The invention relates to the preparation of phenolics derivatives by enzymatic condensation of phenolics selected among pyrocatechol or its derivatives with the glucose moiety of sucrose. The production of said phenolics derivatives is achieved with a glucosyltransferase (EC 2.4.1.5). These O-α-glucosides of selected phenolics are new, have a solubility in water higher than that of their parent polyphenol and have useful applications in cosmetic and pharmaceutical compositions, such as antioxidative, antiviral, antibacterial, immune-stimulating, antiallergic, antihypertensive, antischismic, antiarhythmic, antithrombotic, hypcholesterolemic, antilipidoxidant, hepatoprotective, anti-inflammatory, anticarcinogenic, antimutagenic, antineoplastic, anti-thrombotic and vasodilatory formulations, or in any other field of application.
FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6
Caffeic acid glucoside

4-methylcoumarin glucoside

Chlorogenic acid glucoside

Rosmarinic acid glucoside

Esculetin glucoside

Caffeic acid phenethyl ester glucoside

FIGURE 7
FIGURE 7 (continued)
Verbascoside O-α-D-glucoside
Homoprotocatechuic acid glucoside

Protocatechuic acid ethyl ester glucoside

Protocatechuic acid glucoside

Propyl gallate glucoside

Gallic acid glucoside

3,4 dihydrocaffeic acid glucoside

FIGURE 8
Hamamelitannin glucoside

FIGURE 8 (continued)
Catechin glucoside

Epicatechin glucoside

Gallocatechin glucoside

Epigallocatechin glucoside

Epicatechin gallate glucoside

Epigallocatechin gallate glucoside

FIGURE 9
FIGURE 10 (continued)
3,4-dihydroxybenzaldehyde glucoside

Maclurine glucoside

Pyrocatechol glucoside

Nordihydroguaiaretic acid glucoside

3,4 dihydroxybenzophenone glucoside

Anthrarobin glucoside

3-hydroxydaidzein glucoside

Hydroxytyrosol glucoside

FIGURE 11
FIGURE 11 (continued)
Eriodictyol chalcone glucoside

Maritimein glucoside

3,4-dihydroxyacetophenone glucoside

Salsolinol glucoside

FIGURE 11 (continued)
WATER SOLUBLE AND ACTIVABLE PHENOLICS DERIVATIVES WITH DERMOCOSMETIC AND THERAPEUTIC APPLICATIONS AND PROCESS FOR PREPARING SAID DERIVATIVES

FIELD OF THE INVENTION

The present invention relates to the preparation of phenolics derivatives, pharmacological and cosmetic compositions comprising such phenolics derivatives, and their use for the beauty of the skin and for treating diseases.

BACKGROUND OF THE INVENTION

Phenolic Compounds and their Properties

Phenolic compounds (also called phenolics), or polyphenols, constitute one of the most numerous and widely-distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known. Polyphenols are products of the secondary metabolism of plants. The expression “phenolic compounds” embraces a considerable range of substances that possess an aromatic ring bearing one or more hydroxyl substituents. Most of the major classes of plant polyphenol are listed in Table 1, according to the number of carbon atoms of the basic skeleton. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins (HARBONE J B (1980) Plant phenolics. In: BELL EA, CHARWOOD BV (eds) Encyclopedia of Plant Physiology, volume 8 Secondary Plant Products, Springer-Verlag, Berlin Heidelberg N.Y. Pp: 329-395).

The three important groups for humans are phenolic acids (C6-C1), C6-C2 and C6-C3), flavonoids (C6-C3-C6) and high-molecular weight polyphenols (more than 30 carbon atoms). Indeed, the phenolics, particularly polyphenols, exhibit a wide variety of beneficial biological activities in mammals, including antiviral, antibacterial, immune-stimulating, antiallergic, antihypertensive, antischmerz, anti-inflammatory, antithrombotic, hypcholesterolemic, antilipperoxidant, hepatoprotective, anti-inflammatory, antineoplastic, anti-thrombotic and vasodilatory actions. They are powerful antioxidants in vitro.

TABLE I

<table>
<thead>
<tr>
<th>NUMBER OF CARBON BASIC ATOMS</th>
<th>SKELETON</th>
<th>CLASS</th>
<th>EXAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>C6</td>
<td>Simple phenols</td>
<td>Catechol, hydroquinone</td>
</tr>
<tr>
<td>7</td>
<td>C6-C1</td>
<td>Phenolic acids</td>
<td>Gallic, salicylic</td>
</tr>
<tr>
<td>8</td>
<td>C6-C2</td>
<td>Tyrosine derivatives</td>
<td>Methoxybenzaldehyde</td>
</tr>
<tr>
<td>9</td>
<td>C6-C3</td>
<td>Hydroxybenzamides</td>
<td>Tyrosol</td>
</tr>
<tr>
<td>10</td>
<td>C6-C4</td>
<td>Naphthoquinones</td>
<td>Juglone, plumbagin</td>
</tr>
<tr>
<td>13</td>
<td>C6-C1-C6</td>
<td>Xanthones</td>
<td>Mangiferin</td>
</tr>
<tr>
<td>14</td>
<td>C6-C2-C6</td>
<td>Stilbenes</td>
<td>Resveratrol</td>
</tr>
<tr>
<td>15</td>
<td>C6-C3-C6</td>
<td>Flavanoids</td>
<td>Quercetin, cyanidin</td>
</tr>
<tr>
<td>18</td>
<td>C6-C3-C6</td>
<td>Lignans</td>
<td>Genistein</td>
</tr>
<tr>
<td>30</td>
<td>C6-C3-C6</td>
<td>Flavonoids</td>
<td>Quercetin, cyanidin</td>
</tr>
</tbody>
</table>

Among the phenolic acids, the most important constitute carbon frameworks are the hydroxybenzoic (C6-C1) and hydroxycinnamic (C6-C3) structures. The hydroxybenzoic acid fraction of edible plants is generally very low, with the exception of certain red fruits, black radishes, and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight. Hydroxybenzoic acids are components of complex structures such as hydrolyzable tannins (gallocatein in mangoes and elagitannins in red fruits such as strawberries, raspberries and blackberries). The hydroxycinnamic acids are more common than are the hydroxybenzoic acids and consist chiefly of p-coumaric, caffeic, ferulic and sinapic acids. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilization or fermentation. The bound forms are glycosylated derivatives or esters of quinic acid, shikimic acid and tartaric acid. Caffeic acid and quinic acid combine to form chlorogenic acid, which is found in many types of fruit and in high concentration in coffee. Caffeic acid, both free and esterified, is generally the most abundant phenolic acid and represents between 75% and 100% of the total hydroxycinnamic acid of most fruit (MANACH C, SCALBERT A, MORAND C, REMESY C, JIMENEZ L (2004) Polyphenols: food sources and bioavailability. Am J Clin Nutr 79: 727-747).

The flavonoids consist of a large group of low-molecular weight polyphenolic substances, benzene-2-pyrene derivatives that are diverse in chemical structure; they represent the most common and widely distributed group of plant phenolics. The flavonoids common structure is that of diphe-
nylpropanes (C6-C3-C6); it consists of two aromatic rings (cycles A and B) linked through three carbons that usually form an oxygenated heterocycle (cycle C). FIG. 1 shows the basic structure and the system used for the carbon numbering of the flavonoid nucleus. Structural variations within the rings subdivide the flavonoids into several families: flavonols, flavones, flavanols, isoflavones, anthocyanidins and others. These flavonoids often occur as glycosides, glycosylation rendering the molecule more water-soluble and less reactive toward free radicals. The sugar most commonly involved in glycoside formation is glucose, although galactose, rhamnose, xylose and arabinose also occur, as well as disaccharides such as rutinose. The flavonoid variants are all related by a common biosynthetic pathway, incorporating precursors from both the shikimate and the acetate-malonate pathways (CROZIER A, BURNS J, AZIZ A A, STEWART A J, RAMASZ H S, JENKINS G I, EDWARDS C A, LEAN M J E J (2000) Antioxidant flavonoids from fruits, vegetables and beverages: measurements and bioavailability. Biol Res 33: 79-88). Further modifications occur at various stages, resulting in an alteration in the extent of hydroxylation, methylation, isoprenylation, dimerization and glycosylation (producing O- or C-glycosides). Phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. Indeed, excess levels of metal cations of iron, zinc and copper in the human body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA; by forming complexes with these reactive metal ions, they can reduce their absorption and reactivity. It has to be underlined that though most flavonoids chelate Fe, there are large differences in the chelating activity. In particular, the dihydroflavonol taxifolin chelates more efficiently Fe²⁺ than the corresponding flavonol quercetin (VAN ACKER S A B E, VAN DEN BERG D J, TROMP M J L, GRIFFIOEN D H G, VAN BENNEKOM, VAN DER VIJGH W J E, BAST A (1996) Structural aspects of antioxidant activity of flavonoids. Free Radic Biol Med 20: 331-342).

Flavonoids have ideal structural chemistry for free radical-scavenging activities (several studies have shown the flavonoids to act as scavengers of superoxide anions, singlet oxygen, hydroxyl radicals and lipid peroxyl radicals by rapid donation of a hydrogen atom). One important finding from the studies of the relationship between the structural characteristics of flavonoids and their antiradical activity is that a catechol moiety (3',4'-dihydroxyphenyl) on ring B is required for good scavenging activity. Recently, this statement was confirmed with nevertheless a modulation: in a study about the relationship between the structural characteristics of 29 flavonoids and their antiradical activity, it was indeed observed that the catechol structure in the B ring is not always a conditio sine qua non in achieving high free radical scavenging activity and that highly active flavonoids possess a 3',4'-dihydroxy B ring and/or a 3-OH group (AMIC D, DAVIDOVIC-AMIC D, BESLIO D, TRINAJSTIC N (2003) Structure-radical scavenging activity relationships of flavonoids. Croatica Chem Acta 76: 55-61). Flavonoids have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis (RICE-EVANS C A, MILLER N J, PAGANGA G (1997) Antioxidant properties of phenolic compounds. Trends in Plant Science 2: 152-159). There are also reports of flavonoids inhibiting the activity of enzymes such as oxygenases.

It must be underlined that the hydrophobicity of polyphenols is intermediate between that of vitamin C (highly hydrophilic) and that of vitamin E (highly hydrophobic); polyphenols are thus expected to act at water-lipid interfaces and may be involved in oxidation regeneration pathways with vitamin C and E.

Phenolics Derivatives and their Preparation

Due to their low aqueous solubility and/or high sensitivity toward oxidation, the use of phenolics in pharmaceutical or cosmetic preparations requires adapted and specific formulations. Since these formulations must also satisfy the constraints associated with their final usage, the compromise between acceptability, concentration and stability is often difficult to reach.

More water soluble and/or oxidation resistant forms of phenolics such as the glycosides are not always available in nature and may demand, when they exist, complex procedures of extraction and purification from the plant material. Both chemical and biochemical (enzymatic) approach have been attempted to increase water solubility and/or stability. As phenolic compounds have several free hydroxyl groups, attempts for chemical modifications of phenolic compounds lead to unspecific reactions, generating a panel of different molecules. Further steps of purification are then required to recover the desired product(s).

As far as the biochemical approach is concerned, three ways have been investigated to date to obtain phenolics glycosides and basically flavonoids glycosides.

The first way relies on glycosyltransferases able to transfer the sugar moiety of a sugar nucleotide to an acceptor (in the case of UDP-glucose:glucosyltransferases (UGT)), glucose is transferred from uridine 5'-diphosphoglucose. These enzymes, which contribute in the synthesis of secondary metabolism in plants, have broad acceptor substrate specificities (LIM E K, HIGGINS G S, BOWLES D J (2003) Regioselectivity of glucosylation of caffeic acid by UDP-glucose glucosyltransferase is maintained in planta. Biochem J 373: 987-92; LIM E K, ASHFORD D A, HOU B, JACKSON R G, BOWLES D J (2004) Arabidopsis glycosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glucosides. Biotechnol. Bioeng. 87(5): 623-31). Nevertheless, this approach is impaired by the very high cost of the sugar nucleotides and the regeneration of the sugar nucleotide substrate, which is a way to decrease the substrate cost, is difficult to master at large scale.

The second way relies on saccharide—transferring enzymes able to transfer glucose from an α-glucosyl saccharide. Said enzymes are selected from the hydrolases α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1), and from the transferase cyclodextrin-glucosyltransferase (EC 2.4.1.19). Their substrates are amylose, dextrins, cyclodextrins, maltotriosesaccharides and partial starch hydrolysates, all of them containing mainly or exclusively glucosyl residues linked to each other through an α 1→3 or α 1→α bond. According to this approach, U.S. Pat. No. 5,565,435 states that α-glucosyl quercetin is obtained. It has to be underlined that the starch degrading enzymes link the glucosyl residue to the flavonoid through an α-osidic bond whereas the UDP-glucose:glucosyltransferase investigated by LIM et al. links the glucosyl residue to the flavonoid through a β-osidic bond. It has also to be underlined that in the conditions described in U.S. Pat. No. 5,565,435, the quercetin molecule could be solubilized by adjusting the pH at 8.5 and by maintaining the reaction medium at 60°C. The solubilisation of phenolics in
alkaline media is due to the formation of phenolates; in these pH and temperature conditions, the stability of the substrate was achieved by operating under anaerobic conditions. It thus appears that this mode of preparation is highly difficult to control and manage and that a simple mode of preparation should be valuable.

[0016] The third way involves glucosyltransferases using sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) as glucosyl donor and producing glucans with the release of fructose. Several attempts have been achieved with this class of enzymes to try to get phenolics glucosides. First, the glucosyltransferase from Streptococcus sobrinus (referred to by the authors as strain 6715, serotype g) was proven to catalyze the synthesis of 4′-O-α-D-glucopyranosyl-β-D-catechin in a strictly aqueous medium (catechin at 1 g/L in 100 mM phosphate buffer pH 6.0 containing 2% sucrose) (NAKAIHARA K, KONTANI M, ONO H, KOMADA T, TANAKA T, OOSHIMA T, HAMADA S (1995) Glucosyltransferase from Streptococcus sobrinus catalyzes glycosylation of catechin. Appl. Environ. Microbiol. 61(7): 2768-70). A similar enzyme, the glucosyltransferase-D from Streptococcus mutans GS-5, was proven to be less regioselective, as it catalyzes not only the synthesis of 4′-O-α-D-glucopyranosyl-β-D-catechin but also the synthesis of 4′-O-α-D-glucopyranosyl-β-D-catechin and of the diglycosylated derivative 4′,7-O-α,α-D-diglucopyranosyl-β-D-catechin (MEULENBEIL G H, ZUHLOF H, VAN VELDE HUIZEN A, VAN DEN HEUVEL R H H, HARTMANS S (1999) Enhanced (+)-catechin transglucosylating activity of Streptococcus mutans GS-5 glucosyltransferase-D due to fructose removal. Appl Environ Microbiol 65(9): 4141-7). Though several investigations regarding the acceptor specificity of Streptococcus mutans GS-5 glucosyltransferase lead the authors to infer that aromatic acceptors appear to require two adjacent aromatic hydroxyl groups (MEULENBEIL G H, HARTMANS S (2000) Transglucosylation by Streptococcus mutans GS-5 glucosyltransferase-D: acceptor specificity and engineering action conditions. Biotechnol Bioeng 70(4): 363-9), this statement was counteracted by the identification of glucosylation at position 7 in catechin (MEULENBEIL et al., 1999) and by the synthesis of non-pyrolicetol derivatives. Indeed, pinosylvin and resveratrol, respectively, 3,5-di-hydroxysterilbene and 3,4′,5-trihydroxysterilbene, were glucosylated to a crude glucosyltransferase preparation produced by Streptococcus mutans to form respectively 3-O-α-D-glucopyranosyl-(E)-pinosylvin and 3-O-α-D-glucopyranosyl-(E)-resveratrol (SHIM H, HONG W, AIN Y (2003) Enzymatic preparation of phenolic glucosides by Streptococcus mutans. Bull Korean Chem Soc 24(11): 1680-2). Very recently, it was claimed that the flavonol quercetin and myricetin and the flavone luteolin could be glycosylated by special glucansucrases, namely the Leuconostoc mesenteroides NRRL B-512F glucosyltransferase (sucrose:1,6-α-D-glucopyranosyl-6-α-D-glucosyltransferase, EC 2.4.1.15) and the Leuconostoc mesenteroides NRRL B-23192 alternasucrase (sucrose:1,6 (1,3)-α-D-glucosyltransferase, EC 2.4.1.140) (BERTRAND A, MOREL S, LEFOULON F, ROLLAND Y, MONSAN P, REMAUD-SIMEON M (2006) Leuconostoc mesenteroides glucosyltransferase synthesis of flavonoid glucosides by acceptor reactions in aqueous-organic solvents. Carbohydr Res 341: 855-63). Conventionally, in the presence of sucrose, the former produces a glucan (dextran) in which 95% of the glucosidic bonds are α-(1→6) (skeleton of the polysaccharide) and 5% α-(1→5) (branching points), and the later a glucan (alternan) in which the glucosidic bonds are alternatively α-(1→6) and α-(1→3). The obtained flavonoid derivatives were: luteolin-3′-O-α-D-glucopyranoside, luteolin-4′-O-α-D-glucopyranoside, quercetin-3′-O-α-D-glucopyranoside, quercetin-4′-O-α-D-glucopyranoside, quercetin-3′-4′-O-α-D-diglucopyranoside, myricetin-3′-O-α-D-glucopyranoside and myricetin-4′-O-α-D-glucopyranoside. This work demonstrates that yields of glycosides derivatives synthesis not only rely on the enzyme itself (the synthesis of luteolin-O-glycosides dropped down from 44% to 8% between dextranusease and alternasucrase), but also on slight chemical differences between two acceptors (no conversion was observed with the dextranusease on diosmetin and diosmin).

[0017] From the above significant (though not exhaustive) state of the art regarding the experimented ways to obtain glucosylated derivatives of polyphenols in general (and flavonoids in particular) in order to overcome the main conventional drawbacks of flavonoids (poor water solubility at physiological conditions, in particular at pH ranging from 5 to 7 and 30°C and high sensitivity to autoxidation in these biological conditions), it clearly appears that no precise guidelines can be deduced to set up the enzymatic production of a specific phenolics glycoside. On the contrary, it shows that there is no way for a man of the art to predict which flavonoid can be glucosylated with which enzyme and in which conditions to obtain high glycoside concentrations (see summary in Table 2). Indeed, though attempts have been made in order to establish a relationship between the phenolic structures and the possibility of their use as glucosyl acceptor by glucosyltransferases, it still appears that the obtention of glucosylated phenolics strongly depends on the nature of the phenolic substance and on the enzyme used for the condensation reaction. This is particularly true with glucosyltransferases synthesizing conventionally α-D-glucans from sucrose (EC 2.4.1.5) for which only a very few number of polyphenolic structures have been successfully reported. Furthermore, in the case of the main glucosyltransferases studied, namely S. mutans GS-5 glucosyltransferase D and L. mesenteroides NRRL B-512F dextranusease, it has to be mentioned that the former synthesizes a water-soluble α-glucan in a primer-stimulated and dependent manner (HAMADA N, KURAMITSU H K (1989) Isolation and characterization of the Streptococcus mutans gfrD gene, coding for primer-dependant soluble glucan synthesis. Infect Immun 56: 1999-2005) whereas the later does not (ROBYT J F, WALSETH T F (1978) The mechanism of acceptor reactions of Leuconostoc mesenteroides NRRL B 512F. Carbohydr Res 61: 433-45). These glucosyltransferases have distinct mechanism of action and consequently molecules that are acceptor for an enzyme are not necessarily acceptor for the other; in other words, as shown in the previously cited works, there is no justification to consider that the substances that act as glucosyl acceptor in the case of S. mutans GS-5 glucosyltransferase D act also as glucosyl acceptor in the case of L. mesenteroides NRRL B-512F dextranusease and vice versa.

