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Food Chemistry 95 (2006) 562–565
Food Chemistry
www.elsevier.com/locate/foodchem

Phenolic components of *Olea europea*: Isolation of new tyrosol and hydroxytyrosol derivatives

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Received 18 October 2004; received in revised form 16 December 2004; accepted 18 December 2004

Abstract

Two new phenolic compounds were isolated from fruits of *Olea europaea*, Hojiblanca cultivar. The first compound is the methyl acetal of the aglycone of ligstroside, while the second derivative, not yet reported in the literature, is the β-hydroxytyrosyl ester of methyl malate. These microcomponents may be responsible for hedonistic-sensorial characteristics of olive products.

Keywords: *Olea europaea*; Oleaceae; Ligstroside aglycon; β-Hydroxytyrosyl ester of methyl malate

1. Introduction

Biophenols, typical secondary plant metabolites, constitute an important group of naturally occurring compounds, present as minor molecular components in both olive fruit and derived products (Bianco, Muzzalupo, Uccella, Piperno, & Romeo, 1999a; Bianco, Muzzalupo, Uccella, Piperno, & Romeo, 1999b; Bianco & Uccella, 1998; Esti, Cinquanta, & La Notte, 1998; Romeo & Uccella, 1996). These molecules have pharmacological properties (Scaccini et al., 1992; Visioli & Galli, 1998; Wiseman, Mathot, De Fouw, & Tijburg, 1996), are natural antioxidants (Kohyama, Nagata, Fujimato, & Sekiya, 1997; Saija et al., 1998) and inhibit the Gram positive microorganisms involved in olive fruit fermentation (Brenes, Rejano, Garcia, Sanches, & Garrido, 1995; Briante, La Cara, Tonziello, Febbraio, & Nucci, 2001). Furthermore, the biophenolic profile influences the sensory and functional properties of fresh and processed foods derived from *Olea europaea* L., namely table olives and olive oil (Bianco, Chiacchio, Rescifina, Romeo, & Uccella, 1997; Brenes et al., 1995; Marsilio, Lanza, & Pozzi, 1996).

The ability of biophenols to act as antioxidants depends on the redox properties of their phenolic hydroxyl groups and the structural relationships between the different parts of the chemical structures. The major polyphenolic constituent in olives is oleuropein glycoside, but this compound is almost completely absent from olive oil because of its high water solubility (Paiva-Martins & Gordon, 2001). Benzoic acids and benzylic alcohols, including 3,4-(dihydroxyphenyl)ethanol (hydroxytyrosol) or *p*-hydroxyphenylethanol (tyrosol) have been found in virgin olive oil and table olives, together with secoiridoid derivatives of oleuropein, such as the dihaldeydic form of elenolic acid linked

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0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.12.033

to hydroxytyrosol, and an isomer of the oleuropein aglycone, the 3,4-(dihydroxyphenyl)ethyl ester of elenolic acid (Brenes, Garcia, Garcia, Rios, & Garrido, 1999; Servili et al., 1999). These compounds are the most abundant of those with a phenolic structure and the hydroxytyrosol derivatives are of particular significance because of their strong antioxidant activity in several lipid systems.

Biophenols can be regarded as molecular microcomponents, present in Mediterranean foods, responsible for complex sensorial attributes and for natural defence against pathogens: accordingly, the investigation of their distribution in olive fruits at harvesting could allow the prediction of the molecular composition of the resulting olive products, olive oil and table olives, and rational criteria for the production in relation to quality, authenticity and health effects (Bianco, Melchioni, Ramunno, Romeo, & Uccella, 2004; Bianco et al., 1998a; Bianco et al., 1998b). In this paper, we have continued the investigation on the phenolic compounds present in *O. europaea*. Two new derivatives have been identified: a compound related to the aglycone of ligstroside and a tyrosol ester, in which the acyl residue is constituted of malic acid. To our knowledge, these compounds have not been hitherto reported in the literature.

2. Materials and methods

2.1. Instrumentation

NMR measurements for ^{1}H at 300.12 MHz and for ^{13}C at 75.42 MHz were recorded on a Varian VXR-300 spectrometer, using TMS in deuterochloroform or DDS in D_2O as internal standards. Two-dimensional COSY and inverse mode heteronuclear multiple-bond correlation (HBMC) spectra were determined in absolute value mode.

The extracts were analyzed by TLC on silica gel GF 254 (Merck, Germany) and the spots were detected under UV light (254 nm). Flash chromatography was carried out with Kieselgel 60 (Merck).

