

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2017/182340 A1

(43) International Publication Date
26 October 2017 (26.10.2017)

(51) International Patent Classification:

A61K 8/60 (2006.01) A61K 8/66 (2006.01)
A61Q 5/10 (2006.01)

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/EP2017/058715

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(22) International Filing Date:

12 April 2017 (12.04.2017)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1653598 22 April 2016 (22.04.2016) FR

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(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ,
CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR,
KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

(54) Title: USE OF O-GLYCOSYLATED INDOLE OR INDOLINE DERIVATIVE WITH GLYCOSIDASE FOR DEYING KER-
ATIN FIBERS

(57) Abstract: A subject of the invention is the use of i) hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group, in the presence of at least ii) one enzyme with glycosidase activity, in particular beta-glycosidase activity, more particularly glucosidase activity and even more particularly beta-glucosidase activity, for treating keratin fibres, in particular dyeing human keratin fibres such as the hair, preferably dyeing said fibres. A subject of the invention is also a process for treating keratin fibres using i), ii) and a kit comprising the ingredients i) and ii). The process according to the invention has the advantage of dyeing human keratin fibres, with powerful or chromatic dyeing results that are resistant to washing, perspiration, sebum and light, and that are moreover long-lasting, without impairing said fibres. Furthermore, the colourations obtained from the process give uniform colours from the root to the tip of a fibre (low colouration selectivity). In addition, the compositions comprising the i) hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups degrade very little in air and remain stable with respect to storage.



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USE OF O-GLYCOSYLATED INDOLE OR INDOLINE DERIVATIVES IN THE PRESENCE OF GLYCOSIDASE FOR DYEING KERATIN FIBRES

A subject of the invention is the use of i) hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group, in the presence of at least ii) one enzyme with glycosidase activity, in particular beta-glycosidase activity, more particularly glucosidase activity and even more particularly beta-glucosidase activity, for treating keratin fibres, in particular dyeing human keratin fibres such as the hair, preferably dyeing said fibres. A subject of the invention is also a process for treating keratin fibres using i), ii) and a kit comprising the ingredients i) and ii).

It is known practice to obtain "permanent" colourings with dye compositions comprising oxidation dye precursors, generally known as oxidation bases, such as ortho- or para-phenylenediamines, ortho- or para-aminophenols and heterocyclic compounds. These oxidation bases are colourless or weakly coloured compounds, which, when combined with oxidizing products, may give rise to coloured compounds via a process of oxidative condensation. It is also known that the shades obtained may be varied by combining these oxidation bases with colour modifiers or couplers, the latter being chosen especially from aromatic meta-diamines, meta-aminophenols, meta-diphenols and certain heterocyclic compounds such as indole compounds. This oxidation dyeing process consists in applying, to the keratin fibres, bases or a mixture of bases and couplers with hydrogen peroxide (H_2O_2 or aqueous hydrogen peroxide solution) as oxidizing agent, in leaving to diffuse and in then rinsing the fibres. The colourings which result therefrom are permanent, powerful and resistant to external agents, in particular to light, bad weather, washing operations, perspiration and rubbing actions.

However, the commercial hair dyes which contain them can exhibit disadvantages, such as staining and problems of odour, comfort and degradation of the keratin fibres. This is particularly the case with oxidation dyeing operations.

It is also known to dye keratin fibres with "auto-oxidizable" dyes such as 5,6-dihydroxyindoles or 5,6-dihydroxyindolines, i.e. dyes which gradually oxidize with atmospheric oxygen, in particular in a basic medium. However, the results obtained via that process are not always satisfactory, in particular in terms of intensity of colouring, and the risks of colour homogeneity of the keratin fibres are non-negligible. Furthermore, since these dyes can oxidize in the presence of atmospheric oxygen, this reactivity represents a risk of degradation during storage and reduces their colouring performance on keratin fibres. It should also be noted that auto-oxidizable dyes can mark clothing fabrics, which can be problematic when it is desired to carry out "clean" dyeing.

Moreover, it is known to use, for dyeing the hair, indole derivatives which are O-glycosylated in position 3 and which can be hydrolysed by an enzyme of β -glucosidase type (US 6 656 229). Nevertheless, the colourings obtained are not always satisfactory, in particular in terms of colour intensity or of colouring homogeneity between the root and end

of the keratin fibres, or between fibres with respect to one another.

There is consequently a real need to develop dyeing processes and hair compositions which make it possible to obtain powerful colourings using indole or indoline derivatives which are stable with respect to storage, in particular in a basic medium, which are easy to formulate and the amount of which can be optimized according to the intended dyeing level, while at the same time limiting the bleaching of the keratin fibres. In particular, there is a need to obtain colourations that are sparingly aggressive on the hair and that are simultaneously resistant to external agents (light, bad weather, shampooing) and that are remanent and/or homogeneous, while at the same time remaining powerful and/or chromatic.

This (ese) objective(s) is (are) achieved by virtue of the present invention, of which the first subject is the use of i) hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, in the presence of at least ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, more particularly glucosidase activity and preferentially beta-glucosidase activity iii) preferably at a pH of inclusively between 3 and 8, for treating keratin fibres, in particular dyeing keratin fibres, preferably human keratin fibres such as the hair.

Another subject of the invention is a process for treating keratin fibres, in particular a process for dyeing human keratin fibres such as the hair, using i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, particularly glucosidase activity and even more particularly beta-glucosidase activity, at a pH iii) of inclusively between 3 and 8.

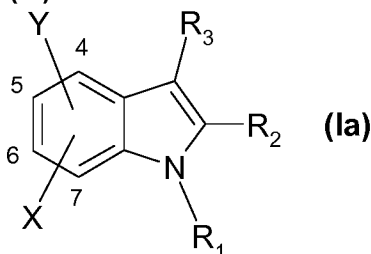
Another subject of the invention is a multicompartiment device comprising the ingredients i) to iii) as defined above, it being understood that the ingredients i) and ii) or ii) and iii) are in separate compartments.

The process according to the invention exhibits the advantage of dyeing keratin fibres, in particular human keratin fibres, with powerful and/or chromatic dyeing results, which are resistant to washing operations, perspiration, sebum and light and which are moreover long-lasting, without detrimentally affecting said fibres. Furthermore, the colourations obtained from the process give uniform colours from the root to the tip of a fibre (low colouration selectivity). In addition, the compositions comprising the i) hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups degrade very little in air and remain stable with respect to storage. They are in addition easy to formulate and the amount can be optimized according to the intended dyeing level.

i) Hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group

According to one particular embodiment of the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention is (are) chosen from the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group in

particular among those of formula **(Ia)** below:



and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof such as hydrates;

5 in which formula **(Ia)**:

- **R₁** and **R₃**, which may be identical or different, represent a hydrogen atom or a C₁-C₄ alkyl radical;

- **R₂** represents a hydrogen atom, or a C₁-C₄ alkyl, -C(O)-OH or -C(O)-OR₄ radical with R₄ representing a (C₁-C₄)alkyl group;

10 - **X** and **Y**, which may be identical or different, denote a hydrogen atom, or an -OH or -O-Sugar group; and

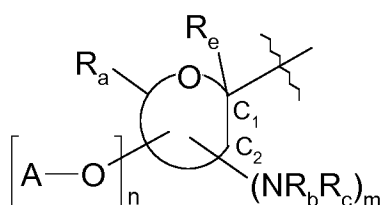
- **Sugar** represents a monosaccharide radical or polysaccharide radical constituted of 2 to 5 saccharide units, in particular of 2 to 3 and preferably of 2 saccharide units,

it being understood that at least one of the groups X and Y represents an -O-Sugar group.

15

For the purposes of the present invention, the term sugar "**S***" is intended to mean a monosaccharide or a polysaccharide radical constituted of 2 to 5 saccharide units, in particular of 2 to 3 and preferably of 2 saccharide units.

20 According to one preferred embodiment, the sugar is attached to the oxygen atom of the -O-Sugar via the C₁ carbon atom of the monosaccharide **S***:



S*

R_a representing a hydrogen atom, a (C₁-C₄)alkyl group such as methyl or a (poly)hydroxy(C₁-C₄)alkyl group such as hydroxymethyl or 1,2-dihydroxyethyl, the hydroxyl function(s) of the (poly)hydroxy(C₁-C₄)alkyl group being substituted with **A** as defined hereinafter-;

it being understood that the **R_a** radical is in the C₅ position if the sugar unit is in pyranose form or in the C₄ position if it is in furanose form;

R_b representing a hydrogen atom or a (C₁-C₄)alkyl group, preferably hydrogen;

R_c representing a hydrogen atom, or a (C₁-C₄)alkyl or R_d-C(X')- group, identical in the case of several hydroxyl functions, with X' representing an oxygen or sulfur atom, in particular an oxygen atom, and **R_d** representing a (C₁-C₄)alkyl group, **R_c** preferably representing an acetyl group CH₃-C(O)-;

R_e representing a hydrogen atom or a -CH₂-O-A group;

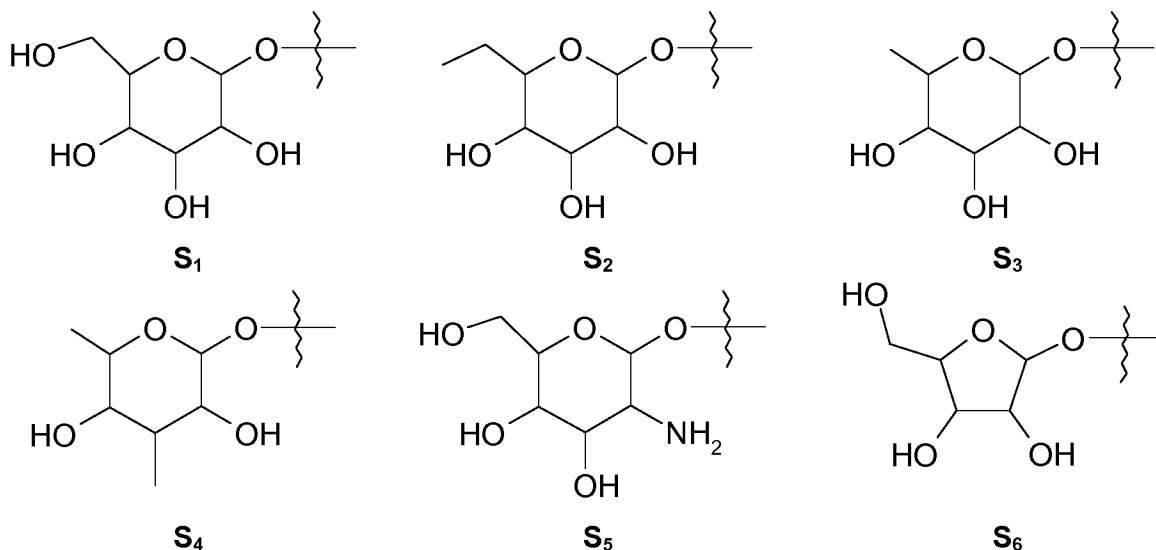
A representing a hydrogen atom, a (C₁-C₆)alkyl group or a hydroxy-function-protecting

group, such as $R_d-C(X')-$ as defined above and in particular acetyl $CH_3-C(O)-$, or else, when n is greater than or equal to 2 and two groups $A-O$ are contiguous, then two A groups can together form a linear or branched (C_1-C_6)alkylene chain; in particular, all the groups which protect **A** are identical, preferably A represents a hydrogen atom; n is equal to 1, 2 or 3 and m is equal to 0 or 1.

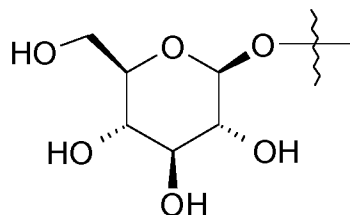
In particular, the "sugar" represents a monosaccharide or polysaccharide, more particularly said sugar is chosen from monosaccharides or polysaccharides composed of the following sugars: pentoses (such as ribose, arabinose, xylose, lyxose, ribulose, or xylulose); hexoses (such as allose, altrose, galactose, glucose, gulose, idose, mannose, talose, fructose, psicose, sorbose or tagatose), hexosamines (such as glucosamine, galactosamine or mannosamine) and deoxyhexoses (such as fucose or rhamnose). In particular, the sugar represents a monosaccharide. Preferably, the monosaccharides may be in D or L configuration, preferentially D configuration.

According to one particular embodiment, said "sugar" represents a monosaccharide chosen from glucose, glucosamine, rhamnose and xylose, preferentially D-glucose, D-glucosamine, D-rhamnose, D-xylose and more preferentially D-glucose.

