Antioxidant Activity of the Main Bioactive Derivatives from Oleuropein Hydrolysis by Hyperthermophilic β-Glycosidase

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The main reaction products obtainable by the hydrolysis of commercially available oleuropein by hyperthermophilic β-glycosidase were purified and structurally characterized by UV and ^{1}H and ^{13}C NMR analyses. Their antioxidant activity, in particular their capacity to inhibit the fatty acid peroxidation rate, was studied. The molecular structures assigned revealed the presence of two elenolic acid forms presenting different antioxidant abilities closely correlated to their molecular structures, as well as an unstable elenolate which is a rearrangement product of the oleuropein aglycon. This molecule, under the reaction conditions (pH 7.0, 60 °C) required for β-glycosidase activity, rapidly gives rise to 3,4-dihydroxy-phenylethanol (hydroxytyrosol).

Keywords: *Oleuropein; 3,4-dihydroxy-phenylethanol; elenolic acid; β-glycosidase; antioxidant activity*

INTRODUCTION

Phenolic compounds in *Olea europaea* fruits are important factors to consider in order to evaluate virgin olive oil quality because they are partly responsible for its autoxidation stability (*1*, *2*) and organoleptic characteristics (*3*). Moreover, these molecules have pharmacological properties (*4*), are natural antioxidants (*5*, *6*), and inhibit the Gram positive microorganisms involved in olive fruit fermentation (*7*, *8*). Oleuropein, the main phenolic compound present in *O. europaea* fruits and leaves, is a 3,4-dihydroxy-phenylethanol (hydroxytyrosol) ester with a β-glucosylated elenolic acid (*9*, *10*) and is responsible for producing bitterness in unripened olives. The aglycon, which is obtainable from oleuropein hydrolysis, is well-known as a pharmacologically active molecule for its potential application as an antimicrobial agent in some fairly common olive tree diseases (*11*). Another interesting product, probably deriving from enzymatic and/or chemical degradation of oleuropein during fruit ripening (*12*), is a simple phenolic compound, hydroxytyrosol. This lipid- and water-soluble molecule, recalling the structure of a cathecol, is present in extra-virgin olive oil either as simple phenol or esterified with elenolic acid (*13*, *14*), and recently was investigated and successfully tested for its free-radical scavenging activity. It was shown to strongly inhibit superoxide production by either a cell-free system or activated human neutrophils (*15*) with a possible connection between the Mediterranean diet and the observed lower incidence of cancer and coronary heart disease (*16*). Furthermore, this molecule was demonstrated to inhibit rat platelet 12-lipoxygenase and rat polymorphonuclear leukocyte 5-lipoxygenase activities (*17*). Recently, the protective role of hydroxytyrosol on oxidative stress in the human system has been investigated (*18*).

Many substances isolated from *Olea europaea* fruits and leaves are thought to originate from oleuropein via an aglycon. To understand the oleuropein transformation pathway, several groups investigated oleuropein enzymatic hydrolysis in vitro by means of an endogenous β-glucosidase activity (*19*, *20*) and by a commercial β-glucosidase from sweet almonds (*13*), obtaining glucose and oleuropein aglycon. During these enzymatic reactions the presence of hydroxytyrosol was never observed.

In a preliminary publication, we described the enzymatic hydrolysis of commercially available oleuropein to produce hydroxytyrosol, using homogeneous recombinant β-glycosidase from the hyperthermophilic archaeon *Sulfolobus solfataricus* expressed in *E. coli* (EcSβgly) immobilized on a chitosan support (*21*). The glycosidases (*O*-glycoside hydrolase, EC 3.2.1.x) are a widespread group of enzymes with significant biochemical, biomedical, and industrial importance that catalyze the hydrolysis of glycosidic bonds in oligo- and polysaccharides. Because of the exceptional number of possible combinations between carbohydrates, there are a large number of glycosidases of varying substrate specificity (*22*). In our laboratory, a β-glycosidase with a remarkable β-glucosidase activity and wide substrate specificity was purified to homogeneity (*23*) from the thermophilic archaeon *Sulfolobus solfataricus*. The gene coding for this enzyme was expressed in *Escherichia coli* (*24*).

In this investigation, we propose a schematic reaction pathway that gives rise to hydroxytyrosol from pure oleuropein hydrolysis by EcSβgly, via the aglycon. We also characterize the antioxidant properties of the main products obtained, testing their radical hydrogen-donor ability and their capacity to inhibit the rate of fatty acid peroxidation.

