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Biological Properties of Hydroxytyrosol and Its Derivatives

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1. Introduction

Polyphenols are a wide family of compounds found in fruits and vegetables, wine, tea, cocoa, and extra-virgin olive oil, which exhibit strong antioxidant activity by scavenging different families of Reactive Oxygen Species (ROS). One of the most effective members of the polyphenol family in terms of free radical scavenging is hydroxytyrosol, 2-(3,4-dihydroxyphenyl)ethanol (Fernández-Bolaños et al., 2008), a simple phenol found predominantly in olive tree (*Olea europaea*).

Hydroxytyrosol (HT) can be found in leaves and fruits of olive, extra virgin olive oil and it is specially abundant in olive oil mill wastewaters from where it can be recovered (Fernández-Bolaños et al., 2008; Sabatini, 2010). Hydroxytyrosol is a metabolite of oleuropein (Fig. 1), another major phenolic component of olive products; they both give to extra-virgin olive oil its bitter and pungent taste (Omar, 2010a). Hydroxytyrosol shows a broad spectrum of biological properties due to its strong antioxidant and radical-scavenging properties. More active than antioxidant vitamins and synthetic antioxidants, hydroxytyrosol exerts its antioxidant activity by transforming itself into a catechol quinone (Rietjens, 2007).

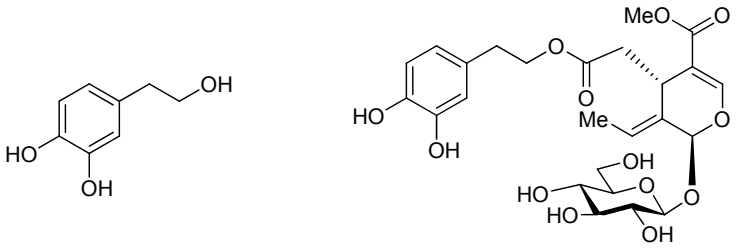


Fig. 1. Structures of hydroxytyrosol and oleuropein

2. Biological activity of hydroxytyrosol

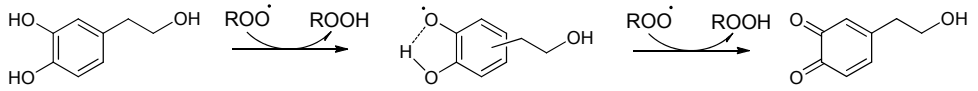
Historically, olive tree leaves were used for traditional therapy by ancient civilizations. Extracts from olive tree leaves were found to have a positive effect on hypertension by the

middle of last century (Scheller, 1955; Perrinjaquet-Moccetti et al. 2008; Susalit et al., 2011), and, since then, the benefits of minor olive components have been extensively investigated (Tripoli et al., 2005).

2.1 Antioxidant activity

The antioxidant activity is the most studied property of olive phenolic compounds. The interest of hydroxytyrosol is based on its remarkable pharmacological and antioxidant activities. Reactive oxygen species, which are continuously being formed as a result of metabolic processes in the organism, may cause oxidation and damage of cellular macromolecules, and therefore, may contribute to the development of degenerative diseases, such as atherosclerosis, cancer, diabetes, rheumatoid arthritis and other inflammatory diseases (Balsano & Alisi, 2009).

The high antioxidant efficiency of HT, attributed to the presence of the *o*-dihydroxyphenyl moiety, is due to its high capacity for free radical scavenging during the oxidation process and to its reducing power on Fe^{3+} (Torres de Pinedo et al., 2007). The antioxidant properties of the *o*-diphenols are associated with their ability to form intramolecular hydrogen bonds between the hydroxyl group and the phenoxy radical (Visioli et al., 1998); therefore, the catechol avoids the chain propagation by donating a hydrogen radical to alkylperoxyl radicals ($ROO^{\cdot}$) formed in the initiation step of lipid oxidation (Scheme 1).



Scheme 1. Mechanism of free radical scavenging by hydroxytyrosol

Oxidation of low-density lipoproteins (LDLs) is a lipid peroxidation chain reaction, which is initiated by free radicals. It has been shown that hydroxytyrosol can inhibit LDL oxidation efficiently due to its capacity to scavenge peroxyl radicals (Arouma et al., 1998; Turner et al., 2005). Hydroxytyrosol reduces oxidation of the low-density lipoproteins carrying cholesterol (LDL-C), which is a critical step in the development of atherosclerosis and other cardiovascular diseases (Gonzalez-Santiago et al., 2010; Vázquez-Velasco et al., 2011); hydroxytyrosol has also a potential protective effect against oxidative stress induced by *tert*-butyl hydroperoxide (Goya et al., 2007).

It has been reported that hydroxytyrosol enhances the lipid profile and antioxidant status preventing the development of atherosclerosis. This compound may also reduce the expression of vascular cell adhesion molecules (Carluccio et al., 2007) and inhibit platelet aggregation in rats (González-Correa et al., 2008a) and hypercholesterolaemia in humans (Ruano et al., 2007).

2.2 Anticancer activity

Numerous studies about the relationship between olive oil consumption and cancer prevention have been carried out (Pérez-Jiménez et al., 2005). Antioxidant compounds supplied in the diet can reduce the risk of cancer due to the fact that they can minimize DNA damage, lipid peroxidation and the amount of ROS generated (Omar, 2010a; Hillestrom, 2006; Manna, 2005).

It has been reported that HT may exert a pro-apoptotic effect by modulating the expression of genes involved in tumor cell proliferation of promyelocytes (HL60 cells) (Fabiani et al., 2006, 2008, 2009, 2011). Moreover, it has been shown that HT inhibits proliferation of human MCF-7 breast cancer cells (Siriani et al., 2010; Bulotta et al. 2011; Bouallagui et al., 2011a), human HT29 colon carcinoma cells (Guichard et al., 2006), human M14 melanoma cells (D'Angelo et al., 2005) and human PC3 prostate cells (Quiles et al., 2002).

Pre-treatment of HepG2 cells with hydroxytyrosol prevented cell damage, what could be due to the fact that hydroxytyrosol may prepare the antioxidant defense system of the cell to face oxidative stress conditions (Goya et al., 2007, 2010).

2.3 Osteoporosis

Hydroxytyrosol may have critical effects on the formation and maintenance of bone, and could be used as an effective remedy in the treatment of osteoporosis symptoms, as it can stimulate the deposition of calcium and inhibit the formation of multinucleated osteoclasts in a dose-dependent manner. HT also suppressed the bone loss of spongy bone in femurs of ovariectomized mice (Hagiwara et al., 2011).

2.4 Antimicrobial activity

Antimicrobial activity of oleuropein, tyrosol and hydroxytyrosol has been studied *in vitro* against bacteria, viruses and protozoa (Bisignano et al., 1999).

The *in vitro* antimycoplasmal activity of HT has been investigated, concluding that this compound might be considered as an antimicrobial agent for treating human infections caused by bacterial strains or casual agents of intestinal or respiratory tract (Furneri et al., 2004).

It has been shown that polyphenols from olive oil are powerful anti-*Helicobacter Pylori* compounds *in vitro* (Romero et al., 2007), a bacteria linked to a majority of peptic ulcers and to some types of gastric cancer.

2.5 Antiinflammatory activity

Inflammation and its consequences play a crucial role in the development of atherosclerosis and cardiovascular diseases. Polyphenols have been shown to decrease the production of inflammatory markers, such as leukotriene B4, in several systems (Biesalski, 2007).

The effect of hydroxytyrosol on platelet function has been tested. Hydroxytyrosol was proven to inhibit the chemically induced aggregation, the accumulation of the pro-aggregant agent thromboxane in human serum, the production of the pro-inflammatory molecules leukotrienes and the activity of arachidonate lipoxygenase (Visioli et al., 2002).

Recently, it has been described that HT-20, an olive oil extract containing about 20% of hydroxytyrosol, inhibits inflammatory swelling and hyperalgesia, and suppresses proinflammatory cytokine in a rat inflammation model (Gong et al., 2009).

2.6 Antiviral activity

Hydroxytyrosol and oleuropein have been identified as a unique class of HIV-1 inhibitors that prevent HIV from entering into the host cell and binding the catalytic site of the HIV-1

integrase. Thus, these agents provide an advantage over other antiviral therapies in which both, viral entry and integration, are inhibited (Lee-Huang et al., 2007a, 2007b, 2009).

HT and its derivatives are also useful, when applied topically, as microbicide for preventing HIV-infection, as well as other sexually transmitted diseases caused by fungi, bacteria or viruses (Gómez-Acebo et al., 2011). Furthermore, it has been reported that hydroxytyrosol inactivated influenza A viruses, suggesting that the mechanism of the antiviral effect of HT might require the presence of a viral envelope (Yamada et al., 2009).

2.7 Hydroxytyrosol as an antinitrosating agent

The antinitrosating properties of hydroxytyrosol and other plant polyphenols of dietary relevance have been investigated (De Lucia et al., 2008). It has been shown that HT reacts with sodium nitrite at pH 3 to give 2-nitrohydroxytyrosol, supporting a protective role of HT as an efficient scavenger of nitrosating species (Fig. 2).