[0018] All the more, prior art information shows that despite the interest and abundance of phenolics, few phenolics glycosides have been obtained by enzymatic reactions.
TABLE 2

<table>
<thead>
<tr>
<th>POLYPHENOL</th>
<th>ENZYME ORIGIN</th>
<th>PRODUCT(S) AND REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid (OH in 3 and 4)</td>
<td>Arabidopsis thaliana</td>
<td>Caffeoyl-3-O-β-glucoside - LIM et al, 2003</td>
</tr>
<tr>
<td>o- and m-coumaric acids (OH in 2 and 3, respectively)</td>
<td>Arabidopsis thaliana</td>
<td>2-O- and 3-O-β-glucosides of o- and m-coumaric acids - LIM et al, 2003</td>
</tr>
<tr>
<td>Isoflavone (OH in 3; OCH3 in 4)</td>
<td>Arabidopsis thaliana</td>
<td>3'O-β-glucoside - LIM et al, 2003</td>
</tr>
<tr>
<td>p-coumaric acid (OH in 4), ferulic acid (OH in 4 and OCH3 in 3) and sinapic acid (OH in 4 and OCH3 in 3 and 5)</td>
<td>Arabidopsis thaliana</td>
<td>No glucoside - LIM et al, 2003</td>
</tr>
<tr>
<td>Quercetin (flavonol; OH in 3, 5, 7, 3' and 4')</td>
<td>Arabidopsis thaliana</td>
<td>3-O-, 7-O-, 3'-O-, 4'-O-monoglucosides and 3,7-di-O and 7,3'-di-O-glicosides LIM et al, 2003; LIM et al, 2004</td>
</tr>
<tr>
<td>Caffeoyl-3-O-B-glucoside - LIM et al, 2003</td>
<td>2-O- and 3-O-B-glucosides of o- and m-coumaric acids - LIM et al, 2003</td>
<td></td>
</tr>
</tbody>
</table>

Enzymes and substrates: Starch degrading enzymes (α-glucosidase, cyclodextrin glucoamylase-transferease or CGTase, α-amylase) and starch and/or starch hydrolysatates

<table>
<thead>
<tr>
<th>POLYPHENOL</th>
<th>ENZYME ORIGIN</th>
<th>PRODUCT(S) AND REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin (flavonol; OH in 5, 7, 3' and 4')</td>
<td>α-glucosidase: pig liver, backswheat seed, Mucor, Penicillium, Saccharomyces CGTase: Bacillus, Klebsiella α-amylase: Aspergillus</td>
<td>α-glucosyl quercetin (U.S. Pat. No. 5,565,435) (OH glucosylated not mentioned)</td>
</tr>
<tr>
<td>Catechin (flavanoid; OH in 3, 5, 7', 3' and 4')</td>
<td>Streptococcus sobrini</td>
<td>4'-O-α-D-glucopyranosyl(+) catechin (NARABARA et al, 1995)</td>
</tr>
<tr>
<td>Renveratrol (OH in 3, 5, 4') and pinosylvin (OH in 3, 5)</td>
<td>Streptococcus mutans</td>
<td>3-O-α-D-glucopyranosyl(+) pinosylvin and 3-O-α-D-glucopyranosyl(+)renveratrol (SITTH et al, 2003)</td>
</tr>
<tr>
<td>Catechin (flavanoid; OH in 3, 5, 7', 3' and 4')</td>
<td>Streptococcus mutans</td>
<td>4'-O-α-D-glucopyranosyl(+) catechin, 7-O-α-D-glucopyranosyl(+)catechin and 4',7-O-α-D-diglucopyranosyl(+)catechin (MEULENBEIJD et al, 1999)</td>
</tr>
<tr>
<td>Catechol (OH in 1 and 2), 3-methoxyacetol (OCH3 in 3), 3-methyletylacetol (CH3 in 3), 4-methyletylacetol (CH3 in 4)</td>
<td>Streptococcus mutans</td>
<td>Glucosides (MEULENBEIJD and HARTMANS, 2000)</td>
</tr>
<tr>
<td>Phenol, 3-hydroxyphenol, benzylicalcohol, 2-hydroxybenzyl alcohol, 2-methoxybenzyl alcohol, 1-phenyl-1,2-ethanediol</td>
<td>Streptococcus mutans</td>
<td>Glucosides (MEULENBEIJD and HARTMANS, 2000)</td>
</tr>
<tr>
<td>Quercetin (flavonoid; OH in 3, 5, 7', 3' and 4'), luteolin (flavone; OH in 5, 7, 3'and 4'), myricetin (flavonoid; OH in 3, 5, 7, 3', 4' and 5')</td>
<td>L. mesenteroides NRRL B-512F</td>
<td>L. mesenteroides NRRL B-512F (BERTRAND et al, 2006)</td>
</tr>
<tr>
<td>Diosmetin (flavonoid; OH in 5 and 3', OCH3 in 4')</td>
<td>L. mesenteroides NRRL B-23192</td>
<td>L. mesenteroides NRRL B-23192 (BERTRAND et al, 2006)</td>
</tr>
</tbody>
</table>

[0019] Another key point to consider in the enzymatic synthesis of phenolics glycosides is the possibility to create phenolics derivatives that enable recovering of the initial phenolics by a hydrolysis reaction in smooth conditions.

[0020] Indeed, for a given polyphenol, the advantageous properties that are presently known correspond to a specific structure and it has thus to be demonstrated that the valuable derivativer with increased water solubility and stability properties can be converted into the saccharide part in one hand and the aglycone part in the other hand. One example of decrease of antioxidant activity due to glycosylation is given by MISHRA et al (MISHRA B, PRIYADARSINl K I, KUMAR M S, UNNIKRISHNAN M K, MOHAN H (2003) Effect of O-glycosylation on the antioxidant activity and free radical
reactions of a plant flavonoid, chrysoeriol. Bioorg Med Chem 11: 2677-85). Chrysoeriol and its glycoside (chrysoeriol-6-O<sub>a</sub>-acetyl-4'-β-D-glucoside) are two flavonoids extracted from the tropical plant Coroponid didymus; chrysoeriol shows better protecting effect than the glycoside when tested for their ability to inhibit lipid peroxidation induced by gamma-radiation, Fe (III) and Fe (II). To date, this reversibility is only known for the α-glucosyl queretin obtained with starch degrading enzymes in vitro (U.S. Pat. No. 5,565,435).

So, if the functionalization of phenolics as glycoside derivatives is a way (i) to facilitate their formulation in cosmetic, pharmaceutical or any other man made preparation due to a higher water solubility than that of the aglycone and (ii) to increase the stability of these phenolics in said formulas, both of them being universal properties of the glucosylated forms of polyphenols, these glycoside derivatives must be hydrolyzable in biological conditions.

[0021] There is therefore a need to create:

[0022] new derivatives of valuable phenolic compounds with increased water solubility (in the same physicochemical conditions (pH, salinity, temperature, . . .)) and stability; and/or,

[0023] new derivatives of valuable phenolic compounds that can be readily converted into their precursor, glucose and phenolic substance, in the place where they have to exert their biological activity and not during their storage in a commercial formula; and/or,

[0024] new derivatives of valuable phenolic compounds that can be obtained through a process in which the synthesis and purification steps can be carried out in a reproducible manner and at any scale dependant on the market demand.

[0025] Owing to the fact that the pyrocatechol structure (presence of two vicinal hydroxyl groups) is recognized as particularly important for the scavenging activity of polyphenols, the phenolic compounds that seem particularly efficient are those containing a catechol structure; among the phenolic compounds that are of particular interest, there are the following compounds:

[0026] protocatechuic acid (3,4-dihydroxybenzoic acid, FIG. 2) and its esters derivatives; and/or,

[0027] caffeic acid (3,4-dihydroxyxinnamic acid, FIG. 3) and its esters derivatives, especially rosamarinic acid (3,4-dihydroxyxinnamic acid (R)-1-carboxy-2-(3,4-dihydroxyphenyl)ethyl ester), chlorogenic acid (3-O-(3,4-dihydroxyxinnamoyl)-D-quinic acid), chicosic acid, echinacoside, verbascoside and caffeic acid phenethyl ester, and its reduced form hydrocaffeic acid and its esters derivatives; and/or,

[0028] special structures not closely related to protocatechuc acid or caffeic acid and containing the pyrocatechol ring: 3,4-dihydroxymandelic acid (FIG. 4) and its related substance 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol with a C2-C6 skeleton, and esculetin (6,7-dihydroxycoumarin, FIG. 5) with a C6-C5 skeleton; and/or,

[0029] the flavonones taxifolin (3,5,7,3',4'-pentahydroxyflavone, FIG. 6), fisatin (3,7,3',4'-tetrahydroxyflavone), eriodictol (5,7,3',4'-tetrahydroxyflavone); and/or,

[0030] the flavonols fisetine (3,7,3',4'-tetrahydroxyflavone) and ruthein (3,5,3',4'-tetrahydroxy-7-methoxyflavone); and/or,

[0031] the flavones cirsiliol and 3',4',7-trihydroxyflavone and the isoflavone 3'-hydroxydaidzein.

[0032] More detailed information on these phenolics of interest is included below.

[0033] Protocatechuic acid (also named 3,4-dihydroxybenzoic acid, or (3,4-dihydroxybenzoic acid)) is found in many edible and medicinal plants, though most of the time at concentrations lower than derivatives of cinnamic acid. Though slightly less potent than caffeic acid, protocatechuic acid showed a time-dependent and dose-dependent inhibitory effect on T47D human breast cancer cell growth. It was also demonstrated that protocatechuic acid and caffeic acid interact directly with the aryl hydrocarbon receptor, inhibit nitric oxide synthase and have a pro-apoptotic effect (KAMP A M, ALEXAKI V I, NOTAS G, NIFL A P, NISTIKAKI A, HATZOGLOU A, BAKOGIEORGOU E, KOUMITZOGLOU E, BLEKAS G, BOSKOU D, GRAVANIS A, CASTANAS E (2004) Antiproliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res 6: R63-R74). LIU et al. (LIU K S, TSAO S M, YIN M C (2005) In vitro antibacterial activity of roselle calyx and protocatechuic acid. Phytother Res 19(11): 942-5) demonstrated in vitro an inhibitory effect of protocatechuic acid on the growth of methicillin-resistant Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumannii. The data from inhibition zone and minimum inhibitory concentration (MIC) values showed that protocatechuic acid inhibited effectively the growth of all tested bacterial pathogens. Recent studies indicate that protocatechuic acid could be used as a protective agent against cardiovascular diseases and neoplasms (SZUMILO J. (2005), Postepy Hig Med Dosw (Online) 59: 608-15). The mechanism of its action is mostly associated with antioxidant activity, including inhibition of generation as well as scavenging of free radicals and upregulating enzymes which participate in their neutralization.

[0034] It was also demonstrated that protocatechuic acid is a possible chemopreventive agent for colon carcinogenesis through the suppression of manifestation of intermediate biomarkers induced by azoxymethane (AOM)-induced colon carcinogenesis in rats (TANKA T, KOJIMA T, SUZU M, MORI H. (1993) Chemoprevention of colon carcinogenesis by the natural product of a simple phenolic compound protocatechuic acid: suppressing effects on tumor development and biomarkers expression of colon tumorigenesis. Cancer Res. September 1; 53(17): 3908-13). Protocatechuic acid is therefore also a valuable active phenolic compound, but its bioavailability should be increased through functionalization to obtain more water soluble derivatives.

[0035] Caffeic acid (also named 3,4-Dihydroxyxinnamic acid), a derivative of trans-cinnamic acid (trans-3-phenylacrylic acid) contains a —CH—CH—COOH group which ensures greater H-donating ability and subsequent radical stabilization than the carboxylic group in benzoic acids (RICE-EVANS C A, MILLER N J, PAGANDA G (1996) Structure—antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20(7): 933-56). In addition to its possible beneficial effects on human health (caffeic and 3-methoxycaffeic or ferulic acids react with nitrite in vitro and inhibit nitrosamine formation in vivo; they also inhibit tyrosine nitration mediated by peroxynitrite), caffeic acid recently proved effective in protecting human skin from UVB-induced erythema (SOBODOVA A, PSOTOVA J, WALTEROVA D (2003) Natural phenolics in the preven-

[0042] Glycosylation being recognized to render, in vegetal cells as well as in vitro, polyphenols more water-soluble and less reactive toward free radicals, if glucosides of these phenolics of particular interest exist, then they might represent polyphenol derivatives with increased water solubility and stability, and thus with increased added value.

[0043] It would also be useful to obtain derivatives from these phenolics which can be converted during their final usage in the metabolizable initial phenolics structure. This objective can be achieved by means of the present invention.

SUMMARY OF THE INVENTION

[0044] The present invention concerns a method for producing a phenolic compound O-α-glucoside comprising incubating sucrose and a glucosucrase from Leuconostoc species, preferably from Leuconostoc mesenteroides NRRL B-512F, preferably in buffered water at pH convenient for the enzymatic activity (well known by a skilled man) or in a buffered water at pH convenient for the enzymatic activity-cosolvent mixture, with a phenolic compound having the following formula:

\[
R_1 \quad R_2 \quad O \quad OH
\]

[0045] wherein

[0046] R2 is H or OH; and

[0047] R1 is selected from the group consisting of

\[
R_3 \quad O \quad R_4
\]

[0048] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH; and

[0049] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH;

\[
R_7 \quad R_8
\]

[0050] wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H11O2, and R11 is H, OH or C6H11O2, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H11O2 then R11 is H;

[0051] (CH2)n—COOR or —(CH2)n—CONHR, with n being an integer from 0 to 2;

[0052] (CR12—CH)—COOR or —(CR12—CH)—CONHR, R12 being H or a C1-C3 linear, branched or cyclic alkyl or aralkyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl;

[0053] (CH2)n—OR or —(CH2)n—NHR with n being an integer from 0 to 2;

[0054] —(CH3)n—COR or —(CH=CH)n—COR with n being an integer from 0 to 2;

[0055] H;

\[
R_9 \quad R_10 \quad R_11
\]

[0056] R11 is selected from the group consisting of H, —OH or —OCOR;
and

[0056] a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom;

[0057] wherein R1 is H or a linear, branched or cyclic, aromatic or not, saturated or unsaturated, C1-C10 hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl or an alkynyl, preferably an alkyl or an alkene, which can be substituted by one or several substituents selected from the group consisting of: an (C2-C6) aryl, an (C3-C6) heterocycle, an (C1-C3)alkoxy, an (C2-C5)acyl, an (C1-C3)alkohol, a carboxylic group (—COOH), an (C2-C5)ester, an (C1-C3) amine, an amino group (—NH2), an amide (—CONH2), an (C1-C3)imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an (C1-C3)halogenoalkyl, a thiol (—SH), a (C1-C3)thioalkyl, a (C1-C3)sulfone, a (C1-C3)sulf oxide and a combination thereof.

[0058] Preferably, the buffered water at a pH convenient for the enzymatic activity used either without cosolvent or in a mixture with a cosolvent consists of sodium or potassium acetate buffer at a concentration ranging from 20 to 500 mM in water but any other buffering substance without any negative effect on the enzymatic activity can be used. Preferably, the buffered water at a pH convenient for the enzymatic activity-cosolvent mixture consists in mixture of water, preferably a buffered water as previously described, and dimethyl sulfoxide (DMSO) with a ratio of less than 35% of DMSO (volume/volume), preferably between 15-25%, more preferably about 15%.

[0059] In a first embodiment, R1 of the phenolic compound is

[0060] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH. In particular, the phenolic compound can be selected from the group consisting of the taxifolin, the eriodictyol, the dihydorobinetin and the fustin.

[0061] In a second embodiment R1 of the phenolic compound is

[0062] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH. In particular, the phenolic compound can be selected in the group consisting of the catechin, the epicatechin, the catechin gallate, the epicatechin gallate, the gallo catechin, the epigallocatechin, the gallo catechin gallate and the epigallocatechin gallate.

[0063] In a third embodiment, R1 of the phenolic compound is

[0064] wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H5O2, and R11 is H, OH or C6H5O2, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H5O2 then R11 is H. In particular, the phenolic compound can be selected from the group consisting of the rhamnetin, the fisetin, the robinetin, the gossypetin, the orientin, the homoorientin and the cirsiliol.

[0065] In a fourth embodiment, R1 of the phenolic compound is —(CH2)n—COOR or —(CH2)n—CONHR with n being an integer from 0 to 2. In particular, the phenolic compound can be selected from the group consisting of the homoprotocatechuic acid, the dihydrocaffeic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitannin (2',5-di-O-galloyl-hamamelose) and the protocatechuic acid.

[0066] In a fifth embodiment, R1 of the phenolic compound is —(CR12—CH)——COOR or —(CR12—CH)——CONHR, R12 being H or a C1-C6 linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl. In
particular, the phenolic compound can be selected from the group consisting of the caffeic acid, the rosmarinic acid, the esculetin, the 4-methylesculetin, the nordihydroguaiaretic acid, the 3-hydroxydaidzein, the oleuropein and the maritine (3',4',6,7-tetrahydroxy-6-O-gluco-sylaurone), respectively.

In a ninth embodiment, R1 of the phenolic compound is a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom.

In particular, the phenolic compound can be selected from the group consisting of

![Chemical structure](attachment:image.png)

Preferably, the phenolic compound can be selected from the group consisting of the anthrarobin and the salsolidol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline).

The present invention also concerns the phenolic compounds O-α-glucosides obtainable by the method of the invention. Consequently, the present invention concerns a phenolic compound O-α-glucoside having the following formula:

![Chemical structure](attachment:image.png)

wherein

A and B, identical or different, are H or a -α-glucosyl residue, with the proviso that at least one of A and B is an -α-glucosyl residue;

R2 is H or OH; and

R1 is selected from the group consisting of
[0080] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH; and

[0081] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH, and at least one among R7 and R8 is OH;

[0082] wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H11O, and R11 is H, OH or C6H12O, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H12O then R11 is H;

[0083] —(CH2)n—COOR or —(CH2)n—CONHRR, with n being an integer from 0 to 2;

[0084] —(CR12—CH)—COOR or —(CR12—CH)—CONHR, R12 being H or a C1-C8 linear, branched or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl;

[0085] —(CH2)n—OR or —(CH2)n—NHR with n being an integer from 0 to 2;

[0086] —(CH2)n—COR or —(CH=CH)n—COR with n being an integer from 0 to 2;

[0087] H;

[0088] a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom;

[0089] wherein R is H or a linear, branched or cyclic, aromatic or not, saturated or unsaturated, C1-C10 hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkynyl, or an alkenyl, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C1-C2)aryls, an (C1-C2)heterecycle, an (C1-C2)alkoxy, an (C1-C2)acyl, an (C1-C2)alcohol, a carboxylic group (—COOH), an (C2-C8)ester, an (C1-C3) amine, an amino group (—NH2), an amide (—CONH2), an (C1-C3)imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), a halogen, an (C1-C3)halogenoalkyl, a thiol (—SH), a (C1-C3)thioalkyl, a (C1-C3)sulfone, a (C1-C3)sulfoxide and a combination thereof.