Preferentially, the $-O$ -Sugar or Sugar- $O-$ group represents a monosaccharide chosen from **S**₁ to **S**₆:



$-O$ -Sugar particularly represents **S**₁, **S**₃, **S**₅, **S**₆, more particularly **S**₁,

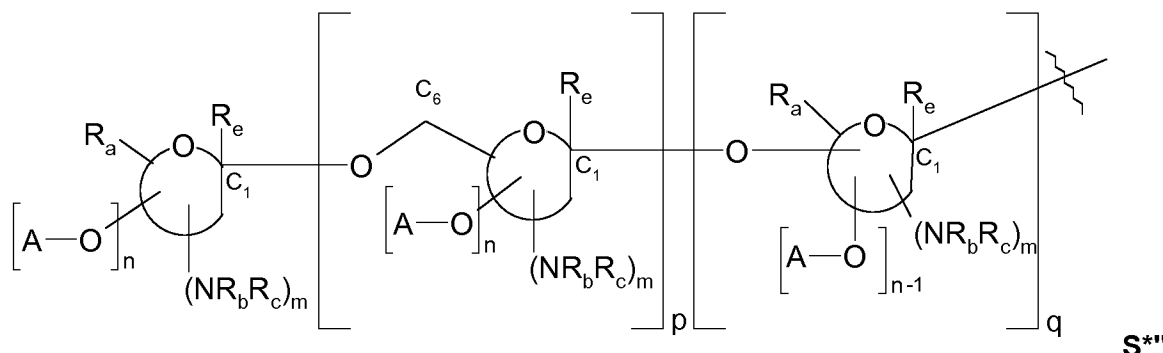


and preferentially $-O$ -Sugar represents:

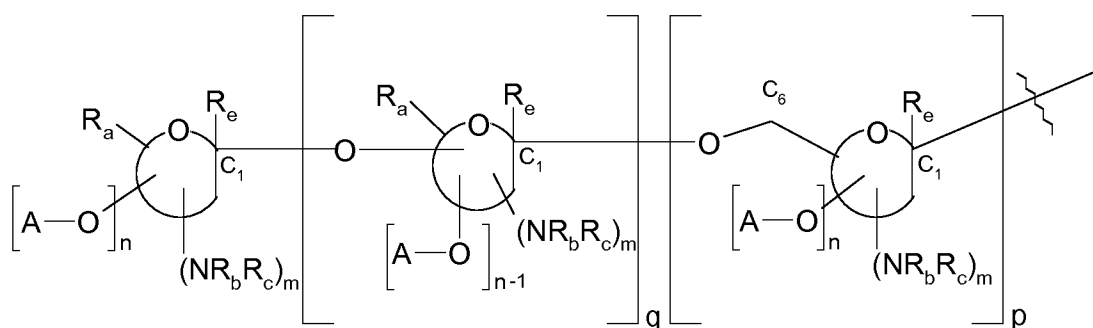
The O-glycosylation is carried out by conventional methods known to those skilled in the art, for example enzymatically or chemically.

According to another embodiment, the sugar radical **S**^{*} represents a polysaccharide radical constituted of 2 to 5 saccharide units, in particular of 2 to 3 and preferably of 2 saccharide

units, linked together via an oxygen atom (oxy), 1→4 (C₁ of one saccharide unit → C₄ of the other saccharide unit) or 1→3 (C₁ of one saccharide unit → C₃ of the other saccharide unit) or 1→6 (C₁ of one saccharide unit → C₆ of the other saccharide unit), each saccharide unit of which is constituted of a heterocycle comprising 4 or 5 carbon atoms, of formula **S**** below:



in which formula **S****, p and q represent integers of inclusively between 0 and 4 with p+q inclusively between 1 and 4, particularly between 1 and 2, preferentially p + q = 1; **R_a**, which may be identical or different, are as defined above for the monosaccharide, **R_b**, which may be identical or different, are as defined above for the monosaccharide, **R_c**, which may be identical or different, are as defined above for the monosaccharide, **R_e**, which may be identical or different, are as defined above for the monosaccharide, **A**, which may be identical or different, are as defined above for the monosaccharide, **m**, which may be identical or different, are as defined above for the monosaccharide, **n**, which may be identical or different, are as defined above for the monosaccharide, it being understood that the two sugar units between the square brackets q and p can intervene, i.e. can represent the chain below:

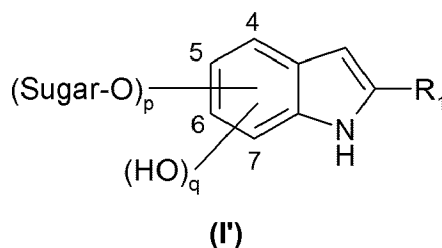


According to this embodiment, the sugar radical **S*** or **S**** represents a disaccharide chosen from lactose, maltulose, palatinose, lactulose, amygdalose, turanose, cellobiose, isomaltose, rutinose and maltose, and more particularly lactose; said disaccharide radical being linked to the rest of the molecule by a bond between the C¹ carbon atom of the sugar or of one of the sugars, this bond possibly being α or β anomeric.

According to one particular embodiment of the invention, the compounds of formula **(Ia)** are such that X is on carbon atom 6 and Y is on carbon atom 5.

According to another embodiment of the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from those of formula **(I')**:

6



and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof such as hydrates;

5 in which formula (I'):

- **R₁** represents a hydrogen atom, or a group chosen from (C₁-C₄)alkyl, -C(O)-OH and -C(O)-OR₄' with R₄' representing a (C₁-C₄)alkyl group such as methyl or ethyl; in particular **R₁** represents a hydrogen atom, or a group chosen from (C₁-C₄)alkyl and -C(O)-OH, more particularly **R₁** represents a hydrogen atom;

10 • **Sugar** is as defined above;

- **p** is equal to 1 or 2; and

- **q** is equal to 0 or 1;

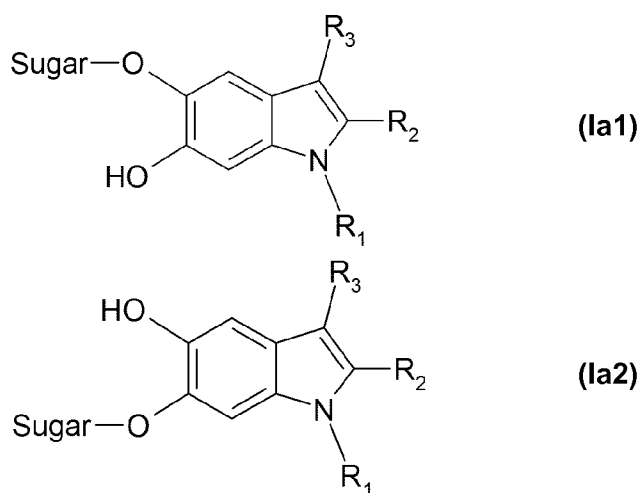
it being understood that the sum p+q is equal to 2.

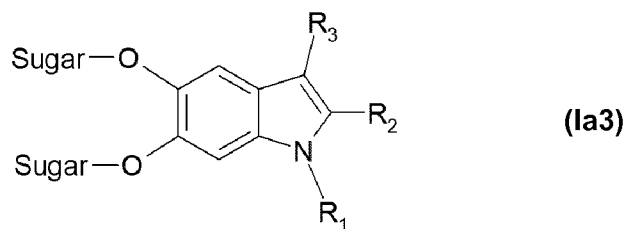
15 According to one particular embodiment of the invention, p is equal to 1 and q is equal to 1 and Sugar-O- is in position 5.

According to another particular embodiment of the invention, p is equal to 1 and q is equal to 1 and Sugar-O- is in position 6.

According to another particular embodiment of the invention, p is equal to 2 and Sugar-O- is in position 5 and 6 and q is equal to 0.

20 According to one preferred embodiment of the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from those of formulae (Ia1), (Ia2) and (Ia3):





and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof such as hydrates;

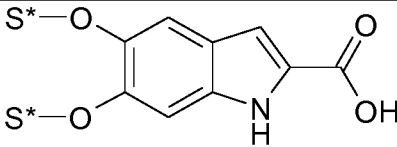
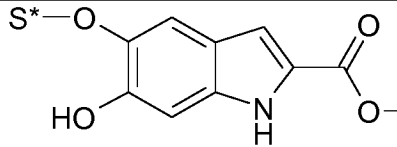
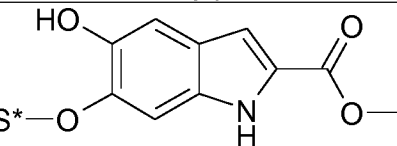
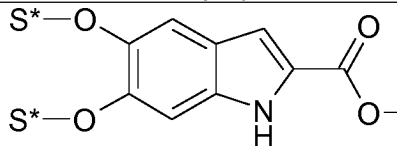
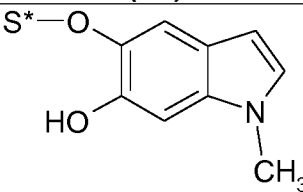
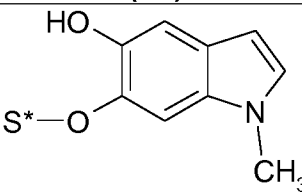
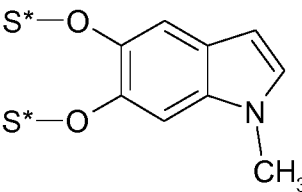
in which formulae **(Ia1)**, **(Ia2)** and **(Ia3)**, **R₁**, **R₂** and **R₃** and **Sugar** are as defined for **(Ia)**, in particular **R₁**, **R₂** and **R₃** represent a hydrogen atom or (C₁-C₄)alkyl, preferably hydrogen.

When several sugar-O groups are present in formulae **(Ia)**, **(I')**, **(Ia1)**, **(Ia2)** and **(Ia3)** of the invention, then the sugar-O groups are preferably identical.

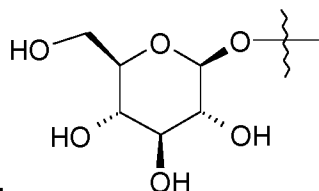
More particularly, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group, in particular those of formulae **(Ia)** and **(Ia2)** as defined above.

According to another variant of the invention, the hydroxyindol(in)e derivative(s) which are glycosylated on at least one hydroxyl group i) are chosen from the compounds **(1)** to **(15)** below:

(1)	(2)
(3)	(4)
(5)	(6)
(7)	(8)

	
(9)	(10)
	
(11)	(12)
	
(13)	(14)
	
(15)	

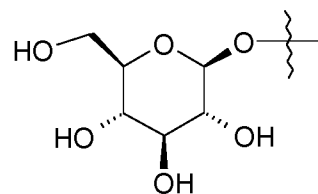
and the organic or mineral acid or base salts thereof, and/or the solvates thereof, with S*-O- representing a monosaccharide chosen from **S**₁ to **S**₆ as defined above, and particularly S*-O- represents a monosaccharide chosen from **S**₁, **S**₃, **S**₅, **S**₆, more particularly **S**₁ and



more preferentially:

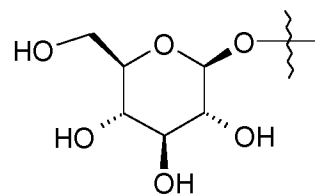
5 Preferably, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention are chosen from the compounds **(1)**, **(2)** and **(3)**. According to a first variant, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention are chosen from the hydroxyindole derivatives **(1)** and **(2)** and preferentially **(1)**, the organic or mineral acid or base salts thereof and/or solvates thereof.

10 More particularly, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention are chosen from the hydroxyindole derivatives **(1)** and **(2)**,



associated with S*-O- which represents **S**₁ and in particular

and



preferentially **(1)** associated with S^*-O- which represents the organic or mineral acid or base salts thereof and/or solvates thereof.

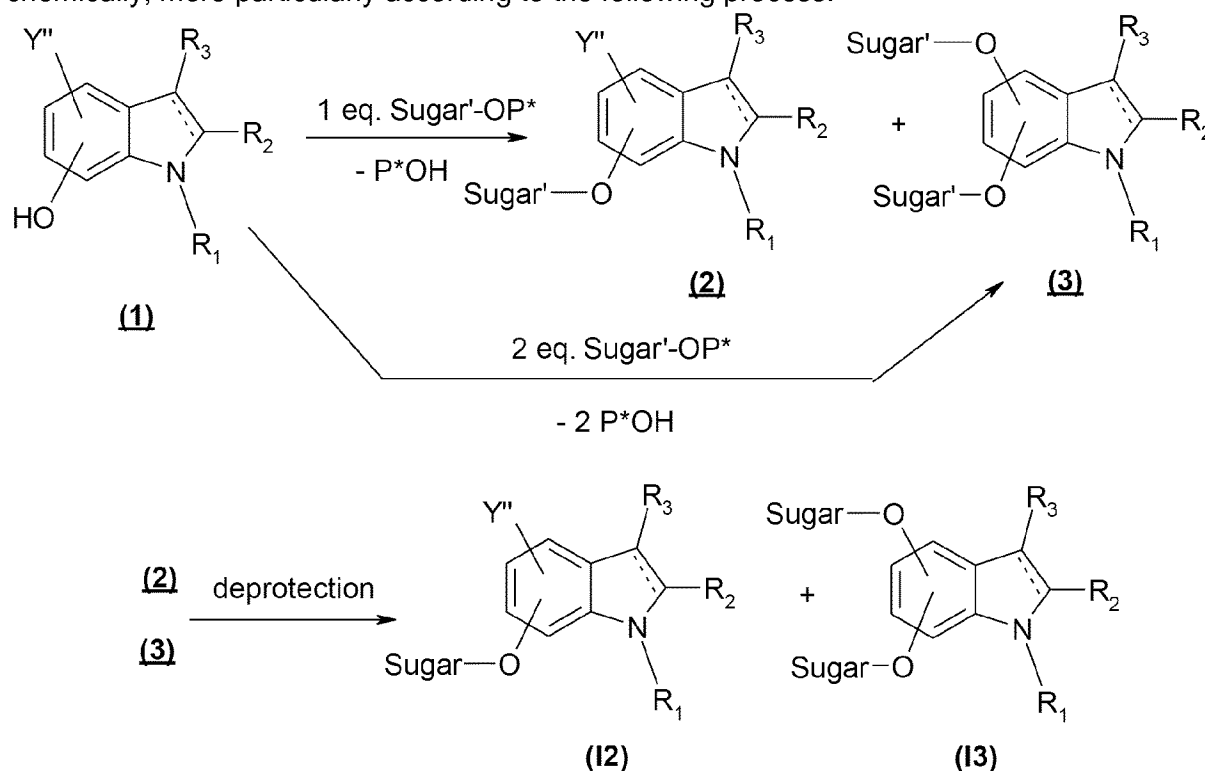
The two groups S^* of the compounds **(3)**, **(6)**, **(9)**, **(12)** or **(15)** of the invention are preferably identical to one another.

