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Preparation and antioxidant activity of tyrosyl and homovanillyl ethers

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ABSTRACT

Preparation of tyrosyl and homovanillyl lipophilic derivatives was carried out as a response to the food industry's increasing demand for new synthetic lipophilic antioxidants. Tyrosyl and homovanillyl ethers were synthesized in high yields by a three-step procedure starting from tyrosol (Ty) and homovanillic alcohol (HMV). The antioxidant activity of these new series of alkyl tyrosyl and homovanillyl ethers was evaluated by the Rancimat test in a lipophilic food matrix and by the FRAP, ABTS and ORAC assays and compared to free Ty and HMV as well as two antioxidants widely used in the food industry, butylhydroxytoluene (BHT) and α-tocopherol. The results pointed out the higher activity of homovanillyl series in comparison with tyrosyl series with all the assayed methods. However, while both synthetic series were less antioxidant than BHT and α-tocopherol in a lipophilic matrix after their Rancimat test evaluation, homovanillyl alkyl ethers showed the best reducing power and radical scavenging activity of all evaluated compounds. This batch of synthetic lipophilic compounds, derived from biologically active compounds such as Ty and HMV, provide interesting and potentially bioactive compounds.

1. Introduction

It is well known that lipid peroxidation decreases food's nutritive value and deteriorates its flavour and taste. Food industry attempts to prevent food oxidation using food additives, in order to improve its quality. In this sense, numerous phenolic antioxidants have the potential to be used in hydrophilic food matrices. Among natural polyphenols, olive oil phenols and, particularly, hydroxytyrosol, (2-(3′,4′-dihydroxyphenyl)ethanol, HTy, **1a**), has the capacity to protect against oxidative stress by scavenging radical species (Goya, Mateos, & Bravo, 2007; Rietjents, Bast & Haenen, 2007) and by inducing antioxidant enzymes (Martín et al., 2010). Furthermore, several studies have demonstrated that HTy has cardioprotective effects (De la Torre-Carbot et al., 2010; Rietjents, Bast, Vente & Haenen, 2007), anti-inflammatory (Bitler, Viale, Damaj, & Crea, 2005) and antiplatelet aggregation activity (Dell'Agli et al., 2008), largely related to its antioxidant properties.

In contrast, Ty (**1b**), another natural olive oil phenol, has significantly lower antioxidant capacity than HTy (Mateos, Domínguez, Espartero, & Cert, 2003) due to the absence of the *ortho*-diphenolic group in its chemical structure. Nevertheless, Ty exerts a protective effect against oxidative injury in cell models (Giovannini et al., 1999) and improves the intracellular antioxidant defence systems (Di Benedetto et al., 2007). In fact, one of the tyrosyl secoiridoid derivatives present in olive oil, oleocanthal, has shown anti-inflammatory activity similar to ibuprofen (Beauchamp et al., 2005). Furthermore, recent studies suggest that specifically *mono*-phenols as Ty or *p*-coumaric acid as well as *o*-diphenolic compounds inhibit homocysteine-induced endothelial cell adhesion, regardless their antioxidant activity, playing a key role in the control of several inflammation-associated processes (Manna, Napoli, Cacciapuoti, Porcelli, & Zappia, 2009).

Taking into account the high potential effectiveness as antioxidants of these virgin olive oil polyphenols and the great interest in the use of phytochemicals as biological ingredients for functional foods with enhanced nutritional value, HTy (**1a**) has already been used as a bioactive ingredient in tomato juice (Larrosa, Espín, & Tomás-Barberán, 2003) and fish products (Pazos, Alonso, Sánchez, & Medina, 2008), showing good results. To our knowledge, no applications has been studied for Ty (**1b**) or HMV (**1c**), which are present in the phenolic fraction of virgin olive oil, and may be effectively recovered from olive oil wastewaters, similarly to HTy (Fernández-Bolaños et al., 2005).