[0090] A first preferred phenolic compound O-α-glucoside of formula (II) has R1, which is

[0091] and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the taxifolin O-α-glucoside, the eriodictyol O-α-glucoside, the dihydromobinetin O-α-glucoside and the fustin O-α-glucoside.
A second preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the second preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the catechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, and the epicatechin gallate O-α-glucoside.

A third preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the third preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the catechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, and the epicatechin gallate O-α-glucoside.

A fourth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the fourth preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the homoprotocatechuic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the propyl gallate O-α-glucoside, the gallic acid O-α-glucoside, the hamamelitnin O-α-glucoside and the protocatechuic acid O-α-glucoside.

In a fifth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the fifth preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is selected from the group consisting of the caffeic acid O-α-glucoside, the rosmarinic acid O-α-glucoside, the esculetin O-α-glucoside, the 4-methylesculetin O-α-glucoside, the neralbergin (6,7-dihydroxycoumarin) glucoside, the chlorogenic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the chlorogenic acid (dicafeoyl tartaric acid) O-α-glucoside, the echinacoside (2- (3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl (1→3)→(beta-D-glucopyranosyl)(1→6)), 4-(3-(3,4-dihydroxyphenyl)-2-propenoyl)-O-α-glucoside, the beta-O-glucopyranoside O-α-glucoside and the verbascoside O-α-glucoside.

A sixth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the sixth preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is the hydroxytyrosol O-α-glucoside.

A seventh preferred phenolic compound O-α-glucoside of formula (II) has R1 which is

![Image of the seventh preferred phenolic compound](image)

and preferably the phenolic compound O-α-glucoside is selected in the group consisting of the maclurine O-α-glucoside, the 3,4-dihydroxybenzaldehyde O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the butein (2',3',4',5'-tetrahydroxylchalcone) O-α-glucoside, the 3,4-dihydroxyacetophenone O-α-glucoside, the marin (2',3',3',4',5'-pentahydroxy-4'-glucosylchalcone) O-α-glucoside and the eriodictyolchalcone (2',3',4',5'-pentahydroxy-4'-glucosylchalcone) O-α-glucoside.

An eighth preferred phenolic compound O-α-glucoside of formula (II) has R1 which is selected from the group consisting of H;
R1, said ring being optionally interrupted by at least one heteroatom. Preferably the phenolic compound O-α-glucoside is selected in the group consisting of the anthrarabino O-α-glucoside and the salasine (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline) O-α-glucoside.

[0103] In a preferred embodiment, the phenolic compound O-α-glucoside of the present invention has a α-glucosyl residue which is a glucose monomer, dimer, trimer or tetramer, preferably a monoglucoside.

[0104] Preferably, the phenolic compound O-α-glucosides of the present invention have a 20 folds higher solubility than the corresponding aglycone in the same physiological conditions.

[0105] The phenolic compound O-α-glucosides of the present invention can be cleaved by an enzyme to release the corresponding aglycone. Said enzyme is an O-α-glucosidase. Preferably, said enzyme is issued from human associated micro-organisms, in particular human micro-organisms associated to skin, mouth, intestinal tract, upper respiratory system or female genital tract, even more preferably skin associated micro-organisms.

[0106] The present invention further concerns phenolic compound O-α-glucosides of the present invention as medicament.

[0107] The present invention also concerns a pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside of the present invention.

[0108] The present invention also concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered topically, orally, rectally, nasally or vaginally, wherein enzymes issued from micro-organisms associated to skin, mouth, intestinal tract, upper respiratory system or female genital tract release the corresponding aglycone.

[0109] The present invention also concerns the use of a phenolic compound O-α-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

[0110] FIG. 1—Flavonoids: basic structure and numbering of carbon atoms.
[0111] FIG. 2—Proocatechuic acid.
[0112] FIG. 3—CaFeic acid (3,4-dihydroxycinnamic acid).
[0113] FIG. 4—3,4-dihydroxymandelic acid.
[0114] FIG. 5—Esculetin (6,7-dihydroxycoumarin).
[0115] FIG. 6—Taxifolin.
[0116] FIG. 7—Glucoside of caffeic acid derivatives.
[0117] FIG. 8—Glucoside of 3,4 dihydroxybenzoic acid and other phenolic acids.
[0118] FIG. 9—Glucoside of flavanol.
[0119] FIG. 10—Glucoside of flavonol, isoflavone, flavone and dihydroflavonol.
[0120] FIG. 11—Glucoside of neutral polyphenol.
[0121] FIG. 12—HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm). Incubation duration: 0.
[0122] FIG. 13—HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm). Incubation duration: 22 hours.

[0123] FIG. 14—Mass spectrum of the substance eluted at around 8.13 minutes. Incubation duration: 22 hours.
[0124] FIG. 15—UV spectrum of the substance eluted at around 8.13 minutes. Incubation duration: 22 hours.
[0125] FIG. 16—Mass spectrum of the substance eluted at around 6.15 minutes. Incubation duration: 22 hours.

DETAILED DESCRIPTION OF THE INVENTION

[0126] Definitions
[0127] Phenolic compound or Phenolics: compound that possess an aromatic ring bearing one or more hydroxyl substituents.

[0128] Flavonoids: polyphenolic compounds possessing 15 carbon atoms, two benzene rings joined by a linear three carbon chain giving a system C6-C3-C6. The first benzene ring (ring A) forms with an oxygen atom and the three carbon atoms joining the two benzene rings a chromane skeleton (rings A and C). The chromane skeleton bears the second aromatic ring B in position 2, 3 or 4. In few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring. Both the oxidation state of the heterocyclic C ring and the position of ring B are important in the classification of flavonoids:

[0129] anthocyanins: ring C is a pyran which participates in a 3-hydroxychromene skeleton substituted in 2.
[0130] catechic substances (flavanols): ring C is a hydroxymethylenepyrrole which participates in a 3-hydroxy or 3,4-dihydroxychromene skeleton substituted in 2 (catechin, epicatechin, gallocatechin and epigallocatechin forming the condensable tannins).

[0131] flavones: ring C is a pyrone substituted in 2.
[0132] flavonols: ring C is a pyrone hydroxylated in 3 and substituted in 2.
[0133] flavanones: ring C is a dihydroxy pyrone substituted in 2.

[0134] dihydroflavonols: ring C is a dihydroxy pyrone hydroxylated in 3 and substituted in 2.
[0135] isoflavones: flavones with the substitution in 3 instead of 2.

[0136] chalcones and dihydrochalcones: ring C is open and with a C2/C3 double bond (chalcones) or not (dihydrochalcones).
[0137] aurones: ring C is a five-membered ring.

[0138] Enzyme: protein molecule that catalyses chemical reactions on molecules (named substrates) to obtain other molecules (named products). A recommended name, a systematic name which stresses the type of reaction and an Enzyme Commission (EC) code number, are assigned to each enzyme. These code numbers, prefixed by EC, contain four elements separated by points. The first number shows to which of the six main divisions (classes) the enzyme belongs: oxidoreductases (EC 1), transferases (EC 2), hydrolases (EC3), lyases (EC4), isomerases (EC5) and liggases (EC6). The second number indicates the subclass, the third the sub-subclass and the fourth is the serial number of the enzyme in its sub-subclass.

[0139] Bioavailability: the degree to which or rate at which a molecule or other substance is absorbed or becomes available at the site of physiological activity after administration or application.

[0140] Glucansucrases: common name of glucosyltransferases with the EC number 2.4.1.5 (see hereafter: KRAJIS, VAN GEEL-SCHUTTEN G H, DONDOREFT MMG, KIRSANOVVS S, VAN DER MAAREL M J E C, DIJKHUIZEN L.
Glucan synthesis in the genus Lactobacillus: isolation and characterization of glucansucrase genes, enzymes and glucan products from six different strains. Microbiology 150: 3681-90).

Glycosyltransferase: enzyme that catalyzes the transfer of glycosyl group(s) from one compound (said donor) to another (said acceptor). Glycosyltransferases are classified as transferases, with the EC number EC 2.4. Transfereases that transfer hexoses (carbohydrate molecules that have six carbon atoms per molecule) are included in the sub-class EC 2.4.4. Transfereases that transfer the glucose moiety of sucrose to an acceptor are EC 2.4.1.4 (sucrase: 1,4-β-D-glucan 4-α-D-glucosyltransferase; recommended name: amylolysisucrase, EC 2.4.1.5 (sucrase: 1,6-α-D-glucan 6-α-D-glucosyltransferase; recommended name: dextrosucrase) and EC 2.4.1.7 (sucrose: orthophosphate α-D-glucosyltransferase; recommended name: sucrose phosphorylase).

Glycine: chemical part of a glycoidic derivative which belongs to the carbohydrate family. If the glycone group is glucose, then the molecule is a glycoside; if it is fructose, then the molecule is a fructoside; if it is glucuronic acid, then the molecule is a glucuronide.

Glycosidic bond: chemical linkage between a glycone and another glycone or an aglycone. Depending on whether the glycosidic bond lies "below" or "above" the plane of the cyclic carbohydrate molecule when considering the HAWORTH projection, glycosides are classified as α-glycosides or β-glycosides.

Aglycone: Chemical part of a glycoidic derivative which is not the glycone one.

Where "comprising" is used, this can preferably be replaced by "consisting essentially of", more preferably by "consisting of".

In the context of the present invention, the term "alkyl" more specifically means a group such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, heicosyl, docosyl and the other isomeric forms thereof. (C₁₋₅)alkyl more specifically means methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl and the other isomeric forms thereof. (C₁₋₅) alkyl more specifically means methyl, ethyl, propyl or isopropyl.

The term "alkenyl" refers to an alkyl group defined hereinabove having at least one unsaturated ethylene bond and the term "alkynyl" refers to an alkynyl group defined hereinabove having at least one unsaturated acetylene bond. (C₂₋₅)alkenyl includes an ethenyl and a propenyl (1-propenyl or 2-propenyl).

The "aryl" groups are mono-, bi- or tri-cyclic aromatic hydrocarbons having from 5 to 9 carbon atoms. Examples include a phenyl, in particular.

"Heterocycle" groups are groups containing 1 to 3 rings comprising one or more heteroatoms, preferably 1 to 5 endocyclic heteroatoms. They may be mono-, bi- or tri-cyclic. They may be aromatic or not. Examples of aromatic heterocycles include pyridine, pyridazine, pyrimidine, pyrazine, furan, thiophene, pyrrole, oxazole, thiazole, isothiazole, imidazole, pyrazole, oxadiazole, triazole, thiadiazole and triazine groups. Examples of bicycles include in particular quinoline, isoquinoline and quinazoline groups (for two 6-membered rings) and indole, benzimidazole, benzoxazole, benzothiazole and indazole (for a 6-membered ring). Nonaromatic heterocycles comprise in particular piperazine, piperidine, etc.

(C₁₋₅)alkoxy includes methoxy, ethoxy, propoxy, and isopropoxy.

(C₂₋₅)acyl includes acetyl, propionyl and isopropionyl.

(C₁₋₅)alkyl includes methanol, ethanol, propanol and isopropanol.

(C₂₋₅)ester includes methyl ester and ethyl ester.

(C₁₋₅)amine includes methylanilne, ethylamine and propylanilne.

(C₁₋₅)amine includes mthylaime, ethylinine and propyline.

The halogen can be Cl, Br, I or F.

(C₁₋₅)halogenoalkyl includes halogenomethyl, halogenoethyl and halogenopropyl.

(C₁₋₅)haloalkyl includes thiomethyl, thioethyl and thioisopropyl.

(C₁₋₅)sulfone includes methylsulfone, ethylsulfone and propylsulfone.

(C₁₋₅)sulfamide includes methylsulfamide, ethylsulfamide, propylsulfamide and isopropylsulfamide.

"Heteroatom" denotes N, S or O.

This invention also relates to a process for preparing O-α-glucosides of phenolic compounds containing a catechol structure and, for instance, selected among protocatechuic acid and its esters derivatives, caffeic acid and its esters derivatives, especially rosmaninic acid, chlorogenic acid and caffeic acid phenethyl ester and hydrocaffeic acid or 3,4-dihydroxyphenylglycol, esculin, taxifolin, fisetin, eriodictyol, fisetin and rhamnetin. In particular, the phenolic compounds containing a catechol structure can be selected from the group consisting of the epicatechin gallate, the eriodictyol, the esculetin, the epicatechin, the fisetin, the fisitin, the homoprotocatechuic acid, the protocatechuic acid, the protocatechuic acid ethyl ester, the hydroxytyrosol, the maculine, the nordihydroguaiaretic acid, the oleuropein, the pyrocatechol, the rhamnetin, the rosmaninic acid, the taxifolin, the 3-hydroxydaidzein, the 3,4-dihydroxybenzophenone, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the catechin, the cirsisiol, the chlorogenic acid, the gossypetin, the orientin, the homoericientin, the 3,4-dihydroxybenzdehydrol, the buetin, the 3,4-dihydroxyacetophenone, the maren, the mariteinin, the eriodictyolalkaline, the 4-methylesculin, the nordalbergin, the salsoholin, the chropic acid, the echinacside, the verbascoside, the anatharbin, the epigallocatechin, the dihydromobinetin, the galocatechin, the gallic acid, the propyl gallate, the epigallocatechin gallate, the hamamelitannin and the robinetin. The process for preparing O-α-glucosides of phenolic compounds containing a catechol structure can also be performed with cirsisiol, 3',4',7-trihydroxyflavone and 3'-hydroxydaidzein (flavones and isoflavones).

For this purpose, an enzymatic reaction is achieved using sucrose, an abundant and rather cheap substance used in the food and feed fields. This reaction consists of the transfer of the glucose part of sucrose on an hydroxyl group of the catechol ring by a glycosyltransferase (EC 2.4.1) or, once a first glucosyl residue has been attached to a hydroxyl group of the catechol ring, the transfer of the glucose part of sucrose to a hydroxyl group of the fixed glucose, the position of this hydroxyl group depends on the enzyme specificity. As each phenolic compound cited above bears two hydroxyl groups on said ring, two derivatives can be obtained by this enzy-
motic reaction. When a population of glycoside derivatives results from the synthesis reaction (by population, it is understood the compounds for which the catechol ring has one of its hydroxyl group substituted or both of its hydroxyl group substituted by one glucosyl residue or an oligosaccharide), the entire population is said to be the product and corresponds to the invention.

[0164] The present invention concerns a method for producing a phenolic compound O-α-glucoside comprising incubating sucrose and a glucansucrase from Leuconostoc mesenteroides NRRL B-512f in buffered water at pH convenient for the enzymatic activity (well known by a skilled man) or in a buffered water at pH convenient for the enzymatic activity-cosolvent mixture with a phenolic compound having the following formula:

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 \quad \text{R}_3 \quad \text{R}_4 \\
\text{O} & \quad \text{O} & \quad \text{O} & \quad \text{O}
\end{align*}
\]

[0165] wherein \( \text{R}_2 \) is H or OH; and

[0166] \( \text{R}_1 \) is selected from the group consisting of

\[
\begin{align*}
\text{R}_5 & \quad \text{R}_6 \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0167] wherein \( \text{R}_3 \) and \( \text{R}_4 \), independently, are H or OH, with the proviso that at least one among \( \text{R}_3 \) and \( \text{R}_4 \) represents OH; and

\[
\begin{align*}
\text{R}_7 & \quad \text{R}_8 \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0168] wherein \( \text{R}_7 \) is selected from the group consisting of \( \text{H}, \text{OH} \) or \( \text{O} \); and

\[
\begin{align*}
\text{R}_9 & \quad \text{R}_{10} \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0169] wherein \( \text{R}_7 \) is selected from the group consisting of \( \text{H}, \text{OH} \) or \( \text{O} \); and

\[
\begin{align*}
\text{R}_{11} & \quad \text{R}_5 \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0170] wherein \( \text{R}_5 \) is \( \text{OH} \) or \( \text{O} \); \( \text{R}_6 \) is \( \text{H} \), \( \text{OH} \), \( \text{R}_9 \) is \( \text{H} \) or \( \text{OH} \), \( \text{R}_{10} \) is \( \text{H}, \text{OH} \), \( \text{C}_2\text{H}_5 \), \( \text{O} \), and \( \text{R}_{11} \) is \( \text{H}, \text{OH} \), \( \text{C}_2\text{H}_5 \), \( \text{O} \), with the proviso that \( \text{R}_6 \) and \( \text{R}_{11} \) cannot be both \( \text{H} \) when \( \text{R}_5 \) and \( \text{R}_6 \) are both \( \text{OH} \) and that when \( \text{R}_10 \) is \( \text{C}_2\text{H}_5 \), then \( \text{R}_{11} \) is \( \text{H} \);

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 = \text{COOR} & \text{OR} & \text{CONH}_2 \quad \text{R}_3 \\
\end{align*}
\]

[0171] wherein \( \text{R}_2 \) is \( \text{H} \); and

\[
\begin{align*}
\text{R}_4 & \quad \text{R}_5 = \text{H} \quad \text{R}_6 = \text{H} \quad \text{R}_7 = \text{H} \\
\end{align*}
\]

[0172] wherein \( \text{R}_1 \) is \( \text{H} \); and

\[
\begin{align*}
\text{R}_2 & \quad \text{R}_3 = \text{H} \quad \text{R}_4 = \text{H} \\
\end{align*}
\]

[0173] wherein \( \text{R}_1 \) is \( \text{H} \); and

\[
\begin{align*}
\text{R}_2 & \quad \text{R}_3 = \text{H} \\
\end{align*}
\]

[0174] wherein \( \text{R}_2 \) is \( \text{H} \); and

\[
\begin{align*}
\text{R}_3 & \quad \text{R}_4 = \text{H} \\
\end{align*}
\]

[0175] wherein \( \text{R}_2 \) is \( \text{H} \); and

\[
\begin{align*}
\text{R}_3 & \quad \text{R}_4 = \text{H} \\
\end{align*}
\]

and

\[
\begin{align*}
\text{R}_5 & \quad \text{R}_6 \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0176] a \( \text{C}_1-\text{C}_{10} \) hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (bi or tricyclic) together with the ortho carbon of \( \text{R}_1 \); and

\[
\begin{align*}
\text{R}_7 & \quad \text{R}_8 \\
\text{O} & \quad \text{O}
\end{align*}
\]

[0177] wherein \( \text{R} \) is \( \text{H} \) or a linear, branched or cyclic, aromatic or not, saturated or unsaturated, \( \text{C}_1-\text{C}_{10} \) hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkyl, or an alkyl, preferably an alkyl or an alkyl, which can be substituted by one or several substituents selected from the group consisting of: an \( \text{C}_1-\text{C}_3 \) aryl, an \( \text{C}_4-\text{C}_9 \) hetereo-
cycle, an (C_{1}-C_{3})alkoxy, an (C_{1}-C_{3})acyl, an (C_{1}-C_{3})alcohol, a carboxylic group (—COOH), an (O_{2}-C_{3})ester, an (C_{1}-C_{3}) amine, an amino group (—NH_{2}), an amide (—CONH_{2}), an (C_{1}-C_{3})imine, a nitrile, an hydroxyl (—OH), an aldehyde group (—CHO), an halogen, an (C_{1}-C_{3})halogenalkyl, a thiol (—SH), a (C_{1}-C_{3})thioalkyl, a (C_{1}-C_{3})sulfonyl, a (C_{1}-C_{3})sulfoxide and a combination thereof.