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Process for preparing hydroxyindol(in)e derivatives glycosylated on at least one hydroxyl group i), in particular those of formula (I'):

10 The hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention can be prepared chemically or enzymatically.

According to one particular embodiment of the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention is (are) prepared chemically, more particularly according to the following process:



15

with R_1 , R_2 , R_3 , as defined above for **(la)** et **(lb)**, --- representing a single bond or double bond and Y'' representing a hydrogen atom or a hydroxyl group, $\text{Sugar}'-OP^*$ represents a monosaccharide or oligosaccharide sugar, preferably monosaccharide sugar, protected by protective groups or PGs conventionally used to protect the hydroxyl and/or amino groups of the sugars, such as i) (hetero)aryl(C_1 - C_4)alkyl such as benzyl, ii) $R-C(Z)-$ with Z representing an oxygen or sulfur atom, R representing a) a hydrogen atom, b) a (C_1 - C_4)alkyl group such as

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methyl, c) (hetero)aryl such as phenyl, d) (hetero)aryl(C₁-C₄)alkyl such as benzyl, e) (hetero)cycloalkyl such as cyclohexyl, or iii) R_aR_bR_cSi- with R_a, R_b and R_c, which may be identical or different, representing a) a (C₁-C₄)alkyl group, b) (hetero)aryl such as phenyl, c) (hetero)aryl(C₁-C₄)alkyl such as benzyl, and P* represents a hydroxyl-group-protecting group PG, preferably borne by the C₁ carbon atom bearing the alpha or beta anomerism, as defined for i) above; by way of examples of hydroxyl-function-protecting groups, mention may be made more particularly of acetyl, THP, TMS, TBDMS, trityle, tosyl, tert-butyl, MOM and benzoate groups;

which process consists in reacting the indol(in)e derivative of formula **(1)** with an equivalent of protected sugar **Sugar'-OP*** or 2 equivalents of **Sugar'-OP*** so as to give the compounds **(2)** and **(3)***; the compounds (2) and (3) may then be deprotected by conventional methods so as to give the compounds of the invention **(I2)** and **(I3)**, with O-Sugar being as defined above, preferably sugar represents S* as defined above.

The protection and deprotection steps on the hydroxyl and/or amino groups of the sugars are known to those skilled in the art. Mention may be made, for example, of the following scientific articles: *Turkish J. Chem.*, D.P. Iga et al., 37, 299-307 (2013); *Carbohydrate Research*, C.Fernandez et al., 327, 353-365 (2000) and "Protective Groups in Organic Synthesis", T. W. Greene, published by John Wiley & Sons, NY, 1981, pp.193-217; "Protecting Groups", P. Kocienski, Thieme, 3rd ed., 2005.

According to another particular embodiment the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention is (are) prepared enzymatically, more particularly according to the following process:

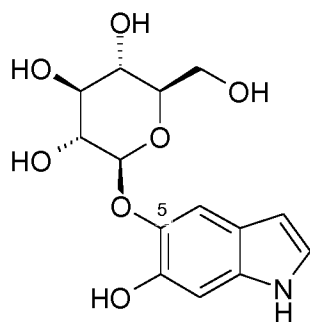
Production of 5,6-dihydroxyindole β-D-glucosylated at C₅ of the indole (Compound 1), 5,6-dihydroxyindole β-D-glucosylated at C₆ of the indole (Compound 2), and di-β-D-glucosylated 5,6-dihydroxyindole (Compound 3) β-D-glucosylated on the carbon atoms in positions 5 and 6 of the indole:

The process for producing 5-hydroxyindole, 6-hydroxyindole and 5,6-dihydroxyindole (or 5,6-DHI) glucosylated with a β-D-glucose is carried out using an *in vitro* reaction which comprises bringing the 5,6-DHI into contact with one or more UGT polypeptides, in the presence of one or more UDP-sugars.

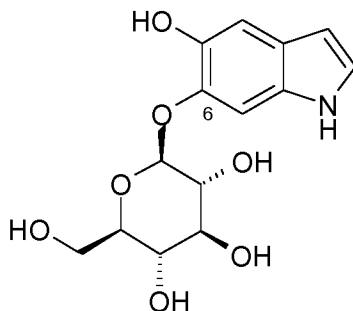
The terms "UDP-glycosyltransferase", "glycosyltransferase" and "UGT" are intended to mean any enzyme capable of transferring a sugar residue, for example galactose, xylose, rhamnose, glucose, arabinose, glucuronic acid, and other amino sugars such as N-acetyl glucosamine of MBHB Ref. No: 15-1854-PRO9 to a receiver molecule. The receiver molecule in this case is 5,6-DHI.

The company Evolva prepared the enzymatic means below for synthesizing the mono- and diglycosylated compounds of the 5,6-dihydroxyindole derivatives.

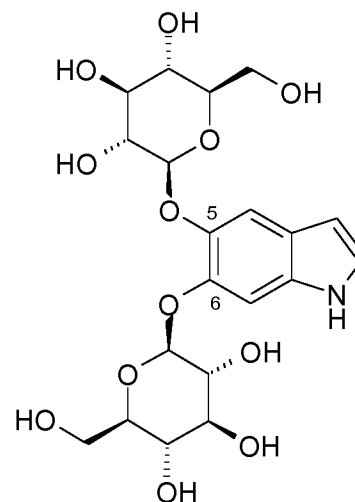
The **compounds 1 to 3** below were entirely characterized by conventional spectrometric and spectroscopic methods:



Compound 1

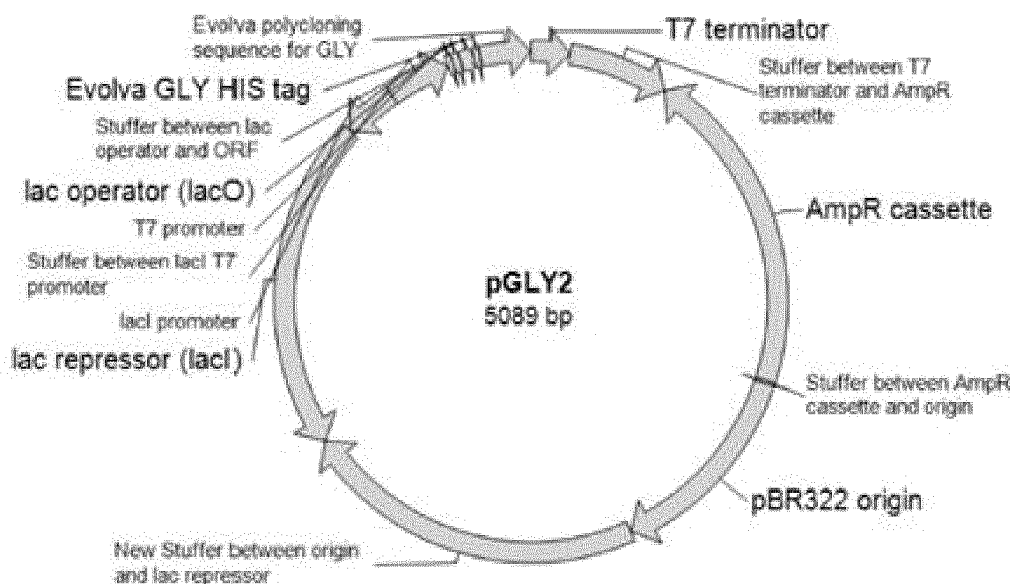


Compound 2



Compound 3

Scheme 1 hereinafter illustrates an *E. coli* expression vector used for the UGT gene expression *in vitro*. The plasmid was synthesized by the company GeneArt™ Gene Synthesis. It carries a T7 transcription promoter and terminator according to Scheme 1 below:



Scheme 1

Stable glucosylated melanin precursors can be produced by isolated UGTs *in vitro*, such as 5,6-DHI glucosylated forms. The 5,6-DHI glucosylated forms can be glucosylated forms alone, such as **Compound 1** or **Compound 2**. In another embodiment, the 5,6-DHI glucosylated forms obtained can be in the doubly glucosylated form i.e. the hydroxyl groups in positions 5 and 6 of the indole are glucosylated (**Compound 3**).

Identification of the UGTs capable of glycosylation of 5,6-DHIs in vitro

UGTs transformed with a melanin-producing yeast strain can spontaneously slow down or stop the polymerization of melanin precursors by the formation of glycosylated melanin precursors (GLYMPs). Consequently, in this example, the UGTs capable of glucosylating the 5,6-DHI melanin precursors so as to form GLYMPs were identified by systematic screening *in vitro*.

The term "glucosylated melanin precursor" or "GLYMP" is intended to mean any form of "melanin precursor" which has been glucosylated. The GLYMPs are more specifically the 5,6-DHIs mono-glucosylated in position 5 (**Compound 1**), or mono-glucosylated in position 6 (**Compound 2**), and those di-glucosylated in positions 5 and 6 (**Compound 3** or "Di-Glc").

The term "melanin precursor" is intended to mean any known molecule entering into the synthesis of melanin (see for example "Current Trends in the Chemistry of Permanent Hair Dyeing", *Chem. Rev.*, 2011, 111, 2537–2561) including L-DOPA, DOPAquinone, LeucoDOPAchome, DOPAchome, 5,6-DHICA, 5,6-DHI, 5,6-indolequinone-CA, 5,6-indolequinone, and melanochrome.

An *in vitro* collection of UGT enzymes purified from plants was used in hi-throughput (HT) screening to identify the enzymes capable of transferring the sugar(s) to the 5,6-DHI provided from UDP-glucose acting as sugar donor.

Methods

In vitro glucosylation reaction

A 50 µl batch of sample was prepared by mixing the following components:

- Enzymes: The UGT genes were cloned into an appropriate E. coli expression vector (synthesized by the company GeneArt™, Gene Synthesis (see Scheme 1 above) and were transformed into and expressed in an E. coli system (100 µl of cultures), purified by conventional methods, and eluted in 300 µl of elution buffer (via 6XHis-tag purification, see Hochuli *et al.*, "Genetic Approach to Facilitate Purification of Recombinant Proteins with a Novel Metal Chelate Adsorbent", *Nature Biotechnology*, 1321-1325, Nov. 1988). A fixed volume of enzyme preparations was added to each reaction (5 µl).
- The UDP-sugar donor sugar was added to each reaction so as to achieve a final concentration of 0.6 mM.
- A reaction buffer: 100 mM of Tris-base, 5,1 mM of MgCl₂, 1 mM of KCl, pH 8.0.
- The substrate: 5,6-DHI dissolved in DMSO was added to each reaction so as to achieve a final concentration of 0.2 mM (3:1 sugar donor: 5,6-DHI molar ratio). The reactions were incubated overnight at 30°C with gentle stirring and directly injected for the LC-MS analysis.

GLYMPs analysis: An analytical method for analysis of the GLYMPs was developed on a

Waters UPLC (Ultra Performance Liquid Chromatography) system equipped with a Waters 2777 sample manager and a PDA detector. The system was also coupled to a Waters® SQD (single quadrupole) mass spectrometer.

Column: Acquity BEH C₁₈, 2.1 x 100 mm, 1.7 µm particle size (part. no.186002352). The column was maintained at 35°C for the duration of the analysis.

Mobile phases: A : Demineralized water + 0.1% of formic acid; B: Acetonitrile + 0.1% of formic acid. Flow rate: 0.4 ml/min. The mobile phase gradient is the following: time (min.) / % of B: 0/1%; 5/50%; 5.5/100%; 7/100%; 7.1/1% and 10/1%.

The enzymes are particularly glucosyltransferases chosen from *Arabidopsis thaliana*:

UDP-glucosyl transferase 71C1, *Arabidopsis thaliana* UDP-glucosyl transferase 71C5, *Arabidopsis thaliana* UDP-glycosyltransferase 72B1, *Arabidopsis thaliana* UDP-glycosyltransferase 72B2, *Arabidopsis thaliana* UDP-glycosyltransferase 74F1, *Arabidopsis thaliana* UDP-glycosyltransferase 75C1, *Arabidopsis thaliana* UDP-glucosyl transferase 76D1, *Arabidopsis thaliana* UDP-glucosyl transferase 76E1, *Arabidopsis thaliana* UDP-glucosyltransferase 76E11, *Arabidopsis thaliana* UDP-glucosyl transferase 85A2 and *Stevia rebaudiana* UDP-glycosyltransferase 71E1.

Plasmid ID	Organism	UGT (Evolva)	SEQ ID No. of gene	SEQ ID No. of protein
pG187* SEQ ID No.3	<i>Arabidopsis thaliana</i> / <i>Stevia rebaudiana</i>	71C125571E1	1	2

* available from Life Sciences

Screening results

5,6-DHI monoglucosylated in position 5 (Compound 1)				
Plasmid ID	UGT name	Area of the peak	Retention time	Relative protein concentration
pG187	71C125571E1	40275	2.17	110.9
5,6-DHI monoglucosylated in position 6 (Compound 2)				
Plasmid ID	UGT name	Area of the peak	Retention time	Relative protein concentration
pG187	71C125571E1	109832	2.46	110.9
5,6-DHI diglucosylated in position 5 and 6 (Compound 3)				
Plasmid ID	UGT name	Area of the peak	Retention time	Relative protein concentration
pG187	71C125571E1	142710	2.03	110.9

Relative protein concentration: Calculated as percentage of 1 µg of BSA standard loaded onto an SDS gel. BLQ: below the limit of quantitation.