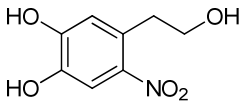


Fig. 2. 2-Nitrohydroxytyrosol formed by nitrosation of HT

3. Hydroxytyrosol derivatives

3.1 Lipophilic hydroxytyrosol esters

Many different hydroxytyrosol lipophilic analogues occur naturally in olive fruit and in virgin olive oil. The amount of these compounds is related to olive variety and ripeness, climate, location, type of crushing machine and oil extraction procedures. As an example, the concentration of hydroxytyrosyl acetate is similar to that of HT in some olive oil varieties such as Arbequina, twice as high in the Picual variety, and between one third and one fourth in the Manzanilla and Hojiblanca oils (Romero et al., 2007).

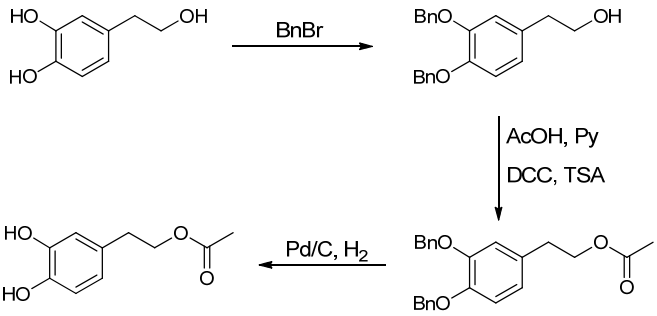
Due to the limited solubility of HT in lipid media, the search for new lipophilic hydroxytyrosol esters with enhanced properties is of great interest, both in food industry and in medicine. Studies on olive polyphenols have shown the importance of the lipophilicity of the antioxidants on the cell uptake and membrane crossing, and on the substrate to be protected (membrane constituents or LDL), (Grasso et al., 2007). These facts explain the efforts made in the development of new synthetic analogues with increased lipophilicity.

3.1.1 Synthetic approaches

Phenolic acids, such as caffeic acid, have been esterified with good chemoselectivity in the presence of strong protic acids (Fischer esterification), but the severe reaction conditions together with the large excess of alcohol required make this strategy of limited applicability (Burke et al., 1995). Under basic catalysis, phenols can be easily deprotonated, so the esterification of phenolic alcohols and phenolic acids via acyl nucleophilic substitution requires previous protection of the phenolic hydroxyl groups, due to the competition between aliphatic and phenolic hydroxyl groups (Appendino et al., 2002; Gambacorta et al., 2007).

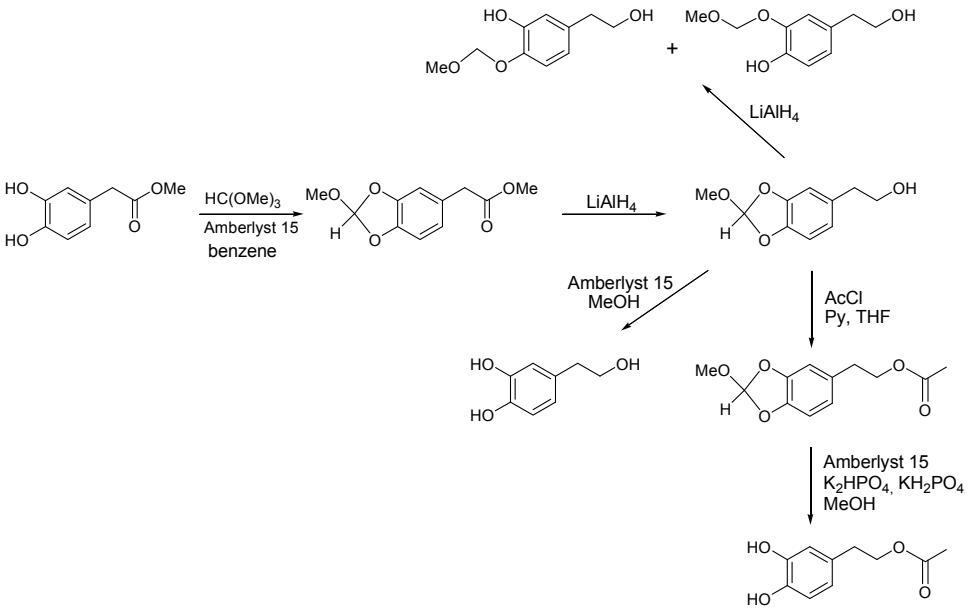
3.1.1.1 Protection of phenolic hydroxyl groups

As an example, benzyl groups have been used to carry out the HT esterification under basic conditions, followed by catalytic hydrogenation to remove the protective groups (Gordon et al., 2001), as depicted in Scheme 2.



Scheme 2. Synthesis of hydroxytyrosyl acetate via benzylation of phenolic hydroxyls

A two-step procedure involving the reaction of methyl orthoformate-protected hydroxytyrosol with acetyl chloride, and hydrolytic deprotection in phosphate buffer under very mild conditions (pH=7.2) to get hydroxytyrosyl acetate (87% overall yield) (Scheme 3) was also described as a successful procedure for the preparation of HT-derived esters (Gambacorta et al., 2007). The key synthetic orthoester intermediate was also used for the synthesis of HT upon reduction with $LiAlH_4$ and acidic deprotection.

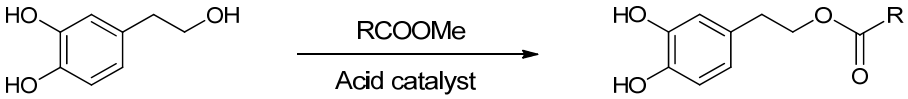


Scheme 3. Synthesis of hydroxytyrosyl acetate via methyl orthoformate-protected hydroxytyrosol.

In order to overcome the problems associated to the protection and deprotection steps of the phenolic hydroxyl groups, different methods for the preparation of hydroxytyrosyl esters by reaction of hydroxytyrosol with various acylating agents have been described, such as esterification with free acids (Appendino et al., 2002), transesterification with methyl or ethyl esters (Alcudia et al., 2004; Trujillo et al., 2006), acyl chlorides (Torregiani et al., 2005) and the use of enzymatic methodologies (Grasso et al., 2007; Mateos et al., 2008; Torres de Pinedo et al., 2005; Buisman, 1998).

3.1.1.2 Acid catalyzed transesterification

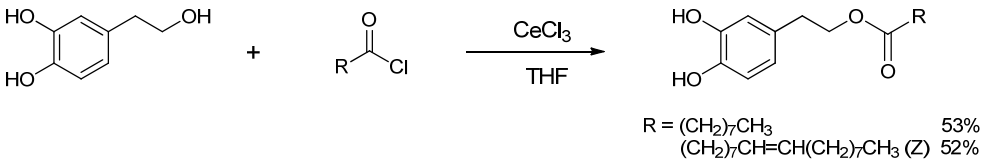
HT transesterification using methyl or ethyl esters and *p*-toluenesulfonic acid as catalyst has been described as a method without the need of protection of the aromatic hydroxyl groups due to its total chemoselectivity (Alcudia et al., 2004; Trujillo et al., 2006). This method involves heating a solution solution of hydroxytyrosol in the corresponding ethyl or methyl ester, containing a catalytic amount of *p*-toluenesulfonic acid (Scheme 4). This protocol has been optimized for HT acetate (86%), and also for longer aliphatic chains like hydroxytyrosyl butyrate, laureate, palmitate, stearate, oleate and linoleate, obtained in acceptable to good yields (62-76%) (Mateos et al., 2008).



Scheme 4. General procedure of acid-catalyzed transesterification

3.1.1.3 Acylation of polyphenolic alcohols with the couple $CeCl_3$–RCOCl

Cerium (III) chloride has been reported to be an efficient promoter for the chemoselective esterification of unprotected polyphenolic alcohols with acyl halides as acyl donors, thereby making it possible to avoid the protection of phenolic hydroxyl groups and providing polyphenolic esters of interest (Torregiani, 2005). This reaction is one example of the so-called Lewis acid catalysis by lanthanide salts (Ishihara et al., 1995). The reaction presumably involves the formation of an electrophilic Lewis adduct between acyl chlorides and cerium (III) chloride, which is quenched by the more nucleophilic aliphatic hydroxyl group of the substrate, with formation of the ester, and regeneration of the lanthanide promoter. The yields obtained are acceptable for HT using nonanoyl and oleoyl chlorides (53 and 52%), respectively (Scheme 5).

