An ecofriendly lipophilization of hydroxytyrosol present in industrial *Olea europaea* L. fractions obtained from olive oil by-products

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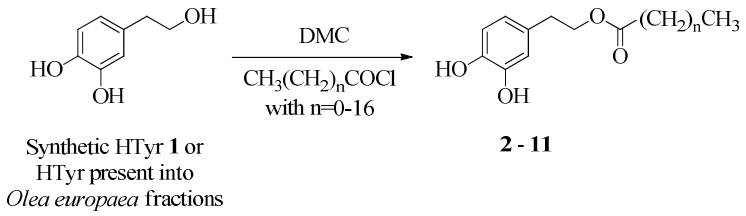
Abstract. Hydroxytyrosol [2-(3,4-dihydroxyphenyl)ethanol, HTyr] is a simple phenol found in olive tree tissues (*Olea europaea* L.). Despite its well-known beneficial properties for human health, several applications in hydrophobic/lipid media are limited because it is strongly hydrophilic, and this property makes difficult its incorporation into fats and oils. In this communication, we report a simple and ecofriendly procedure to derivatize synthetic HTyr and HTyr present into *Olea europaea* L. fractions deriving from olive oil by-products into the corresponding lipophilic esters, using acyl chlorides in dimethyl carbonate as solvent without any catalyst.

Introduction. Hydroxytyrosol (HTyr) is present in leaves and fruits of the olive tree and in the extra virgin olive oil. It exhibits a broad spectrum of biological properties, such as antioxidant, anticancer, antimicrobial, anti-inflammatory and antiviral activities [1, 2]. Among them, the antioxidant activity, e.g. the ability to scavenger different families of Reactive Oxygen Species (ROS), is the most remarkable effect, mainly attributed to the *ortho*-dihydroxyphenyl moiety. Due to the high solubility in water, during the olive fruits processing, abundant amounts of HTyr end up into olive oil mill wastewaters, and, then, such by-products become precious source of this high value compound. On the other hand, the poor solubility of HTyr in lipid media is a limiting factor for its incorporation into oils, fats and, then, for cosmetic formulations. Because of these considerations, the search for ecofriendly processes to obtain lipophilic HTyr derivatives is of great interest for industrial applications.

Materials and Methods. All reagents were of the highest grade available (Sigma Aldrich, Milan, Italy). HTyr was synthetized in our laboratories by *ortho*-selective oxygenation of tyrosol **1** with 2-iodoxybenzoic acid (IBX) [3]. *Olea europaea* L. fractions were obtained by olive oil by-products (biological olive oil mill waste) using an innovative process of separation, based on membrane technologies defined as BAT (Best Available Technology) and recognized from EPA (Environmental Protection Agency) [4]. Chromatographic purifications were performed on columns packed with Merck silica gel 60, 230-400 mesh. Thin layer chromatography (TLC) was carried out using Merck platen Kieselgel 60 F254. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz. Mass spectra were recorded with a GC Shimadzu GC-17A equipped with an electron beam of 70 eV, an SPB column (25 m-0.30 mm and 0.25 mm film thickness) and a mass-selective detector QP 6000. The injector temperature was 280°C. An isothermal temperature profile of 60°C for 5 min, followed by a 10°C/min temperature gradient to 250°C for 10 min was used. Chromatographic grade helium was used as the carrier gas. The HPLC/DAD/MS analysis were performed using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP 1100 MSD API-electrospray (Agilent Technologies) operating in negative ionization mode. A four-step linear solvent gradient starting from 100% H_2O up to 100% CH_3CN was performed with a flow rate of 0.8 mL min^{-1} during a 88 min period. The column was a Lichrosorb C18 250×4.60 mm, 5µm (LichroCART, Merck) operating al 26°C. The eluents were H_2O adjusted to pH 3.2 by HCOOH/CH_3CN. Mass spectrometer operating conditions were: gas temperature 350°C at a flow rate of 10.0 L min^{-1}, nebulizer pressure 30 psi, quadrupole temperature 30°C and capillary voltage 3500 V.

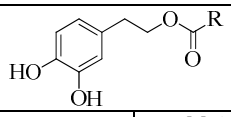
Results and Discussion. Firstly, we optimized the experimental conditions of esterification of HTyr using pure samples in combination with acyl chlorides showing different length (C2-C18, even number) in dimethyl carbonate, an ecofriendly solvent (**Scheme 1**). The best yields of the corresponding esters **2-11** were obtained after 24 h at room temperature (**Table 1**). In order to control the purity and efficiency of the synthetic modifications, all lipophilic derivatives were analyzed by GC-MS, NMR and HPLC/DAD/MS.

Finally, the *Olea waste* fraction, PHENOLEA-OH-TY, containing HTyr (60 mg HTyr/gram of extract) and derivatives (OH-TY glycol and glucoside), tyrosol, secoiridoids and trace amount of caffeic acid derivatives (verbascoside), was employed as substrate and treated with acyl chlorides in dimethyl carbonate under the same experimental conditions. Yields of the esters into derivatized fractions were calculated by HPLC/DAD/MS (**Table 1**).



Scheme 1. Esterification of HTyr **1**.

Table 1. Yields (%) of HTyr esters.



R	Yield (%)[a]	Yield (%)[b]
2: CH_3	59	53
3: $CH_3(CH_2)_2$	54	42
4: $CH_3(CH_2)_4$	60	39
5: $CH_3(CH_2)_6$	63	32
6: $CH_3(CH_2)_8$	55	30
7: $CH_3(CH_2)_{10}$	54	33
8: $CH_3(CH_2)_{12}$	57	10
9: $CH_3(CH_2)_{14}$	58	12
10: $CH_3(CH_2)_{16}$	57	10
11: $CH_3(CH_2)_7CH{=}CH(CH_2)_7$	60	58

[a] Calculated after chromatographic purifications starting from synthetic HTyr.
[b] Calculated by HPLC/DAD/MS and referred to unreacted HTyr present into *Olea europaea* L. fractions.

As reported in **Table 1**, the esterification of HTyr proceeded with satisfactory yields from synthetic HTyr and with lower yields starting from *Olea europea* L. fractions. These results are reasonable in consideration of the presence of other phenolic compounds into natural fractions which could be derivatized under the same experimental conditions. Although some data require a further optimization (e.g reactions with 10-12% yields), these results seem to be interesting and encouraging.
Pure HTyr esters and the corresponding esterified *Olea* fractions will be tested in biomedical applications such as on tumoral cell lines. Further tests will be carried out to investigate their potential applications in crop protection, and as food/feed supplements.
The work is in progress in our laboratories to evaluate the antioxidant, antitumoral and other biological activities of the derivatized extracts and their industrial applicability in pharmaceutical and agronomic field.

References

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