All chemicals were analytical grade and used without further purification.

2.2. Extraction and analysis

The isolation of the biophenolic fraction was performed according to the reported procedure (Bianco et al., 1999a). Thus, Hojiblanca green olives (250 g) were diced, homogenized in 200 ml of methanol/acetone (1:1), saturated with sodium disulfite, in an Ultraturrax homogenizer (Janke & Kunkel, IKA, Labortechnik, Germany) at 0 °C for 5 min and centrifuged at 5000*g* for 20 min at 4 °C. The supernatant was separated; the pellet was resuspended (four times) in 200 ml of methanol/acetone (1:1) and saturated with sodium bisulphite, until a colorless solution was obtained. The combined supernatants were evaporated at a reduced pressure; the obtained residue was solubilized in water at pH 2 and extracted five times with hexane to remove free fatty acids and other lipid contaminants. Finally, biophenols were then extracted six times with ether/ethyl acetate (1:1); the combined extracts were dehydrated with sodium sulphate, and evaporated to dryness under vacuum. The residue (2.9 g) was subjected to flash chromatography on a silica gel column, with chloroform/methanol 95:5 as eluant. The first eluted fractions (0.6 g) were a mixture of compounds **1** and **2**.

2.2.1. Compound *1*

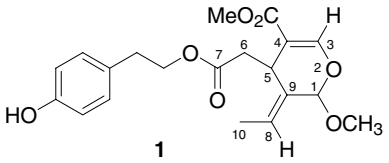
^{1}H NMR (δ, $CDCl_3$): 1.53 (d, 3H, $J = 7.2$ Hz, CH_3), 2.64 (dd, 1H, $J = 8.5$ and 4.5 Hz, H-6), 2.77 (dd, 1H, $J = 4.5$ and 3.5 Hz, H-6), 2.79 (t, 2H, $J = 6.2$ Hz, CH_2), 3.40 (s, 3H, O–CH_3), 3.74 (s, 3H, O–CH_3), 3.92 (dd, 1H, $J = 8.5$ and 3.5 Hz, H-5), 4.11 (t, 2H, $J = 6.2$ Hz, CH_2), 5.07 (s, 1H, H-1), 5.70 (q, 1H, $J = 7.2$ Hz, H-8), 6.70 (d, 2H, $J = 7.1$ Hz, aromatic protons), 6.75 (d, 2H, $J = 7.1$ Hz, aromatic protons), 7.50 (s, 1H, H-3). ^{13}C NMR: 13.25 (C-10), 28.63 (C-5), 34.51 (CH_2Ph), 38.54 (C-6), 51.76 (Me), 56.15 (Me), 65.02 (CH_2O–), 104.62 (C-1), 108.56 (C-4), 114.85 (aromatic carbon), 117.09 (aromatic carbon), 121.16 (aromatic carbon), 128.73 (C-8), 128.83 (aromatic carbon), 129.90 (aromatic carbon), 130.58 (C-9), 143.16 (aromatic carbon), 153.24 (C-3), 168.30 (C=O), 171.87 (C=O).

2.2.2. Compound *2*

^{1}H NMR (δ, $CDCl_3$): 2.68 (t, 2H, $J = 6.3$ Hz, H-1), 2.78 (dd, 1H, $J = 4.5$ and 13.5 Hz, H-5a), 2.82 (dd, 1H, $J = 4.9$ and 13.5 Hz, H-5b), 3.75 (s, 3H, O–CH_3), 3.76 (t, 2H, $J = 6.3$ Hz, H-2), 4.45 (dd, 1H, $J = 4.5$ and 4.9 Hz, H-6), 6.61 (dd, 1H, $J = 8.1$ and 0.9 Hz, aromatic proton), 6.70 (d, 1H, $J = 0.9$ Hz, aromatic proton), 6.75 (d, 1H, $J = 8.1$ Hz, aromatic proton). ^{13}C NMR: 26.90 (C-1), 38.27 (C-5), 52.96 (C-2), 63.68 (O–Me), 66.99 (C-6), 115.45 (aromatic carbon), 116.09 (aromatic carbon), 121.32 (aromatic carbon), 131.50 (aromatic carbon), 141.02 (aromatic carbon), 142.33 (aromatic carbon), 173.74 (C=O), 174.15 (C=O).