In response to the lack of antioxidants to be used in lipidic foods, new lipophilic derivatives have been investigated in the last

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years. In this sense, several chemical (Alcudia, Cert, Espartero, Mateos, & Trujillo, 2004; Appendino, Minassi, Daddario, Bianchi, & Tron, 2002; Bernini, Mincione, Barontini, & Crisante, 2008; Capasso, Sannino, De Martino, & Manna, 2006; Gordon, Paiva-Martins, & Almeida, 2001; Palozza et al., 2008; Tofani, Balducci, Gasperi, Incerpi, & Gambacorta, 2010; Torregiani, Seu, Minassi, & Appendino, 2005; Trujillo et al., 2006) or enzymatic (Alcudia et al., 2004; Bouallagui et al., 2011; Buisman et al., 1998; Grasso, Siracusa, Spatafora, Renis, & Tringali, 2007; Lucas et al., 2010; Mateos et al., 2008; Torres de Pinedo, Peñalver, & Morales, 2007; Torres de Pinedo, Peñalver, Pérez-Victoria, Rondón, & Morales, 2007; Torres de Pinedo, Peñalver, Rondon, & Morales, 2005) synthesis reactions of lipophilic esters derivatives of hydroxytyrosol, homovanillic alcohol and/or tyrosol esters have been reported and recently reviewed (Chillemi, Sciuto, Spatafora, & Tringali, 2010; Fernández-Bolanos, Lopez, Fernández-Bolanos, & Rodríguez-Gutiérrez, 2008). The antioxidant effects of the new series of esters derivatives, containing lipophilic acyl chains of different length with increasing lipophilicity, have been tested using different methods. Remarkable antioxidant capacity has been observed when the new compounds were tested in cell lines (Bouallagui et al., 2011; Grasso et al., 2007; Tofani et al., 2010) and in food matrices, such as oils and oil-in-water emulsions (Lucas et al., 2010; Mateos et al., 2008; Medina, Lois, Alcántara, Lucas, & Morales, 2009; Torres de Pinedo, Peñalver, & Morales, 2007; Torres de Pinedo, Peñalver, Pérez-Victoria et al., 2007; Trujillo et al., 2006). Having been evaluated using different methods, it may be concluded that these new esters derivatives possess slightly higher or comparable antioxidant activity than their respective precursors.

Recently, a new group of lipophilic hydroxytyrosyl derivatives, hydroxytyrosyl ethers (Madrona et al., 2009), with linear alkyl side chains of variable length, have been synthesised by our group. These new derivatives of HTy (**1a**) showed comparable or even higher antioxidant capacity than free HTy (Pereira-Caro et al., 2009) and higher bioavailability at both intestinal (Pereira-Caro et al., 2010) and hepatic levels (Pereira-Caro, Bravo, Madrona, Espartero, & Mateos, 2010). Taking into account the enhanced antioxidant properties of alkyl hydroxytyrosyl ethers in comparison with their precursor HTy (**1a**) and the promising biological activity described for Ty (**1b**), the synthesis of alkyl tyrosyl derivatives could be an interesting alternative to meet the food industry needs. Moreover, homovanillyl ethers have a great potential as phytochemicals, considering that their precursor homovanillic alcohol (HMV, **1c**) presents biological activity against oxidative kidney cell injury (Incani et al., 2010), and is one of the identified metabolites after human virgin olive oil intake (Caruso, Visioli, Patelli, Galli, & Galli, 2001; Miró-Casas et al., 2003; Visioli et al., 2000; Vissers, Zock, Roodenburg, Leenen, & Katan, 2002). Bearing this in mind, in the present work the synthesis of tyrosyl (**4h–n**) and homovanillyl ethers (**4o–u**) and their oxidative stability in lipid matrices spiked with these new synthetic compounds (**4h–u**) by Rancimat test, was carried out. Moreover, reducing power by FRAP assay and radical scavenging activity by ABTS and ORAC assays of tyrosyl and homovanilly ethers (**4h–u**) were also assessed.

2. Materials and methods

2.1. Materials

All solvents and reagents were of analytical grade unless otherwise stated. -Tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were from Aldrich (Madrid, Spain). Benzyl bromide was from Fluka (Steinheim, Germany). Sodium hydroxide, sodium hydrogen phosphate and potassium dihydrogen phosphate were from Panreac (Madrid, Spain). Tyrosol (**1b**), homovanillic alcohol (**1c**) and the alkyl iodides (methyl, ethyl, *n*-propyl, *n*-butyl, *n*-hexyl, *n*-octyl and *n*-dodecyl iodides) were from Aldrich (Steinheim, Germany). Fluorescein, methylated β-cyclodextrin (RMCD), 2, 2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2′-azino bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (98%), 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) were from Sigma (Madrid, Spain).