MATERIALS AND METHODS

Materials. *N*,*N*-dimethyl-*p*-phenylenediamine dihydrochloride (DMPD) and ferric chloride were obtained from Fluka (Fluka Chimica, Milan, Italy); *p*-nitrophenyl-β-D-glucopyrano-

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10.1021/jf001342r CCC: $20.00
Published on Web 06/09/2001

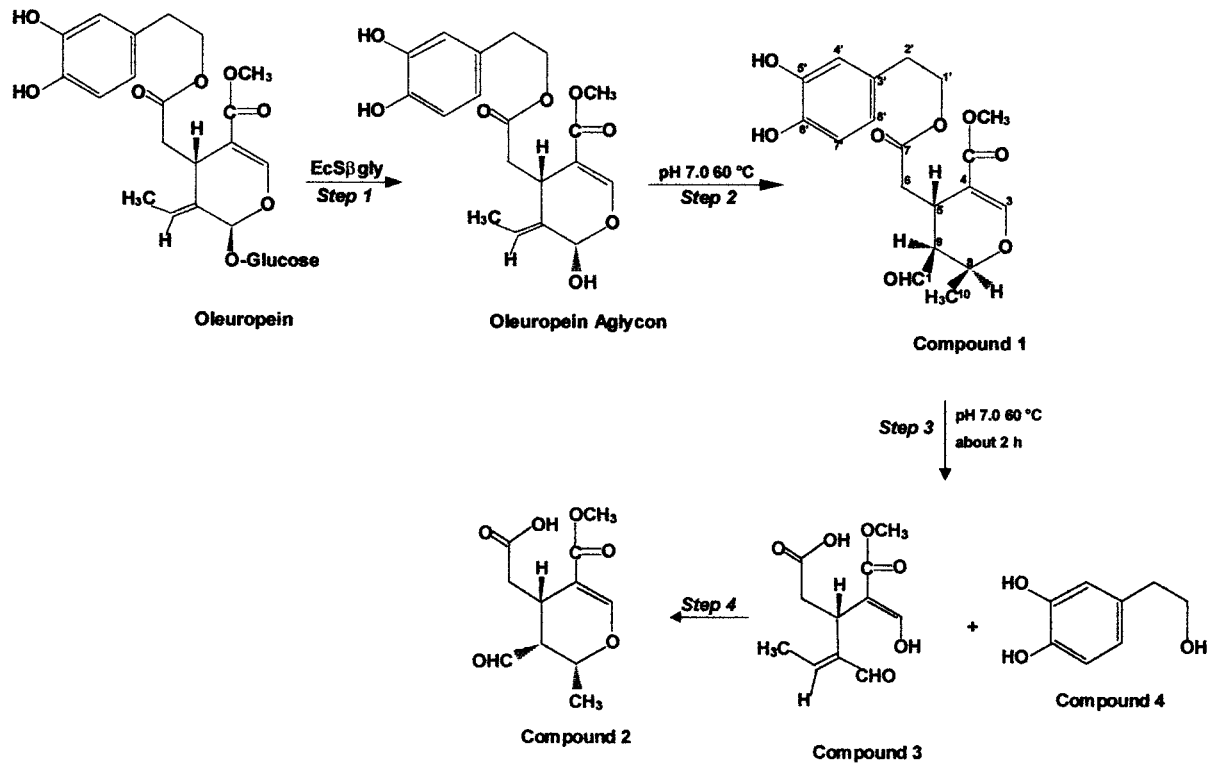

Figure 1. The main reaction products obtainable from oleuropein hydrolysis by EcSβgly at pH 7.0 at 60 °C. Rearrangement product of oleuropein aglycon (compound **1**), two forms of elenolic acid (compounds **2** and **3**), hydroxytyrosol (compound **4**).

side (PNPG), linoleic acid, arachidonic acid and bis-trimethylsilyl-trifluoroacetamide + 1% trimethyl-chlorosilane were from Sigma (St. Louis, MO); L-ascorbic acid and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Aldrich (Germany); $CHCl_3$, CH_3OH, their deuterated forms, silica gel 60 F_{254} plates, and silica gel 60 (0.0063−0.200 mm) were purchased from Merck (Bracco S.p.A., Milano, Italy); 2,2′-azobis(2-amidinopropane) dihydrochloride (ABAP) was obtained from Waco; and oleuropein was purchased from Extrasynthèse (Genay-France). All chemicals used were of analytical grade.