[0178] In a first embodiment, R2 is H. In this embodiment, the phenolic compound can be, for example, the epicatechin gallate, the eriodictyol, the esculetin, the epicatechin, the fisetin, the fisetin, the homoprotocatechuic acid, the protocatechuic acid, the protocatechuic acid ethyl ester, the hydroxytyrosol, the maclurine, the nordihydroguaiaretic acid, the oleanolic, the pyrocatechol, the rhamnetin, the rosmarinic acid, the taxifolin, the 3-hydroxyxaladzein, the 3,4-dihydroxynaphtho-phenone, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the catechin, the cirsiliol, the chlorogenic acid, the gossypetin, the orientin, the homoorientin, the 3,4-dihydroxybenzaldehyde, the butein, the 3,4-dihydroxyacetophenone, the marein, the maritaine, the eriodictyol, the 4-methyleuscelutin, the nocardbergin, the salisol, the chioric acid, the echinacoside, the verbascoside and the anthorobin.

[0179] In an alternative embodiment, R2 is OH. In this embodiment, the phenolic compound can be, for example, the epigallocatechin, the dihydrorobinetin, the gallate, the gallic acid, the propyl gallate, the epigallocatechin gallate, the hamamelitannin and the robinetin.

[0180] In a particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

[0181] wherein

[0182] R2 is H or OH; and

[0183] R1 is

[0184] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH.

[0185] In a preferred embodiment, R3 and R4 are OH. In another preferred embodiment, R3 is H and R4 is OH. In a further preferred embodiment, R3 is OH and R4 is H. In a particularly preferred embodiment, R2 is H and R3/R4 are selected in the following combinations: H/OH; H/OH; OH/H; OH/H; OH/OH; OH/OH; OH/H; OH/H; OH/OH; OH/OH. Preferably, the phenolic compound is selected from the group consisting of the taxifolin, the eriodictyol, the dihydrorobinetin and the fisetin.

[0186] In another particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

[0187] wherein

[0188] R2 is H or OH; and

[0189] R1 is

[0190] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH. In a preferred embodiment, R8 is OH and R7 is OH or OCOR. In a more preferred embodiment, R7 and R8 are both OH. In another preferred embodiment, R7 is —OCOR and R8 is OH. In a particular preferred embodiment, R2 is H and R3/R4 are selected in the following combinations: H/OH, OH/H, OH/OH and OCOR/OH. In another particular preferred embodiment, R2 is OH and R3/R4 are selected in the following combinations: H/OH, OH/H, OH/OH and OCOR/OH. More preferably, R is

[0191] Preferably, the phenolic compound is selected from the group consisting of the catechin, the epicatechin, the catechin gallate, the epicatechin gallate, the gallic acid, the epigallocatechin, the gallic acid, the epigallocatechin gallate.

[0192] In a further particular embodiment of the method according to the present invention, the phenolic compound has the following formula:
wherein R2 is H or OH; and

R1 is

wherein R5 is OH or OCH₃; R6 is H or OH; R9 is H or OH; R10 is H, OCH₃, or C₆H₅O₂; and R11 is H, OH or C₆H₅O₂, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C₆H₅O₂ then R11 is H. In particular, R6, R5 and R11 can be selected from the following combinations:

- a) R6 is OH and R5 is OCH₃ and R11 is H;
- b) R6 is OH and R5 is OH and R11 is OH;
- c) R6 is OH and R5 is OH and R11 is C₆H₅O₂;
- d) R6 is H and R5 is OH and R11 is H; and
- e) R6 is H and R5 is OH and R11 is H;

In a preferred embodiment, R9 is OH, R10 is H and R11 is H, whereas R6 is OH and R5 is OCH₃ or R6 is H and R5 is OH. Preferably, R2 is H. Alternatively, R2 is OH.

In another preferred embodiment, R9 is H and R10 is OCH₃ or C₆H₅O₂. In a particular aspect of this embodiment, R9 and R11 are H, R10 and R5 are OCH₃, and R6 is OH.

In an preferred additional embodiment, R5 and R6 or both OH; R9 is H or OH, R10 is OH or C₆H₅O₂ and R11 is H, OH or C₆H₅O₂ with the proviso that when R10 is C₆H₅O₂ then R11 is H. In another preferred embodiment, R5 and R6 are both OH; R9 is H or OH, R10 is H and R11 is OH or C₆H₅O₂.

In another preferred embodiment, R9 is H and R10 is H. In a further preferred embodiment, R9 is H, R10 and R5 are OCH₃, and R6 is OH.

In a particular embodiment, R2, R5, R6, R9, R10 and R11 can be selected from the above mentioned combinations.

<table>
<thead>
<tr>
<th>R2</th>
<th>R5</th>
<th>R6</th>
<th>R9</th>
<th>R10</th>
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<tr>
<td>H</td>
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<td>H</td>
<td>OCH₃</td>
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<td>C₆H₅O₂</td>
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<tr>
<td>H</td>
<td>OCH₃</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

Preferably, the phenolic compound is selected from the group consisting of the rhamnetin, the fisetin, the robinetin, the gossypetin, the orientin, the homoorientin and the cirsiliol.

In a further particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein

R2 is H or OH; and R1 is —(CH₂)ₙ—COOR or —(CH₂)ₙ—CONHR with n being an integer from 0 to 2. In a preferred embodiment, R2 is H. Alternatively, R2 is OH.
Preferably, R is selected from the group consisting of H, a C₁-C₃ alkyl, preferably methyl, ethyl or propyl and

In a first more preferred embodiment, n is 0 and R is preferably H. In a second more preferred embodiment n is 1 and R is preferably H. In a third more preferred embodiment, n is 2 and R is preferably H. In another preferred embodiment, n is 0 and R is a C₁-C₃ alkyl, preferably methyl, ethyl or propyl or

In a preferred embodiment, R₁ is —(CH₂)—COOR. In a preferred embodiment, R is H.

Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid, the dihydrocaffeic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitinin (2',5-di-O-galloyl-hamamelose) and the protocatechuic acid.

In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

wherein

R₂ is H or OH; and R₁ is —(CR₁₂=CH)—COOR or —(CR₁₂=CH)—CONHR, R₁₂ being H or a C₁-C₆ linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl. Preferably R₁ is —(CH=CH)—COOR or —(CH=CH)—CONHR. In a preferred embodiment, R₂ is H. Alternatively, R₂ is OH. In a preferred embodiment, R₁ is —(CH=CH)—COOR. In a preferred embodiment, R is selected in the group consisting of H;

and a bond attached to the phenyl group of formula (I) at the carbon in ortho of R₁.

When R is a bond attached to the phenyl group of formula (I) at the carbon in ortho of R₁, R₁₂ can be in particular selected from the group consisting of H, methyl and phenyl. Then, the phenolic compound can have the following formula:
[0220] R12 being H or a C1-C6 linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl.

[0221] Preferably, the phenolic compound is selected from the group consisting of the caffeic acid, the rosmarinic acid, the esculetin, the 4-methylesculetin, the nardalbergin (6,7-dihydroxyphenylecoumarin), the chlorogenic acid, the caffeic acid phenethyl ester, the chicoic acid (dicaffeoxy tartaric acid), the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1→3)-O-(beta-D-glucopyranosyl-(1→6))-4-(3-(3,4-dihydroxyphenyl)-2-propenoate), beta-D-glucopyranoside) and the verhascoside.

[0222] In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

\[
\begin{align*}
\text{R1} & \quad \text{R2} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

[0223] wherein

[0224] R2 is H or OH; and R1 is \(-(CH_2)_n-OR\) with n being an integer from 0 to 2. In a preferred embodiment n is 2. Preferably, the phenolic compound is the hydroxytyrosol.

[0225] In an additional particular embodiment of the method according to the present invention, the phenolic compound has the following formula:

\[
\begin{align*}
\text{R1} & \quad \text{R2} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

[0226] wherein

[0227] R2 is H or OH; and R1 is \-(CH_2)_n-COR\ or \-(CH=CH)_n-COR\, with n being an integer from 0 to 2.

[0228] In a preferred embodiment, n is 0 or 1 and R is selected in the group consisting of H; a C1-C5 alkyl, preferably methyl, ethyl or propyl, more preferably a
Preferably, the phenolic compound is selected from the group consisting of the pyrocatechol, the nordihydroguaiaretic acid, the 3-hydroxydaidzein, the oleuropine and the maritunein (3',4',6,7-tetrahydroxy-6-O-glucosylaurone).

In this embodiment, R1 of the phenolic compound is a C₁₋C₁₀ hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (bi or tricyclic) together with the ortho carbon of R1. In particular, the phenolic compound can be selected from the group consisting of amino group (—NH₂), an amide (—CONH₂), an (C₁₋C₃) imine, a nitrile, an hydroxyl (—OH), a aldehyde group (—CHO), an halogen, an (C₁₋C₃)halogenoalkyl, an thiol (—SH), a (C₁₋C₃)thioalkyl, a (C₁₋C₃)sulfone, a (C₁₋C₃)sulf oxide and a combination thereof. In a particular preferred embodiment, the phenolic compound is

Nature and Source of the Enzyme

The enzymes that can be used for this condensation reaction are glycosyltransferases, more preferably hexosyltransferases (EC 2.4.1), and in a preferred manner glucansucrases (EC 2.4.1.5).

In a preferred embodiment, the enzyme used for the desired condensation of these phenolic compounds with glucose is a glucansucrase from a bacterial species, more precisely from a Leuconostoc species and more preferably from Leuconostoc mesenteroides NRRL B-512F.

Alternative sources of enzyme may be the glucansucrase(s) from Leuconostoc mesenteroides NRRL B-742, Leuconostoc mesenteroides NRRL B-1299, Leuconostoc mesenteroides NRRL B-1355 or Leuconostoc mesenteroides NRRL B-23192.

Such enzymes can be obtained by a natural fermentation of the producing strains followed by cell treatments and enzyme recovery and purification. Since glucansucrases are mainly extracellular large enzymes in solution in the culture broth or cells associated, the techniques that can be used for the recovery of the enzyme include but are not limited to centrifugation and tangential microfiltration and, if it is a cell associated enzyme, the techniques aiming at cell disruption include, but are not limited to, French press homogenization, glass beads, sonification or any equivalent method. The techniques aiming at enzyme concentration include, but are not limited to, ultrafiltration with a molecular weight cut off ranging from 10 kDa to 300 kDa and the techniques that can be used for enzyme purification include, but are not limited to, phase partition with polyethylene glycol, gel permeation chromatography. An alternative solution consists in the recombinant expression of said enzymes in well known expression hosts such as E. coli, S. cerevisiae, Baculovirus, Y. lipolytica, Bacillus sp., Pseudomonas sp., H. polymorpha or mammalian cells (see as one reference “Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems” Wiley 2004—Gerd Gelissem Ed.), optionally followed by a purification step(s) using well known methods from man of the art.

The enzyme can also be obtained through well known from the man of the art random mutagenesis, directed mutagenesis or directed evolution methods (MIYAZAKI K, ARNOLD F H, (2004), In vitro DNA recombination. In Phage Display: A practical approach. Clarkson T and Lowman H, editors. New York/Oxford University Press Inc., 43-60). These technologies could enable to obtain enzymes...
with higher specific activity, lower products inhibition, dedicated region, chemio and stereo selectivity, better stability, or any combination thereof.

The process of the invention can thus be carried out with either whole cells or with natural or recombinant crude or purified enzyme. The enzyme can be used under its "free" form or as an immobilized catalyst. Such immobilisation procedures include but are not limited to gel encapsulation (calcium alginate), resin adsorption, glutaraldehyde reticulation, spray drying in the presence eventually of an adequate adjuvant to obtain an insoluble form of the enzyme, membrane reactors or any combination thereof and are well known from the man of the art. The choice of one immobilisation approach relies on its economical cost and on the final yield of the process involving said immobilized enzyme.

The amount of enzymatic activity of an enzyme preparation can be estimated using the hydrolysis of sucrose and the measurement of the released reducing sugar (fructose) by means of colorimetric methods (such as the one involving 3,5-dinitro-salicylic acid; SUMMER J B, HOWELL S T F (1935) A method for determination of invertase activity. J Biol Chem 108: 51-4). This enzymatic activity is expressed in units, wherein one unit (U) corresponds to the amount of enzyme that releases 1 μmol of fructose per minute at 30°C, pH 5.2 (sucrose: 100 g/L; sodium acetate buffer: 50 mM; calcium chloride dihydrate: 10 mg/L).

**Reaction Conditions**

The reaction can be achieved in buffered water or in buffered water/cosolvent(s) mixture. Indeed, the inventors surprisingly observed that the enzyme is able to glucosylate in absence of cosolvents.

Preferably, the buffered water at a pH convenient for the enzymatic activity used either without cosolvent or in a mixture with a cosolvent consists of sodium or potassium acetate buffer at a concentration ranging from 20 to 500 mM in water but any other buffering substance without any negative effect on the enzymatic activity can be used. Preferably, the buffered water at a pH convenient for the enzymatic activity-cosolvent mixture consists in mixture of water, preferably a buffered water as previously described, and dimethyl sulfoxide (DMSO) with a ratio of less than 35% of DMSO (volume/volume), preferably between 15-25%, more preferably about 15%.

The reaction can be achieved in a water/cosolvent(s) mixture that enables both a proper activity of the enzyme and a good level of solubility of the phenolic compounds and of the glucose donor, i.e. sucrose. Such cosolvents can be the following water-miscible organic solvents dimethyl sulfoxide, dioxane, dimethyl formamide, ethanol, n-propanol, isopropanol, ethylene glycol, glycerol, 1,2-propanediol, sulfolane, tetramethylethylene, ethyl-lactate, diethyl ether of diethylene glycol and dimethyl ether of triethylene glycol used at different weight/volume ratio. In addition to these simple organic solvents, ionic liquids (imidazolium, pyridinium, phosphonium and ammonium salts) can also be envisaged. The cosolvents can also be the following water-immiscible organic solvents ethyl acetate, methyl ethyl ketone, methyl-2 butanol-2 and a combination of water-miscible organic solvent(s) with water-immiscible organic solvent(s).

In a preferred embodiment, the mixture is made of water and dimethyl sulfoxide (DMSO), with DMSO concentrations ranging from 5 to 70% (volume/volume). In a preferred embodiment, DMSO concentrations are between 5 and 50% (volume/volume). In a most preferred embodiment, DMSO concentration is between 10 to 35% (volume/volume). Indeed, the inventors surprisingly found that the reaction is highly more efficient when proceeding at a ratio of DMSO lower than 40%. The higher rate of product has been registered for a ratio of 15%. Therefore, a preferred ratio of the method according to the present invention is comprised between 15-25%, preferably about 15% (+/-3%).

Each phenolic compound is incubated in this reaction mixture with sucrose and the enzyme in pH and temperature conditions that allow the enzyme to be active and to synthesize the maximum possible of desired glucoside. Preferably, the reaction medium contains, in addition, calcium cations in the form of calcium chloride (or in the form of any water soluble salt of calcium) to improve the stability of the enzyme. The condensation reaction can be performed at a pH ranging from 4 to 8, and preferably from 5 to 7 by introducing a low amount of acetic buffer in the reaction medium. The temperature of the synthesis medium is maintained at a value ranging from 10 to 40 degrees Celsius, and preferably approximately 25 to 33 degrees Celsius.

Typical reaction conditions with the glucansucrase from Leuconostoc mesenteroides NRRL B-512F consists of a mixture of acetic buffer at 10 mM to 100 mM, DMSO at 10 to 35% (volume/volume), sucrose at 100 mM to 900 mM and phenolic compound at 2 to 200 mM, calcium salts at 0.5 mg to 1 g/l and the enzyme for a final concentration of 0.5 to 5 U/ml. This reaction is incubated at 30°C for several hours (e.g., 10 to 48 hours) and the synthesis of the phenolic compound derivative, as well as the disappearance of said phenolic compound upon time, is followed by HPLC analysis. A better characterization of the products can be achieved by high performance liquid chromatography coupled with a photodiode array detector coupled with a mass spectrometer to directly estimate the number of glucose moiety attached to the phenolic compound and thus have a good analytical characterization of the synthesized derivatives.

In one embodiment of the present invention, such conditions allowing the analytical characterization of the synthesized derivatives can be as follows:

The synthesis media can be analyzed by high performance liquid chromatography coupled with a photodiode array detector (FDA Waters® 956) and a mass spectrometer (Micromass ZQ 2000, Waters®).

**Operating conditions for chromatography:**

**Column**: KROMASIL C18 5μ, 250 mm x 4.6 mm (reference: K2185; A.I.T: Chromato; 117 rue de Stalingrad; 78800 Houilles).

**Elution (method 1):**

- **solvent A**: deionized water containing 1% v/v acetic acid
- **solvent B**: HPLC grade methanol containing 1% v/v acetic acid
- **0 to 10 minutes**: 90% to 80% A (linear); 10% to 20% B (linear); 1 ml/minute
- **10 to 25 minutes**: 80% to 50% A (linear); 20% to 50% B (linear); 1 ml/minute
- **25 to 30 minutes**: 50% A; 50% B; 1 ml/minute
- **30 to 35 minutes**: 50% to 90% A (linear); 50% to 10% B (linear); 1 ml/minute
- **45 minutes**: next injection

**Column temperature**: 30°C.

**Injection volume**: 10 μL.
The invention relates to O-α glucosides of phenolic compounds containing a catechol structure and for instance selected among protocatechuic acid and its esters derivatives, caffeic acid and its esters derivatives, especially rosmarinic acid, chlorogenic acid and caffeic acid phenethyl ester and hydrocaffeic acid or 3,4-dihydroxyhydrocinamic acid, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol, esculentin, taxifolin, fistin, eriodictyol, fisetin and rhamnetin. In particular, the invention relates to O-α glucosides of phenolic compounds containing a catechol structure and selected from the group consisting of the epicatechin gallate, the eriodictyol, the esculentin, the fisetin O-α-gluco side, the fistin, the homoprotocatechuic acid, the protocatechuic acid, the protocatechuic acid ethyl ester, the hydroxytyrosol, the maclurine, the nordihydroguaiaretic acid, the oleuropein, the pyrocatechol, the rhamnetin, the rosmarinic acid, the taxifolin, the 3-hydroxydaidzein, the 3,4-dihydroxybenzophenone, the caffeic acid, the dihydrocaffeic acid, the caffeic acid phenethyl ester, the cirsiliol, the chlorogenic acid coside, the anthurabin, the epigallocatechin, the dihydrorobinetin, the gallocatechin, the gallic acid, the propyl gallate and the robini netin. These new phenolic compounds derivatives have a better bioavailability through an improved solubility in water and/or on in situ release of the aglycones during their usage through their hydrolysis by human natural microorganisms and more specifically of human skin micro-organisms, or by a selected α-glucosidase such as the α-glucosidase produced by the yeast Saccharomyces cerevisiae.