NMR spectra were recorded on a Bruker Avance 500 spectrophotometer operating at 500.13 MHz (^{1}H) and 125.75 MHz (^{13}C). Samples were dissolved (0.1 mmol/ml) in hexadeuterated methylsulfoxide (DMSO-d_6), and spectra were recorded at 303 K. Chemical shifts are given in ppm with the residual solvent signals (2.49 ppm for ^{1}H and 39.5 ppm for ^{13}C) as references. Elemental analyses were made on a Leco CHNS-932 apparatus. High-resolution EI, CI and FAB mass spectra were obtained on a Micromass AUTOSPECQ spectrometer.

2.2. Synthetic procedures

2.2.1. General procedure for alkylation of *2b* and *2c*

A mixture of **2b** (Ajao, Bird, & Chauhan, 1985) or **2c** (Battersby, Le Count, Garratt, & Thrift, 1961) (1 mmol), KOH (335 mg) and the corresponding alkyl iodide (3 mmol) in methylsulfoxide (12 ml) was stirred at room temperature until completion of reaction (thin layer chromatography, TLC). 3 M HCl (25 ml) was added and the mixture was extracted with $CHCl_3$ (3 × 25 ml). The organic phase was further washed with 2% $NaHSO_3$ (25 ml) and water (25 ml), dried over anhydrous Na_2SO_4, filtered and evaporated. The crude products were purified by flash column chromatography over silica gel yielding the desired products **3h–u**.

1-(Benzyloxy)-4-(2-methoxyethyl)benzene (**3h**): colourless liquid (85% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2*Ph* in pos. 6), 3.46 (t, J = 6.9 Hz, 2H, H_1), 3.21 (s, 3H, $H_{1'}$), 2.71 (t, J = 6.9 Hz, 2H, H_2); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.7 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.5 (C_5), 72.9 (C_1), 69.1 (CH_2Ph in pos. 6), 57.7 ($C_{1'}$), 34.4 (C_2). Elem. Anal. Calcd for $C_{16}H_{18}O_2$: C, 79.31; H, 7.49; found: C, 79.27; H, 7.27; HRMS, 242.1302 (2 ppm).

1-(Benzyloxy)-4-(2-ethoxyethyl)benzene (**3i**): colourless liquid (89% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.39 (q, J = 7.0 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.1 Hz, 2H, H_2), 1.07 (t, J = 7.0 Hz, 3H, $H_{2'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.5 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.9 (C_5), 71.1 (C_1), 69.1 (CH_2Ph in pos. 6), 65.1 ($C_{1'}$), 34.7(C_2), 15.0 ($C_{2'}$). Elem. Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86; found: C, 79.46; H, 7.95; HRMS, 256.1465 (0.7 ppm).

1-(Benzyloxy)-4-(2-propoxyethyl)benzene (**3j**): colourless liquid (89% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.1 Hz, 2H, H_1), 3.31 (t, J = 6.6 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.1 Hz, 2H, H_2), 1.47 (m, 2H, $H_{2'}$) 0.83 (t, J = 7.4 Hz, 3H, $H_{3'}$); ^{13}C-NMR δ ppm 156.6 (C_6), 137.2 (C_{ipso}, *Bn* group), 131.1 (C_3), 129.6 (C_4), 128.3–127.5 (C_2–C_4, *Bn* group), 114.4 (C_5), 71.5 ($C_{1'}$), 71.0 (C_1), 69.1 (CH_2Ph in pos. 6), 34.6 (C_2), 22.3 ($C_{2'}$), 10.4 ($C_{3'}$). Elem. Anal. Calcd for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20; found: C, 80.17; H, 8.18; HRMS, 270.1630 (3.8 ppm).

1-(Benzyloxy)-4-(2-butoxyethyl)benzene (**3k**): colourless liquid (87% yield); ^{1}H-NMR δ ppm 7.37 (m, 5H, *Ph*), 7.12 (d, J = 8.7 Hz, 2H, H_5), 6.90 (d, J = 8.7 Hz, 2H, H_4), 5.05 (s, 2H, CH_2Ph in pos. 6), 3.49 (t, J = 7.0 Hz, 2H, H_1), 3.34 (t, J = 6.5 Hz, 2H, $H_{1'}$), 2.71 (t, J = 7.0 Hz, 2H, H_2), 1.44 (m, 2H, $H_{2'}$) 1.27 (m, 2H, $H_{3'}$), 0.84