This shows that the UGTs can glucosylate, in particular in positions 5 and 6, the hydroxyl groups of 5,6-DHI. As UGTs capable of glycosylating the 5,6-DHIs, mention may be made of the UGTs 71C125571E1 chosen for the *in vitro* production of mono- and diglucosylated 5,6-DHI respectively **Compound 1**, **Compound 2** and **Compound 3**.

The hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention can occur naturally. It is then possible to carry out the invention by using one or more natural extracts of animal origin or plant origin, such as certain extracts of beetroot
5 (*Beta vulgaris*), or of bacterial or fungal origin, comprising one or more hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention, optionally followed by enzymatic or chemical O-glycosylation known by those skilled in the art.

According to the invention, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention as defined above are preferably present at a
10 concentration ranging from 0.0005% to 10% by weight relative to the total weight of the dye composition containing them. Even more preferentially, this concentration ranges from 0.005% to 5% by weight and better still from 0.01% to 4% by weight relative to the total weight of the dye composition containing them.

Preferably, the concentration i) of hydroxyindol(in)e derivative(s) glycosylated on at least
15 one hydroxyl group of the invention is inclusively between 0.1% and 1% by weight of the total weight of the composition which contains it (them), such as 0.5%.

For the purposes of the present invention and unless otherwise indicated:

The saturated or unsaturated and optionally fused rings can also be optionally substituted.

20 The term "hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group" is intended to mean a compound comprising at least one indole or indoline unit which comprises one or more hydroxyl groups, on the benzo part of the indole or indoline, at least one of the hydroxyl groups of which is O-glycosylated with a sugar group so as to give an –O-sugar group.

25 The term "–O-sugar" is intended to mean a hydroxyl group which has been O-glycosylated with a sugar, said sugar being a monosaccharide or oligosaccharide (2 to 5 saccharide units), in particular a disaccharide (diholoside) which may be in furanose or pyranose or dextrorotatory (D) or levorotatory (L) form, in alpha (α) and/or beta (β) anomeric form, said mono or polysaccharide radical being linked to the rest of the molecule by a bond
30 between the oxygen atom bonded to the C₁ carbon atom of one of the sugars of said mono or polysaccharide radical, it being possible for this bond to be α or β anomeric. Said sugar may have a hydroxyl group which has been substituted with a group chosen from i) (di)(C₁–C₆)(alkyl)amino, ii) aryl(C₁–C₄)alkylamino such as benzylamino, iii) R–C(X)–N(R')– with R and R', which may be identical or different, representing a hydrogen atom or a (C₁–C₄)alkyl group,
35 and X representing an oxygen or sulfur atom, the hydroxyl group of sugar preferably being substituted with an amino group NH₂. Said sugar may have one or more hydroxyl groups which are O-protected for example by an R_d–C(X') group, and which are identical in the case of several protected hydroxyl functions, with X' representing an oxygen or sulfur atom, in particular an oxygen atom, and R_d representing a (C₁–C₄)alkyl group, R_d–C(X') preferably
40 representing an acetyl group CH₃–C(O)–.

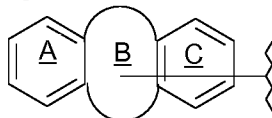
The term "protective group" or "PG" of the "hydroxyl" or "amino" function is intended to mean those known by those skilled in the art; mention may be made of the two books "*Protective Groups in Organic Synthesis*", T. W. Greene, published by John Wiley & Sons, NY, 1981, pp.193-217; "*Protecting Groups*", P. Kocienski, Thieme, 3rd ed., 2005.

In particular, the protective group is chosen from:

- (C₁-C₆)alkyl(thio)carbonyl such as formyl, acetyl or t-butylcarbonyl;
- (di)(tri)halo(C₁-C₆)alkyl(thio)carbonyl such as trifluoroacetyl (TFA);
- (C₁-C₆)alkoxy(thio)carbonyl such as methoxycarbonyl, ethoxycarbonyl, isobutyloxycarbonyl, t-butyloxycarbonyl (BOC), vinyloxycarbonyl, allyloxycarbonyl;
- (di)(tri)halo(C₁-C₆)alkoxy(thio)carbonyl such as 2,2,2-trichloroethylcarbonyl;
- (C₁-C₆)alkylthiothiocarbonyl;
- (di)(tri)halo(C₁-C₆)alkylthiothiocarbonyl;
- (di)(C₁-C₆)(alkyl)aminocarbonyl;
- (di)(C₁-C₆)(alkyl)aminothiocarbonyl;
- optionally substituted arylcarbonyl such as phenylcarbonyl or 2,4,6-trimethylphenylcarbonyl;
- optionally substituted aryloxycarbonyl such as p-nitrophenoxycarbonyl;
- optionally substituted aryl(C₁-C₆)alkoxycarbonyl such as benzyloxycarbonyl or Cbz, p-methoxybenzyloxycarbonyl, 3,4-dimethoxybenzyloxycarbonyl, o-nitrobenzyloxycarbonyl, p-nitrobenzyloxycarbonyl, 2-bromobenzyloxycarbonyl (2-bromo-Z) and 2-chlorobenzyloxycarbonyl (2-chloro-Z), 4-nitrobenzyloxycarbonyl (nitro-Z),
- heteroaryl(C₁-C₆)alkoxycarbonyl such as 9-fluorenylmethoxycarbonyl (Fmoc) or nicotinoyl;
- (di)(C₁-C₆)(alkyl)aminocarbonyl such as dimethylaminocarbonyl;
- (C₁-C₆)(alkyl)arylaminocarbonyl;
- carboxyl;
- optionally substituted aryl such as phenyl, dibenzosuberyl or 1,3,5-cycloheptatrienyl;
- optionally substituted heteroaryl; especially including the cationic or non-cationic heteroaryls comprising from 1 to 4 heteroatoms below:
 - i) 5-, 6- or 7-membered monocyclic groups such as furanyl or furyl, pyrrolyl or pyrrol, thiophenyl or thienyl, pyrazolyl, oxazolyl, oxazolium, isoxazolyl, isoxazolium, thiazolyl, thiazolium, isothiazolyl, isothiazolium, 1,2,4-triazolyl, 1,2,4-triazolium, 1,2,3-triazolyl, 1,2,3-triazolium, 1,2,4-oxazolyl, 1,2,4-oxazolium, 1,2,4-thiadiazolyl, 1,2,4-thiadiazolium, pyrylium, thiopyridyl, pyridinium, pyrimidinyl, pyrimidinium, pyrazinyl, pyrazinium, pyridazinyl, pyridazinium, triazinyl, triazinium, tetrazinyl, tetrazinium, azepine, azepinium, oxazepinyl, oxazepinium, thiepinyl, thiepinium, imidazolyl, imidazolium;
 - ii) 8- to 11-membered bicyclic groups such as indolyl, indolinium, benzimidazolyl, benzimidazolium, benzoxazolyl, benzoxazolium, dihydrobenzoxazolyl,

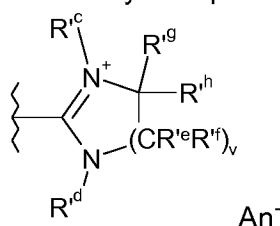
benzothiazolyl, benzothiazolium, pyridoimidazolyl, pyridoimidazolium, thienocycloheptadienyl, these monocyclic or bicyclic groups being optionally substituted with one or more groups such as (C₁-C₄)alkyl, for instance methyl, or polyhalo(C₁-C₄)alkyl, for instance trifluoromethyl;

iii) or the following tricyclic ABC group:



in which the two rings A and C optionally comprise a heteroatom, and ring B is a 5-, 6- or 7-membered ring, particularly a 6-membered ring, and contains at least one heteroatom, for instance piperidyl or pyranyl;

- optionally cationic, optionally substituted heterocycloalkyl, the heterocycloalkyl group in particular representing a saturated or partially saturated 5-, 6- or 7-membered monocyclic group comprising from 1 to 4 heteroatoms chosen from oxygen, sulfur and nitrogen, such as di/tetrahydrofuranyl, di/tetrahydrothiophenyl, di/tetrahydropyrrolyl, di/tetrahydropyranyl, di/tetra/hexahydrothiopyranyl, dihydropyridyl, piperazinyl, piperidynyl, tetramethylpiperidyl, morpholinyl, di/tetra/hexahydroazepinyl, di/tetrahydropyrimidinyl, these groups being optionally substituted with one or more groups such as (C₁-C₄)alkyl, oxo or thioxo, preferably tetrahydropyranyl THP; or the heterocycle represents the following group:

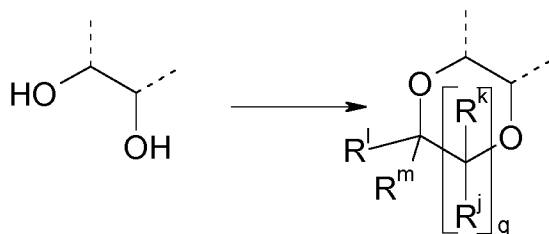


in which R^c, R^d, R^e, R^f, R^g and R^h, which may be identical or different, represent a hydrogen atom or a (C₁-C₄)alkyl group, or alternatively two groups R^g with R^h, and/or R^e with R^f form an oxo or thioxo group, or alternatively R^g with R^e together form a cycloalkyl; and v represents an integer between 1 and 3 inclusive; preferentially, R^c to R^h represent a hydrogen atom; and An⁻ represents a counterion;

- isothiuronium -C(NR^cR^d)=N⁺R^eR^f; An⁻ with R^c, R^d, R^e and R^f, which may be identical or different, representing a hydrogen atom or a (C₁-C₄)alkyl group; preferentially, R^c to R^f represent a hydrogen atom; and An⁻ represents a counterion;
- isothiourea -C(NR^cR^d)=NR^e; with R^c, R^d and R^e as defined above;
- optionally substituted (di)aryl(C₁-C₄)alkyl or triaryl(C₁-C₄)alkyl such as 9-anthracenylmethyl, phenylmethyl (benzyl), diphenylmethyl or triphenylmethyl optionally substituted with one or more groups, in particular chosen from halogen, (C₁-C₄)alkyl, (C₁-C₄)alkoxy such as methoxy, hydroxyl, (C₁-C₄)alkylcarbonyl, (di)(C₁-C₄)(alkyl)amino such as dimethylamino, nitro;

- 5 ▪ optionally substituted (di)heteroaryl(C₁-C₄)alkyl or triheteroaryl(C₁-C₄)alkyl, the heteroaryl group in particular being cationic or noncationic, 5- or 6-membered monocyclic comprising from 1 to 4 heteroatoms chosen from nitrogen, oxygen and sulfur, such as pyrrolyl, furanyl, thiophenyl, pyridyl, pyridyl N-oxide such as 4-pyridyl or 2-pyridyl-N-oxide, pyrylium, pyridinium or triazinyl groups, optionally substituted with one or more such as alkyl, particularly methyl; advantageously, the (di)heteroaryl(C₁-C₄)alkyl is (di)heteroarylmethyl or (di)heteroarylethyl;
- 10 ▪ CR¹R²R³ with R¹, R² and R³, which may be identical or different, representing a halogen atom, such as (tri)(di)halo(C₁-C₄)alkyl such as 2,2,2-trichloroethyl or a group chosen from:

 - (C₁-C₄)alkyl such as methyl;
 - (C₁-C₄)alkoxy;
 - optionally substituted aryl such as phenyl optionally substituted with one or more groups, for instance (C₁-C₄)alkyl, (C₁-C₄)alkoxy or hydroxyl;
 - 15 - optionally substituted heteroaryl such as thiophenyl, furanyl, pyrrolyl, pyranlyl or pyridyl, optionally substituted with a (C₁-C₄)alkyl group;
- 20 ▪ P(Z¹)R¹R²R³ with R¹ and R², which may be identical or different, representing a hydroxyl, (C₁-C₄)alkoxy or alkyl group, R³ representing a hydroxyl or (C₁-C₄)alkoxy group, and Z¹ representing an oxygen or sulfur atom;
- 25 ▪ (C₂-C₆)alkylene, in particular allyl H₂C=CH-CH₂-;
- 30 ▪ optionally substituted arylsulfonyl such as p-toluenesulfonyl (Tos);
- sterically hindered cycloalkyl such as the adamantyl group;
- sterically hindered cycloalkyloxy(thio)carbonyl such as 1-adamantyloxycarbonyl (Adoc) or 1-(adamantyl)-1-methylethoxycarbonyl (Adpoc);
- optionally substituted (C₁-C₄)alkoxy(C₁-C₄)alkyl such as methoxymethyl (MOM), ethoxyethyl (EOM) and isobutoxymethyl;
- (tri)(di)halo(C₁-C₄)alkyl such as 2,2,2-trichloroethyl;
- R_eR_fR_gSi- with R_e, R_f, et R_g, which may be identical or different, representing a (C₁-C₆)alkyl group, optionally substituted aryl group, optionally substituted (di)aryl(C₁-C₄)alkyl group, optionally substituted triaryl(C₁-C₄)alkyl group, such as benzyl, in particular chosen from trimethylsilyl or TMS, triethylsilyl, isopropyl dimethylsilyl, tert-butyl dimethylsilyl or TBDMS, (triphenylmethyl)dimethylsilyl, t-butyl diphenylsilyl, methyl diisopropylsilyl, methyl(di-t-butyl)silyl, tribenzylsilyl, tri-p-xylylsilyl, triisopropylsilyl, triphenylsilyl;
- 35 ▪ or else two contiguous hydroxyl groups can be protected with an alkylene group *-C(R^l)(R^m)-(C(R^k)(R^j))_q-* as drawn below:



with R^j , R^k , R^l , and R^m , which may be identical or different, representing a hydrogen atom or a (C₁-C₄)alkyl, (poly)halo(C₁-C₄)alkyl, optionally substituted aryl such as phenyl, aryl(C₁-C₄)alkyl such as benzyl, (poly)halo(C₁-C₄)alkoxy, (C₁-C₄)alkoxy, halogen, (di)(C₁-C₄)(alkyl)amino or hydroxyl group, or else two R^l and R^k and/or R^l , and R^m groups form, together with the carbon atom which bears them, an oxo group or a (hetero)cycloalkyl group such as clohexyl or cyclopropyl; q is 0, 1, 2 or 3, preferably $^*-C(R^l)(R^m)-(C(R^k)(R^j))_q-^*$ represents a methylene, ethylene, propylene, dimethylmethylene, $^*-C(CH_3)_2-^*$ or diphenylmethylene $^*-C(Ph)_2-^*$, cyclopentylidene, cyclohexylidene, cycloheptylidene, benzylidene, p-methoxybenzylidene, 2,4-dimethoxybenzylidene, methoxymethylene and ethoxymethylene.