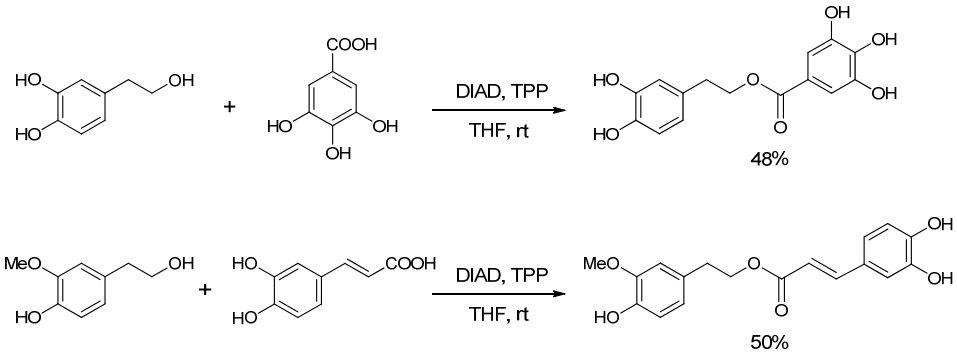


Scheme 5. Acylation of hydroxytyrosol with acyl chlorides and Ce(III)

3.1.1.4 Esterification with free acids: Mitsunobu esterification

The Mitsunobu reaction has been also applied to the chemoselective esterification of phenolic acids with phenolic alcohols (Appendino et al., 2002) as demonstrated by the condensation of hydroxytyrosol with gallic acid, and of vanillyl alcohol with caffeic acid in a one step

procedure with 48% and 50% yields, respectively. The esterification is carried out using DIAD (diisopropyl azodicarboxylate) and TPP (triphenylphosphine) in THF (Scheme 6). The removal of byproducts arising during the Mitsunobu reaction, a major problem of this type of reactions, could be solved by gel-permeation chromatography on Sephadex LH-20.

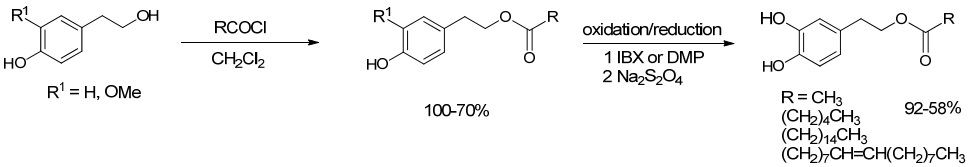


Scheme 6. Mitsunobu esterification of hydroxytyrosol and vanillyl alcohol

3.1.1.5 Syntheses of hydroxytyrosol esters from tyrosol and homovanillyl alcohol

The syntheses previously described in the previous sections had all in common hydroxytyrosol as a precursor of its esters, but some efforts have also been done to get hydroxytyrosyl esters starting from different and cheaper reagents. In this context, the syntheses of hydroxytyrosol esters from tyrosol and homovanillyl alcohol have been proposed (Bernini et al., 2008b). This procedure involves the selective esterification of tyrosol and homovanillyl alcohol with acyl chlorides in dicholoromethane as solvent, to give tyrosyl and homovanillyl acetates in 90% and quantitative yields, respectively, by using only a little excess of acetyl chloride in dichloromethane without any catalysts. The authors suggested acid catalysed acylation due to traces of hydrochloric acid derived from the hydrolysis of the acetyl chloride under the experimental conditions. A similar selectivity was observed by using several saturated or unsaturated acyl chlorides with longer chains such as hexanoyl, palmitoyl, oleoyl and linoleoyl chlorides.

The subsequent oxidation with 2-iodoxybenzoic acid (IBX) or Dess-Martin periodinane reagent (DMP) and *in situ* reduction with sodium dithionite ($Na_2S_2O_4$) of tyrosyl and homovanillyl esters led to the corresponding hydroxytyrosol derivatives. In general, the oxidation of tyrosol derivatives proceeded with higher yields (92-77%) compared to those of homovanillyl derivatives (88-58%). The use of DMP gave similar results to those obtained with IBX. The procedure of oxidation/reduction with IBX/$Na_2S_2O_4$ to obtain the different esters is under protection of two patents (Bernini et al. 2007, 2008c).



Scheme 7. Synthesis of hydroxytyrosol esters from tyrosol and homovanillyl alcohol

3.1.1.6 Lipase-catalyzed transesterification

The use of enzymes, like lipases, as catalysts in non-aqueous solvents to prepare lipophilic derivatives directly from HT has been widely described in the last few years (Grasso et al., 2007; Torres de Pinedo et al., 2005; Mateos et al., 2008; Buisman et al., 1998). This procedure avoids the use of toxic reagents and allows mild reaction conditions.

The esterification of phenols with carboxylic fatty acids and lipases as biocatalysts was firstly investigated by Buisman et al., (1998), using hydroxytyrosol, octanoic acid in hexane, and immobilized lipases from *Candida antartica* (CAL-B). Furthermore, a strong dependence of the yield on the solvent used was observed; so, in diethyl ether a conversion of 85% was obtained within 15 hours (35 ºC), while conversions of roughly 20% were found in the case of solvents like chloroform, dichloromethane or THF. Yields of 70–80% were observed using *n*-pentane and *n*-hexane, in spite of the poor solubility of HT in such solvents.

Different enzymes have been tested on hydroxytyrosol (Grasso et al., 2007) including lipases from *A. niger, C. cylindracea, M. javanicus, P. cepacia, M. miehei, C. viscosum, P. fluorescens, R. arrhizus, R. niveus, C. antarctica*, porcine pancreas and wheat germ, using vinyl acetate as reagent and *tert*-butyl methyl ether as solvent. The best results were obtained with *C. antarctica* in terms of short reaction time, chemioselective conversion and good yield. *C. antarctica* lipase (CAL) was selected for acylation of hydroxytyrosol and homovanillic alcohol with vinyl esters of different acyl chains on a preparative scale, as shown in Table 1. The use of *C. antarctica* with increasing alkyl chain length required longer reaction times. The homovanillyl alcohol and its esters were found to exhibit scarce effectiveness both as radical scavengers and antioxidant agents.

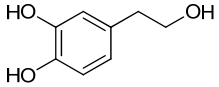
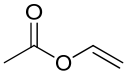
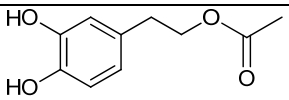
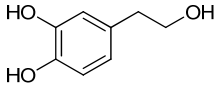
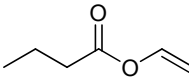
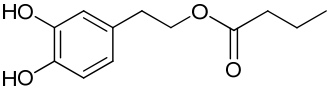
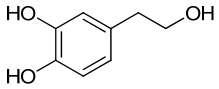
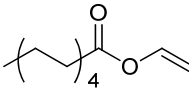
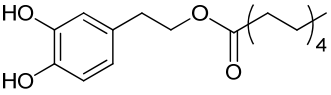
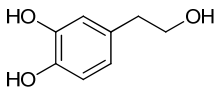
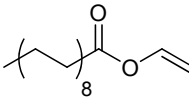
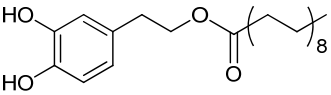
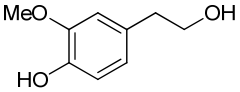
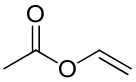
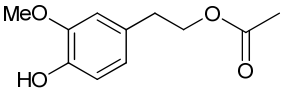
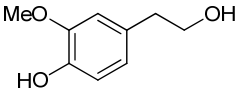
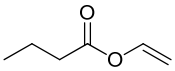
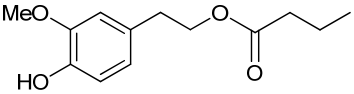
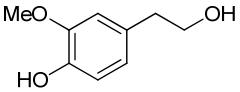
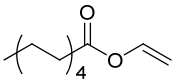
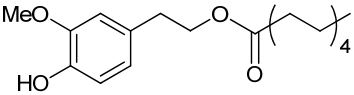
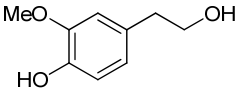
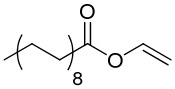
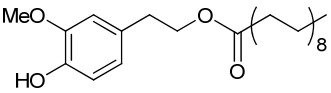
Transesterification of HT with ethyl saturated, mono- and poly-unsaturated fatty acid esters, catalized by Novozym® 435 (immobilized *C. antarctica* lipase B), in vacuum under solventless conditions, has been successfully developed (Torres de Pinedo et al. 2005). This procedure gave hydroxytyrosyl esters in 59-98% yield for the saturated fatty acid esters, and 32-97% yield for the mono- and poly-unsaturated fatty acid esters.

3.1.2 Biological activity

3.1.2.1 Antioxidant activity

The antioxidant activity of hydroxytyrosyl esters has been measured with different methods, including DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2'-azino-bis(3-etilbenzotiazolin-6-sulfonic acid), FRAP (ferric reducing antioxidant power) and Rancimat (Mateos et al., 2008; Gordon et al., 2001; Bouallagui et al., 2011b). The Rancimat test is a method commonly used to evaluate the antioxidant power in lipophilic food matrices, such as oils and fats, while the ABTS and FRAP assays are used for the evaluation of antioxidant activity in hydrophilic medium; the ABTS assay evaluating the radical-scavenging capacity, and the FRAP method determining the reducing activity.