In particular, the present invention concerns a phenolic compound O-α glucoside having the following formula:

![Chemical Structure](image)

wherein A and B, identical or different, are H or a -α-glucosyl residue, with the proviso that at least one of A and B is a -α-glucosyl residue.

R2 is H or OH; and,

R1 is selected from the group consisting of

![Chemical Structure](image)

wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH.
[0293] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH and at least one among R7 and R8 is OH;

[0294] wherein R5 is OH or OCH3; R6 is H or OH, R9 is H or OH, R10 is H, OCH3 or C6H5O2 and R11 is H, OH or C6H5O2, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H5O2 then R11 is H;

[0295] (CH2)n—COOR or (CH2)n—CONH2, with n being an integer from 0 to 2;

[0296] (CR12—CH)—COOR or (CR12—CH)—CONHR, R12 being H or a C2—C6 linear, branched or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl;

[0297] (CH2)n—OR or (CH2)n—NHR with n being an integer from 0 to 2; (CH2)n—COR or (CH—CH)n—COR with n being an integer from 0 to 2; H;

and,

[0298] a C1—C10 hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom;

[0299] wherein R is H or a linear, branched or cyclic, aromatic or not, saturated or unsaturated, C1—C10 hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl or an alkylnyl, preferably an alkyl or an alkenyl, which can be substituted by one or several substituents selected from the group consisting of: an (C2—C6)aryl, an (C2—C6)hetercycle, an (C1—C6)alkyl, an (C1—C6)acyl, an (C1—C6)alkol, a carboxylic group (—COOH), an (C2—C6)ester, an (C1—C6) amine, an amino group (—NH2), an amide (—CONH2), an (C1—C6)imin, a nitrile, an hydroxyl (—OH), a aldehyde (—CHO), an halogen, an (C1—C6)halogenalkyl, a thiol (—SH), a (C1—C6)thiokyl, a (C1—C6)sulfone, a (C1—C6)sulfoxide and a combination thereof.

[0300] In a first embodiment, R2 is H. In this embodiment, the phenolic compound O-α-glucoside can be, for example, the epicatechin gallate O-α-glucoside, the eriodictyol O-α-glucoside, the esculetin O-α-glucoside, the fisetin O-α-glucoside, the quercetin O-α-glucoside, the homoprotocatechuic acid O-α-glucoside, the protocatechuic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the hydroxytyrosol O-α-glucoside, the machurine O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the oleanone O-α-glucoside, the pyrocate chol O-α-glucoside, the rhamnetin O-α-glucoside, the rosmarinic acid O-α-glucoside, the taxifolin O-α-glucoside, the 3-hydroxydaidzein O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the caffeic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the caaffeic acid phenethyl ester O-α-glucoside, the cirsiliol O-α-glucoside, the chlorogenic acid O-α-glucoside and the anthrarobin O-α-glucoside.

[0301] In an alternative embodiment, R2 is OH. In this embodiment, the phenolic compound O-α-glucoside can be, for example, the epigallocatechin O-α-glucoside, the dihydrobiobetin O-α-glucoside, the gallatechin O-α-glucoside, the gallic acid O-α-glucoside, the propyl gallate O-α-glucoside and the robinetin O-α-glucoside.

[0302] In a particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:
[0303] wherein 
[0304] A and B, identical or different, are H or a -O-glucosyl residue, with the proviso that at least one of A and B is a -α-glucosyl residue; 
[0305] R2 is H or OH; and 
[0306] R1 is 

[0307] wherein R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH. 
[0308] In a particular embodiment, R2 is H. In another embodiment R2 is OH. 
[0309] In a preferred embodiment, R3 and R4 are OH. In another preferred embodiment R3 is H and R4 is OH. In a further preferred embodiment, R3 is OH and R4 is H. In a particularly preferred embodiment, R2 is H and R3/R4 are selected in the following combinations: OH/OH; H/OH; OH/H. In another preferred embodiment, R2 is OH and R3/R4 are selected in the following combinations: OH/OH; H/OH; OH/H. 
[0310] In particular, R2 is H, R3 is H and R4 is OH (resulting in eriodictyol O-α-glucoside). Alternatively, R2 is H, R3 is OH and R4 is H (resulting in fustin O-α-glucoside). In a preferred embodiment, R2 is H and both R3 and R4 are OH (resulting in taxifolin O-α-glucoside). 
[0311] Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the taxifolin O-α-glucoside, the eriodictyol O-α-glucoside, the dihydrorobinetin O-α-glucoside and the fustin O-α-glucoside. 
[0312] In another particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula: 

[0331] wherein 
[0332] A and B, identical or different, are H or a -α-glucosyl residue, with the proviso that at least one of A and B is a -α-glucosyl residue; 
[0333] R2 is H or OH; and 
[0334] R1 is 

[0335] wherein R7 is selected from the group consisting of H, —OH or —OCOR and R8 is H or OH, with the proviso that, when R2 is H, R7 and R8 are not both OH and at least one among R7 and R8 represents OH. 
[0336] In a particular embodiment, R2 is H. In another embodiment R2 is OH. 
[0337] In a preferred embodiment, R2 is OH, R8 is OH and R7 is OH or OCOR. In a more preferred embodiment, R7 and R8 are both OH. In another preferred embodiment, R2 is H, R8 is OH and R7 is OCOR. In a further preferred embodiment, R2 is H or OH, R7 is —OCOR and R8 is OH. More preferably, R is 

[0338] Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the epigallocatechin O-α-glucoside, the gallocatechin O-α-glucoside and the epicat-echin gallate O-α-glucoside. 
[0339] In a further particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula: 

[0340] wherein 
[0341] A and B, identical or different, are H or a -α-glucosyl residue, with the proviso that at least one of A and B is a -α-glucosyl residue; 
[0342] R2 is H or OH; and 
[0343] R1 is 

[0344] Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the epigallocatechin O-α-glucoside, the gallocatechin O-α-glucoside and the epicat-echin gallate O-α-glucoside. 
[0345] In a further particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:
wherein R5 is OH or OCH3; R6 is H or OH; R9 is H or OH; R10 is H, OCH3 or C6H11O5; and R11 is H, OH or OCH3, with the proviso that R10 and R11 can not be both H when R5 and R6 are both OH and that when R10 is C6H11O5 then R11 is H. In particular, R6, R5 and R11 can be selected from the following combinations:

- **[0327]** a) R6 is OH and R5 is OCH3 and R11 is H;
- **[0328]** b) R6 is OH and R5 is OH and R11 is OH;
- **[0329]** c) R6 is OH and R5 is OH and R11 is C6H11O5; and,
- **[0330]** d) R6 is H and R5 is OH and R11 is H; and
- **[0331]** R9 is H or OH, and R10 is H or OCH3 or C6H11O5.

**[0332]** with the proviso that when R10 is C6H11O5, R11 is H.

**[0333]** In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

**[0334]** In a preferred embodiment, R9 is OH, R10 is H and R11 is H, whereas R6 is OH and R5 is OCH3 or R6 is H and R5 is OH. Preferably, R2 is H. Alternatively, R2 is OH.

**[0335]** In another preferred embodiment, R9 is H and R10 is OCH3 or C6H11O5. In a particular aspect of this embodiment, R9 and R11 are H, R10 and R5 are OCH3, and R6 is OH.

**[0336]** In an additional preferred embodiment, R5 and R6 are both OH, R9 is H or OH, R10 is OH or C6H11O5, and R11 is H, OH or C6H11O5, with the proviso that when R10 is C6H11O5 then R11 is H. In another preferred embodiment, R5 and R6 are both OH, R9 is H or OH, R10 is H and R11 is OH or C6H11O5.

**[0337]** In another preferred embodiment, R9 is H and R10 is H. In a further preferred embodiment, R9 is H, R10 and R5 are OCH3, and R6 is OH.

**[0338]** In a particular embodiment, R2, R5, R6, R9, R10 and R11 can be selected from the above mentioned combinations.

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<th>R9</th>
<th>R10</th>
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**[0339]** In a particular embodiment, R2 is H and R1 is OH.

**[0340]** wherein either R6 is OH and R5 is OCH3 (resulting in rhamnetin O-α-glucoside) or R6 is OH and R5 is OH (resulting in fisetin O-α-glucoside).

**[0341]** Preferably, the phenolic compound O-α-glucose is selected from the group consisting of the rhamnetin O-α-glucoside, the fisetin O-α-glucoside, the robinetin O-α-glucoside, the gossypetin O-α-glucoside, the orientin O-α-glucoside, the homoeroorientin O-α-glucoside and the cirsiliol O-α-glucoside.

**[0342]** In a further particular embodiment of the present invention, the phenolic compound O-α-glucose has the following formula:

![Diagram](image)

**[0343]** wherein

**[0344]** A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

**[0345]** R2 is H or OH; and

**[0346]** R1 is —(CH2)n—COOR or —(CH2)n—CONHR with n being an integer from 0 to 2.

**[0347]** In a particular embodiment, R2 is H. In another embodiment, R2 is OH.

**[0348]** Preferably, R is selected from the group consisting of H, a C1-C3 alkyl, preferably methyl, ethyl or propyl, and
In a first more preferred embodiment, n is 0 and R is preferably H. In a second more preferred embodiment, n is 1 and R is preferably H. In a third more preferred embodiment, n is 2 and R is preferably H. In another preferred embodiment, n is 0 and R is a C₁-C₃ alkyl, preferably methyl, ethyl or propyl or preferably H.

In a preferred embodiment, R₁ is —(CH₂)ₙ—COOR. In a preferred embodiment, R is H.

Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid, the dihydrocaffeic acid, the protocatechuic acid ethyl ester, the propyl gallate, the gallic acid, the hamamelitannin (2,5-di-O-galloyl-hamamelse) and the protocatechuic acid.

In a preferred embodiment, R₂ is H and R₁ is —COOH (resulting in protocatechuic acid O-α-glucoside). In another preferred embodiment, R₂ is H and R₁ is —(CH₃)₂—COOH (resulting in hydrocaffeic acid O-α-glucoside).

The present invention contemplates the ester thereof and the pharmaceutically acceptable salts thereof.

Preferably, the phenolic compound is selected from the group consisting of the homoprotocatechuic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the propyl gallate O-α-glucoside, the gallic acid O-α-glucoside, the hamamelitannin (2,5-di-O-galloyl-hamamelse) O-α-glucoside and the protocatechuic acid O-α-glucoside.

In an additional particular embodiment of the present invention, the phenolic compound O-α-glucose has the following formula:

[Formula (I)]

wherein A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

R₂ is H or OH; and

R₁ —(CR₁₂—CH) —COOR or —(CR₁₂—CH) —CONHR, R₁₂ being H or a C₁-C₅ linear or cyclic alkyl or alkenyl, preferably methyl, ethyl, propyl, cyclohexyl or phenyl, more preferably methyl or phenyl. Preferably R₁ is —(CH₁—CH) —COOR or —(CH₁—CH) —CONHR. In a preferred embodiment, R₂ is H. Alternatively, R₂ is OH.

In a preferred embodiment, R₁ is —(CH—CH) —COOR. In a preferred embodiment, R is selected in the group consisting of H; and a bond attached to the phenyl group of formula (I) at the carbon in ortho of R₁.

In a particular embodiment, R₂ is H and R₁ is —(CH—CH) —COOH (resulting in caffeic acid O-α-glucoside). The present invention contemplates the ester thereof and the pharmaceutically acceptable salts thereof. In particular, when R₁ is —(CH—CH) —COOR, R is selected from 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid and being...
attached at position 3 (resulting in chlorogenic acid O-\(\alpha\)-glucoside), (R)-1-carboxy-2-(3,4-dihydroxyphenyl)ethyl (resulting in rosmarinic acid O-\(\alpha\)-glucoside) and phenethyl (resulting in caffeic acid phenethyl ester O-\(\alpha\)-glucoside). In particular, when \(R_1\) is \(-\text{(CR}12=\text{CH})\text{-COOR}\), \(R\) is a bond attached to the phenyl group of formula (II) by the carbon in meta of \(O\) giving the following formula:

![Chemical structure](image)

\(\text{[0362]}\) (i.e., when \(R_{12}\) is \(H\), then esculetin O-\(\alpha\)-glucoside, when \(R_{12}\) is methyl, then 4-methylesculetin O-\(\alpha\)-glucoside and when \(R_{12}\) is phenyl, then nardalbergin O-\(\alpha\)-glucoside). In a particular embodiment, \(R_{12}\) is \(H\).

\(\text{[0363]}\) Preferably, the phenolic compound O-\(\alpha\)-glucose is selected from the group consisting of the caffeic acid O-\(\alpha\)-glucoside, the rosmarinic acid O-\(\alpha\)-glucoside, the esculetin O-\(\alpha\)-glucoside, the 4-methylesculetin O-\(\alpha\)-glucoside, the nardalbergin (6,7-dihydroxyphenylcoumarin) O-\(\alpha\)-glucoside, the chlorogenic acid O-\(\alpha\)-glucoside, the caffeic acid phenethyl ester O-\(\alpha\)-glucoside, the chionic acid (dicafeeoyl tartaric acid) O-\(\alpha\)-glucoside, the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1\(\rightarrow\)3)-O-(beta-D-galactopyranosyl(1\(\rightarrow\)6))-\(\rightarrow\)4-(3-(3,4-dihydroxyphenyl)-2-propenoyl) O-\(\alpha\)-glucoside, beta-D-galactopyranoside O-\(\alpha\)-glucoside and the verbascoside O-\(\alpha\)-glucoside.

\(\text{[0364]}\) In an additional particular embodiment of the present invention, the phenolic compound O-\(\alpha\)-glucose has the following formula:

![Chemical structure](image)

\(\text{[0372]}\) wherein

\(\text{[0373]}\) \(A\) and \(B\), identical or different, are \(H\) or a \(\alpha\)-glucosyl residue, with the proviso that at least one of \(A\) and \(B\) is a \(\alpha\)-glucosyl residue;

\(\text{[0374]}\) \(R_2\) is \(H\) or \(OH\); and

\(\text{[0375]}\) \(R_1\) is \(-\text{(CH}_2)_n\text{-COR}\) or \(-\text{(CH}=\text{CH})_n\text{-COR}\) with \(n\) being an integer from 0 to 2.

\(\text{[0376]}\) In a particular embodiment, \(R_2\) is \(H\). In another embodiment, \(R_2\) is \(OH\).

\(\text{[0377]}\) In a preferred embodiment, \(n\) is 0 or 1 and \(R\) is selected in the group consisting of

\(\text{[0378]}\) \(H\); a \(C_1-C_3\) alkyl, preferably methyl, ethyl or propyl, more preferably a methyl;

![Chemical structure](image)

Preferably, \(n\) is 0. Alternatively, \(n\) is 1.

\(\text{[0379]}\) Preferably, the phenolic compound O-\(\alpha\)-glucoside is selected from the group consisting of the machurine O-\(\alpha\)-glucoside, the 3,4-dihydroxybenzaldehyde O-\(\alpha\)-glucoside, the 3,4-dihydroxybenzophenone O-\(\alpha\)-glucoside, the butein (2',3,4,4-tetrahydroxylchalcone) O-\(\alpha\)-glucoside, the 3,4-dihydroxyacetophenone O-\(\alpha\)-glucoside, the marcin (2',3,3',4,4'-pentahydroxy-4'-glucosylethallone) O-\(\alpha\)-glucoside and the eriodictyolchalcone (2',4',6',3,4-pentahydroxychalcone) O-\(\alpha\)-glucoside.

\(\text{[0380]}\) In an additional particular embodiment of the present invention, the phenolic compound O-\(\alpha\)-glucoside has the following formula:

![Chemical structure](image)
[0381] wherein

[0382] A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

[0383] R2 is H or OH; and

[0384] R1 is selected from the group consisting of H;

[0385] Preferably, the phenolic compound O-α-glucoside is selected from the group consisting of the oleuropein O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the pyrocatechol O-α-glucoside, the 3-hydroxydaidzein O-α-glucoside and the marinetrin (3',4',6,7-tetrahydroxy-6-O-glucosylaurone) O-α-glucoside.

[0386] In an additional particular embodiment of the present invention, the phenolic compound O-α-glucoside has the following formula:

[0387] wherein

[0388] A and B, identical or different, are H or a α-glucosyl residue, with the proviso that at least one of A and B is a α-glucosyl residue;

[0389] R2 is H or OH; and

[0390] R1 is a C1-C10 hydrocarbon group which forms with the represented ring of formula (I) a fused aromatic ring (bi or tricyclic) together with the ortho carbon of R1. In particular, the phenolic compound O-α-glucoside can be selected from the group consisting of

[0391] said fused ring can be optionally interrupted by at least one heteroatom and can be substituted by one or several substituents selected from the group consisting of: an (C1-C3) alkoxy, an (C2-C6) acyl, an (C1-C6) alcohol, a carboxylic group (—COOH), an (C2-C6) ester, an (C1-C6) amine, an amino group (—NH2), an amide (—CO(NH2)), an (C1-C6) imine, a nitrile, an hydroxyl (—OH), a aldehyde group (—CHO), an halogen, an (C1-C6) halogenalkyl, a thiol (—SH), a (C1-C6) thiolalkyl, a (C1-C6) sulfone, a (C1-C6) sulfoxide and a combination thereof. In a particular preferred embodiment, the phenolic compound O-α-glucoside is
The O-α-glucosyl residue refers herein to a glucose monomer, dimer, trimer, tetramer, pentamer or more. Preferably, the O-α-glucosyl residue is a glucose monomer, dimer or trimer, namely glucosyl, diglucosyl or triglucosyl. Still preferably, the O-α-glucosyl residue is a glucose monomer. In a particular embodiment the O-α-glucosyl residue is attached to the phenolic compound by the carbon in position 1. In a preferred embodiment, OA is OH and OB is a O-α-glucosyl residue. In another preferred embodiment, OB is OH and OA is a O-α-glucosyl residue.

In a particular embodiment, R can be a monosaccharide. In an other particular embodiment, R is a (C,−C,₃) alkyl or a (C,₃,−C,₅) alkyl.

Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable base addition salts, pharmaceutically acceptable metal salts and ammonium and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, perchloric and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutically acceptable salts listed in J. Pharm. Sci. 1977, 66, 2, and in Handbook of Pharmaceutical Salts: Properties, Selection, and Use edited by P. Heinrich Stahl and Camille G. Wermuth 2002. Examples of metal salts include lithium, sodium, potassium, magnesium salts and the like. Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium, tetramethylammonium and the like. Examples of organic bases include lysine, arginine, guanidine, diethanolammonelne and the like.

In Situ Release of the Aglycones.

Surprisingly, the inventors found that the phenolic compound O-α-glucosides of the present invention can be cleaved by α-glucosidases leading to in situ releasing of the phenolic compounds. All the phenolic compounds O-α-glucosides of the present invention have at least one O-α-glucoside bond. This bond can be specifically hydrolyzed by enzymes, such as α-glucosidases (EC 3.2.1.20), to release the glucosyl residue and the aglycone part. When achieved in situ, this liberation has several advantages:

It enables to release the poorly soluble aglycone (which may be more active than the glycoside derivative) after their administration/injection/application under a soluble glycoside form, and/or

the in situ release can be time dependant (if achieved by enzymes expressed by micro-organisms, the amount of releasing enzyme will be correlated to the number of micro-organisms: the more dense the bacterial population will be, the more aglycones release will occur), and/or

the in situ release can be controlled by an in situ administration/injection/application of an α-glucosidase or of a micro-organism expressing such enzymatic activity.

These advantages are important in the formulation of phenolics in cosmetics or dermocosmetics preparations. In a preferred embodiment of the present invention, said phenolic compound O-α-glucosides can be in situ activated by enzyme(s) expressed by human associated micro-organisms, and more preferentially by human skin associated micro-organisms. Known and non exhaustive examples of such human commensal or non commensal micro-organisms include Streptococcus species, Staphylococcus species, Enterococcus species, Escherichia coli, Bacilli, Corynebacterium species and Propionibacterium species. When applied on skin, the phenolic compounds O-α-glucosides of the present invention are converted by skin associated micro-organisms into the aglycones part and the glucosyl residue. Such bacteria can be found in human beings in mouth, intestinal tract, genital tract and upper respiratory system.

In another preferred embodiment of the present invention, said phenolic compounds O-α-glucosides can be in situ activated by an α-glucosidase (EC 3.2.1.20), such as the α-glucosidase from Saccharomyces cerevisiae.

So phenolic compounds O-α-glucosides of the present invention have a pro-drug status as the active part of the molecule (the aglycones) can be released in situ.

Therefore, the present invention concerns a pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside of the present invention or a pharmaceutically acceptable salt thereof. The present invention also concerns a phenolic compound O-α-glucosides of the present invention or a pharmaceutically acceptable salt thereof as a medicament. The medicament can be therapeutic or prophylactic. Phenolic compound O-α-glucosides of the present invention have several activity among which antiviral, antimicrobial, immune-stimulating, antiallergic, antihypertensive, antiischemic, antihypercholesterolemic, antiplatelet, hepatoprotective, anti-inflammatory, antineoplastic, antineoplastics and vasodilatory actions.

In a particular embodiment, the composition can further comprise a O-α-glucosidase (EC 3.2.1.20) or a micro-organism expressing O-α-glucosidase activity. Preferably, the O-α-glucosidase is from Saccharomyces cerevisiae. In particular, the O-α-glucosidase (EC 3.2.1.20) or a microorganism expressing O-α-glucosidase activity is present in the composition in an inactivated form and the O-α-glucosidase is activated just at the moment of administration. For instance, the composition can be formulated in dried form, the absence of water leading to the inactivation of O-α-glucosidase; after water addition, the enzyme will become active and will then be able to hydrolyze the glucosidic bond. The enzyme and the phenolic compound O-α-glucosides can be
put in two different liquid preparations that will be mixed just at the moment of administration. If the enzyme and the phenolic compound $\alpha$-glucosides are put into the same solution, it is possible to use an enzyme irreversible inhibitor that will be diluted after administration, thus allowing the enzyme to recover its ability to hydrolyze the phenolic compound $\alpha$-glucosides. Phenolic compound $\alpha$-glucosides of the present invention and the $\alpha$-glucosidase or a micro-organism expressing $\alpha$-glucosidase activity can also be physically separated (e.g., microcapsule). [0406]

The present invention concerns the use of a phenolic compound $\alpha$-glucoside of the present invention or a pharmaceutically acceptable salt thereof for preparing a pharmaceutical or cosmetic composition for treating or preventing a cancer, cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder. In particular, the cancer is a solid tumor, for example a breast or colon cancer. In particular, the allergy can be allergic rhinoconjunctivitis. Therefore, the present invention also concerns a method for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder comprising administering a phenolic compound $\alpha$-glucoside of the present invention or a pharmaceutically acceptable salt thereof. In addition, the method can further comprise the step of administering sequentially or simultaneously a $\alpha$-glucosidase (EC 3.2.1.20) or a micro-organism expressing $\alpha$-glucosidase activity. Preferably, the $\alpha$-glucosidase (EC 3.2.1.20) or a micro-organism expressing $\alpha$-glucosidase activity is administered by the same route. [0407]

In a particular embodiment, the present invention concerns the use of a phenolic compound $\alpha$-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered topically (i.e., on skin), wherein enzymes issued from skin-associated micro-organisms release the corresponding aglycone. In addition, the present invention concerns the use of a phenolic compound $\alpha$-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered orally, wherein enzymes issued from mouth and intestinal tract-associated micro-organisms release the corresponding aglycone. The present invention also concerns the use of a phenolic compound $\alpha$-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered nasally, wherein enzymes issued from upper respiratory system-associated micro-organisms release the corresponding aglycone. The present invention further concerns the use of a phenolic compound $\alpha$-glucoside of the present invention for preparing a pharmaceutical or cosmetic composition to be administered vaginally, wherein enzymes issued from female genital tract-associated micro-organisms release the corresponding aglycone. [0408]

The present invention also concerns a combination of a phenolic compound $\alpha$-glucosides of the present invention or a pharmaceutically acceptable salt thereof with a $\alpha$-glucosidase (EC 3.2.1.20) or a micro-organism expressing $\alpha$-glucosidase activity for a simultaneous or sequential administration. When simultaneously administration is performed, the phenolic compound $\alpha$-glucosides of the present invention or a pharmaceutically acceptable salt thereof and the $\alpha$-glucosidase (EC 3.2.1.20) or a micro-organism expressing $\alpha$-glucosidase activity can be administered in the same or different compositions. [0409]

Such a composition can comprise pharmaceutically acceptable carrier, stabilizers or excipients. [0410]

Use of Phenolic Compounds as Key Intermediates for the Development of Other Derivatives [0411]

Phenolic compound $\alpha$-glucosides of the present invention can be directly used as active ingredients as cosmetics or as active substances alone or in combination with other products, including other active molecules with synergistic or complementary activities or with stabilizers or excipients. These phenolic compounds derivatives can also be used as starting materials for additional chemical, physical or enzymatic modification(s) in order to produce second generation derivatives. As the enzymatic reaction used in the present invention concerns specific hydroxyl positions on the catechol ring of the phenolic compound, the other hydroxyl groups can for example be used in a chemical reaction to create ester bonds, acyl bonds, sulphate or phosphate bonds. Such modifications can improve already existing properties of the phenolic compounds $\alpha$-glucosides of the present invention or provide new properties for specific applications (higher therapeutic efficiency, lower cytotoxicity, higher stability after release of the glycone part by micro-organisms, ...).

[0412] Formulation of Said Derivatives for Cosmetic or Therapeutic Applications [0413]

The compositions of the present invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intraduodenal and intracranial injection or infusion techniques. Preferably, the composition of the present invention are administered orally, by inhalation spray, topically, rectally, nasally, buccally or vaginally. In a preferred embodiment, the pharmaceutical or cosmetic composition is administered topically. [0414]

New types of cosmetic products are constantly being developed and new raw materials are adding to the cosmetic chemist's selection of personal care ingredients. The phenolic compounds $\alpha$-glucosides described in the present invention can easily be incorporated in a large panel of cosmetic products. Such preparations are well known from the man of the art: it can be creams, sticks, shampoo, shower gels, lotions, soaps, emulsions, gels. These formulations can include other ingredients such as but not limited to: deionized water, magnesium, aluminium silicate, xanthan gum, nylon-12, sodium PCA, propylene glycol, red iron oxides, talc, yellow iron oxides, black iron oxides, titanium dioxide, glyceryl stearate, stearic acid, DEA-cetyl phosphate, methylparaben, butylparaben, ethylparaben, propylparaben, isoearyl neopentanoate, isopropyl palmitate, ethylene/proplpylene/styrene, copolymers, butylene/ethylene/styrene copolymer, isopropyl palmitate, phenoxyethanol tocopheryl acetate, glycerin, triethanolamine, stearic acid, propylene glycol stearate, mineral oil, butylene/ethylene/styrene copolymer, diazolidinyl urea, hydrogenated polysobutylene, octyl palmitate, tridecyl neopentanoate, isoamyl isostearate, isopropylparaben, isobutylparaben, octyldecoh styrene copolymers, tocopheryl acetate, fragrance, octyl methoxycinnamate, ben...
Zophenone, octyl salicylate, isopropyl isostearate, propylene glycol isoceteth-3 acetate or any combinations thereof.

For their use in therapeutic applications, phenolic compounds O-α-glucosides of the present invention can be incorporated in different galenic preparations such as pills, tablets, syrups, creams, lotions, gels using for example packing, standardisation, blending/homogenisation, sterile and nonsterile micronization, granulation/compacting, sieving or any combination thereof. Preparations of said phenolic compounds O-α-glucosides can include some excipients of the following non exhaustive list: talc, lactose, magnesium stearate, glycerol monostearate, colloidal silicon dioxide, precipitated silicon dioxide, crosslinked polyvinyl pyrrolidone, dibasic calcium phosphate dihydrate, micro crystalline cellulose, corn starch, povidone, sodium carboxy-methyl cellulose, polysorbate 80, lactic acid, carbomer, cethyl alcohol, isopropyl myristate, isopropyl palmitate, glucose, dextrose, triethanolamine, glycerine, fructose, sucrose, polymers and nanostructures.

The compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, the compositions of this invention may be administered in the form of suppositories for rectal administration. These can be prepared by mixing the agent with a suitable non-irritating excipient that is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials include cocoa butter, beeswax and polyethylene glycols.

The compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, including diseases of the eye, the skin or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

For topical applications, the compositions may be formulated in a suitable ointment containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetaryl alcohol, 2-octyldecanol, benzyl alcohol and water.

For ophthalmic use, the compositions may be formulated as micronized suspensions in isotonic, pH adjusted sterile saline or, preferably, as solutions in isotonic, pH adjusted sterile saline, either with or without a preservative such as benzylalkonium chloride. Alternatively, for ophthalmic uses, the compositions may be formulated in an ointment such as petrolatum.

The compositions of this invention may also be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other conventional solubilizing or dispersing agents.

Sterile injectable forms of the compositions of this invention may be aqueous or an oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example as a solution in 1,3-butandiol. Among the acceptable vehicles and solvents that may be employed are water, Ringer’s solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils such as olive oil or castor oil, especially in their polyoxyethyalted versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens. Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid or other dosage forms may also be used for the purposes of formulation.

ADVANTAGES OF THE PRESENT INVENTION

The advantages of the method of the present invention over pre-existing methods appears clearly from the previous descriptions and embodiments. A non exhaustive list of others advantages of the present invention are described below.

The present invention describes new original phenolic compounds O-α-glucosides of

- proteocatechuic acid and its esters derivatives,
- caffeic acid and its esters derivatives, especially rosmarinic acid, chlorogenic acid and caffeic acid phenethyl ester and hydrocaffeic acid or 3,4-dihydroxydrocinnamic acid, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylglycol,
- esculetin,
- taxifolin,
- fisetin,
- eriodictyol,
- fustin,
- rhamnetin.

Preferably, the new original phenolic compounds O-α-glucosides of the present invention are selected in the group consisting of the epicatechin gallate O-α-glucoside, the eriodictyol O-α-glucoside, the esculetin O-α-glucoside, the fisetin O-α-glucoside, the fustin O-α-glucoside, the
homoprotocatechuic acid O-α-glucoside, the protocatechuic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the hydroxytyrosol O-α-glucoside, the maclurine O-α-glucoside, the nordihydroguaiaretic acid O-α-glucoside, the oleanolic O-α-glucoside, the pyrocathecol O-α-glucoside, the rutin O-α-glucoside, the rosmarinic acid O-α-glucoside, the taxifolin O-α-glucoside, the 3-hydroxy-
daidzein O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the caffeic acid O-α-glucoside, the dihydro-
caffeic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the cirsiliol O-α-glucoside, the chologenic acid O-α-glucoside, the anthrarohin O-α-glucoside, the epig-
gallocatechin O-α-glucoside, the dihydronobinetin O-α-
glucoside, the galloカテchin O-α-glucoside, the gallic acid O-α-
glucoside, the propyl gallate O-α-glucoside and the robinetin O-α-
glucoside.

[0434] These phenolic compounds O-α-glucosides, of high interest in the fields of cosmetic and therapy, shows improved water solubility. Indeed, an increase by at least 20, 30 or 50 folds of the solubility has been observed in comparison with the corresponding aglycone in the same physiological conditions.

[0435] These phenolic compounds O-α-glucosides have an increased bioavailability. These phenolic compounds O-α-
glucosides can be “in situ activated” through their hydrolysis into the initial phenolic structure by human commensal micro-organisms, giving them a “pro-drug” status of high interest for both cosmetic and therapy applications. They can also be activated with an α-glucosidase, such as the α-glucosidase produced by the yeast Saccharomyces cerevisiae.

[0436] These new phenolic compounds O-α-glucosides are obtained through a proven, reliable, low cost, “green chem-
istry” enzymatic process that ensures high quality of these products (due to the specificity and selectivity of the enzyme used).

EXAMPLES

[0437] Any other embodiments and advantages of the present invention will appear from the following examples that are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Example 1

Synthesis of Glucosylated Taxifolin; Solubility in Water of Highly Purified Glucosylated Taxifolin and Stability of the Glucosylated Derivative Molecule at Temperatures Ranging from 4°C to 45°C.

[0438] The conditions that were carried out for the synthesis of glucosylated Taxifolin are as follows (amounts for 1 liter of reaction medium):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Origin</th>
<th>Amount</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution of Taxifolin at</td>
<td>Sigma T 4512</td>
<td>100 ml</td>
<td>Taxifolin: 9 g/L</td>
</tr>
<tr>
<td>90 g/L in pure DMSO</td>
<td>Riedel de Haen</td>
<td>250 ml</td>
<td>Total DMSO: 350 ml/L</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>Prolabo 20104.298</td>
<td>40 ml</td>
<td>Sodium acetate: 20 mM</td>
</tr>
<tr>
<td>Water</td>
<td>Deionized</td>
<td>170 ml</td>
<td>3.1 U/ml</td>
</tr>
</tbody>
</table>

[0439] The reaction medium without the enzyme was first obtained by mixing the various solutions in the order reported in the table. The mixture was incubated at 30°C during a period of time sufficient to attain the desired temperature of 30°C (plus or minus 0.2°C). Then the reaction was started by introducing the enzyme preparation. The reaction medium may be moderately agitated.

[0440] The enzyme preparation has been obtained as follows: the culture broth of Leuconostoc mesenteroides NRRL B512-L titrating an enzyme activity ranging from 4 to 6 U/ml is centrifuged in order to completely separate the microbial cells from the liquid containing the enzyme. The centrifugation supernatant was then concentrated 4 to 10 times by tangential ultrafiltration (molecular weight cut off of 100 kDa). The retentate was then diluted 4 times with 20 mM acetate buffer pH 5.2 containing calcium chloride dihydrate at 10 mg/L and then concentrated 4 times in order to extensively remove the residual low molecular weight components of the cell culture medium containing the enzyme. The purified enzyme preparation has then been stored in a freeze-dried form (~20°C) or freeze dried up to several months without loss of activity. As a general procedure, the activity of the enzyme preparation is adjusted by intensifying the concentration of the retentate in order that the volume of the enzyme preparation will not be higher than 20% of the final volume of the synthesis reaction medium.

[0441] The reaction medium was incubated at 30°C (plus or minus 0.2°C) during 22 hours. An aliquot of the reaction medium was taken off from the reaction medium and diluted 50 times with a solution containing methanol and water in the proportions of 40:60. The methanolic solution was then analyzed by HPLC.

[0442] The analysis conditions were those as previously described except that the profile of the methanol concentration was as follows (Method 2):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Solvent A: deionized water containing 1% v/v acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent B: HPLC grade methanol containing 1% v/v acetic acid</td>
<td></td>
</tr>
<tr>
<td>0 to 10 minutes: 60% A; 40% B; 1 ml/minute</td>
<td></td>
</tr>
<tr>
<td>10 to 12 minutes: 60% to 20% A (linear); 40% to 80% B (linear); 1 ml/minute</td>
<td></td>
</tr>
<tr>
<td>12 to 14 minutes: 20% A; 80% B; 1 ml/minute</td>
<td></td>
</tr>
</tbody>
</table>
[0448] 14 to 16 minutes: 20% to 60% A (linear); 80% to 40% B (linear); 1 ml/minute
[0449] 16 to 25 minutes: 60% A; 40% B; 1 ml/minute
[0450] 25 minutes: next injection
[0451] FIG. 7 shows the HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm) at just the beginning of the incubation. The major pic at 8.15 minutes corresponds to Taxifolin.
[0452] FIG. 8 shows the HPLC chromatogram of the reaction medium containing Taxifolin as glucoside acceptor (289 nm) after 22 hours of incubation. A pic with a retention time of 6.15 minutes is observed.
[0453] FIG. 9 shows the mass spectrum and FIG. 10 the UV spectrum of the pic eluted at around 8.15 minutes: the substance is Taxifolin (m/z [M-H]: 302.96 and m/z [M-H—H₂O]: 284.96) which molecular weight is 304.
[0454] FIG. 11 shows the mass spectrum and FIG. 12 the UV spectrum of the pic eluted at around 6.15 minutes: the corresponding substance is Taxifolin glucoside (m/z [M-H]: 464.98) since its molecular weight is 466.
[0455] The substances eluted at 9.33 and 12.75 minutes are polyphenolic substances found in the Taxifolin preparation.
[0456] FIG. 13 shows a HPLC chromatogram of an aqueous solution containing Taxifolin and Taxifolin glucoside after carrying out purification to remove the enzyme, dextran, fructose and DMSO and a fraction of residual Taxifolin. The eluting conditions are those previously described in which the initial content of methanol is 10% (method 1). Taxifolin is eluted at 24.01 minutes and Taxifolin glucoside at 22.33 minutes.
[0457] Taxifolin glucoside has been purified extensively to reduce as much as possible the Taxifolin concentration. It was finally obtained a solution titrating more than 93 mM of Taxifolin glucoside with a Taxifolin residual concentration of less than 2 mM (FIG. 14: Taxifolin eluted at 8.95 minutes and Taxifolin glucoside eluted at 6.55 minutes).
[0458] Concentrations of Taxifolin glucoside were determined as follows: after having established the relationship between the molar concentration of Taxifolin and the pic areas with a precisely characterized Taxifolin preparation (SIGMA), concentrations of Taxifolin glucoside were determined by applying the relationship between area and concentration to Taxifolin glucoside, since Taxifolin and Taxifolin glucoside have the same UV spectra. Then, concentrations in g/L were obtained by multiplying the molar concentration by the value of the Taxifolin glucoside molecular weight (466).
Whereas the Taxifolin solubility in water at 25°C is measured at 1.19 g/L (3.91 mM), the solubility of Taxifolin glucoside in water at 25°C is higher than 43.5 g/L (93.2 mM).
[0459] It is thus possible, according to the described method, to synthesize a new substance, Taxifolin glucoside with a molecular weight of 466 and a solubility in water at around 25°C higher than 93 mM, corresponding to an increase in water solubility regarding the Taxifolin residue higher than 23. Taxifolin glucoside can be purified according to the techniques previously mentioned (resin adsorption, elution, concentration, liquid extraction, solvent removal and concentration and eventually drying).
[0460] The Taxifolin glucoside solution can be stored during a long period of time without loss of the glucoside bond and with a quite satisfactory resistance to oxidation.
[0461] Accelerated shelf-life studies were performed using temperature chambers at 4°C, 22°C, 37°C and 45°C for 4 months. The Taxifolin glucoside content was frequently measured and color and odor were roughly controlled. The Taxifolin glucoside content was determined by HPLC as previously described (500 fold dilution of an aliquot of the solution and analysis using method 2; detection: 210-400 nm).
[0462] The following table describes the observed quantity of Taxifolin glucoside versus the storage time at different storage temperatures.