Enzyme Purification. The EcSβgly overexpressed in *E. coli* (BL21de3 strain) was purified according to Moracci et al. (*24*).

Enzyme Assay. The enzyme activity toward PNPG as a substrate was evaluated as in ref *23*. All spectroscopic assays were followed using a double-beam Cary 1E thermostated spectrophotometer (Varian, Victoria, Australia) equipped by Peltier effect for controlling temperature.

Purification of the Main Reaction Products obtained by Oleuropein Enzymatic Hydrolysis. The rearrangement product of the oleuropein aglycon (compound **1**), two elenolic acid forms (compounds **2** and **3**), and hydroxytyrosol (compound **4**) (Figure 1) were obtained by enzymatic hydrolysis of commercially available oleuropein performed by EcSβgly. The enzyme (0.8 mg = 164 units) was added to a solution of 10 mL of 50 mM sodium phosphate, pH 7.0, containing 20 mM oleuropein with a final molar oleuropein/enzyme ratio of 5.9×10^4:1. The reaction mixture was incubated at 70 °C for 2 h. The reaction products were purified on a silica gel column for successive gravimetric analysis. For a sample of 100 mg of the crude hydrolyzed mixture, a column of 1.5 cm i.d. and 20 g of silica gel suspended in $CHCl_3/CH_3OH$ (8:2) was used. The crude hydrolyzed mixture was extracted three times with $CHCl_3$. The aqueous phase was adsorbed on dry silica gel, successively dried in vacuo, and then applied on the top of a silica gel column suspended in $CHCl_3$, and the elution was performed isocratically by $CHCl_3/CH_3OH$ (95:5). The chloroform phase was chromatographed on a preparative TLC eluted with $CHCl_3/CH_3OH$ (8:2). The purified products were identified by ^{1}H NMR, ^{13}C NMR, UV, and TLC methods and were immediately used for testing their antioxidant activities in order to avoid autoxidation.

Thin-Layer Chromatography. Analytical and preparative TLC were carried out on silica gel 60 F_{254} plates eluted with $CHCl_3/CH_3OH$ (8:2). These techniques were used to monitor the reaction time-course by product formation, to confirm the purity of the products isolated, and to purify the reaction products.

The spots on silica plates were visualized (a) under UV light (254 nm); (b) by spraying with cerium sulfate followed by heating at 105 °C for 5 min; and (c) by leaving the plates exposed to the air and evaluating spots browning after 24 h of exposure at room temperature.

The rearrangement product of the oleuropein aglycon (compound **1**) had R_f 0.7; elenolic acid closed ring (compound **2**), R_f 0.5; elenolic acid open ring (compound **3**), R_f 0.8; and hydroxytyrosol (compound **4**), R_f 0.55.

UV Analysis. UV spectra were obtained in CH_3OH from 190 to 400 nm in quartz cuvettes.

Compound **1.** λ_{max}: 205 nm, aromatic group; 230 nm, α,β-unsaturated ester group; 282 nm, dihydroxyphenyl group. Compound **2.** λ_{max}: 239 nm. Compound **3.** λ_{max}: 235 nm.

NMR Analysis. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 MHz on a Bruker AMX 500 instrument (500.13 MHz for ^{1}H and 125.75 for ^{13}C). All chemical shifts are expressed in ppm, using solvent signals as an internal standard ($CDCl_3$ for compounds **1**, **2**, and **3**, 7.26 ppm; CD_3OD for compound **3**, 3.31 ppm). The structural identity of oleuropein subjected to hydrolysis was performed by ^{1}H NMR analysis using $CDCl_3$ and CD_3OD (99.8% in deuterium) as standards.