3. Results and discussion

The less polar fraction (0.6 g), isolated as an oily product, revealed a mixture of two components **1** and **2**, showing a nearly 2:1 relative ratio, as determined in $CDCl_3$ solution from the ^{1}H NMR analysis.



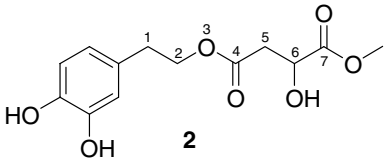
The structure of **1**, the methyl acetal of the aglycone of ligstroside, was determined on the basis of proton and carbon magnetic resonance experiments. Thus, the presence of the tyrosol moiety is clearly indicated by the two proton doublets at 6.70 and 6.75 ppm; the $–CH_2–CH_2–$ methylene part resonates as triplets centred at 2.79 and 4.11 ppm. Compound **1** contains a methyl group, as a doublet, linked to a sp^2 carbon atom, as shown from the examination of ^1H and ^{13}C spectra and relative integrals. Moreover, the spectrum indicates the presence of a singlet at 7.50 ppm, attributed to the vinyl hydrogen atom H-3, linked to a carbon bearing an oxygen atom, as in compound **1**. In fact, the chemical shift of C-3 (153.24 ppm) suggests its linkage to oxygen. The singlet at 5.07 ppm is due to the acetal proton at C-1.

One-dimensional ^1H and ^{13}C NMR spectral data and the phase-sensitive DQF–COSY reveal the two proton spin systems, from H-3 (s, 7.50 ppm) to H-10 (d, 1.53 ppm) trough H-5 (dd, 3.92 ppm) and H-8 (q, 5.70 ppm) and between H-5 and H-6 (dd, 2.77 and 2.64 ppm).

Furthermore, $^1H–^1H$ and $^1H–^{13}C$ long-range couplings between H-8 and H-1, H-8 and C-1 (104.62 ppm), H-8 and C-3 (153.24 ppm), H-8 and C-5 (28.63 ppm), H-5 and C-4 (108.56 ppm) and H-6 and C-4, prove the relationship between these two sequences and the acetal group at C-1.

Compound **1** is the methyl acetal of the aglycone of ligstroside, a minor component of olive oil. This is the first report of its presence in olive fruits; the occurrence, as methyl acetal, suggest that this compound could be formed from ligstroside in the extraction process, by exchange with methanol, which blocks the compound in the methyl acetal form, so avoiding the subsequent steps of transformation to the reported dialdehydic derivatives (Bianco et al., 1999a; Marsilio et al., 1996).

The detection of the acetal **1** indicates that this compound can be an intermediate of an enzymatic hydrolysis sequence starting from ligstroside, similar to that reported for oleuropein.



The structure of the second component **2** has been assembled as follows. The ^1H NMR spectrum shows the typical resonances of the hydroxytyrosol moiety, with the aromatic proton signals in the range 6.59–6.70 ppm and the $–CH_2–CH_2–$ methylene part as triplets at 2.78 and 3.76 ppm. Detailed analysis of the $^1H–^{13}C$ HBMC spectrum reveals long-range couplings from H-5a (dd, 2.80 ppm) H-5_b (dd, 2.82 ppm) and H-6 (dd, 4.45 ppm) to the carboxylic carbon atom C-4 (173.74 ppm) and between H-2 (t, 3.76 ppm) and C-4; these data, together with the COSY between H-1 and H-2, indicate that the $–CH_2–CH_2–$ hydroxytyrosol moiety is bonded to the ester function at C-5. Furthermore, the long-range coupling between H-6 and the estereal C-7 (174.15 ppm), together with the COSY between H-5 and H-6, confirm the structural determination; compound **2** is the β-hydroxytyrosyl ester of methyl malate.

The occurrence of malic acid and Krebs cycle acids in the olive pulp has already been reported (Donaire, Sanchez, Lopez-Gorge, & Recalde, 1975; Panagou, Tassou, & Katsaboxakis, 2003). The isolation of compound **2** is sound with the detection of the oleic ester of hydroxytyrosol (Bianco et al., 2004) and of its modified derivatives (Paiva-Martins & Gordon, 2001) and indicates a widespread presence of hydroxytyrosol in *O. europaea* in both esterified and glucosylated forms (Bianco et al., 1998a, 1998b).

The presence, in table olives, of hydroxytyrosol derivatives, with well known antioxidant activities, may be correlated with the texture and organoleptic features of the food product.

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