The Rancimat test revealed a lower activity for ester derivatives compared to HT, in agreement with the so-called polar paradox, according to which hydrophilic antioxidants are more effective in less polar media, such as bulk oils, whereas lipophilic antioxidants are more effective in relatively more polar media, such as in oil-in-water emulsions or liposomes (Frankel et al., 1994; Shahidi & Zhong, 2011).

Phenol	Acylating agent	Product	Time (min)	Yield (%)
			35	95.0
			35	96.5
			75	93.3
			180	92.3
			60	96.8
			90	90.9
			90	97.5
			240	98.0

Phenol: acylating agent 1:20, *C. antarctica* lipase, *t*-BuOMe, 40 ºC

Table 1. Enzymatic esterification of HT and homovanillyl alcohol (Grasso et al., 2007)

The order of the scavenging activities toward the ABTS radical was hydroxytyrosyl esters ≥ α-tocopherol > hydroxytyrosol > tyrosyl >tyrosyl esters ≅ BHT. In a similar trend, comparison of FRAP values obtained for the free hydroxytyrosol and tyrosol with the corresponding esters revealed that while hydroxytyrosyl esters showed a significantly higher reducing activity than their precursor, all the tyrosyl esters showed a lower antioxidant activity than that of tyrosol. The same conclusion was obtained from DPPH assay of the radical scavenging activity (Grasso et al., 2007).

In connection with the size of the acyl chain, the reported literature seems to conclude that the antioxidant capacity of hydroxytyrosyl esters is better for medium-sized (C4–C9) alkyl chains in comparison with HT, whereas further elongation of the acyl chain does not improve the antioxidant activity. This confirms that antioxidant capacity does not depend

only on lipophilicity. A possible explanation could be related to the fact that the conformational freedom of the ester chain increases with the acyl chain length, and this could result in folded structures in which catechol hydroxyls are shielded (Tofani et al., 2010; Pereira-Caro et al., 2009; Medina et al., 2009).

This antioxidant activity has also been proved in biological assays, in order to check the ability of hydroxytyrosyl esters to protect proteins and lipids against oxidation caused by peroxyl radicals, using a brain homogenate as an *ex vivo* model (Trujillo et al., 2006) and cumene hydroperoxide to induce oxidation. The results obtained showed a protective effect in these systems, which was more effective in preventing the generation of carbonyl groups in proteins than the generation of malondialdehyde in lipid; hydroxytyrosyl linoleate showed the greatest activity. This fact proves that the introduction of a lipophilic chain in the hydroxytyrosol molecule increases both protein and lipid protection.

Dichlorodihydrofluorescein (DCF) fluorometric assay on whole cells, carried out to check the antioxidant activity of a large serie of hydroxytyrosyl esters (Tofani et al., 2010) on rat muscle cells, showed that hydroxytyrosol esters had a better antioxidant activity compared to HT due to the better penetration into the cells of the lipophilic derivatives.

Hydroxytyrosol fatty acid esters have shown a nonlinear tendency in antioxidant capacity in fish oil-in-water emulsions (Lucas et al., 2010), where a maximum of antioxidant efficiency appeared for hydroxytyrosol octanoate in a study of hydrosytyrosyl esters with alkyl chains varying from C2 to C18. These results seem to be in disagreement with the antioxidant polar paradox.

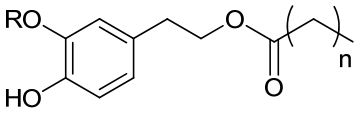
3.1.2.2 Cardiovasvular diseases

Platelet aggregation is considered one of the main events in arterial thrombosis; therefore aggregation prevention is a major goal of cardiovascular research. It has been proved that hydroxytyrosol acetate inhibits platelet aggregation induced by ADP, collagen or arachidonic acid and stimulates nitric oxide production, more efficiently than hydroxytyrosol, and as effectively as acetylsalicylic acid; the latter is the most widely used drug in the world to prevent ischaemic cardiovascular diseases because of its antiplatelet aggregating action. This conclusion has been achieved *in vivo* in a study of oral administration of this ester to rats (González-Correa et al., 2008b), and *in vitro* in both human whole blood and platelet-rich plasma (González-Correa et al., 2009).

3.1.2.3 DNA damage oxidative protection

The atypical Comet test on whole blood cells has been applied to several hydroxytyrosyl esters to check their capacity to counteract the oxidative stress caused by H_2O_2 and the basal DNA damage.

The results obtained show that antidamaging properties on DNA of HT acetate and propanoate are comparable to those of HT, whereas the protective effect progressively decreases in the order butanoate < decanoate ≅ estearate (Fig. 3). This behavior was not observed for the lipophilic analogues of homovanillyl alcohol which appear to be scarcely protective, indicating that *o*-diphenols are more effective antioxidants than simple phenols (Grasso et al., 2007).



R = H, Me n = 0,1,2,8,16

Fig. 3. Hydroxytyrosol lipophilic analogues

3.1.2.4 Prevention of oxidative stress

The ability of hydroxytyrosol and its esters to prevent iron-induced oxidative stress has been studied on human cervical cells (HeLa cells) by the TBARS protocol (Bouallagui et al., 2011b). Pre-incubation of HeLa cells in the presence of 100 µM phenolic compounds led to a significant improvement of the oxidative status. In fact, thiobarbituric acid-reactive substance (TBARS) production was decreased by 30%, 36% and 38% with hydroxytyrosol, hydroxytyrosyl acetate and hydroxytyrosyl oleate, respectively.

3.1.2.5 Transport, absorption and metabolism

The study of the metabolism of hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate has been carried out using human hepatoma cells (HepG2) as a model system of the human liver (Mateos et al., 2005). The results showed extensive uptake and metabolism of hydroxytyrosol and scarce metabolism of tyrosol, while hydroxytyrosyl acetate showed an interesting behavior, with formation of deacetylated hydroxytyrosol after only 2 h. Because hydroxytyrosyl acetate was stable in the culture medium, the hydroxytyrosol detected in the extracellular medium should be attributed to the action of the hepatic cells.

3.1.2.6 Neuroprotective effect of hydroxytyrosyl and hydroxytyrosol acetate

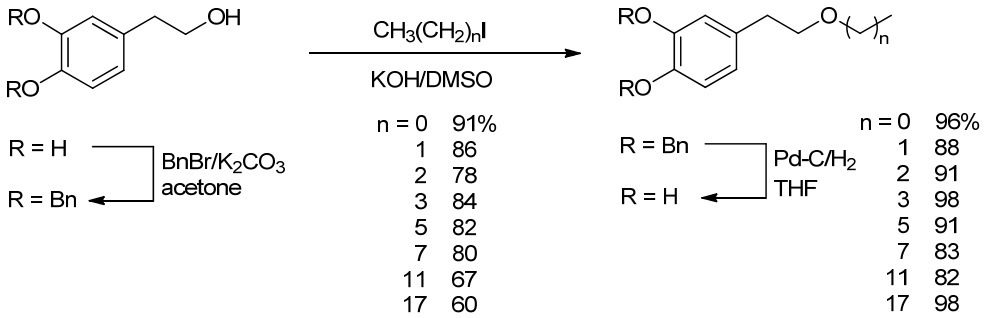
Neuroprotection exerted by HT derivatives has been investigated in rat brain slices subjected to hypoxia-reoxygenation, both *in vitro* and after oral administration (González-Correa et al., 2008). This study was carried out to confirm to the previously demonstrated neuroprotective effects of virgin olive oil in rats (González-Correa et al., 2007). Although the studies gave positive results in the neuroprotective activity of both HT and hydroxytyrosyl acetate, mechanisms that underlie this effect are still unknown.

3.2 Lipophilic hydroxytyrosyl alkyl ethers

3.2.1 Synthetic approaches

Hydroxytyrosyl alkyl ethers have been obtained (Madrona et al., 2009) in a three-step procedure starting from hydroxytyrosol isolated from olive oil waste waters (Scheme 8). This procedure requires first the selective protection of the aromatic hydroxyl groups *via* benzylation with benzyl bromine in the presence of K_2CO_3, and then the addition of an alkyl iodide under basic conditions, and the subsequent deprotection by catalytic hydrogenation (Pd/C) to obtain the corresponding ethers.

The yield for the alkylation step varies depending on the length of the alkyl chain; as depicted in Scheme 8, the yields decrease as the length of the alkyl chain increases, due to the reduced solubility of the corresponding long chain alkyl iodides in the solvent (DMSO).