By way of examples of protective groups, mention may more particularly be made of acetyl, THP, TMS, TBDMS, trityl, tosyl, tert-butyl, MOM and benzoate groups.

The "alkyl" radicals are saturated, linear or branched, generally C₁-C₂₀, particularly C₁-C₁₀, hydrocarbon-based radicals, preferably C₁-C₆ alkyl radicals, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl and hexyl.

The "aryl" radicals are fused or non-fused monocyclic or polycyclic carbon-based radicals, preferentially comprising from 6 to 30 carbon atoms, and of which at least one ring is aromatic; the aryl radical is preferentially a phenyl, biphenyl, naphthyl, indenyl, anthracenyl or tetrahydronaphthyl, more preferentially a phenyl radical.

The "alkoxy" radicals are alkyl-oxy radicals with alkyl as defined previously, preferably C₁-C₁₀, such as methoxy, ethoxy, propoxy and butoxy.

The "alkoxyalkyl" radicals are preferably (C₁-C₂₀)alkoxy(C₁-C₂₀)alkyl radicals, such as methoxymethyl, ethoxymethyl, methoxyethyl, ethoxyethyl, and the like.

The "cycloalkyl" radicals are generally C₄-C₈ cycloalkyl radicals, preferably cyclopentyl and cyclohexyl radicals. The cycloalkyl radicals can be substituted cycloalkyl radicals, in particular substituted with alkyl, alkoxy, carboxylic acid, hydroxyl, amine and ketone groups.

The "alkyl" radicals, when they are optionally substituted, can be substituted with at least one substituent carried by at least one carbon atom, chosen from:

- a halogen atom;
- a hydroxyl group;
- a C₁-C₂ alkoxy radical;
- a C₁-C₁₀ alkoxycarbonyl radical;
- a (poly)hydroxy(C₂-C₄)alkoxy radical;
- an amino radical;

- a quaternary ammonium group $-N^+R'R''R'''$, M^- for which R' , R'' and R''' , which may be identical or different, represent a hydrogen atom or a C_1 - C_4 alkyl group; and M^- represents the counterion of the corresponding organic or mineral acid or of the corresponding halide;

- a 5- or 6-membered heterocycloalkyl radical;

- an optionally cationic 5- or 6-membered heteroaryl radical, preferentially imidazolium, optionally substituted with a (C_1-C_4) alkyl radical, preferentially methyl;

- an amino radical substituted with one or two identical or different C_1 - C_6 alkyl radicals, optionally bearing at least:

* a hydroxyl group;

* an amino group optionally substituted with one or two optionally substituted C_1 - C_3 alkyl radicals, it being possible for said alkyl radicals to form, with the nitrogen atom to which they are attached, a saturated or unsaturated and optionally substituted 5- to 7-membered heterocycle optionally comprising at least one other heteroatom different or not different from nitrogen;

* a quaternary ammonium group $-N^+R'R''R'''$, M^- as defined previously;

* or an optionally cationic 5- or 6-membered heteroaryl radical, preferably an imidazolium radical, optionally substituted with a (C_1-C_4) alkyl radical, preferably a methyl radical;

- an acylamino radical $(-NR-C(O)R')$ in which the radical R is a hydrogen atom, a C_1 - C_4 alkyl radical optionally bearing at least one hydroxyl group and the radical R' is a C_1 - C_2 alkyl radical; a carbamoyl radical $((R)_2N-CO-)$ in which the radicals R , which may be identical or different, represent a hydrogen atom, a C_1 - C_4 alkyl radical optionally bearing at least one hydroxyl group; an alkylsulfonylamino radical $(R'S(O)_2-NR-)$ in which the radical R represents a hydrogen atom, a C_1 - C_4 alkyl radical optionally bearing at least one hydroxyl group and the radical R' represents a C_1 - C_4 alkyl radical or a phenyl radical; an aminosulfonyl radical $((R)_2N-S(O)_2-)$ in which the radicals R , which may be identical or different, represent a hydrogen atom or a C_1 - C_4 alkyl radical optionally bearing at least one hydroxyl group;

- a carboxyl radical in the acid or salified (preferably salified with an alkali metal or a substituted or unsubstituted ammonium) form;

- a cyano group;

- a nitro group;

- a carboxyl or glycosylcarbonyl group;

- a phenylcarbonyloxy group optionally substituted with one or more hydroxyl groups;

- a glycosyloxy group; and

- a phenyl group optionally substituted with one or more hydroxyl groups.

The "aryl" or "heteroaryl" or "heterocyclic" radicals or the "aryl" or "heteroaryl" or "heterocyclic" part of the radicals, when they are optionally substituted, may be substituted with at least one substituent borne by at least one carbon atom, chosen from:

- a C_1 - C_{10} and preferably C_1 - C_8 alkyl radical optionally substituted with one or more

radicals chosen from the following radicals: hydroxyl, C₁-C₂ alkoxy, (poly)hydroxy(C₂-C₄)alkoxy, acylamino, amino substituted with two identical or different C₁-C₄ alkyl radicals optionally carrying at least one hydroxyl group, or it being possible for the two radicals to form, with the nitrogen atom to which they are attached, a saturated or unsaturated and optionally substituted 5- to 7-membered and preferably 5- or 6-membered heterocycle optionally comprising another heteroatom identical to or different from nitrogen;

- a halogen atom;
- a hydroxyl group;
- a C₁-C₂ alkoxy radical;
- a C₁-C₁₀ alkoxy carbonyl radical;
- a (poly)hydroxy(C₂-C₄)alkoxy radical;
- an amino radical;
- a 5- or 6-membered heterocycloalkyl radical;
- an optionally cationic 5- or 6-membered heteroaryl radical, preferentially imidazolium, optionally substituted with a (C₁-C₄)alkyl radical, preferentially methyl;
- an amino radical substituted with one or two identical or different C₁-C₆ alkyl radicals, optionally bearing at least:
 - * a hydroxyl group;
 - * an amino group optionally substituted with one or two optionally substituted C₁-C₃ alkyl radicals, it being possible for said alkyl radicals to form, with the nitrogen atom to which they are attached, a saturated or unsaturated and optionally substituted 5- to 7-membered heterocycle optionally comprising at least one other heteroatom different or not different from nitrogen;
 - * a quaternary ammonium group -N⁺R'R''R''', M' for which R', R'' and R''' which may be identical or different, represent a hydrogen atom or a C₁-C₄ alkyl group; and M' represents the counterion of the corresponding organic or mineral acid or of the corresponding halide;
 - * or an optionally cationic 5- or 6-membered heteroaryl radical, preferably an imidazolium radical, optionally substituted with a (C₁-C₄)alkyl radical, preferably a methyl radical;
- an acylamino radical (-NR-C(O)R') in which the radical R is a hydrogen atom, a C₁-C₄ alkyl radical optionally bearing at least one hydroxyl group and the radical R' is a C₁-C₂ alkyl radical; a carbamoyl radical ((R)₂N-CO-) in which the radicals R, which may be identical or different, represent a hydrogen atom, a C₁-C₄ alkyl radical optionally bearing at least one hydroxyl group; an alkylsulfonylamino radical (R'S(O)₂-NR-) in which the radical R represents a hydrogen atom, a C₁-C₄ alkyl radical optionally bearing at least one hydroxyl group and the radical R' represents a C₁-C₄ alkyl radical or a phenyl radical; an aminosulfonyl radical ((R)₂N-S(O)₂-) in which the radicals R, which may be identical or different, represent a hydrogen atom or a C₁-C₄ alkyl radical optionally bearing at least one hydroxyl group;

- a carboxyl radical in the acid or salified (preferably salified with an alkali metal or a substituted or unsubstituted ammonium) form;
- a cyano group;
- a nitro group;
- 5 - a polyhaloalkyl group, preferentially trifluoromethyl;
- a carboxyl group;
- a phenylcarbonyloxy group optionally substituted with one or more hydroxyl groups;
- a glycosyloxy or O-sugar group; and
- a phenyl group optionally substituted with one or more hydroxyl groups.

10 The term "glycosyl" is intended to mean a radical derived from a monosaccharide or from an oligosaccharide comprising from 2 to 5 units, preferentially derived from a monosaccharide or from a disaccharide.

The radicals comprising one or more silicon atoms are preferably polydimethylsiloxane, polydiphenylsiloxane, polydimethylphenylsiloxane or stea-
15 dimethicone radicals.

The "heterocyclic" radicals are generally cyclic, saturated or unsaturated 3- to 22-membered radicals, comprising in at least one ring one or more heteroatoms chosen from O, N and S, preferably O or N, optionally substituted especially with one or more alkyl, alkoxy, carboxylic acid, hydroxyl, amine or ketone groups. These rings may contain one or more oxo
20 groups on the carbon atoms of the heterocycle of the non-aromatic part. The heterocycles include heteroaryl, heterocycloalkyl or heterocycloalkenyl groups.

The "heterocycloalkyl" radicals represent saturated monocyclic or polycyclic, fused or non-fused, optionally cationic, 3- to 22-membered and preferentially 3- to 7-membered groups, such as morpholinyl, thiomorpholinyl, piperidyl, piperazinyl, pyrrolidinyl, tetrahydrofuryl or azepanyl, preferentially pyrrolidinyl and morpholinyl;
25

The "heterocycloalkenyl" radicals represent unsaturated monocyclic or polycyclic, fused or non-fused, optionally cationic, 3- to 22-membered and preferentially 5- to 7-membered groups, which comprise from 1 to 3 conjugated or unconjugated double bonds; particularly, the heterocycloalkenyls are piperazenyls such as piperazin-2-en-4-yl, optionally
30 substituted especially with two carboxyl groups in positions 2 and 6 of said heterocycloalkenyl;

The "heteroaryl" radicals represent fused or non-fused, optionally cationic, 5- to 22-membered monocyclic or polycyclic groups, comprising from 1 to 6 heteroatoms chosen from nitrogen, oxygen, sulfur and selenium atoms, and at least one ring of which is aromatic;
35 preferentially, a heteroaryl radical is chosen from acridinyl, benzimidazolyl, benzobistriazolyl, benzopyrazolyl, benzopyridazinyl, benzoquinolyl, benzothiazolyl, benzotriazolyl, benzoxazolyl, pyridyl, tetrazolyl, dihydrothiazolyl, imidazopyridyl, imidazolyl, indolyl, isoquinolyl, naphthoimidazolyl, naphthooxazolyl, naphthopyrazolyl, oxadiazolyl, oxazolyl, oxazolopyridyl, phenazinyl, phenoxazolyl, pyrazinyl, pyrazolyl, pyrilyl, pyrazoyltriazyl, pyridyl, pyridinoimidazolyl, pyrrolyl, quinolyl, tetrazolyl, thiadiazolyl, thiazolyl, thiazolopyridyl, thiazoylimidazolyl, thiopyrylyl, triazolyl, xanthyl and the ammonium salt thereof;
40

Among the "heterocyclic" radicals that may be used, mention may be made of the furyl, pyranyl, pyrrolyl, imidazolyl, pyrazolyl, pyridyl and thienyl groups.

More preferably, the "heterocyclic" groups are fused groups, such as benzofuranyl, chromenyl, xanthenyl, indolyl, isoindolyl, quinolyl, isoquinolyl, chromanyl, isochromanyl, indolinyl, isoindolinyl, coumarinyl or isocoumarinyl groups, it being possible for these groups to be substituted, in particular by one or more OH groups.

The term "salt of an organic or mineral acid" is intended to mean a salt derived, for example, from i) hydrochloric acid HCl, ii) hydrobromic acid HBr, iii) sulfuric acid H₂SO₄, iv) alkylsulfonic acids: Alk-S(O)₂OH such as methanesulfonic acid and ethanesulfonic acid; v) arylsulfonic acids: Ar-S(O)₂OH such as benzenesulfonic acid and toluenesulfonic acid; vi) citric acid; vii) succinic acid; viii) tartaric acid; ix) lactic acid; x) alkoxysulfinic acids: Alk-O-S(O)OH such as methoxysulfinic acid and ethoxysulfinic acid; xi) aryloxysulfinic acids such as tolueneoxysulfinic acid and phenoxysulfinic acid; xii) phosphoric acid P(O)(OH)₃; xiii) acetic acid CH₃C(O)OH; xiv) triflic acid CF₃S(O)₂OH; and xv) tetrafluoroboric acid HBF₄.