Compound **1**. ^{1}H NMR ($CDCl_3$), δ: 9.60 (aldehydic proton, H-1), 7.65 (1H, s, H-3); 6.86−6.55 (3H, m, phenyl protons); 4.37 (1H, m, Ha-1′); 4.25 (1H, m, H-8); 4.20 (1H, m, Hb-1′); 3.74 (3H, s, COOMe); 3.35 (1H, dd, H-5); 2.86 (1H, dd; H-6a); 2.82 (2H, m, H-2′); 2.62 (1H, m, H-9); 2.20 (1H, dd; H-6b); 1.55 (3H, d, H-10). ^{13}C NMR: 199.89 (C-1); 157.24 (C-3); 130.33 (C-4); 28.07 (C-5); 38.93 (C-6); 171.02 (C-7); 69.65 (C-8); 50.90 (C-9); 17.84 (C-10); 51.67 (COO*Me*); 167.2 (*C*OOMe); 65.46 (C-1′); 34.17 (C-2′); 106.43 (C-3′); 115.09 (C-4′); 143.14 (C-5′); 142.9 (C-6′); 116.58 (C-7′); 121.19 (C-8′).

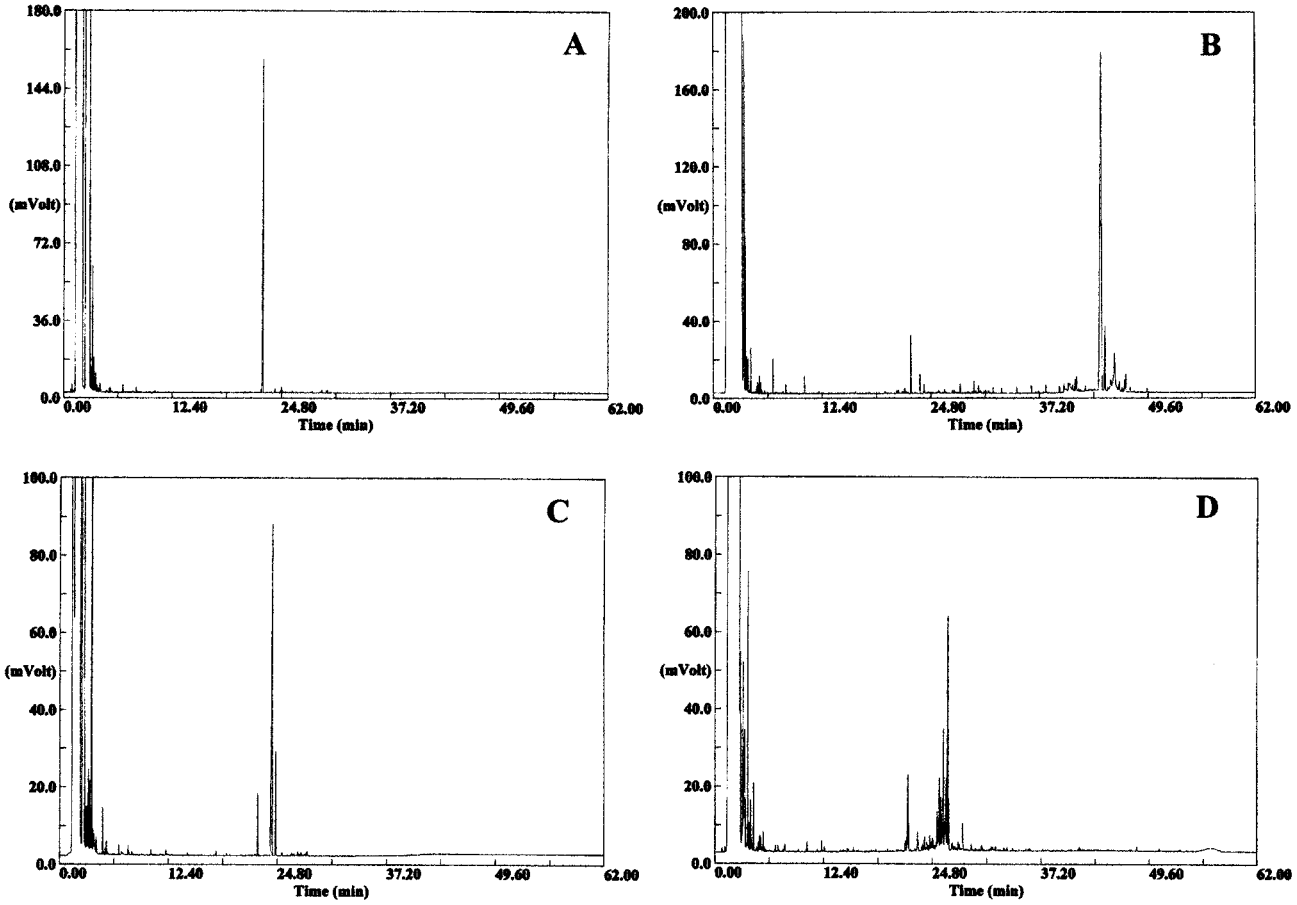

Figure 2. GC chromatogram of TMS-derivatized reaction compounds from oleuropein hydrolysis. Panel A, hydroxytyrosol; panel B, compound **1**; panel C, compound **2**; panel D, compound **3**.