Scheme 8. Synthesis of hydroxytyrosyl alkyl ethers by alkylation with alkyl iodides

The oxidative stability of lipid matrix in the presence of these compounds, measured by the Rancimat method, has shown that these derivatives retain the high protective capacity of free hydroxytyrosol and similar induction times, having higher induction times than butylhydroxytoluene (BHT) and α-tocopherol (Madrona et al., 2009). These results are in agreement with those obtained in the case of hydroxytyrosyl esters, covered in the previous section (Mateos et al., 2008). The antioxidant activity has been checked by the DPPH, FRAP and ABTS assays in a hydrophilic medium (Pereira-Caro et al., 2009). The antioxidant activity of the lipophilic hydroxytyrosyl ethers was slightly lower in bulk oils and higher in hydrophilic media in comparison with their reference HT, supporting the polar paradox. The length of the alkyl chain did have a positive influence in hydrophilic medium for ethers with a short alkyl chain (methyl, ethyl, propyl), while ethers with longer alkyl chains (from butyl to octadecyl) maintained or decreased their antioxidant activity, probably due to the steric effect of the hydrocarbon chains.

3.2.2 Biological activity

In order to evaluate the safety and potential biological activity of these ethers, studies of their transport, absorption and metabolism in cellular and animal models have been developed (Pereira-Caro et al., 2010a, 2010b) using a human hepatoma cell line (HepG2) as a model system of the human liver and human enterocyte-like Caco-2/TC7 cells, which are commonly used to characterize the intestinal absorption of a range of drugs, nutrients, and other xenobiotics.

The results showed a direct relationship between the lipophilic nature of each compound and the level of metabolization; as an example, hydroxytyrosyl butyl ether biotransformation was complete after 18 h, whereas small amounts of the others remained after the same time. Furthermore, an intestinal absorption increase was observed from methyl to *n*-butyl ethers.

Protective effects against oxidative stress have also been studied (Pereira-Caro et al., 2011) using HepG2 cells, the ones previously employed to assess the metabolism of the synthesized HT ethers. The results obtained show the potential to prevent cell damage induced by *tert*-butyl hydroperoxide (*t*-BuOOH) and the ability to maintain unaltered cellular redox status, partially after 2 hours of pretreatment and almost completely after 20 hours. These results are in accordance with those obtained with hydroxytyrosol (Martín et al., 2010), but they also show the relevance of the role of the lipophilic character of the

phenolic compounds on their antioxidant potential against cell damage: HT methyl and ethyl ethers are less effective than HT propyl and butyl ethers.

3.3 Hydroxytyrosol-derived isochromans

Isochroman fragment is a ubiquitous scaffold that can be found in natural products, drugs and agrochemicals (Larghi & Kaufman, 2006). Access to dihydroxyisochromans derived from HT can be achieved by using the oxa-Pictet-Spengler reaction, by reaction of arylethanols with aldehydes, ketones or masked-carbonyl derivatives (Guiso et al. 2001). The reaction is highly regioselective, as intramolecular cyclization takes place mainly in the less hindered position, as it can be deduced from its reaction mechanism, shown in Scheme 9. Two of the synthetized isochromans (Fig. 4) have been detected in olive oil (Bianco et al., 2001).

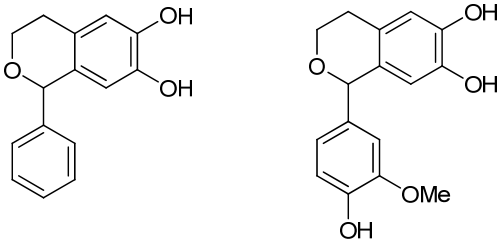
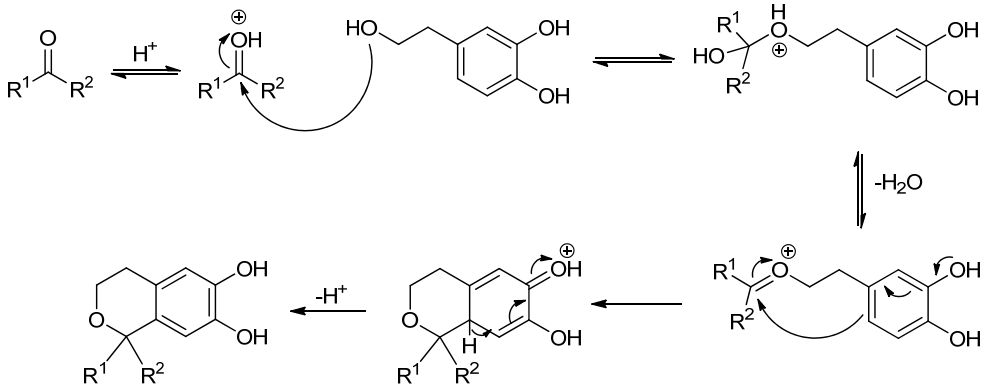


Fig. 4. Isochromans naturally present in olive oil



Scheme 9. Synthesis of hydroxytyrosol isochroman derivatives

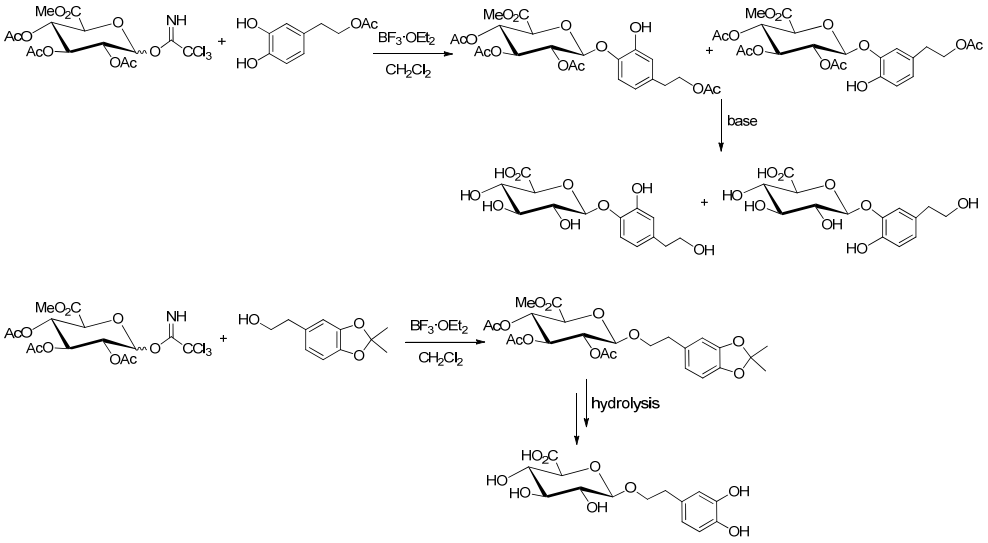
Hydroxytyrosol isochroman derivatives shown in Fig. 4 were effective free radical scavengers able to inhibit platelet aggregation and thromboxane release (Togna, 2003).

3.4 Hydroxytyrosol glucoronide derivatives

One of the major metabolic pathways found *in vivo* for dietary phenolic compounds such as hydroxytyrosol is *O*-conjugation via glucuronidation and sulfation. Therefore, it is of interest to study these metabolites and their biological activities.

Biocatalyzed syntheses of hydroxytyrosol and other phenolic glucuronides have been developed using porcine liver microsomes (Khymenets et al., 2006, 2010). This type of glucuronides has also been synthesised stereoselectively (Lucas et al., 2009) in the phenolic or aliphatic hydroxyl groups using efficient chemical method from *O*-partially protected hydroxytyrosol and glucuronosyl trichloroacetimidate donors (Scheme 10).

The antioxidant activities of hydroxytyrosol conjugates have been evaluated, concluding that none of these glucuronides displayed significant antioxidant activities at the concentration tested (Khymenets et al., 2010).



Scheme 10. Synthesis of hydroxytyrosol glucuronides

3.5 Hydroxytyrosol glucosides

The three isomers of hydroxytyrosol β-D-glucopyranosides (Fig.5) have been reported to be present in olives (Bianco et al., 1998). The 4-glucoside (Romero et al., 2004) and the 1-glucosides (Medina et al., 2007) have been found in table olive brines, and have been analysed as antimicrobial compounds against *Lactobacillus pentosus* with negative results. It has been recently shown that hydroxytyrosol 4-glucoside was the main phenolic compound in the aqueous phase of fresh alpeorujo, followed by hydroxytyrosol, and hydroxytyrosol 1-glucoside.