The term "salt of an organic or mineral base" is intended to mean a salt derived, for example, from mineral bases such as i) sodium hydroxide NaOH, ii) potassium hydroxide KOH, or from organic bases such as iii) aqueous ammonia; iv) amines and hydroxyamines such as (tri)(C₁-C₆)alkylamine, (tri)hydroxy(C₁-C₆)alkylamine; or v) salts derived from alkali metals or alkaline-earth metals.

For the purposes of the present invention, the "solvates" comprise conventional solvates such as those formed during the final step of preparation of said compounds because of the presence of solvents. Mention may be made, by way of example, of the solvates due to the presence of water (hydrates) or of linear or branched alcohols, such as ethanol or isopropanol.

ii) glycosidase enzymes

The glycosidases of the invention are glycoside hydrolase enzymes which catalyse the hydrolysis of glycosidic bonds by releasing at least one glycoside compound.

For the purposes of the present invention, the glycosidases can be in powder form, in solution or immobilized on a solid support, i.e. a support bearing ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity.

The term "support" bearing one or more enzyme(s) is intended to mean a "matrix" on which one or more enzymes have been immobilized; said enzymes have retained their catalytic functions. Said enzymes are immobilized on a support by means of chemical or physical immobilization methods and remain in a defined space within which they can be used several times in a row or continuously. As methods for immobilization of said enzymes, mention may be made of the conventional methods known to those skilled in the art (see for example "Enzyme immobilization: an overview on techniques and support materials, 3 Biotech"; 3(1): 1-9 (2013 Feb), Doctoral thesis, Ecole Nat. Sup. Chimie Montpellier, H.

Jarrar, "Bioélectrodes enzymatiques pour des applications en biocapteurs et en biopiles" ["Enzymatic bioelectrodes for applications in biosensors and in biocells"] (16/12/2011) in particular chap. 1.7), such as adsorption, crosslinking, covalent grafting, encapsulation, or recognition by affinity/bioaffinity such as recognition of antigen/antibody type. The matrices
5 used are those known to those skilled in the art; mention may be made of i) organic polymers such as agarose, cellulose, dextrans, polymers such as polyvinyl chloride, acrylates, nylons, polystyrene, ii) inorganic materials: silica in the form of microporous glass beads and of silica gel and iii) hybrids of the two matrices above i) and ii) such as agarose-acryl amide and polymer-coated silica.

10 In particular, the matrix used for immobilizing, optionally by encapsulation, the enzyme(s) of the invention is alginate. Alginate is an extract of an alga composed of chains which alternate alpha-L-guluronic acid and beta-D-mannuronic acid residues. The alginate supports are preferably prepared by crosslinking the carboxylic group of the alpha-L-guluronic acid with a cationic ligand such as calcium chloride, barium chloride or poly(L-lysine) (see for example *Carbohydrate Polymers* 56(4), 483-488 (2004); *Journal of Applied Polymer Science* 132(26), 42125/1-42125/15 (2015); *Chitosan-Based Hydrogels* 339-406 (2012); *Shiyou Huagong* 39(1), 7-12 (2010); *Surface Science*, 648, 53-59 (2016); *Bioorganic and Medicinal Chemistry Letters*, 18 (6), 1922-1925 (2008); *Enzyme Research* Vol 2011 (2011), Article ID 642460 and FR2393810).

20 The glycosidases ii) are chosen according to the nature of the glycosylated radical borne by the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) in order to be suitable for the hydrolysis of the bond between the glycosylated radical **S*** and the hydroxyindol(in)e radical(s) as defined above.

25 The i) hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group as defined above is (are) used in the presence of one or more ii) enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, more particularly glucosidase activity and preferentially beta-glucosidase activity.

The enzyme(s) with glycosidase activity may be of vegetable, animal, fungal or bacterial origin.

30 Preferentially, the glycosidases of the invention are glucosidases and in particular beta-glucosidases.

The latter hydrolyse an -O-glucoside bond to beta-glucoside of a monosaccharide or oligosaccharide comprising a glucose portion.

35 These enzymes are known as gentiobiase, cellobiase, emulsin, elaterase, aryl-beta-glucosidase, beta-D-glucosidase, beta-glucoside glucohydrolase, arbutinase, amygdalinase, p-nitrophenyl beta-glucosidase, primeverosidase, amygdalase, linamarase, salicilase, and beta-1,6-glucosidase (EC number 3.2.1.21 - Locus Chr. 4 p15.31). The latter are also known as amygdalin beta-glucosidase, prunasin beta-glucosidase, vicianin beta-glucosidase, glucosylceramidase, and enzymes of cellulase type produced essentially by fungi, bacteria,
40 and protozoa which catalyse cellulose hydrolysis.

According to one preferred embodiment of the invention, the glycosidase(s) used

represent from 0.0001% to 10% by weight approximately of the total weight of the composition(s) containing this or these glycosidases, and even more preferentially from 0.0005% to 0.1% by weight approximately. By way of examples of glycosidases, mention may be made for example of the O-glycan-peptide hydrolase sold by Sigma Aldrich under the name O-Glycosidase (EC 3.2.1.97).

By way of example of glucosidases, mention may be made for example of those chosen from:

- the beta-primeverosidase sold by the company Amano under the name Aromase®;
- the cellulase sold by the company Amano under the name Cellulase DS®; and
- the beta-glucosidases sold by the company DuPont under the names Multifect® CX 15L and Optimase® CX 15L.

iii) pH of the composition(s)

The pHs of the compositions of the invention may be adjusted to the desired value by means of acidifying or basifying agents usually used in the dyeing of keratin fibres, or alternatively with the aid of standard buffer systems. Mention may be made, among the acidifying agents for the compositions used in the invention, by way of example, of mineral or organic acids, such as hydrochloric acid, orthophosphoric acid, sulfuric acid, carboxylic acids, such as acetic acid, tartaric acid, citric acid or lactic acid, or sulfonic acids. Among the basifying agents, mention may be made of those mentioned in point iv) below.

When reference is made to pH for the use of the invention, it is intended to mean that the ingredients i) and/or ii) are in an aqueous cosmetic composition, iii) at the pH in question, i.e. at a pH of inclusively between 3 and 8; more particularly at a pH of inclusively between 4 and 7 and preferably between 4.5 and 6.

According to one particular embodiment, the pH of the cosmetic composition comprising the ingredient i) as defined above is inclusively between 3 and 8; more particularly at a pH inclusively between 4 and 7 and preferably between 4.5 and 6.

According to another particular embodiment of the invention, the pH of the cosmetic composition comprising the ingredient ii) as defined above is inclusively between 3 and 8; more particularly at a pH inclusively between 4 and 7 and preferably between 4.5 and 6.

In particular, the pH of the "final" composition comprising the ingredients i) and ii) is at a pH inclusively between 3 and 8; more particularly at a pH inclusively between 4 and 7 and preferably between 4.5 and 6.

In particular, i) the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) of the invention is (are) used with ii) one or more glycosidase(s) as defined above in an aqueous cosmetic composition, iii) at a pH of inclusively between 3 and 8; more particularly at a pH of inclusively between 4 and 7 and preferably between 4.5 and 6.

According to one particular mode of the invention, the pH of the cosmetic composition containing ii) the glycosidase(s) and i) at least one hydroxyindol(in)e derivative glycosylated

on at least one hydroxyl group as defined above is inclusively between 4 and 7 and preferably between 4.5 and 6.

After the implementation of the dyeing process according to the invention involving the ingredients i), ii) and iii), the dyeing process of the invention can implement the application of an alkaline aqueous cosmetic composition comprising one or more basifying agents, in particular those as defined in point iv).

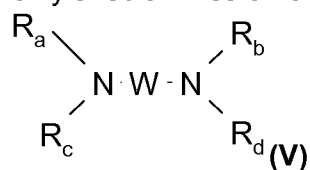
After the use of the ingredients i), ii) and iii) for treating, in particular dyeing, keratin fibres, in particular human keratin fibres such as the hair, an alkaline aqueous cosmetic composition comprising one or more basifying agents, in particular those as defined in point iv), can be used.

The pH of the alkaline composition is greater than 7, in particular inclusively between 8 and 11, preferably between 8.5 and 10.

iv) basifying agents

The basifying agent(s) may be mineral or organic.

Among the organic basifying agents, mention may be made of organic amines and in particular alkanolamines such as monoethanolamine, diethanolamine, triethanolamine, 2-amino-2-methyl-1-propanol, amino acids, in particular basic amino acids such as lysine and arginine, and optionally substituted alkylenediamines of formula **(V)** below:



in which W is a linear or branched (C₁-C₆)alkylene group optionally substituted with one or more hydroxyl groups or a C₁-C₄ alkyl radical; R_a, R_b, R_c and R_d, which may be identical or different, represent a hydrogen atom or a C₁-C₄ alkyl or C₁-C₄ hydroxyalkyl radical; for instance diamine compounds such as diaminopropane.

Preferably, the organic basifying agent(s) are chosen from basic amino acids. More preferentially, the organic basifying agent is arginine.

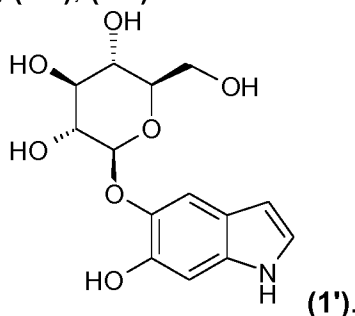
Among the mineral basifying agents, mention may be made of aqueous ammonia, alkali metal or alkaline-earth metal hydroxides, phosphates, monohydrogen phosphates and (bi)carbonates.

According to the invention, the basifying agent(s) used preferably represent from 0.001% to 10% by weight relative to the total weight of composition, and even more preferentially from 0.005% to 5% by weight.

The pH of the alkaline composition is greater than 7, in particular inclusively between 8 and 11, preferably between 8.5 and 10.

Compositions

Another subject of the invention relates to the compositions, in particular cosmetic compositions, in particular aqueous compositions (C1) for dyeing keratin fibres, in particular human keratin fibres such as the hair, which contain at least one hydroxyindole derivative glycosylated on at least one hydroxyl group, of formula **(la)**, **(la1)**, **(la2)**, **(la3)**, as defined above, in particular those for which the sugar S* denotes a monosaccharide and in particular a monosaccharide of structure S₁ to S₆ as defined above, it being understood that the compounds of formula **(la)**, **(la1)**, **(la2)**, **(la3)** are different from the compound **(1')** below:



More particularly, the compositions (C1) contain at least one compound of structure **(1)** to **(15)** defined above, in particular those for which the sugar S* denotes a monosaccharide and in particular a monosaccharide of structure S₁ to S₆, in particular S₁, as defined above, it being understood that **(1)** cannot represent **(1')**.

Even more particularly, the compositions (C1) contain at least one compound chosen from the compound of structure **(1)** with S* representing S₂, S₃, S₄, S₅ and S₆, and the compounds **(2)** and **(3)**, for which the sugar S* denotes a monosaccharide of structure S₁ to S₆, in particular S₁, as defined above.

Another subject of the invention relates to a composition, in particular a cosmetic composition, in particular an aqueous composition (C2) for dyeing keratin fibres, in particular human keratin fibres such as the hair, containing i) at least one hydroxyindol(in)e derivative glycosylated on at least one hydroxyl group, as defined above, and ii) at least one enzyme with glycosidase activity and in particular beta-glycosidase activity, more particularly glucosidase activity and preferentially beta-glucosidase activity.

In particular, the compositions (C2) contain ii) at least one enzyme with beta-glycosidase activity and i) at least one compound of formula **(la)**, **(la1)**, **(la2)**, **(la3)**, as defined above, in particular those for which the sugar S* denotes a monosaccharide of structure S₁ to S₆ as defined above. More particularly, the compositions (C2) contain ii) at least one enzyme with beta-glycosidase activity and at least one compound of structure **(1)** to **(15)** as defined above, in particular those for which the sugar S* denotes a monosaccharide of structure S₁ to S₆, in particular S₁, as defined above. Even more particularly, the compositions (C2) contain ii) at least one enzyme with beta-glycosidase activity and i) at least one compound chosen from the compounds of structure **(1)**, **(2)**, **(3)**, for which the sugar S* denotes a monosaccharide and in particular a monosaccharide of structure S₁ to S₆, in particular S₁, as defined above.

Another subject of the invention relates to a composition, in particular a cosmetic composition, in particular an aqueous composition (C3) for dyeing keratin fibres, in particular

human keratin fibres such as the hair, containing i) at least one hydroxyindol(in)e derivative glycosylated on at least one hydroxyl group, of formula (I), iii) at a pH of between 0 and 3 limits not included and 8 and 12 limits not included and not containing enzymes with beta-glycosidase activity.

Another subject of the invention relates to a composition, in particular a cosmetic composition, in particular an aqueous composition (C4) containing the compound of structure (1) with S* representing S₁ (corresponds to the compound (1') as defined above) and at least one cosmetic adjuvant chosen from anionic, cationic, non-ionic, amphoteric and zwitterionic surfactants or mixtures thereof, anionic, cationic, non-ionic, amphoteric and zwitterionic polymers or mixtures thereof, mineral or organic thickeners, in particular anionic, cationic, non-ionic and amphoteric polymeric associative thickeners, antioxidants, sequestering agents, fragrances, dispersants, conditioning agents such as, for example, modified or non-modified, volatile or non-volatile silicones, film-forming agents, ceramides, preservatives, and opacifiers.

