both conjugates of hydroxytyrosol with the sugar or sulfuric acid (the monosulfate conjugate is expected to be in anionic form under physiological conditions and that is why the dissociated form was incorporated in the calculations), $\mathbf{5_f}$ and $\mathbf{5_g}$ are expected to be inactive according to BDE values, a finding that cannot explain how the first one was found more active than hydroxytyrosol (almost 5×) toward the DPPH• (*22*). Because the strong hydrophilic character of the tested compounds led the investigators to dissolve the stable radical in a mixture of ethanol/water instead of ethanol, the experimental findings may need some reevaluation.

Experimental conditions, such as the environment of the reaction, may affect significantly the performance of the molecules (*24*, *29*) whereas BDE values calculated in the gas phase reflect only the effect of structural characteristics. Because these compounds are expected to act in an aqueous environment, only the in-solvent IP values are discussed. No obvious conclusions could be obtained on the basis of IP values, as was

also observed for the compounds of **Table 1**, not even in the case of $\mathbf{5_i}$, expected to be inactive since no hydrogen atoms are available for donation.

The quantum chemical calculation of BDE values can provide information on the radical scavenging potential of the compounds especially when experimental data cannot be easily obtained as it was evidenced in the present study. Moreover, the BDE values may help reevaluation of the activity of certain compounds the structure of which cannot support high (or low) activity. The lack of experimental data for the most of the investigated compounds supports the usefulness of the present work for those interested in the antioxidant potential of phenolic compounds encountered in *O. europaea* products such as raw olives, table olives, leaves, and virgin olive oil.

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