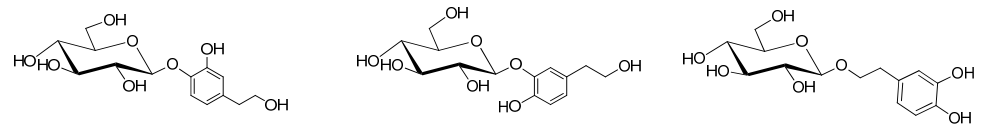
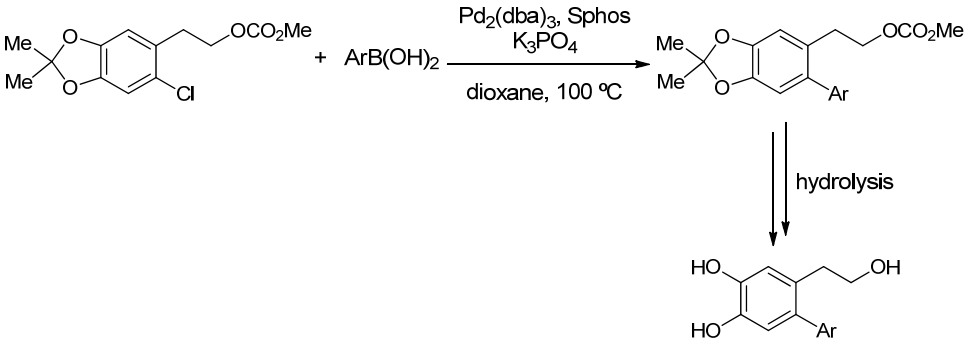


Fig. 5. The three isomers of hydroxytyrosol • –Δ–γλυχοπψρανοσιδεσ

3.6 Arylhydroxytyrosol derivatives

The synthesis of 2-arylhydroxytyrosols from 2-halohydroxytyrosol derivatives has been described (Bernini et al., 2008a). The reaction of the corresponding 2-chloro precursors via Suzuki-Miyaura cross-coupling reaction with arylboronic acids containing electron-donating, electron-withdrawing, as well as *ortho* substituents, yielded this family of compounds in high to excellent yields (Scheme 11).



Scheme 11. Synthesis of 2-arylhydroxytyrosol derivatives

3.7 Complexation of hydroxytyrosol with β-cyclodextrins

The complexation of hydroxytyrosol with commercially-available β-cyclodextrin (β-CD) (López-García et al., 2010; Rescifina et al. 2010) and hydroxypropyl-β-cyclodextrin (HP-β-CD) (López-García et al., 2010) in aqueous solutions has been studied. The stoichiometries, the association constants and the geometry of the complexes have been determined by NMR techniques. The stoichiometries of both complexes are 1:1 and the association constants are 93±7 M^{-1} for HT/β-CD complex and 43±1 M^{-1} for HT/HP-β-CD complex (López-García et al., 2010). In both cases, the insertion of the catechol moiety took place by directing the hydroxyalkyl chain to the primary rim. The postulated geometry of the 1:1 HT/β-CD inclusion complex is depicted in Fig. 6.

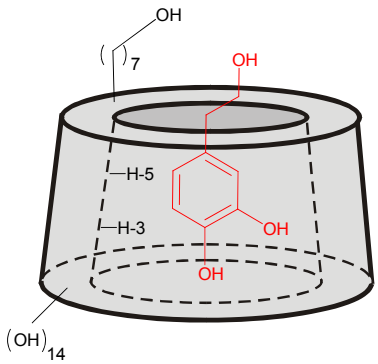


Fig. 6. Postulated geometry of the 1:1 HT/β-CD inclusion complex

Moreover, the antioxidant activity of encapsulated HT, together with the photoprotection effect of β-CD on HT, has been evaluated by scavenging of the stable DPPH radical. It has been proven that β-Cyclodextrin acts as a secondary antioxidant and provides a moderate improvement of the radical scavenging activity of HT measured by the DPPH assay.

β-Cyclodextrin exerts a strong photoprotection of HT upon UV irradiation, which could be deduced from the EC_{50} values (Table 2). For equimolecular mixtures of HT and β-CD at 1.2 mM, the observed degradation after 24 h and 48 h is similar to the degradation found for HT at the same concentration and time (entries 4 and 5) showing no protection at 24 h and only a slight protection after 48 h. However, using 1:4 mixtures of HT (1.2 mM) and β-CD (4.8 mM), a remarkable reduction of the degradation rate was observed when compared with pure HT. In this way, the complexation of HT with cyclodextrins might enhance stability, improve its performance as antioxidant and extend its storage life (López-García et al., 2010).

Entry	Antioxidant	[HT] (mM)	Irradiation time (h)	EC_{50} (g HT / kg DPPH)
1	HT	1.2	12	119.0 ± 1.4
2	HT	1.2	24	353.6 ± 23.4
3	HT	1.2	48	1436.1 ± 73.2
4	HT-βCD (1:1)	1.2	24	357.2 ± 30.7
5	HT-βCD (1:1)	1.2	48	1011.6 ± 171.9
6	HT-βCD (1:4)	1.2	12	112.4 ± 6.4
7	HT-βCD (1:4)	1.2	24	198.0 ± 4.6
8	HT-βCD (1:4)	1.2	48	387.2 ± 13.3

Table 2. Effect of the encapsulation of HT on its photostability

4. Conclusions

Hydroxytyrosol is a phenolic compound that can be isolated from olive oil mill wastewaters. The remarkable biological properties of this compound, mainly due to its strong antioxidant activity, has stimulated the synthesis of a series of derivatives, some of them are also naturally-occurring in the olive tree. Among these derivatives hydroxytyrosyl esters and ethers are of great interest, as some of them show strong antioxidant activity and improved bioavailability.

5. Acknowledgement

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6. References

Alcudia F, Cert A, Espartero JL, Mateos R, & Trujillo M. (2004) Method of preparing hydroxytyrosol esters, esters thus obtained and use of same. PCT WO 2004/005237.

Appendino G, Minassi A, Daddario N, Bianchi F, & Tron GC. (2002) Esterification of phenolic acids and alcohols. Org Lett 4: 3839–3841.

Aruoma O, Deiane M, Jenner A, Halliwell B, Kaur M, Banni S, et al. (1998) Effect of hydroxytyrosol found in extra virgin olive oil on oxidative DNA damage and on low-density lipoprotein oxidation. J Agric Food Chem 46: 5181–5187.

Balsano C & Alisi A. (2009) Antioxidant effects of natural bioactive compounds. Curr Pharm Des 15: 3063-3073.

Bernini R, Mincione E, Barontini M, & Crisante F. (2007) Procedimento per la preparazione di derivati dell'idrossitirosolo e di idrossitirosolo via demetilazione ossidativa MI2007 A001110.

Bernini R, Cacchi S, Fabrizi G, & Filisti E. (2008a) 2-Arylhydroxytyrosol derivatives via Suzuki-Miyaura cross-coupling. Org Lett, 10: 3457–3460.

Bernini R, Mincione E, Barontini M, & Crisante F. (2008b) Convenient synthesis of hydroxytyrosol and its lipophilic derivatives from tyrosol or homovanillyl alcohol. J Agric Food Chem 56: 8897–8904.

Bernini R, Mincione E, Barontini M, & Crisante, F. (2008c) Method for preparing hydroxytyrosol and hydroxytyrosol derivatives. PCT/IB2008/000598.

Bianco A, Mazzei RA, Melchioni C, Romeo G, Scarpati ML, Soriero A, et al. (1998) Microcomponents of olive oil III. Glucosides of 2(3,4-dihydroxy-phenyl)ethanol. Food Chem 63, 461–464.

Bianco, A., Coccioli, F., Guiso, M. & Marra, C. (2001) The occurrence in olive oil of a new class of phenolic compounds: hydroxy-isochromans. Food Chem. 77: 405–411.

Biesalski HK. (2007) Polyphenols and inflammation: basic interactions. Curr Opin Clin Nutr Metab Care 10: 724–728.

Bisignano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N, & Saija A. (1999) On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. J Pharm Pharmacol 51: 971–974.

Bouallagui Z, Han J, Isoda H, & Sayadi S. (2011a) Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. Food Chem Toxicol 49: 179–184.

Bouallagui Z, Bouaziz M, Lassoued S, Engasser JM, Ghoul M, & Sayadi S. (2011b) Hydroxytyrosol acyl esters: Biosynthesis and Activities. Appl Biochem Biotechnol 163: 592–599.

Buisman GJH, van Helteren CTW, Kramer GFH, Veldsink JW, Derksen JTP, & Cuperus FP. (1998) Enzymatic esterifications of functionalized phenols for the synthesis of lipophilic antioxidants. Biotechnol Lett 20: 131–136.

Bulotta S, Corradino R, Celano M, D'Agostino M, Maiuolo J, Oliverio M, et al. (2011) Antiproliferative and antioxidant effects on breast cancer cells of oleuropein and its semisynthetic peracetylated derivatives. Food Chem 127: 1609–1614.

Burke TR Jr, Fesen MR, Mazumder A, Wang J, Carothers AM, Grunberger D, et al. (1995) Hydroxylated aromatic inhibitors of HIV-1 integrase. J Med Chem 38: 4171–4178.

Carluccio MA, Ancora MA, Massaro M, Carluccio M, Scoditti E, Distante A, et al. (2007) Homocysteine induces VCAM-1 gene expression through NF-kB and NAD(P)H oxidasa activation: protective role of Mediterranean diet polyphenolic antioxidants. Am J Physiol Heart Circ Physiol 293: 2344–2354.

D'Angelo S, Ingrosso D, Migliardi V, Sorrentino A, Donnarumma G, Baroni A, et al. (2005) Hydroxytyrosol, a natural antioxidant from olive oil, prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. Free Radic Biol Med 38: 908–919.