Compound **2**. ^{1}H NMR ($CDCl_3$), δ: 9.60 (aldehydic proton, H-1), 7.63 (1H, s, H-3); 4.20 (1H, q, H-8); 3.75 (3H, s, COOMe); 3.35 (1H, m, H-5); 2.85 (1H, dd; H-6a); 2.69 (1H, s, H-9); 2.32 (1H, dd; H-6b); 1.49 (3H, d, H-10). ^{13}C NMR: 199.4 (C-1); 156.66 (C-3); 105.9 (C-4); 28.9 (C-5); 37.8 (C-6); 170.2 (C-7); 69.62 (C-8); 50.96 (C-9); 17.88 (C-1); 167.34 (*C*OOMe); 51.48 (COO*Me*).

Compound **3**. ^{1}H NMR ($CDCl_3$), δ: 9.62 (aldehydic proton, H-1); 7.61 (1H, s, H-3); 4.47 (1H, dq, H-8); 3.75 (3H, s, COOMe); 3.40 (1H, dd, H-5); 2.94 (1H, dd, Ha-6); 2.36 (1H, dd, Hb-6); 1.41 (3H, d, H-10).

3,4-Dihydroxy-phenylethanol (**4**). ^{1}H NMR (CD_3OD), δ: 6.80−6.50 (3H, m, phenyl protons H-3, H-4, H-6); 4.19 (2H, t, CH_2−O), 2.76 (2H, t, CH_2).

Gas Chromatography. GC analyses were carried out on a Supelco fused silica capillary SE-54 (30 m length; 0.25 mm i.d.; 0.25 μm film thickness) by a Carlo Erba 8000 TOP (C. Erba, Milano, Italy) equipped with a splitless injection system and FID detector. The carrier gas was He at 150 kPa. The injector and detector temperatures were maintained at 290 °C. A linear temperature gradient was applied to the oven from 80 to 290 °C at 5 °C/min. The samples, evaporated under N_2 flow, were derivatized for 30 min by bis-trimethylsilyl-trifluoroacetamide + 1% trimethyl-chlorosilane with a final molar ratio of 1:50.

We obtained evidence of a single peak for hydroxytyrosol at t_R 22.8 (Figure 2, panel A), for compound **1** at t_R 44.3 (Figure 2, panel B) and for compound **2** at t_R 24.8 (Figure 2, panel C). The GC chromatogram of compound **3** (Figure 2D) appeared more complex because of its high instability at the GC operating temperature, therefore we attribute the more intense peak at t_R 26.6 to this molecule.

Measurement of Antioxidant Activity by the DMPD Method. Antioxidant activities of aqueous solutions of Trolox, ascorbic acid, oleuropein, hydroxytyrosol, and compounds **1**, **2**, and **3** were tested colorimetrically using the red radical cation DMPD $^{\bullet+}$ (*25*).

DMPD (1.0 mM) was prepared in a 0.1 M sodium acetate buffer, pH 5.25, and the red radical cation (DMPD$^{\bullet+}$) was obtained by adding an aqueous $FeCl_3$ solution, 0.1 mM final concentration. A 1-mL aliquot of this solution was placed directly in a 1-cm light path quartz cuvette and its absorbance at 505 nm at 25 °C was measured; the optical density obtained represents the uninhibited signal and is constant up to 12 h at room temperature. Antioxidant activity was measured by adding different concentrations of the above compounds to 1 mL of DMPD$^{\bullet+}$ solution in a spectrophotometric cuvette; absorbance at 505 nm after 10 min at 25 °C was measured. Dose−response curves (AE) were derived for the above antioxidants by plotting absorbance at 505 nm as a percentage of uninhibited radical cation solution absorbance (blank), according to the equation

$$\text{AE (\%)} = [1 - (A_{AO}/A_0)] \times 100$$

where A_0 is uninhibited radical cation absorbance and A_{AO} is absorbance measured 10 min after the antioxidant samples addition.

