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Enzymatic acylation as an efficient tool for an easy access to specific acyl derivatives of the natural antioxidants verbascoside, teupolioside and echinacoside

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ABSTRACT

The natural antioxidants phenylpropanoids glycosides echinacoside (**1**), verbascoside (**2**) and teupolioside (**3**) were efficiently and regiospecifically monoacylated by means of the enzyme lipase PS. While acylation of teupolioside (**3**) and of echinacoside (**1**) occurred at a sugar primary OH in the "lower" or in the "upper" part of the molecule, respectively, verbascoside (**2**) was acetylated at one of its sugars secondary OHs. At variance to enantioselectivity, which can be rationalized in terms of steric effects due to substituents bulkiness, our new results confirm that enzyme regioselectivity is mainly dictated by the electrostatic interactions of the different OHs of the substrates with the amino acids of the enzyme.

1. Introduction

Phenylpropanoids glycosides (PPGs, also commonly referred to as phenylethanol glycosides) belong to the largest group of secondary metabolites widely distributed in the organs of superior plants, mainly in response to biotic or abiotic stresses such as infections, wounding, UV irradiation, exposure to ozone, pollutants, and other hostile environmental conditions [1].

From the structural point of view they are characterized by the presence of a derivative of cinnamic acid (usually caffeic, ferulic or, more rarely, cinnamic acid itself, almost always in the *trans* form), and of a derivative of phenylethanol (usually hydroxytyrosol) linked to the same molecule of glucopyranose by an ester bond at any free OH and by a glucosidic bond, respectively.

The glucose moiety which is the central core of these phenylpropanoids and acts as a bridge between the two aromatic structures, is commonly decorated by the presence of additional sugars, such as apiose, arabinose, galactose and glucose (always attached with a β-glycosidic bond), or rhamnose and xylose (always with an α-glycosidic bond), thus giving rise to di- and trisaccharide phenylpropanoids, depending on the number of added sugars.

The first PPGs isolated were echinacoside (**1**), obtained by Stoll from the roots of *Echinacea angustifolia* [2] and verbascoside (**2**), found in *Verbascum sinuatum* by an Italian group [3]. This latter compound was subsequently isolated by other researchers [4] and called acteoside. It was not until 1982 that the structure of verbascoside and acteoside were unequivocally found to be identical [5]. Verbascoside has a vast distribution in plant kingdom in the species of the Lamiales order and in particular it is present in the extracts from the fruits (not from the leaves) of the Mediterranean plant *Olea europea* L., a basic constituent of the Mediterranean diet.

Both molecules have become quite popular for their biological and pharmacological effects. In addition to antioxidant activity, many other properties have also been ascertained such as antimicrobial, antiviral and anti-hepatotoxic activities. Echinacoside was shown to have anti-inflammatory and cicatrizing actions. In the case of verbascoside, anti-inflammatory, anticancer and neuroprotective effects have been demonstrated, together with analgesic and anti-hypertensive properties, probably linked to its inhibitory action on the proteine kinase C (PKC) [6], on the aldose reductase and on the formation of 15-hydroxyeicosatetraenoic acid (15-HETE).

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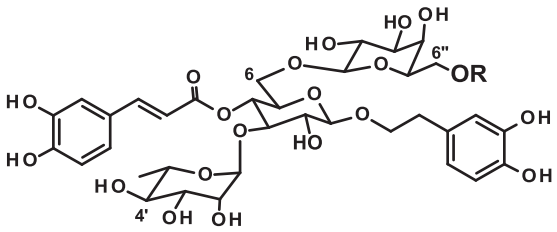
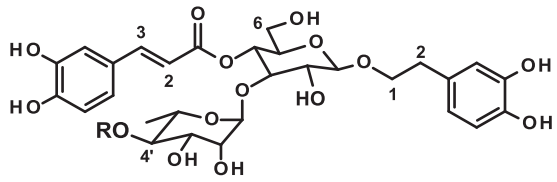
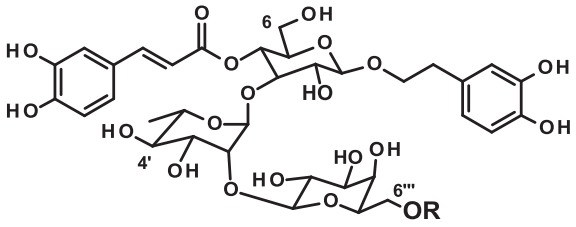


echinacoside 1 , R = H
1a , R = Ac



verbascoside 2 , R = H
2a , R = Ac
2b , R = But

Another PPG of interest is the trisaccharide teupolioside (**3**) isolated from the first time from *Teucrium polium* [7]. It was found as the most abundant metabolite in *Ajuga reptans*, a plant belonging to the family of Labiates and known since the middle ages for anti-haemorrhagic and anti-inflammatory properties and for the peculiar cicatrizing activity on cuts (it is called "Wundkraut" in Germany).



teupolioside 3 , R = H
3a , R = Ac
3b , R = But
3c , R = Laur

Many herbal extracts containing the above-mentioned compounds have been used in traditional medicine [8] and are present on the market as bench-top products and claimed to be used for a wide number of preventions. However it is well known that many problems are associated with the commercial use of these extracts, mainly due to the presence of environmental, chemical and microbiological contaminants deriving from the extraction from plants and, more importantly, to the undefined and sometime troublesome qualitative and quantitative characterization of the active principles present (if any).

PPGs are usually isolated as complex mixtures and single pure products can be obtained in small amount after tedious and complex chromatographic separations. Moreover isolation is hampered by instability to oxygen and to peroxidases contained in the plant tissues. To put on the marked preparations containing active principles devoid of contaminants and of defined activity, an Italian biotechnological industry has successfully developed a cell culture production of PPGs. Specifically, verbascoside and echinacoside are produced from cell cultures of *Syringa vulgaris* and *A. reptans* [9], and teuopolioside from *A. reptans* [10]. This productively simple and environmentally friendly bio-technological methodology allows a fast and unlimited production of these compounds and of their simple derivatives (i.e., the polyacylated ones), and their use as additives in dermo-cosmetic and pharmaceutical topical formulations [11].

Previously we have developed an attractive methodology for the regiospecific acylation of polyhydroxylated substrates based on the use of activated esters in presence of suitable enzymes in polar organic solvent(s). In this way selected esters of flavonoid glycosides [12], colchicoside and thiocolchicoside [13], ginsenosides [14], ecdysteroids [15], stevioside [16], digitonin [17], asiaticoside [18] and others have been successfully obtained. This research was carried out not only with the aim to mimic nature but because it might provide opportunities to develop compounds with potential biological activities. It was suggested by the observation that many biologically active polyhydroxylated compounds are present in nature esterified at specific OHs with simple or complex acids, with the result to change the lipophilic or hydrophilic character and eventually the bioavailability and pharmacological properties with respect to the parent compound.

The biological activities of verbascoside, echinacoside and teupolioside as well as their achieved availability suggested us to apply our experience in biocatalysis in order to obtain specific esters of these compounds that are not available by chemical manipulation. In fact and as an example, chemical acetylation of verbascoside under standard conditions and in the presence of an excess of acylating agent resulted in the esterification of all the OHs, giving rise to the formation of a nonaacetate ester in quantitative yield [11,19]. Moreover the use of stoichiometric amount of acylating agent furnished a complex mixture of mono- di- and polyacylated products.

Here we wish to report our results on the regioselective enzymatic acylation of these three PPGs. Biological activities of these esters in comparison with that of the starting material will be reported elsewhere.