In the compositions of the invention, in particular (C1), (C2), (C3) and (C4), the concentration i) of hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group of the invention is preferably from 0.0005% to 10% by weight relative to the total weight of the composition containing them. Even more preferentially, this concentration ranges from 0.005% to 5% by weight and better still from 0.01% to 4% by weight, relative to the total weight of the composition containing them.

Preferably, the concentration i) of hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group of the invention is inclusively between 0.1% and 1% by weight of the total weight of the composition which contains it (them), such as 0.5%.

The compositions of the invention are cosmetic, i.e. are cosmetically acceptable, i.e. they comprise a dye support that generally contains water or a mixture of water and of one or more organic solvents or a mixture of organic solvents.

The term "organic solvent" is intended to mean an organic substance that is capable of dissolving or dispersing another substance without chemically modifying it.

Preferably, all the compositions of the invention are cosmetic and aqueous, more preferentially they contain between 10% and 99.9% of water.

Organic solvents:

Mention may be made, as organic solvent, for example, of lower C₁-C₄ alkanols, such as ethanol and isopropanol; polyols and polyol ethers, such as 2-butoxyethanol, propylene glycol, propylene glycol monomethyl ether, diethylene glycol monoethyl ether, diethylene glycol monomethyl ether or hexylene glycol; and also aromatic alcohols, such as benzyl alcohol or phenoxyethanol.

The organic solvents are present in proportions preferably of between 1% and 40% by weight approximately and more preferably still between 5% and 30% by weight approximately, relative to the total weight of the dyeing composition.

Adjuvants:

The composition(s) of the dyeing process in accordance with the invention may also contain various adjuvants conventionally used in hair dyeing compositions, such as anionic, cationic, non-ionic, amphoteric or zwitterionic surfactants or mixtures thereof, anionic, cationic, non-ionic, amphoteric or zwitterionic polymers or mixtures thereof, mineral or organic thickeners, and in particular anionic, cationic, non-ionic and amphoteric polymeric associative thickeners, antioxidants, penetrants, sequestrants, fragrances, buffers, dispersants, conditioning agents, for instance volatile or non-volatile, modified or unmodified silicones, film-forming agents, ceramides, preserving agents and opacifiers.

Said adjuvants are preferably chosen from surfactants such as anionic or non-ionic surfactants or mixtures thereof and mineral or organic thickeners.

The above adjuvants are generally present in an amount for each of them of between 0.01% and 40% by weight relative to the weight of the composition, and preferably between 0.1% and 20% by weight relative to the weight of the composition.

Of course, those skilled in the art will take care to choose this or these optional additional compounds so that the advantageous properties intrinsically attached to the composition or to the compositions of use in the dyeing process in accordance with the invention are not, or not substantially, detrimentally affected by the envisioned addition or additions.

Additional dyes:

The process and the composition using the ingredients i) to iii) as defined above together or sequentially can further use or comprise one or more additional direct dyes. These direct dyes are chosen, for example, from those conventionally used in direct dyeing, and among which mention may be made of all commonly used aromatic and/or non-aromatic dyes, such as neutral, acidic or cationic nitrobenzene direct dyes, neutral, acidic or cationic azo direct dyes, natural direct dyes other than ortho-diphenols, neutral, acidic or cationic quinone and in particular anthraquinone direct dyes, azine, triarylmethane, indoamine, methine, styryl, porphyrin, metalloporphyrin, phthalocyanine and methine cyanine direct dyes, and fluorescent dyes. All these additional dyes are other than the ortho-diphenol derivatives according to the invention.

Mention may be made, among the natural direct dyes, of lawsone, juglone, indigo, isatin, curcumin, spinulosin, apigenidin, orceins and polyphenols. Use may also be made of extracts or decoctions comprising these natural dyes and in particular henna-based poultices or extracts.

The additional direct dye(s) used in the composition(s) preferably represent from 0.001% to 10% by weight approximately of the total weight of the composition(s) comprising them and more preferentially still from 0.05% to 5% by weight approximately.

The compositions of the process using ingredients i) to iii) as defined previously may also use or comprise one or more oxidation bases and/or one or more couplers conventionally used for the dyeing of keratin fibres.

Mention may be made, among the oxidation bases, of para-phenylenediamines, bis(phenyl)alkylenediamines, para-aminophenols, bis-para-aminophenols, ortho-aminophenols, heterocyclic bases and their addition salts.

Among these couplers, mention may be made especially of meta-phenylenediamines, meta-aminophenols, meta-diphenols, naphthalene-based couplers and heterocyclic couplers, and the addition salts thereof.

The oxidation base(s) present in the composition(s) are generally each present in an amount of between 0.001% and 10% by weight of the total weight of the corresponding composition(s).

The composition(s) of the invention may be in various presentation forms, such as a powder, a lotion, a foam, a cream or a gel, or in any other form that is suitable for dyeing keratin fibres. They may also be packaged in a propellant-free pump-action bottle or under pressure in an aerosol container in the presence of a propellant and form a foam.

vi) Dyeing processes

Another subject of the invention is a process for dyeing keratin fibres of the invention comprising the application to the fibres of a composition comprising one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above.

According to one particular embodiment of the invention, the process uses i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above, and ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined above.

According to a more particular embodiment of the invention, the process for treating keratin fibres, in particular a process for dyeing human keratin fibres such as the hair, uses i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined above, ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, particularly with glucosidase activity and more particularly beta-glucosidase activity, and iii) preferentially at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

According to one particular embodiment of the invention, the process is a dyeing process in which keratin fibres, more particularly human keratin fibres such as the hair, are treated:

- with a composition (A) comprising:
 - i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above;
 - iii) optionally at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6; then
- with a composition (B) comprising ii) one or more enzyme(s) with glycosidase activity

and in particular beta-glycosidase activity, optionally in aqueous solution, preferably at a pH of between 3 and 8.

- it being understood that at least one of the compositions is at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

5 The leave-on time, after the composition (A) has been brought into contact with the composition (B), is fixed at a period of between 5 minutes and 1 hour, preferentially between 15 and 45 minutes and more particularly between 20 and 40 minutes.

10 According to another particular embodiment of the invention, the process is a dyeing process in which keratin fibres, more particularly human keratin fibres such as the hair, are treated:

- with a composition (B) comprising ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, optionally in aqueous solution, preferably at a pH of between 3 and 8, then

- 15 ▪ with a composition (A) comprising:

- i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined above;

- iii) optionally at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6; then

- 20 ▪ it being understood that at least one of the compositions is at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

The leave-on time, after the composition (B) has been brought into contact with the composition (A), is fixed at a period of between 5 minutes and 1 hour, preferentially between 15 and 45 minutes and more particularly between 20 and 40 minutes.

25 According to one preferred embodiment of the invention, the process is a dyeing process in which keratin fibres, more particularly human keratin fibres such as the hair, are treated:

- with a composition (A) comprising:

- 30 i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined above;

- iii) at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6; and simultaneously

- 35 ▪ with a composition (B) comprising ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, optionally in aqueous solution, preferably at a pH of between 3 and 8,

it being understood that the pH of the resulting composition (A) + (B) is inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

The leave-on time, after the composition (A) has been brought into contact with the composition (B), is fixed at a period of between 5 minutes and 1 hour, preferentially between 15 and 45 minutes and more particularly between 20 and 40 minutes.

According to one advantageous variant of the process for dyeing keratin fibres, said

fibres are treated with a cosmetic composition comprising:

- i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above;
- ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, and
- iii) at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

The leave-on time of the composition comprising i) to iii) is fixed between 5 minutes and 1 hour, preferentially between 15 and 45 minutes and more preferentially between 20 and 40 minutes.

According to one preferred process of the invention, the keratin fibres are treated with a composition resulting from mixing the two compositions (A) and (B) in which: the composition A comprises:

- i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above;
- and

the composition B comprises:

- ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined above; or else
- a support bearing ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined above;

it being understood that at least one of the compositions (A) and/or (B) is an aqueous solution and that the pH of the mixture (A) + (B) is of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

According to another preferred embodiment of the invention, the process is a dyeing process in which the keratin fibres, more particularly human keratin fibres such as the hair, are treated with a composition resulting from mixing the two compositions (A) and (B) in which:

- the composition (A) comprises:
 - i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined above, optionally in aqueous solution; and
 - iii) optionally at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6;
- the composition (B) comprises:
 - ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, optionally in aqueous solution and optionally at a pH of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6;
 - or else
 - a support bearing ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, which are optionally in aqueous solution, particularly at a pH of

inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6;

it being understood that at least one of the compositions (A) and/or (B) is an aqueous solution and that the pH of the mixture (A) + (B) is of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

In particular, the composition resulting from mixing (A) + (B) is left on the keratin fibres for a period ranging from 5 minutes to 1 hour, more particularly from 15 to 45 minutes and preferentially from 20 to 40 minutes.

According to one particular mode of the process above, the composition resulting from mixing (A) + (B) is obtained after mixing the ingredients i) and ii), the pH of said mixture (A) + (B) being inclusively between 3 and 8, for a period (D) of between 5 minutes and 1 hour, more particularly between 15 and 45 minutes and preferentially between 20 and 40 minutes, and is then applied to the keratin fibres for a period (D) as defined above.

In particular, the composition resulting from mixing (A) + (B) can be obtained in the absence of air. According to one variant, this composition resulting from mixing (A) + (B) is applied to dry or wet keratin fibres immediately after the time (D) as defined above.

According to one advantageous variant, the composition resulting from mixing (A) + (B) after the time (D) is separated from the enzymes ii) for example by filtration and then applied to the keratin fibres, in particular human keratin fibres such as the hair, for a period ranging from 5 minutes to 1 hour, more particularly from 15 to 45 minutes and preferentially from 20 to 40 minutes.

According to one particularly appreciated embodiment of the invention, said process comprises a step of separating the enzymes as defined above before application to the keratin fibres.

According to one particular embodiment of the invention, the process after implementation of i), ii) and iii) comprises the application iv) of an alkaline composition at a pH above 7, in particular of inclusively between 8 and 11, preferably of inclusively between 8 and 10, more preferentially between 8.5 and 10 as defined above.

The leave-on time of the alkaline composition iv) is fixed between 5 minutes and 1 hour, preferentially between 15 and 45 minutes and more preferentially between 20 and 40 minutes.

The keratin fibres may or may not be moistened beforehand.

According to one particular dyeing process of the invention, said process may be followed by post-treatment steps v) such as shampooing using a standard shampoo, rinsing, for example with water, and/or drying the keratin fibres in the open air or by heat treatment as defined below.

Irrespective of the application method, the application temperature is generally inclusively between ambient temperature (25°C) and 60°C and particularly between 15 and 50°C, more particularly between 20 and 40°C, preferentially 25 and 35°C.

Thus, after applying the composition according to the invention, the head of hair may advantageously be subjected to a heat treatment by heating at a temperature of between

30°C and 60°C. In practice, this operation may be performed using a hairstyling hood, a hairdryer, an infrared ray dispenser or other standard heating appliances.

Use may be made, as a means for both heating and for straightening the head of hair, of a heating iron at a temperature of between 60°C and 220°C and preferably between 120°C and 200°C, preferably after the application of the alkaline composition iv) as defined above.

A particular embodiment of the invention relates to a dyeing process which is performed at ambient temperature (25°C).

In all the particular modes and variants of the processes described previously, the mentioned compositions (A) and (B) are ready-to-use compositions that may result from the extemporaneous mixing of two or more compositions and especially of compositions present in dyeing kits.

vii) Dyeing device or "kit":

Another subject of the invention is a multi-compartment dyeing device or "kit". Advantageously, this kit comprises from 2 to 5 compartments containing from 2 to 5 compositions in which the ingredients i) to iii) as defined above are distributed, it being understood that the ingredients i) and ii) are in separate compartments; and optionally in another separate compartment is an alkaline composition iv) which contains one or more alkaline agents as defined above, preferably comprising aqueous ammonia.

According to one particularly advantageous embodiment, the device also comprises a separate compartment which comprises an alkaline composition iv) which comprises one or more alkaline agents as defined above, preferably comprising aqueous ammonia.

The compositions of the device according to the invention are packaged in separate compartments, optionally accompanied by suitable application means which may be identical or different, such as fine brushes, coarse brushes or sponges.

This device mentioned above may also be equipped with a means for dispensing the desired mixture onto the hair, for example such as the devices described in patent FR 2 586 913.

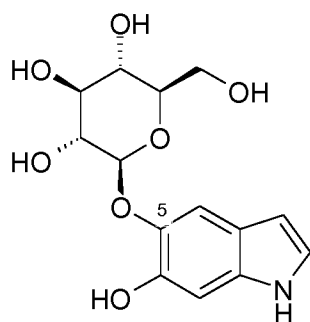
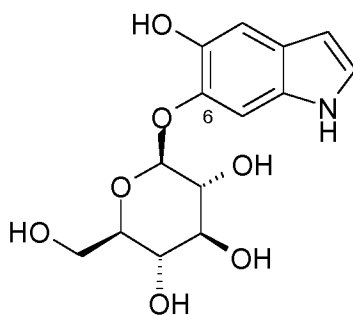
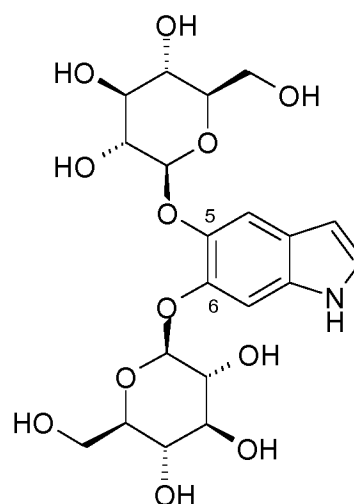
DYEING EXAMPLES**Preparation of the solutions**

- 5 • Preparation of the citrate/phosphate buffer solution, pH 5: Introduce 24.3 ml of solution A and 25.7 ml of solution B into a 100 ml flask and make up the volume with distilled water. The pH is 5.01.
- Solution A: 0.1 M citric acid solution (19.21 g/l);
- 10 - Solution B: 0.2 M dibasic sodium phosphate solution (53.65 g/l of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$);
- Solution of 5,6-dihydroxyindole in a buffer at pH 5 (12.0 g/l, 80 mM);
- Solution of 5,6-dihydroxyindole glycosylated at C₅ (**Compound 1**)* in a buffer at pH 5 (25 g/l, 80 mM);
- Solution of Optimase® CX15L (DuPont) in a buffer at pH 5 (25 g/l);
- 15 • Solution of aqueous ammonia at 2%.