The relative antioxidant efficiency (RAE) of each compound was obtained by the equation

$$\text{RAE (\%)} = (\text{Slope}_{AO}/\text{Slope}_e) \times 100$$

where Slope_{AO} and Slope_e are the slopes of linear plot of AE values versus μg/mL of antioxidants in reaction mixture. We used as reference the slopes (Slope_e) of the linear plots calculated for ascorbic acid and Trolox solutions.

Measurement of Antiperoxidative Activity by the ABAP Method. The antiperoxidant activities of aqueous solutions of ascorbic acid, oleuropein, hydroxytyrosol, and

Table 1. Antioxidant Activity of Bioactive Molecules from Oleuropein Hydrolysis Determined by the DMPD and ABAP Methods

	DMPD method			ABAP method on linoleic acid		ABAP method on arachidonic acid	
	slope (μg/mL)	RAE[a]	RAE[b]	slope (μg/mg acid)	RAE[a]	slope (μg/mg acid)	RAE[a]
Trolox	5.23	0.54	1.00				
ascorbic acid	9.61	1.00	1.84	0.65	1.00	0.56	1.00
oleuropein	3.15	0.33	0.60	0.75	1.15	0.57	1.01
compound **1**	3.04	0.32	0.58	1.22	1.86	1.00	1.77
hydroxytyrosol	4.16	0.43	0.79	2.62	4.01	0.96	1.71
compound **2**	1.19	0.12	0.23	0.18	0.27	0.06	0.11
compound **3**	0.11	0.01	0.021	0.12	0.18	0.06	0.10

[a] Relative to ascorbic acid. [b] Relative to Trolox.

compounds **1**, **2**, and **3** were determined by a slight modification of the method previously described (*26*, *27*).

A 1-mL portion of an aqueous solution of 46.35 μM linoleic acid plus 0.0125 μL/mL Tween 20, as a detergent, in a 50 mM sodium phosphate buffer, pH 7.0, was pipetted into a quartz cuvette and heated at 50 °C. After 10 min of equilibration, 15 μL of ABAP aqueous solution, 129 μM final concentration, was added in order to monitor peroxidation at 234 nm for 20 min. After this time had elapsed, different antioxidant concentrations were added and the kinetics were monitored for a further 20 min. The ratio between slope of the linear plot of absorbance versus time (d*A*/d*t*) after and before the addition of antioxidant solutions gives the antioxidant efficiency (AE):

$$\text{AE (\%)} = [1 - (S_{AO}/S_{ABAP})] \times 100$$

where S_{ABAP} and S_{AO} are slopes after and before the addition of antioxidant samples, respectively.

The relative antioxidant efficiency (RAE) of each compound was expressed as a percentage ratio of the ascorbic acid calculated slope ($Slope_{VitC}$) and the slope obtained by plotting AE values versus antioxidative sample concentrations (μg/mL) referring to 1 mg of linoleic acid ($Slope_{AO}$):

$$\text{RAE (\%)} = (Slope_{AO}/Slope_{VitC}) \times 100$$

The measurements of antioxidant activity (AE) and relative antioxidant efficiency (RAE) were repeated in the same reaction conditions using arachidonic acid as a substrate and monitoring peroxidation at 238 nm.

RESULTS AND DISCUSSION

Purification and Identification of the Main Reaction Products obtained by Oleuropein Enzymatic Hydrolysis. Oleuropein hydrolysis reaction products were identified by TLC, UV, and ^{1}H and ^{13}C NMR analyses, and their structures are shown in Figure 1. Compounds were numbered on the basis of their biogenetic origin from oleuropein. We purified unhydrolyzed oleuropein, hydroxytyrosol (compound **4**), and elenolic acid with a closed ring (compound **2**) in aqueous phase by silica gel chromatography; the rearrangement product of the oleuropein aglycon (compound **1**) and elenolic acid with a open ring (compound **3**) were purified in chloroform by preparative TLC.

The ^{1}H and ^{13}C NMR data of compounds **1** and **2** were in good agreement with those described for one of the rearrangement products of the oleuropein aglycon and elenolic acid (*12*, *28*); these data support the structural and stereochemical identity of the secoiridoid moiety of the substances **1** and **2**. The typical saturated aldehydic group signal at 9.6 δ is recognizable in their ^{1}H NMR spectra, and ^{13}C NMR data indicate that only one olefinic carbon is present at 157.24 for compound **1** and at 156.66 for compound **2**.