<table>
<thead>
<tr>
<th>Days</th>
<th>Stored at +4°C</th>
<th>Stored at +22°C</th>
<th>Stored at +37°C</th>
<th>Stored at +45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>37</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>63</td>
<td>100</td>
<td>95</td>
<td>96</td>
<td>79</td>
</tr>
<tr>
<td>118</td>
<td>100</td>
<td>100</td>
<td>91</td>
<td>73</td>
</tr>
</tbody>
</table>

[0463] No colour or odour changes have been observed whatever the temperature of storage.
[0464] Therefore, the glucoside bond between Taxifolin and the glucose moiety is stable in the above tested conditions. At 37°C and 45°C, a slight degradation for the Taxifolin glucoside has been observed probably due to oxidation: indeed, no Taxifolin concentration increase that would indicate a hydrolysis of the glucoside bond was observed in the corresponding solutions. In the above mentioned conditions, half life of Taxifolin glucoside is estimated at 1.6 year at 37°C and 0.67 year at 45°C.
[0465] This example demonstrates that Taxifolin glucoside has a high chemical stability even in harsh storage conditions.

Example 2

Influence of the DMSO Concentration on the Efficiency of the Synthesis of Taxifolin Glucoside

[0466] Taxifolin glucoside enzymatic synthesis was carried out as described in Example 1 with the following exceptions:
[0467] enzyme concentration was 1 U/ml
[0468] DMSO concentration was 35%, 25%, 15% or 5%.
[0469] After 22 hours of incubation, relative Taxifolin glucoside concentrations in the four reaction medium are reported in the following table.

<table>
<thead>
<tr>
<th>DMSO, %</th>
<th>35</th>
<th>25</th>
<th>15</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taxifolin glucoside (relative concentration), %</td>
<td>100</td>
<td>133</td>
<td>161</td>
<td>17</td>
</tr>
</tbody>
</table>

[0470] The optimal DMSO concentration for the synthesis of Taxifolin glucoside appears to be at a value significantly lower than 30% and close to 15%.

Example 3

Activation of Taxifolin Glucoside by Human Skin Micro-Flora

[0471] Cutaneous flora was separately collected from 5 donors. The forearms and forehead of each donor were
scraped with a cotton-wool swab saturated with NaCl solution (v=5 mL, 8 g/l). After each scraping, the swab was divided into the remaining NaCl solution and squeezed to deliver the sampled material. After two cycles of scraping/squeezing on both forearms and three on the forehead, the obtained trouble preparation was filtered (40 μm) to eliminate squama and finally centrifuged (4°C, 5000 g, 15 min). The microbial pellets were resuspended in a NaCl solution (v=1 mL, 8 g/l) and characterized by OD at 600 nm.

**[0472]** The five microbial samples were mixed to form the final microbial suspension used for the test. Microbial cells were cultivated using the Hickey-Tresner culture medium (yeast extract at 1.0 g/L, meat extract at 1.0 g/L, casein peptone at 2.0 g/L, starch at 10.0 g/L, cobalt chloride hexahydrate at 20 mg/L; pH = 6). Microbial growth was carried out in 100 ml Erlenmeyer flasks at 37°C under continuous agitation (100 rpm). The sterile culture broth (20 ml) was inoculated with 0.1 mL of suspension. Microbial growth was controlled by measuring the OD at 600 nm.

**[0473]** Taxifolin glucoside was obtained as described in example 1 (highly purified preparation corresponding to the HPLC chromatogram reported in FIG. 14). Taxifolin glucoside was added or not at day 0 (V=0.5 mL of 0.20 μm sterilized solution). Control was made by growing the final microbial suspension without Taxifolin glucoside.

**[0474]** After centrifugation of an aliquot of cell culture media, the supernatant was diluted 4-fold with a solution containing methanol and water in the proportions of 40/60. Taxifolin glucoside and taxifolin concentrations in the supernatants were determined by HPLC (method 2).

**[0475]** FIG. 15 shows the apparent bacterial growth during a week in the Hickey-Tresner culture medium. From day 3 to day 7, the apparent biomass production is higher in presence of Taxifolin glucoside than in its absence. This might be explained by a higher concentration of carbon and energy source due to the liberation of the glucose from the taxifolin glucoside under the bacterial hydrolysis.

**[0476]** In FIG. 16, the hydrolysis of Taxifolin glucoside can not be detected during the first three days. After three days of incubation, probably when the carbon and energy source becomes limiting, the concentration of Taxifolin glucoside diminishes in a significant manner and the aglycone flavonoid, Taxifolin, appears concomitantly. The nutritional stress undergone by the bacterial community originating from the human cutaneous flora might stimulate the liberation of the glucosyl residue through the action of the secreted enzymes.

**[0477]** This example demonstrates that human cutaneous flora recognizes and is able to hydrolyze the flavonoid glucosidic bond with a high output, offering a new pathway for the delivery of active ingredients.

**Example 4**

**Activation of Taxifolin Glucoside by an α-Glucosidase Preparation**

**[0478]** Taxifolin glucoside was incubated in the presence of an α-glucosidase enzyme in the following conditions:

**[0479]** Taxifolin glucoside obtained as described in example 1 (highly purified preparation corresponding to the HPLC chromatogram reported in FIG. 14); 0.25 mL.

**[0480]** α-glucosidase (from Saccharomyces cerevisiae; FLUKA 70797, lot 0641337/1; activity: 5.8 U/mg): 50.1 mg in 5 ml of potassium phosphate buffer 0.1 M, pH 7.3; no enzyme in the control reaction medium;

**[0481]** Temperature: 30°C;

**[0482]** Moderate agitation.

**[0483]** The reaction media were analysed by HPLC (method 2) after a 2-fold dilution of an aliquot with methanol.

**[0484]** After 18 hours of incubation, the Taxifolin glucoside molecule remained unchanged in the medium which did not contain the α-glucosidase enzyme whereas the Taxifolin glucoside molecule was totally converted into Taxifolin in the presence of the α-glucosidase enzyme.

**[0485]** These results show that an isolated enzyme specific for the hydrolysis of α-glucosidic bonds is able to hydrolyse the Taxifolin glucoside molecule: this indicates that the Taxifolin glucoside molecule contains Taxifolin and glucose with glucose being linked to a hydroxyl group of Taxifolin through an α-glucosidic bond. For this reason, the synthesized new glucoside derivatives are claimed O-α-D-glucoside derivatives.

**Example 5**

**Enzymatic Synthesis of O-α-D-Glycosides of Pyro catechol, Protocatechuic Acid and Protocatechuic Acid Ethyl Ester**

**[0486]** Reaction media were prepared as described in example 1. Taxifolin being replaced by Pyrocatechol (SIGMA, reference C 9510), by Protocatechic acid (ALDRICH, reference D10, 980-0) or by Protocatechic acid ethyl ester (ALDRICH, reference E 2, 485-9).

**[0487]** After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

**[0488]** The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>m/z</th>
<th>Identification (theoretical molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrocatechol</td>
<td>13.78</td>
<td>108.74</td>
<td>Pyrocatechol (110)</td>
</tr>
<tr>
<td>(chromatogram at 276 nm)</td>
<td>16.80</td>
<td>271.01</td>
<td>Pyrocatechol monoglucoside (272)</td>
</tr>
<tr>
<td></td>
<td>14.88</td>
<td>433.65</td>
<td>Pyrocatechol diglucoside (434)</td>
</tr>
<tr>
<td></td>
<td>13.22</td>
<td>595.66</td>
<td>Pyrocatechol triglucoside (556)</td>
</tr>
<tr>
<td></td>
<td>11.87</td>
<td>919.35</td>
<td>Pyrocatechol pentaglucoside (920)</td>
</tr>
<tr>
<td>Protocatechic acid</td>
<td>11.26</td>
<td>152.88</td>
<td>Protocatechic acid (154)</td>
</tr>
<tr>
<td>(chromatogram at 294 nm)</td>
<td>8.25</td>
<td>315.05</td>
<td>Protocatechic acid monoglucoside (316)</td>
</tr>
<tr>
<td></td>
<td>7.89</td>
<td>477.00</td>
<td>Protocatechic acid diglucoside (478)</td>
</tr>
<tr>
<td></td>
<td>7.15</td>
<td>801.26</td>
<td>Protocatechic acid tetraglucoside (802)</td>
</tr>
<tr>
<td>Protocatechic acid ethyl ester</td>
<td>28.28</td>
<td>180.96</td>
<td>Protocatechic acid ethyl ester (182)</td>
</tr>
<tr>
<td>(chromatogram at 295 nm)</td>
<td>27.30</td>
<td>343.02</td>
<td>Protocatechic acid ethyl ester monoglucoside (344)</td>
</tr>
<tr>
<td></td>
<td>24.99</td>
<td>505.65</td>
<td>Protocatechic acid ethyl ester diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>20.54</td>
<td>829.30</td>
<td>Protocatechic acid ethyl ester tetruglucoside (830)</td>
</tr>
</tbody>
</table>

**[0489]** It is thus possible, according to the described method, to synthesize the new glucosylated derivatives of Pyrocatechol, Protocatechic acid and Protocatechuic acid.
ethyl ester: the resulting products are a family of substances containing at least monoglucosylated, diglucosylated, triglucosylated and tetraglucosylated derivatives.

Example 6
Enzymatic Synthesis of O-α-D-Glycosides of Caffeic Acid, 3,4-Dihydroxyhydrocinamic Acid (Hydrocaffeic Acid) and Rosmarinic Acid

[0490] Reaction media were prepared as described in example 1, Taxifolin being replaced by Caffeic acid (SIGMA, reference C 0625), by 3,4-Dihydroxyhydrocinamic acid (ALDRICH, reference D10, 980-0) or by Rosmarinic acid (FLUKA, reference 44699; the concentration of Rosmarinic acid in the reaction medium was 1 g/L).

[0491] After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

[0492] The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>Identification (theroretical Glucosyl acceptor minutes m/z M-H molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid</td>
<td>19.53</td>
<td>178.97 Caffeic acid (180)</td>
</tr>
<tr>
<td></td>
<td>15.46</td>
<td>341.09 Caffeic acid monoglucoside (342)</td>
</tr>
<tr>
<td></td>
<td>14.62</td>
<td>503.16 Caffeic acid diglucoside (504)</td>
</tr>
<tr>
<td>3,4-Dihydroxyhydrocinamic acid</td>
<td>18.72</td>
<td>343.02 Hydrocaffeic acid monoglucoside (344)</td>
</tr>
<tr>
<td></td>
<td>17.93</td>
<td>505.05 Hydrocaffeic acid diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>17.80</td>
<td>180.98 Hydrocaffeic acid (182)</td>
</tr>
<tr>
<td></td>
<td>17.50</td>
<td>343.02 Hydrocaffeic acid monoglucoside (344)</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>17.06</td>
<td>687.21 Hydrocaffeic acid triglucoside (668)</td>
</tr>
<tr>
<td></td>
<td>16.23</td>
<td>829.25 Hydrocaffeic acid tetraglucoside (830)</td>
</tr>
<tr>
<td></td>
<td>16.01</td>
<td>505.05 Hydrocaffeic acid diglucoside (506)</td>
</tr>
<tr>
<td></td>
<td>15.70</td>
<td>992.36 Hydrocaffeic acid pentaglucoside (992)</td>
</tr>
<tr>
<td></td>
<td>14.70</td>
<td>687.21 Hydrocaffeic acid hexaglucoside (1154)</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>13.92</td>
<td>829.39 Hydrocaffeic acid triglucoside (830)</td>
</tr>
<tr>
<td></td>
<td>13.22</td>
<td>991.50 Hydrocaffeic acid pentaglucoside (992)</td>
</tr>
<tr>
<td></td>
<td>12.61</td>
<td>1153.60 Hydrocaffeic acid hexaglucoside (1154)</td>
</tr>
<tr>
<td></td>
<td>12.08, 11.21</td>
<td># Harmanization degree higher than 6</td>
</tr>
<tr>
<td></td>
<td>28.36</td>
<td>359.09 Rosmarinic acid (360)</td>
</tr>
<tr>
<td></td>
<td>27.18</td>
<td>521.16 Rosmarinic acid monoglucoside (322)</td>
</tr>
</tbody>
</table>

[0493] It is thus possible, according to the described method, to synthesize the new glucosylated derivatives of Caffeic acid, Hydrocaffeic acid and Rosmarinic acid: the resulting products are a family of substances containing at least monoglucosylated, diglucosylated, triglucosylated and tetraglucosylated derivatives. As far as Hydrocaffeic acid is concerned, it clearly appears that both hydroxyl groups have been substituted: indeed, it can be seen at least two series of derivatives, both containing at least monoglucosylated (344), diglucosylated (506), triglucosylated (668), tetraglucosylated (830) and pentaglucosylated (992) derivatives. This shows that in some cases that can not be predicted by a skilled man, both hydroxylated groups can accept a glucose moiety.

Example 7
Enzymatic Synthesis of O-α-D-Glycosides of 3,4-Dihydroxymandel acid, Esculetin and Esculin

[0494] Reaction media were prepared as described in example 1, Taxifolin being replaced by 3,4-Dihydroxymandel acid (ALDRICH, reference 151610), or by Esculetin (ALDRICH, reference 24, 657-3) or Esculin (SIGMA, reference E 8250).

[0495] After 21 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed by HPLC (method 1).

[0496] The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, minutes</th>
<th>Identification (theroretical Glucosyl acceptor minutes m/z M-H molecular weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,4-Dihydroxymandel acid</td>
<td>14.68</td>
<td>136.82 Unknown</td>
</tr>
<tr>
<td></td>
<td>5.32</td>
<td>136.164 Unknown</td>
</tr>
<tr>
<td></td>
<td>4.10</td>
<td>182.95 3,4-Dihydroxymandel acid (184)</td>
</tr>
<tr>
<td></td>
<td>3.45</td>
<td>341.03 Unknown</td>
</tr>
<tr>
<td></td>
<td>2.79</td>
<td>341.03 Unknown</td>
</tr>
<tr>
<td></td>
<td>2.49</td>
<td>140.80 Unknown</td>
</tr>
<tr>
<td>Esculetin</td>
<td>18.36</td>
<td>176.91 Esculetin (178)</td>
</tr>
<tr>
<td></td>
<td>15.65</td>
<td>339.03 Esculetin monoglucoside (340)</td>
</tr>
<tr>
<td></td>
<td>14.74</td>
<td>501.06 Esculetin diglucoside (502)</td>
</tr>
<tr>
<td></td>
<td>12.25</td>
<td>987.40 Esculetin pentaglucoside (988)</td>
</tr>
<tr>
<td></td>
<td>11.686</td>
<td>1149.55 Esculetin diglucoside (1150)</td>
</tr>
<tr>
<td>Esculin or Esculetin 6-O-β-D-glucopyranoside</td>
<td>18.30</td>
<td>176.91 Esculetin (178)</td>
</tr>
<tr>
<td></td>
<td>13.69</td>
<td>338.99 Esculin or Esculetin 6-O-β-D-glucopyranoside</td>
</tr>
<tr>
<td></td>
<td>11.38</td>
<td>501.06 Esculin monoglucoside (502)</td>
</tr>
<tr>
<td></td>
<td>10.73</td>
<td>663.15 Esculin diglucoside (664)</td>
</tr>
<tr>
<td></td>
<td>9.38</td>
<td>1149.48 Esculin tetraglucoside (1150)</td>
</tr>
</tbody>
</table>

[0497] 3,4-Dihydroxymandel acid contains a pyrocatechol structure as Taxifolin. Pyrocatechol, Protocatecnergid acid, Caffeic acid: nevertheless, no glucosylated derivative of 3,4-Dihydroxymandel acid has been synthesized in the present conditions.

[0498] In an unexpected manner, the 6,7-dihydroxycoumarin skeleton is also a glucoside acceptor which leads to a series of glucosylated Esculetin. It has to be underlined that the synthesized Esculetin monoglucoside has a retention time of 15.65 minutes whereas the natural glucosylated Esculetin (Esculin or Esculetin 6-O-β-D-glucopyranoside) has a retention time of 13.69 minutes: this has to be attributed that the osidic bond in the case of the natural molecule is of the α-type whereas the osidic bond in esculin is of the type-[β].
A sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40:60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min</th>
<th>Identification</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>10.40</td>
<td>Gallic acid O-α-glucoside</td>
<td>G6</td>
</tr>
<tr>
<td>5.95</td>
<td>Gallic acid O-β-glucoside</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>Propyl Gallate</td>
<td>27.22</td>
<td>Propyl Gallate O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>Epigallocatechin Gallate</td>
<td>15.53</td>
<td>Epigallocatechin Gallate O-α-glucoside</td>
<td>G1</td>
</tr>
</tbody>
</table>

Analysis conditions:
G6: flow rate 1 ml/min; from 0 to 10 min; B increases linearly from 10 to 20% from 10 to 25 min; B increases linearly from 20 to 50% from 25 to 30 min; B is stable at 50% from 30 to 35 min; B decreases linearly from 50 to 10%.

It is thus possible, according to the described method, to synthesize the new glucosylated derivatives of Gallic acid, Propyl Gallate and Epigallocatechin Gallate: the resulting products are a family of substances containing at least a monoglucosylated derivative.