De Lucia M, Panzella L, Pezzella A, Napolitano A, & D'Ischia M. (2008) Plant catechols and their *S*-glutathionyl conjugates as antinitrosating agents: expedient synthesis and remarkable potency of 5-*S*-Glutathionylpiceatannol Chem Res Toxicol 21: 2407–2413.

Fabiani R, De Bartolomeo A, Rosignoli P, Servili M, Selvaggini R, Montedoro GF, et al. (2006) Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differenciation. J Nutr 136: 614–619.

Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Servili M, Montedoro GF, et al. (2008) Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. J Nutr 138: 1411–1416.

Fabiani R, Fuccelli R, Pieravanti F, De Bartolomeo A, & Morozzi G. (2009) Production of hydrogen peroxide is responsible for the induction of apoptosis by hydroxytyrosol on HL60 cells. Mol Nutr Food Res 53: 887–896.

Fabiani R, Rosignoli P, De Bartolomeo A, Fuccelli R, Servili M, & Morozzi G. (2011) The production of hydrogen peroxide is not a common mechanism by which olive oil phenol compounds induce apoptosis on HL60 cells. Food Chem 125: 1249–1255.

Fernández-Bolaños JG, López O, Fernández-Bolanos J, & Rodríguez- Gutiérrez G. (2008) Hydroxytyrosol and derivatives: Isolation, synthesis, and biological properties. Curr Org Chem 12: 442–463.

Frankel EN, Huang SW, Kanner J, & German JB. (1994) Interfacial phenomena in the evaluation of antioxidants: Bulk oils versus emulsions. J Agric Food Chem 42:1054–1059.

Furneri PM, Piperno A, Sajia A, & Bisignano G. (2004) Antimycoplasmal activity of hydroxytyrosol. Antimicrob Agents Chemother 48: 4892–4894.

Gambacorta A, Tofani D, & Migliorini A. (2007) High-yielding synthesis of methyl orthoformate-protected hydroxytyrosol and its use in preparation of hydroxytyrosyl acetate. Molecules 12: 1762–1770.

Gómez-Acebo E, Alcami Pertejo J, & Aunon Calles D. (2011) Topical use of hydroxytyrosol and derivatives for the prevention of HIV infection. Pat. Appl. Publ. WO 2011067302 A1.

Gong D, Geng C, Jiang L, Cao J, Yoshimura H, & Zhong L. (2009) Effects of hydroxytyrosol-20 on carrageenan-induced acute inflammation and hyperalgesia in rats. Phytoth Res 23: 646–650.

González-Correa JA, Muñoz-Marín J, Arrebola MM, Guerrero A, Carbona F, López-Villodres J, et al. (2007) Dietary virgin olive oil reduces oxidative stress and cellular damage in rat brain slices subjected to hypoxia-reoxygenation Lipids 42: 921–929.

González-Correa JA, Navas MD, Lopez-Villodres JA, Trujillo M, Espartero JL, & de la Cruz, JP. (2008a) Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia-reoxygenation. Neurosci Lett 446: 143–146.

González-Correa JA, Navas MD, Muñoz-Marín J, Trujillo M, Fernádez-Bolaños J, & de la Cruz JP. (2008b) Effects of hydroxytyryosol and hydroxytyrosol acetate

administration to rats on platelet function compared to acetylsalicylic acid. J Agric Food Chem 56: 7872–7876.

González Correa JA, López-Villodres JA, Asensi R, Espartero JL, Rodríguez-Gutiérrez G, & de la Cruz JP. (2009) Virgin olive oil polyphenol hydroxytyrosol acetate inhibits in vitro platelet aggregation in human whole blood: comparison with hydroxytyrosol and acetylsalicylic acid. Br J Nutr 101: 1157–1164.

Gonzalez-Santiago M, Fonolla J, & Lopez-Huertas E. (2010) Human absorption of a supplement containing purified hydroxytyrosol, a natural antioxidant from olive oil, and evidence for its transient association with low-density lipoproteins. Pharmacol Res 61: 364–370.

Gordon MH, Paiva-Martins F, & Almeida M. (2001) Antioxidant activity of hydroxytyrosol acetate compared with other olive oil polyphenols. J Agric Food Chem 49: 2480−2485.

Goya L, Mateos R, & Bravo L. (2007) Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells protection against oxidative stress induced by *tert*-butylhydroxyperoxide. Eur J Nutr 46: 70–78.

Goya L, Mateos R, Martin MA, Ramos S, & Bravo L. (2010) Uptake, metabolism and biological effect of the olive oil phenol hydroxytyrosol by human HepG2 cells, In: Olives and olive oil in health and disease prevention. Preedy VR and Watson RR, (Eds.), Oxford: Academic Press, Elsevier (USA), pp. 1157-1165, ISBN 978-0-12-374420-3.

Grasso S, Siracusa L, Spatafora C, Renis M, & Tringali C. (2007) Hydroxytyrosol lipophilic analogues: Enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. Bioorg Chem 35: 137–152.

Guichard C, Pedruzzi E, Fay M, Marie JC, Braut-Boucher F, Daniel F, et al. (2006) Dihydroxyphenylethanol induces apoptosis by activating serine/threonine protein phosphatase PP2A and promotes the endoplasmic reticulum stress response in human colon carcinoma cells. Carcinogenesis 27: 1812–1827.

Guiso, M., Marra, C. & Cavarischia, C. (2001) Isochromans from 2-(3,4-dihydroxy)phenylethanol. Tetrahedron Lett 42: 6531–6534.

Hagiwara K, Goto T, Araki M, Miyazaki H, & Hagiwara H. (2011) Olive polyphenol hydroxytyrosol prevents bone loss. Eur J Pharmacol 662: 78–84.

Hillestrom PR, Covas MI, & Poulsen HE. (2006) Effect of dietary virgin olive oil on urinary excretion of etheno-DNA adducts. Free Radic Biol Med 41: 1133–1138.

Ishihara K, Kubota M, Kurihara H, & Yamamoto H. (1995) Scandium trifluoromethanesulfonate as an extremely active acylation catalyst. J Am Chem Soc 117: 4413–4414.

Khymenets O, Joglar J, Clapés P, Parella T, Covas M-I, & de la Torre R. (2006) Biocatalyzed synthesis and structural characterization of monoglucuronides of hydroxytyrosol, tyrosol homovanillic alcohol and 3-(4′-hydroxyphenyl)propanol. Adv Synth Catal 34: 2155–2162.

Khymenets O, Clapés P, Parella T, Covas M-I, de la Torre R, & Joglar J. (2009) Biocatalyzed synthesis of monoglucuronides of hydroxytyrosol, tyrosol homovanillic alcohol and 3-(4′-hydroxyphenyl)propanol using liver cells microsomal frations. In: Practical methods for biocatalysis and biotransformations, Wittall J, Sutton P (eds.), John Wiley& Sons, Ltd, pp. 245–250.

Khymenets O, Fito M, Taurino S, Muñoz-Aguayo D, Pujadas M, Torres JL, et al. (2010) Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. Drug Metab Dispos 38: 1417–1421.

Larghi EL & Kaufman TS. (2006) The oxa-Pictet-Spengler cyclization: Synthesis of isochromans and related pyran-type heterocycles. Synthesis 187–220.

Lee-Huang S, Lin Huang P, Zhang D, Wook Lee J, Bao J, Sun Y, et al. (2007a) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part I. Integrase inhibition. Biochem Biophys Res Commun 354: 872–878.

Lee-Huang S, Lin Huang P, Zhang D, Wook Lee J, Bao J, Sun Y, et al. (2007b) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: Part II. Integrase inhibition. Biochem Biophys Res Commun. 354: 879–884.

Lee-Huang S, Huang PL, Huang PL, Zhang D, Zhang JZH, Chang YT, et al. (2009) Compositions and methods for treating obesity, obesity related disorders and for inhibiting the infectivity of human immunodeficiency virus. US Pat Appl Publ US 20090061031 A1 20090305.

López-García MÁ, López Ó, Maya I, & Fernández-Bolaños JG. (2010) Complexation of hydroxytyrosol with β-cyclodextrins: an efficient photoprotection. Tetrahedron 66: 8006–8011.

Lucas R, Alcantara D, & Morales JC. (2009) A concise synthesis of glucuronide metabolites of urolithin-B, resveratrol and hydroxytyrosol. Carbohydr Res 344: 1340–1346.

Lucas R, Comelles F, Alcántara D, Maldonado OS, Curcuroze M, Parra JL, et al. (2010) Tyrosol and hydroxytyrosol fatty acid esters: a potential explanation for the nonlinear hypothesis of the antioxidant activity in oil-in-water emulsions. J Agric Food Chem 58: 8021–8026.

Madrona A, Pereira-Caro G, Mateos R, Rodríguez G, Trujillo M, Fernández-Bolaños J, et al. (2009) Synthesis of hydroxytyrosyl alkyl ethers from olive oil waste waters. Molecules, 14: 1762–1772.