The UV spectrum of compound **1** confirms the presence of a α,β-unsaturated ester group, being the band absorbing at 330 nm attributable to n$-\pi^*$ transitions. The band at 282 nm is due to the dihydroxyphenyl group, whereas the band at 205 nm is due to aromatic $\pi-\pi^*$ transitions. The UV spectrum of compound **2**, in agreement with that described for monoaldehydic elenolic acid with closed enolic ring (*29*), shows a maximum at 239 nm.

For compound **3**, the typical ^{1}H NMR aldehyde signal at 9.60 δ and the appearance of H-8 as a double quartet, while H-5 remains a double doublet, suggests that the mono-aldehydic structure derived from the elenolic ring opening may be attributed to compound **3**. This is an unstable molecule, which under our reaction conditions gives rise to the more stable compound **2**.

Because, under our reaction conditions, the rearrangement product of oleuropein aglycon (compound **1**) is subject to a fast hydrolysis to the stable hydroxytyrosol (*21*), and the two forms of elenolic acid have a different stability, we propose an oleuropein transformation pathway into hydroxytyrosol by means of enzymatic and chemical hydrolysis, as shown in Figure 1. In the first step, oleuropein is readily hydrolyzed by the enzyme to oleuropein aglycon (step 1), which undergoes a fast chemical rearrangement in aqueous solution at 60 °C, leading to the monoaldehydic elenolate compound **1** (step 2). In the biomimetic hydrolysis of oleuropein by the endogenous β-glucosidase present in olive juice, compound **1** was observed after several days at room temperature in aqueous solutions, whereas in the presence of a lipid/water interface ($D_2O/CDCl_3$ mixture) at 60 °C, a rapid rearrangement into final compound **1** was verified (*20*). In both cases, hydroxytyrosol was never observed. Using Ec$S\beta$gly for oleuropein hydrolysis in an aqueous solution at pH 7.0 at 60 °C, we obtained a large amount of compound **1** after a few minutes (*21*) which, according to our results, is converted into hydroxytyrosol and compound **3** by chemical hydrolysis in about 2 h at 60 °C (step 3) and after 12 h at a low temperature (20 °C) (data not shown). On the other hand, the compound **3** under our reaction conditions gave rise to the enolic species **2** (step 4).

Measurement of Antioxidant Activity by the DMPD Method. At acidic pH in the presence of a suitable oxidant solution ($FeCl_3$), DMPD can form a stable red radical cation ($DMPD^{\bullet+}$). Antioxidant compounds, which transfer a hydrogen atom to $DMPD^{\bullet+}$, quench the red color and produce a bleaching of the solution proportional to their amounts. This reaction is fast and its stable end point is taken as a measure of antioxidant activity; therefore, this assay reflects the radical hydrogen-donor ability to scavenge the single electron from $DMPD^{\bullet+}$. The relative antioxidant efficiency values obtained by the DMPD method are shown in Table 1. All data are obtained in aqueous solution.

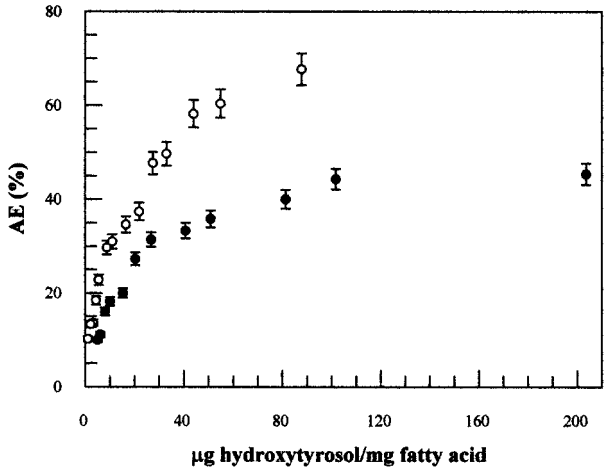

Figure 3. AE (%) values versus hydroxytyrosol concentration for linoleic (○) and arachidonic (●) acid peroxidation, calculated by the ABAP method.