### Example 9

**Enzymatic Synthesis of O-α-D-Glycosides of Caffeic Acid Phenethyl ester, Chlorogenic Acid and 3,4-Dihydroxybenzophenone**

Reaction media were prepared as described in example 1, Taxifolin being replaced by Caffeic acid Phenethyl ester (SIGMA, reference C8221), by Chlorogenic acid (SIGMA, reference 03878) or by 3,4-dihydroxybenzophenone (ALDRICH, reference 579815) and the DMSO concentrations were 15% and 25% v/v.

### Example 10

**Enzymatic Synthesis of O-α-D-Glycosides of Catechin, Erdictyol, Fisetin, Oleuropein and Nordihydroguaiaretic Acid**

Reaction media were prepared as described in example 1, Taxifolin being replaced by catechin (FLUKA, reference 22110), by Erdictyol (EXTRASYNTHES, reference 0056), by Fisetin (SIGMA, reference F4043), by Oleuropein (EXTRASYNTHES, reference 0204) or by Nordihydroguaiaretic acid (EXTRASYNTHES, reference 6135) and the DMSO concentrations were 15% and 25% v/v.

After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40:60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor (DMSO 15 and 25%)</th>
<th>Retention time, min</th>
<th>Identification</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeic acid Phenethyl ester</td>
<td>20.15</td>
<td>Caffeic acid Phenethyl ester</td>
<td>G2</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>15.53</td>
<td>Chlorogenic acid</td>
<td>G1</td>
</tr>
<tr>
<td>3,4-Dihydroxybenzophenone</td>
<td>32.35</td>
<td>3,4-Dihydroxybenzophenone</td>
<td>G1</td>
</tr>
</tbody>
</table>

Analysis conditions:
G1: see example 8
G2: flow rate 1 ml/min; from 0 to 20 min; B increases linearly from 40 to 80%; from 20 to 22 min; B is stable at 80%; from 22 to 27 min; B decreases linearly from 80 to 40%.

It is thus possible, according to the described method, to synthesize the new glucosylated derivatives of Caffeic acid Phenethyl ester, Chlorogenic acid and 3,4-Dihydroxybenzophenone: the resulting products are a family of substances containing at least a monoglucosylated derivative.
1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor (DMSO 15 and 25%)</th>
<th>Retention time, min (Identification)</th>
<th>Retention time, min (Identification)</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>14.07</td>
<td>12.60 Catechin O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>Eriodictyol</td>
<td>30.10 Eriodictyol</td>
<td>27.18 and 26.00 Eriodictyol O-α-glucoside and Eriodictyol di-O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>Fisetin</td>
<td>29.37 Fisetin</td>
<td>26.05 Fisetin O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>Oleuropein</td>
<td>28.28 Oleuropein</td>
<td>26.45 and 24.68 Oleuropein O-α-glucoside and Oleuropein di-O-α-glucoside</td>
<td>G1</td>
</tr>
<tr>
<td>Nordsilydroglauceric acid</td>
<td>18.53 Nordsilydroglauceric acid</td>
<td>16.97 and 16.40 majority products</td>
<td>G2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>O-α-glucosides of Nordsilydroglauceric acid</td>
</tr>
</tbody>
</table>

Analysis conditions:
- G1: see example 8
- G2: see example 9

It is thus possible, according to the described method, to synthesize the new glucosylated derivatives of Catechin, Eriodictyol, Fisetin, Oleuropein and Nordsilydroglauceric acid: the resulting products are a family of substances containing at least a monoglucosylated derivative.

Example 11

Enzymatic Synthesis of O-α-D-Glycosides of Catechin, 3,4-Dihydroxybenzoic Acid, Gallic Acid, Rosmarinic Acid, Caffeic Acid and Chlorogenic Acid in Strictly Aqueous Media

Reaction media were prepared as described in example 1, Taxifolin being replaced by Catechin (FLUKA, reference 22110) at a concentration of 7.5 g/L, by 3,4-Dihydroxybenzoic acid (ALDRICH, reference D10, 980-0) at a concentration of 9.0 g/L, by Gallic acid (FLUKA, reference 48630) at a concentration of 9.0 g/L, by Rosmarinic acid (FLUKA, reference 44609) at a concentration of 7.5 g/L, by Caffeic acid (SIGMA, reference CO925) at a concentration of 9.0 g/L or by Chlorogenic acid (SIGMA, reference C3878) at a concentration of 7.5 g/L. The DMSO was omitted whereas the sodium acetate buffer concentration was increased to 100 mM and the enzyme activity was reduced to 1.0 U/ml.

After 6 hours of incubation, a sample of each reaction medium was diluted 5 times with a solution containing methanol and water in the proportions of 40/60 and then analysed using the HPLC equipment previously described with a combination of eluant A (deionized water containing 1% v/v acetic acid) and eluant B (HPLC grade methanol containing 1% v/v acetic acid) as reported hereafter.

The results are reported in the following table.

<table>
<thead>
<tr>
<th>Glucosyl acceptor</th>
<th>Retention time, min (Identification)</th>
<th>Retention time, min (Identification)</th>
<th>Analysis conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ellagic acid</td>
<td>27.42 Ellagic acid</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G1</td>
</tr>
<tr>
<td>Rutin</td>
<td>26.33 Rutin</td>
<td>No other pic and thus no O-α-glucoside of Ellagic acid</td>
<td>G1</td>
</tr>
</tbody>
</table>
We claim:

1. A method for producing a phenolic compound O-α-glucoside comprising incubating sucrose and a glucansucrase from _Leuconostoc_ species with a phenolic compound having the following formula:

   ![Chemical Structure](image)

   wherein
   - R2 is H or OH; and
   - R1 is selected from the group consisting of

   ![Chemical Structure](image)

   wherein
   - R3 and R4, independently, are H or OH, with the proviso that at least one among R3 and R4 represents OH;

   ![Chemical Structure](image)

   wherein
   - R7 is selected from the group consisting of H, or
   - OH and R8 is H or OH, with the proviso that at least one among R7 and R8 represents OH.

[0519] Though the tested substances contain a pyrocatechol structure the substituents of the ring do not allow their recognition by the enzyme. In the case of rutin, the saccharide part of quercetin 3-O-rutinoside appears to be very important for the enzyme recognition since quercetin is glucosylated in the 3' and/or 4' position (BERTRAND et al.) whereas rutin is not.
wherein R5 is OH or OCH₃; R6 is H or OH, R9 is H or OH, R10 is H, OCH₃ or C₆H₅O₂-, and R11 is H, OH or C₆H₅O₂-, with the proviso that R10 and R11 cannot be both H when R5 and R6 are both OH and that when R10 is C₆H₅O₂- then R11 is H;

—(CH₂)ₙ—COOR or —(CH₂)ₙ—CONH₂, with n being an integer from 0 to 2;

—(CR₁₂=CH)ₙ—COOR or —(CR₁₂=CH)ₙ—CONH₂R₁₂, R₁₂ being H or a C₁₋₇ linear or cyclic alkyl or alkenyl;

—(CH₂)ₙ—COR or —(CH₂)ₙ—COOR with n being an integer from 0 to 2;

—H;

and,

a C₁₀₋₁₄ hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of R1, said ring being optionally interrupted by at least one heteroatom;

wherein R is H or a linear, branched, or cyclic, aromatic or not, saturated or unsaturated, C₁₋₁₀ hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an alkenyl, or an alkynyl, which can be substituted by one or several substituents selected from the group consisting of: an (C₂₋₅-C₅)aryl, a (C₂₋₅-C₅)heterocycle, an (C₁₋₇) alkoxy; an (C₂₋₅-C₅)acyl, an (C₁₋₇)alkoxycarbonyl, a carboxylic group (—COOH), an (C₂₋₇)ester, an (C₂₋₇)amine, an amino group (—NH₂), an amide (—CONH₂), an (C₁₋₇) imine, a nitrile, a hydroxyl (—OH), an aldehyde group (—CHO), a halogen, a (C₁₋₇) halogenoalkyl, a thiol (—SH), a (C₁₋₇)thioalkyl, a (C₁₋₇)sulfone, a (C₁₋₇)sulfoxide, and a combination thereof.

2. The method according to claim 1, wherein:

a) R₁ of the phenolic compound is

wherein R₃ and R₄, independently, are H or OH, with the proviso that at least one among R₃ and R₄ represents OH; or

b) R₁ of the phenolic compound is

wherein R₇ is selected from the group consisting of H, or —OH and R₈ is H or OH, with the proviso that at least one among R₇ and R₈ represents OH; or
e) R₁ of the phenolic compound is

wherein R₅ is OH or OCH₃; R₆ is H or OH, R₉ is H or OH, R₁₀ is H, OCH₃ or C₆H₅O₂-, and R₁₁ is H, OH or C₆H₅O₂-, with the proviso that R₁₀ and R₁₁ cannot be both H when R₅ and R₆ are both OH and that when R₁₀ is C₆H₅O₂- then R₁₁ is H;

d) R₁ of the phenolic compound is —(CH₂)ₙ—COOR or —(CH₂)ₙ—CONH₂R₁₂, R₁₂ being H or a C₁₋₇ linear, branched or cyclic alkyl or alkenyl, or

e) R₁ of the phenolic compound is —(CR₁₂=CH)ₙ—COOR or —(CR₁₂=CH)ₙ—CONH₂R₁₂, R₁₂ being H or a C₁₋₇ linear, branched or cyclic alkyl or alkenyl, or
f) $R_1$ of the phenolic compound is $-(CH_2)_n-COR$ or $-(CH=CH)_n-COR$ with $n$ being an integer from 0 to 2; or
g) $R_1$ of the phenolic compound is H; or
h) $R_1$ of the phenolic compound is

![Diagram of phenolic compound with $R_1$](image)
or
i) $R_1$ of the phenolic compound is

![Diagram of phenolic compound with another $R_1$](image)

j) $R_1$ of the phenolic compound is

![Diagram of phenolic compound with yet another $R_1$](image)

k) $R_1$ of the phenolic compound is

![Diagram of phenolic compound with another $R_1$](image)

l) $R_1$ of the phenolic compound is a C$_x$-C$_y$ hydrocarbon group which forms with the represented ring of formula

![Diagram of phenolic compound with a hydrocarbon group](image)

(I) a fused ring (bi or tricyclic) together with the ortho carbon of $R_1$, said ring being optionally interrupted by at least one heteroatom; or

m) phenolic compound is selected from the group consisting of

![Chemical structures](image) and

3. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of the taxifolin, the eriodictyol, the dihydroquercetin and the fustin.

4. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of catechin, epicatechin, catechin gallate, epicatechin gallate, gallatechin, epigallocatechin, and gallocatechin gallate.

5. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of rhamnetin, tisinetin, robinetin, gossypetin, orientin, homoorientin and teirstiol.

6. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of homoprotocatechuic acid, dihydrocaffeic acid, protocatechuic acid ethyl ester, propyl gallate, tiglic acid, hamamelitannin (2',5-di-[ß]-galloyl-hamamelose) and protocatechuic acid.

7. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of caffeic acid, rosmarinic acid, esculetin, 4-methylesculetin, norelabergin (6,7-dihydroxyphenylcoumarin), chlorogenic acid, caffeic acid phenethyl ester, choric acid (dicafeoyl tartaric acid), echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl-(1→3)—O—(beta-D-glucopyranosyl-(1→6))- 4-(3-(3,4-dihydroxyphenyl)-2-propanoate), beta-D-glucopyranoside) and verbascoside.

8. The method according to claim 2, wherein the phenolic compound is selected from the group consisting of maclurin, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzophenone, butein (2',3,4,4'-tetrahydroxychalcone), 3,4-dihydroxyacetophenone, marein (2',3,3',4,4'-pentahydroxy-4'-glucosylchalcone), and eriodictyolchalcone (2',4',6',3,4-pentahydroxychalcone).
9. A phenolic compound O-α-glucoside having the following formula:

\[
\begin{align*}
R_1 & \quad R_2 \\
O & \quad OB \quad OA \\
\text{wherein} \\
A \text{ and } B, \text{ identical or different, are } H \text{ or an } \alpha-\text{glucosyl residue, with the proviso that at least one of } A \text{ and } B \text{ is an } \alpha-\text{glucosyl residue; } \\
R_2 & \text{ is } H \text{ or } OH; \text{ and,} \\
R_1 & \text{ is selected from the group consisting of:}
\end{align*}
\]

\[
\begin{align*}
R_3 & \quad O \\
R_4 & \quad \text{wherein } R_3 \text{ and } R_4, \text{ independently, are } H \text{ or OH, with the proviso that at least one among } R_3 \text{ and } R_4 \text{ represents OH;} \\
R_7 & \quad R_8 \\
\text{wherein } R_7 \text{ is selected from the group consisting of } H, \text{ or } OH \text{ or OH, with the proviso that, when } R_2 \text{ is } H, \text{ } R_7 \text{ and } R_8 \text{ are not both OH, and at least one among } R_7 \text{ and } R_8 \text{ is OH;} \\
R_5 & \quad R_6 \\
\text{wherein } R_5 \text{ is OH or OCH}_3; \text{ R}_6 \text{ is } H \text{ or OH; } R_9 \text{ is } H \text{ or OH, } R_{10} \text{ is } H, \text{ OCH}_3 \text{ or } C_9H_{11}O_5, \text{ and } R_{11} \text{ is } H, \text{ OH or } C_9H_{11}O_5, \text{ with the proviso that } R_{10} \text{ and } R_{11} \text{ cannot be both } H \text{ when } R_5 \text{ and } R_6 \text{ are both OH and that when } R_{10} \text{ is } C_9H_{11}O_5 \text{ then } R_{11} \text{ is } H; \quad -(CH_2)_n-COOR \text{ or } -(CH_2)_n-CONH_R, \text{ with } n \text{ being an integer from } 0 \text{ to } 2; \\
-(CR_{12}=CH)-COOR \text{ or } -(CR_{12}=CH)-CONHR, \text{ R}_{12} \text{ being } H \text{ or a } C_1-C_6 \text{ linear, branched or cyclic alkyl or alkynyl;}
\end{align*}
\]

\[
\begin{align*}
\text{a } C_1-C_{10} \text{ hydrocarbon group which forms with the represented ring of formula (I) a fused ring (bi or tricyclic) together with the ortho carbon of } R_1, \text{ said ring being optionally interrupted by at least one heteroatom;} \\
\text{wherein } R \text{ is } H \text{ or a linear, branched, or cyclic, aromatic or } \text{not, saturated or unsaturated, } C_1-C_{10} \text{ hydrocarbon group, optionally interrupted by at least one heteroatom, wherein said hydrocarbon group comprises an alkyl, an \alkenyl, or an alkynyl, which can be substituted by one or several substituents selected from the group consisting of: an } (C_2-C_9) \text{aryl, a } (C_2-C_9) \text{heterocycle, an } (C_1-C_3) \text{alkoxy, an } (C_2-C_3) \text{acyl, an } (C_1-C_3) \text{alcohol, a carboxylic group (} -COOH, \text{ an } (C_2-C_6) \text{ester, an } (C_1-C_3) \text{amine, an amino group (} -NH_2, \text{ an amide (} -CONH_2, \text{ an } (C_1-C_3) \text{amine, a nitrile, a hydroxyl (} -OH, \text{ an aldehyde group (} -CHO, \text{ a halogen, a } (C_1-C_3) \text{halogenoalkyl, a thiol (} -SH, \text{ a } (C_1-C_3) \text{thioalkyl, a } (C_1-C_3) \text{sulfone, a } (C_1-C_3) \text{sulfoxide, and a combination thereof.}
\end{align*}
\]
The phenolic compound O-α-glucoside according to claim 9, wherein:

a) R1 is and the phenolic compound O-α-glucoside is selected from the group consisting of the taxifolin O-α-glucoside, the eriodictyol O-α-glucoside, the dihydrorobinetin O-α-glucoside and the fustin O-α-glucoside; or

b) R1 is

and the phenolic compound O-α-glucoside is selected from the group consisting of the catechin gallate O-α-glucoside, the epicatechin gallate O-α-glucoside, the gallolic acid O-α-glucoside, the epigalloacetechin O-α-glucoside, the gallocatechin gallate O-α-glucoside; or
c) R1 is

and the phenolic compound O-α-glucoside is selected from the group consisting of the rhamnetin O-α-glucoside, the fisetin O-α-glucoside, the robinetin O-α-glucoside, the gossypetin O-α-glucoside, the orientin O-α-glucoside, the homoerocitrin O-α-glucoside and the cirsiirol O-α-glucoside; or
d) R1 is —(CH2)n—COOR or —(CH2)n—CONHR with n being an integer from 0 to 2, and the phenolic compound O-α-glucoside is selected from the group consisting of the homoprotocatechuic acid O-α-glucoside, the dihydrocaffeic acid O-α-glucoside, the protocatechuic acid ethyl ester O-α-glucoside, the propyl gallate O-α-glucoside, the gallic acid O-α-glucoside, the humamellatin O-α-glucoside and the protocatechic acid O-α-glucoside; or
e) R1 is —(CR12=CH)—COOR or —(CR12=CH)—CONHR, R12 being H or a C1–C6 linear or cyclic alkyl or alkenyl and the phenolic compound O-α-glucoside is selected from the group consisting of the caffeic acid O-α-glucoside, the rosmarinic acid O-α-glucoside, the esculetin O-α-glucoside, the 4-methylesculetin O-α-glucoside, the nordalbergin (6,7-dihydroxyphenylcoumarin) O-α-glucoside, the chlorogenic acid O-α-glucoside, the caffeic acid phenethyl ester O-α-glucoside, the chicoric acid (dihydrocaffeic acid O-α-glucoside, the echinacoside (2-(3,4-dihydroxyphenyl)ethyl O-6-deoxy-alpha-L-mannopyranosyl(1→3)—O—(beta-D-glucopyranosyl(1→6))−, 4-(3,4-dihydroxyphenyl)-2-propenoate) O-α-glucoside, beta-D-glucoxyranoside O-α-glucoside and the verbascoside O-α-glucoside; or
f) R1 is —(CH2)n—COR or —(CH2)n—CONH—COR with n being an integer from 0 to 2, and the phenolic compound O-α-glucoside is selected in the group consisting of the maclurin O-α-glucoside, the 3,4-dihydroxybenzaldehyde O-α-glucoside, the 3,4-dihydroxybenzophenone O-α-glucoside, the butein (2,3,4,4'-tetrahydrochalcone) O-α-glucoside, the 3,4-dihydroxycacetophenone O-α-glucoside, the marein (2,3,3',4',4'-pentahydroxy-4'-glucosylchalcone) O-α-glucoside, and the epidietyolchalcone (2',4',6',5,3,4-pentahydroxychalcone) O-α-glucoside; or
g) R1 is selected from the group consisting of H;
being optionally interrupted by at least one heteroatom, and the phenolic compound O-α-glucoside is selected in the group consisting of the anthraquinone O-α-glucoside and the salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydropyridazine) O-α-glucoside.

11. A pharmaceutical or cosmetic composition comprising a phenolic compound O-α-glucoside according to claim 9.

12. A method for treating or preventing a cancer, a cardiovascular disease, a bacterial infection, a UVB-induced erythema, an allergy, an inflammatory or immune disorder in a subject, comprising administering to the subject an effective amount of phenolic compound O-α-glucoside according to claim 9.

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