Manna C, Migliardi V, Sannino F, De Artino A, & Capasso R. (2005) Protective effects of synthetic hydroxytyrosol acetyl derivatives against oxidative stress in human cells. J Agric Food Chem 53: 9602-9607.

Martín MA, Ramos S, Granado-Serrano AB, Rodríguez-Ramiro I, Trujillo M, Bravo L, & et al. (2010) Hydroxytyrosol induces antioxidant/detoxificant enzymes and Nrf2 translocation via extracellular regulated kinases and phosphatidylinositol-3-kinase/protein kinase B pathways in HepG2 cells. Mol Nutr Food Res 54: 1–11.

Mateos R, Goya L, & Bravo L. (2005) Metabolism of the olive oil phenols hydroxytyrosol, tyrosol, and hydroxytyrosyl acetate by human hepatoma hepG2 cells. J Agric Food Chem 53: 9897−9905.

Mateos R, Trujillo M, Pereira-Caro G, Madrona A, Cert A, & Espartero JL. (2008) New lipophilic tyrosyl esters. Comparative antioxidant evaluation with hydroxytyrosyl esters. J Agric Food Chem 56:10960–10966.

Medina E, Romero C, de los Santos B, de Castro A, García A, Romero F, & Brenes M. (2011) Antimicrobial activity of olive solutions from stored alpeorujo against plant pathogenic microorganisms. J Agric Food Chem 59: 6927–6932.

Medina E, Brenes M, Romero C, Garcia A, & de Castro A. (2007) Main antimicrobial compounds in table olives. J Agric Food Chem 55: 9817–9823.

Medina I, Lois, S, Alcántara D, Lucas R, & Morales JC. (2009) Effect of lipophilization of hydroxytyrosol on its antioxidant activity in fish oils and fish oil-in-water emulsions. J Agric Food Chem 57: 9773–9779.

Omar SH. (2010a) Cardioprotective and neuroprotective roles of oleuropein in olive. Saudi Pharm J 18: 111–121.

Omar SH. (2010b) Oleuropein in olive and its pharmacological effects. Sci Pharm 78: 133-154.

Pereira-Caro G, Madrona A, Bravo L, Espartero JL, Alcudia F, Cert A, & Mateos A. (2009) Antioxidant activity evaluation of alkyl hydroxytyrosyl ethers, a new class of hydroxytyrosol derivatives. Food Chem 115: 86–91.

Pereira-Caro G, Bravo L, Madrona A, Espartero JL, & Mateos R. (2010a) Uptake and metabolism of new synthetic lipophilic derivatives, hydroxytyrosyl ethers, by human hepatoma HepG2 cells. J Agric Food Chem 58: 798–806.

Pereira-Caro G, Mateos R, Saha, S, Madrona A, Espartero JL, Bravo L, et al. (2010b) Transepithelial transport and metabolism of new lipophilic ether derivatives of hydroxytyrosol by enterocyte-like Caco-2/TC7 cells. J Agric. Food Chem 58: 11501–11509.

Pereira-Caro G, Sarriá B, Madrona A, Espartero JL, Goya L, Bravo L, & et al. (2011) Alkyl hydroxytyrosyl ethers show protective effects against oxidative stress in HepG2 cells. J Agric Food Chem 59: 5964–5976.

Pérez-Jiménez F, Álvarez de Cienfuegos G, Badimon L, Barja G, Battino M, Blanco A, et al. (2005) International conference on the healthy effect of virgin olive oil. Eur J Clin Invest 35: 421–424.

Perrinjaquet-Moccetti T, Busjahn A, Schmidlin C, Schmidt A, Bradl B, & Aydogan C. (2008) Food supplementation with an olive (*Olea europaea* L.) leaf extract reduces blood pressure in borderline hypertensive monozygotic twins. Phytother Res 22: 1239–1242.

Quiles JL, Farquharson AJ, Simpson DK, Grant I, & Wahle KWJ. (2002) Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. Br J Nutr. 88: 225–234.

Rescifina A, Chiacchio U, Iannazzo D, Piperno A, & Romeo G. (2010) β-Cyclodextrin and caffeine complexes with natural polyphenols from olive and olive oils: NMR, thermodynamic, and molecular modeling studies. J Agric Food Chem 58: 11876–11882.

Rietjens SJ, Bast A, & Haenen GRMM. (2007) New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. J Agric Food Chem 55: 7609–7614.

Romero C, Brenes M, García P, García A, Garrido A. (2004) Polyphenol changes during fermentation of naturally black olives. J Agric Food Chem 52:1973–1979.

Romero C, Medina E, Vargas J, Brenes M, & de Castro A. (2007) In vitro activity of olive oil polyphenols against Helicobacter pylori. J Agric Food Chem 55: 680–686.

Ruano J, López-Miranda J, de la Torre R, Delgado-Lista J, Fernández J, Caballero J, et al. (2007) Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. J Clin Nutr 86: 341–346.

Sabatini N. (2010) Recent patents in olive oil industry: new technologies for the recovery of phenols compounds from olive oil, olive oil industrial by-products and waste waters. Recent Pat Food Nutr Agric 2: 154–159.

Scheller EF. (1955) Treatment of hypertension with standardized olive leaf extract. Med Klin 50: 327–329.

Shahidi F & Zhong Y. (2011) Revisiting the polar paradox theory: A critical overview. J Agric Food Chem 59: 3499–3504.

Siriani R, Chimento A, De Luca A, Casaburi I, Rizza P, Onofrio A, et al. (2010) Oleuropein and hydroxytyrosol inhibit MCF-7 breast cancer cell proliferation interfering with ERK1/2 activation. Mol Nutr Food Res 54: 833–840.

Susalit E, Agus N, Effendi I, Tjandrawinata RR, Nofiarny D, Perrinjaquet-Moccetti T, et al. (2011) Olive (*Olea europaea*) leaf extract effective in patients with stage-1 hypertension: Comparison with captopril. Phytomedicine 18: 251–258.

Tofani, D, Balducci V, Gasperi T, Incerpi S, & Gambacorta, A. (2010) Fatty acid hydroxytyrosyl esters: structure/antioxidant activity relationship by ABTS and in cell-culture DCF assays. J Agric Food Chem 58: 5292–5299.

Togna GI, Togna AR, Franconi M, Marra C, & Guiso M. (2003) Olive oil isochromans inhibit human platelet reactivity. J Nutr 2532–2536.

Torregiani E, Seu G, Minassi A, & Appendino G. (2005) Cerium(III) chloride-promoted chemoselective esterification of phenolic alcohols. Tetrahedron Lett 46: 2193–2196.

Torres de Pinedo A, Peñalver P, Rondón P & Morales JC. (2005) Efficient lipase-catalyzed synthesis of new lipid antioxidants based on a catechol structure. Tetrahedron 61: 7654–7660.

Torres de Pinedo A, Peñalver P, & Morales JC. (2007) Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship. Food Chem 103: 55-61.

Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, & La Guardia M. (2005) The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutr Res Rev 18: 98–112.

Trujillo M, Mateos R, Collantes de Terán L, Espartero JL, Cert R, Jover M, et al. (2006) Lipophilic hydroxytyrosyl esters. Antioxidant activity in lipid matrices and biological systems. J Agric Food Chem 54: 3779−3785.

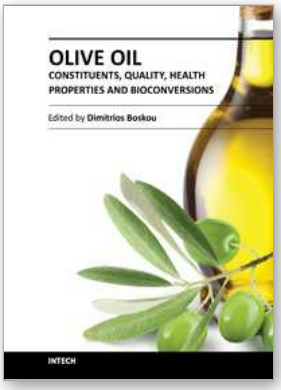
Turner R, Etienne N, Alonso MG, de Pascual-Teresa S, Minihane AM, Weinberg PD, et al. (2005) Antioxidant and anti-atherogenic activities of olive oil phenolics. Int J Vitam Nutr Res. 75: 61–70.

Vázquez-Velasco M, Esperanza Díaz L, Lucas R, Gómez-Martínez S, Bastida S, Marcos A, et al. (2011) Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors. Br J Nutr 105: 1448–1452.

Visioli F, Bellomo GF, & Galli C. (1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 247: 60–64.

Visioli F, Poli A, & Galli C. (2002) Antioxidant and other biological activities of phenols from olives and olive oil. Med Res Rev 22: 65–75.

Yamada K, Ogawa H, Hara A, Yoshida Y, Yonezawa Y, Karibe K, et al. (2009) Mechanism of the antiviral effect of hydroxytyrosol on influenza virus appears to involve morphological change of the virus. Antiviral Res 83: 35–44.



Olive Oil - Constituents, Quality, Health Properties and Bioconversions

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The health-promoting effects attributed to olive oil, and the development of the olive oil industry have intensified the quest for new information, stimulating wide areas of research. This book is a source of recently accumulated information. It covers a broad range of topics from chemistry, technology, and quality assessment, to bioavailability and function of important molecules, recovery of bioactive compounds, preparation of olive oil-based functional products, and identification of novel pharmacological targets for the prevention and treatment of certain diseases.

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