Hydroxytyrosol exhibits RAE values of about 0.4 and 0.8 in comparison to those of reference antioxidants ascorbic acid and Trolox, respectively. It should be noted that hydroxytyrosol, a lipid- and water-soluble molecule, shows a molar antioxidant activity comparable to that of hydrophilic molecules. Contrasting data on the same antioxidant effectiveness by the 2,2-diphenyl-1-picrylhydrazyl radical decoloration method in ethanol have been published by Visioli et al. (*16*) and we interpret this discrepancy in terms of different reaction conditions and method utilized.

Both oleuropein and compound **1** RAE values are comparable, whereas the two forms of elenolic acid show the lowest RAE values. These data suggest that radical hydrogen donor ability is primarily attributable to compounds containing a phenolic ring and to a lesser extent, to the closed ring of elenolic acid, as the open ring form has a less potent effect.

Measurement of Antiperoxidative Activity by the ABAP Method. This investigation was performed in order to compare, in the presence of different antioxidant compounds, the fatty acid peroxidation rate under equal conditions of oxidative stress. The peroxyl radical is generated using the azo radical initiator ABAP at 50 °C and the fatty acid peroxidation rate is monitored by hydroperoxide formation.

AE and RAE values obtained by the ABAP method on linoleic and arachidonic acids are shown in Table 1. As indicated by these data, compounds containing a phenolic ring present additional activities, among the antioxidant properties, that may include chain-breaking in radical propagation by trapping peroxyl radicals (*30*) and/or radical scavenging in lipidic peroxidation (*15*). Furthermore, fatty acid oxidation susceptibility is thought to be dependent on their degree of unsaturation: in fact, the disappearance rate of linoleic acid is higher than that of arachidonic acid (*31*). Our data also show that the antioxidant properties of the molecules tested are related to the degree of unsaturation of fatty acid. In the presence of hydroxytyrosol, the slope in the linear range of AE values versus phenolic concentration for linoleic acid peroxidation is about three times higher than that of arachidonic acid (Table 1), the latter showing a lower saturation AE value in the presence of higher hydroxytyrosol concentration (Figure 3). These data suggest that, in the presence of hydroxytyrosol, the lipid peroxide production rate is lower for linoleic acid than for arachidonic acid. In addition, for the peroxidation of arachidonic acid, the RAE values of hydroxytyrosol and compound **1** prove comparable, whereas for linoleic acid peroxidation, hydroxytyrosol exhibits a stronger radical-scavenging activity (Table 1). We suggest that this capacity is clearly attributable to the interaction of a phenolic ring, which is not bound to the elenolic structure, with a peroxyl radical and especially with the carbon-centered radical derived from the fatty acid. As a consequence, the RAE values of oleuropein and compound **1** were comparable for both fatty acids, and the two forms of elenolic acid have a very slight effect on fatty acid peroxidation.

The characterization of antioxidant properties of the main hydrolysis products obtainable from oleuropein may clarify the role that these molecules play in biochemical processes during the ripening of olive fruits and their importance for improving olive oil quality; in fact, some of these compounds were identified in *Olea europaea* fruits and leaves (*28*, *32*) as well as in olive oil (*33*). Furthermore, as fatty acid oxidation plays an important role in food spoilage development by producing unpleasant off-flavors and rancidity, the natural complexity of phenolic compounds in olive oil leads not only to delaying oxidation phenomena in bulk oil during storage or cooking, but also to protecting other components present in complex food systems (e.g., tomato-based products, vegetable soups, and canned products with added virgin olive oil). In addition, the characterization of antioxidant properties of the oleuropein hydrolysis products would also cast more light on olive oil organoleptic moieties and the beneficial effects to human health, given that olive oil is the main component of the Mediterranean diet.

ABBREVIATIONS USED

Hydroxytyrosol, 3,4-dihydroxyphenylethanol; EcSβgly, β-glycosidase from *Sulfolobus solfataricus* expressed in *E. coli*; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABAP, 2,2′-azobis(2-amidinopropane) dihydrochloride; PNPG, *p*-nitrophenyl-β-D-glucopyranoside; AE, antioxidant efficiency; RAE, relative antioxidant efficiency.

ACKNOWLEDGMENT

We thank V. Mirra of the Istituto per la Chimica di Molecole di Interesse Biologico-CNR for ^{1}H and ^{13}C NMR analyses.

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Received for review November 14, 2000. Revised manuscript received May 7, 2001. Accepted May 8, 2001. The work presented from our laboratory was supported by grants from the European Community P.O.P "Ricerca, Sviluppo Tecnologico ed Alta Formazione".

JF001342R