* compound accessible via the extraction, separation and synthesis processes described in the literature: *Shipin Gongye Keji* 34(7), 300-304 (2013); *Rapid Commun. Mass Spectrum.*, 19, 2603-2616 (2005); *Zeitschrift für Naturforschung, C: Journal of Biosciences*, 56(5/6), 343-348 (2001); and *Helvetica Chimica Acta*, 67(5), 1348-55 (1984) or enzymatically according to the process described above.

20 The same solutions in a buffer at pH 5 were prepared with the 5,6-dihydroxyindole glycosylated at C₆ and the 5,6-dihydroxyindole diglycosylated at C₅ and C₆. The kinetics of deglycosylation carried out in the presence of Optimase® enzyme on the three molecules - 5,6-dihydroxyindole glycosylated at C₅ (**compound 1**), 5,6-dihydroxyindole glycosylated at C₆ (**compound 2**)** and 5,6-dihydroxyindole diglycosylated at C₅ and C₆ (**compound 3**)** –

25 clearly showed the gradual release of 5,6-dihydroxyindole.

**Compound 1****Compound 2****Compound 3**

**accessible enzymatically, in particular according to the process described above.

Example 1 (comparative)

The locks used weigh 0.25 g and are treated with 2.5 ml of solution (bath ratio (BR) of 10/1).

1/ At T_0 , introduce 1.05 ml of 5,6-dihydroxyindole solution + 1.05 ml of buffer solution at pH 5 into a flask, then continue with magnetic stirring at 30°C.

2/ At T_{30} , (30 min after T_0) spread the solution onto a lock of permanent-waved (sensitized) Caucasian grey hairs (90%). Then apply 0.4 ml of 2% aqueous ammonia solution and then leave to act for 30 min at 32°C.

3/ At T_{60} , (60 min after T_0) rinse, shampoo, rinse and dry the lock in the open air.

4/ At T_{6h} , measure the values of L, a, b of the lock.

Example 2 (invention)

The locks used weigh 0.25 g and are treated with 2.5 ml of solution ((BR) of 10/1).

1/ At T_0 , introduce 1.05 ml of solution of **Compound 1** + 1.05 ml of Optimase® CX15L into a flask, then continue with magnetic stirring at 30°C.

2/ At T_{30} , (30 min after T_0) spread the solution onto a lock of permanent-waved (sensitized) Caucasian grey hairs (90%). Then apply 0.4 ml of 2% aqueous ammonia solution and then leave to act for 30 min at 32°C.

3/ At T_{60} , (60 min after T_0) rinse, shampoo, rinse and dry the locks.

4/ At T_{6h} , measure the values of L, a, b of the locks.

The colouration of the hair is read on a Minolta spectrophotometer (CM3600d, illuminant D65, angle 10°, SCI values) for the L^* , a^* , b^* colorimetric measurements. In this $L^*a^*b^*$ system, L^* represents the intensity of the colour, a^* indicates the green/red colour axis and b^* indicates the blue/yellow colour axis. The higher the value of a^* , the redder the shade, and the higher the value of b^* , the yellower the shade.

Chromaticity C^* :

The chromaticity in the CIE L^* , a^* , b^* system is calculated according to the following equation:

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

The higher the value of C^* , the more chromatic the colouration obtained.

	a	b	C^*
Example 1 (comparative 1)	1.22	2.66	2.92
Example 2 (invention)	0.73	2.83	2.92

The composition of Example 2 of the invention is very stable with respect to atmospheric oxygen, whereas the comparative composition is not stable with respect to atmospheric oxygen (see Example 3 below). Moreover, on the linen used with the composition of the

invention, no marks were noted on white towels, whereas, with the comparative composition, some brown marks, which appear after a few minutes, are to be noted. Furthermore, it appears, according to the colorimetric measurements, that the results obtained, in particular in terms of chromaticity, are identical to those obtained with the comparative composition.

5

Example 3: stability test

Preparation of the citrate/phosphate buffer solution, pH 5.2

10 Introduce 24.3 ml of solution A and 25.7 ml of solution B into a 100 ml flask and make up the volume with distilled water. The pH is 5.2.

- Solution A: 0.1 M citric acid solution (19.21 g/l);
- Solution B: 0.2 M dibasic sodium phosphate solution (53.65 g/l of $\text{Na}_4\text{HPO}_4 \cdot 7\text{H}_2\text{O}$).

Preparation of the stock solutions (SS):

- **Compound 1**: 50.12 mg qs 2 ml of citrate/phosphate buffer, pH 5.2 (SS1)
- 15 - 5,6-dihydroxyindole: 22.44 mg qs 2 ml of citrate/phosphate buffer, pH 5.2 (SS2).

Preparation of the daughter solutions:

1/ Test A: introduce 0.8 ml of solution SS1 into a flask. Add 0.8 ml of buffer and 0.3 ml of a 2% aqueous ammonia solution and then continue with magnetic stirring at ambient
20 temperature. The pH of the solution is 9.2.

2/ Test B: introduce 0.8 ml of solution SS2 into a flask. Add 0.8 ml of buffer and 0.3 ml of a 2% aqueous ammonia solution and then continue with magnetic stirring at ambient temperature. The pH of the solution is 9.2.

Each test is sampled at $t = 5$ min, $t = 6$ h and $t = 24$ h, and diluted by a factor of 20 in water,
25 and then the percentage of residual compound is determined by UPLC/UV (297 nm).

UPLC/UV materials and methods

The analysis of the samples is carried out on an Acquity UPLC H-Class system (Waters)
30 comprising a diode array detector:

<i>Column</i>	Acquity BEH C18	
	Length	: 50 mm
	Internal diameter	: 2,6 mm
	V_{column}	: 0.18 ml
	Particle diameter	: 1,7 μm
<i>Mobile phase</i>	A = H_2O containing 0.1% of HC(O)OH	
	B = CH_3CN containing 0.1% of HC(O)OH	

<i>Gradient 1</i>	T(min)	% A	% B	P (psi)
	0	99	1	6750
	3	90	10	
	4	0	100	
	4.7	0	100	
	5	99	1	
	7	99	1	
<i>Flow rate</i>	0.6 ml/minute			
<i>Temperatures</i>	T _{column} = 35°C		T _{sample} = 25°C	
<i>Detection</i>	Diode array (DAD)			

The chromatogram is recorded in MaxPlot on the range of wavelengths of inclusively between 191 nm and 700 nm.

5

The percentage values in the graphs correspond to the residual values of the compounds.

time	T = 5 min	T = 6 h	T = 24 h
Test A according to the invention (Compound 1)	98.1%	75.6%	21.8%
Comparative test B (5,6-DHI)	91.8%	50.8%	3.3%

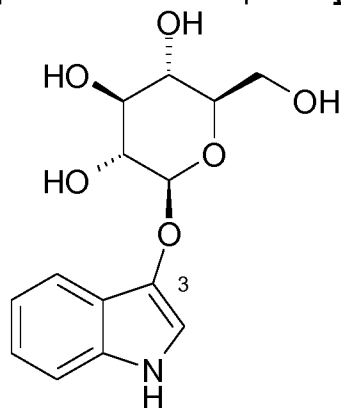
% corresponds to the residual percentage of the compounds after a time T

- 10 Thus, the results of tests A and B demonstrate the greater stability of the glycosylated 5,6-dihydroxyindoles according to the invention compared with non-glycosylated 5,6-dihydroxyindole.

Example 4

15

The comparative was carried out with an equimolar amount of **compound 1** according to the invention compared with the compound of the comparative US 6 656 229 glycosylated in position 3: the compound **(A)**:



Compound **(A)**

20

Prepare the following compositions:

Citrate/phosphate buffer solution, pH 5:

Introduce 24.3 ml of 0.1 M citric acid solution A' (19.21 g/l) and 25.7 ml of 0.2 M dibasic sodium phosphate solution B' (53.65 g/l of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) into a 100ml flask and make up the volume with distilled water. The pH is 5.01.

Comparative composition comprising the compound **(A)** in buffer pH 5 (23.7 g/l, 80 mM):

Introduce 238 mg of compound **(A)** into 10 ml of the buffer solution.

The substrate of the compound **(A)** is the enzyme CAS 487-60-5; M = 295 g/mol; sourcing Aldrich.

Composition according to the invention comprising **Compound 1** in the buffer pH 5 (25 g/l, 80 mM).

Introduce 250 mg into 10 ml of buffer.

The substrate of **Compound 1**, M = 311 g/mol solution of Optimase CX15L® in the buffer pH 5 (25 g/l). Introduce 0.5 mg of enzyme into 20 ml of the buffer solution.

Solution of aqueous ammonia at 2%.

Experiments

The locks used weigh 1 g and are treated with 10 ml of composition comprising **compound 1** or **(A)**.

1/ At T_0 , introduce the compositions below into flasks and then continue with magnetic stirring at 30°C.

- 4.2 ml of composition comprising a **Compound 1** + 4.2 ml of Optimase CX15L®
- 4.2 ml of composition comprising comparative compound **(A)** + 4.2 ml of Optimase CX15L®

2/ At T_{30} , (30 min after T_0) spread each solution onto a lock of natural Caucasian grey hairs (90%) and a lock of permanent-waved (sensitized) Caucasian grey hairs (90%). Then apply to each lock 1.6 ml of 2% aqueous ammonia solution and then leave to act for 30 min at 32°C.

3/ At T_{60} , (60 min after T_0) rinse, shampoo, rinse and dry the locks.

4/ At T_{6h} , measure the ΔE and ΔL values of the locks.

Selectivity ΔE results obtained

The value of the selectivity (ΔE) is calculated using the following formula:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

In this equation, ΔE represents the difference in colour between two locks of hair.

L^* , a^* , b^* represent respectively the values relative to the natural hair containing 90% grey hairs (NG 90) that have been dyed and L_0^* , a_0^* , b_0^* represent respectively the values relative

to the permanent-waved hair containing 90% grey hairs (PG 90) that have been dyed. The lower the ΔE , the lower the selectivity and the more homogeneous the colour on the keratin fibres.

Use	hair	L	a	b	ΔE
Compound 1 + enzyme (Invention)	NG 90	44.9	1.27	5.18	3.61
	PG 90	42.4	1.24	7.78	
Compound (A) + enzyme (Comparative)	NG 90	42.7	-8.16	0.22	8.16
	PG 90	35.25	-5.59	2.34	

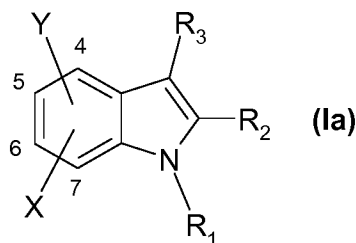
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It appears, according to the table of results above, that the colouration obtained on the keratin fibres with **compound 1** combined with the enzyme makes it possible to obtain a significantly less selective colouration than that obtained with comparative **compound (A)**.

CLAIMS

1. Use of i) hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, in the presence of at least ii) one enzyme with glycosidase activity, for dyeing keratin fibres.

2. Use according to the preceding claim, in which i) the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from those of formula (Ia) below:

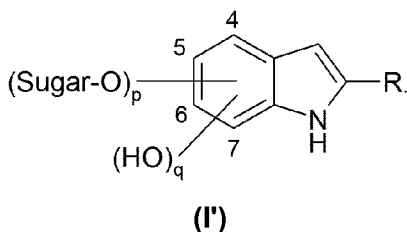


and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof;

in which formula (Ia):

- **R₁** and **R₃**, which may be identical or different, represent a hydrogen atom or a C₁-C₄ alkyl radical;
- **R₂** represents a hydrogen atom, or a C₁-C₄ alkyl, -C(O)-OH or -C(O)-OR₄ radical with R₄ representing a (C₁-C₄)alkyl group;
- **X** and **Y**, which may be identical or different, denote a hydrogen atom, or a hydroxyl or -O-Sugar group;
- **Sugar** represents a monosaccharide radical or polysaccharide radical constituted of 2 to 5 saccharide units, in particular of 2 to 3 and preferably of 2 saccharide units, it being understood that at least one of the groups X and Y represents an -O-Sugar group.

3. Use according to the preceding claim, in which i) the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from those of formula (I') below:



and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof such as hydrates; in which formula (I'):

- **R₁** represents a hydrogen atom, or a group chosen from (C₁-C₄)alkyl, -C(O)-OH

and $-\text{C}(\text{O})-\text{OR}_4'$ with R_4' representing a (C_1-C_4) alkyl group such as methyl or ethyl; in particular R_1 represents a hydrogen atom, or a group chosen from (C_1-C_4) alkyl and $-\text{C}(\text{O})-\text{OH}$, more particularly R_1 represents a hydrogen atom;

- **Sugar** is as defined above;

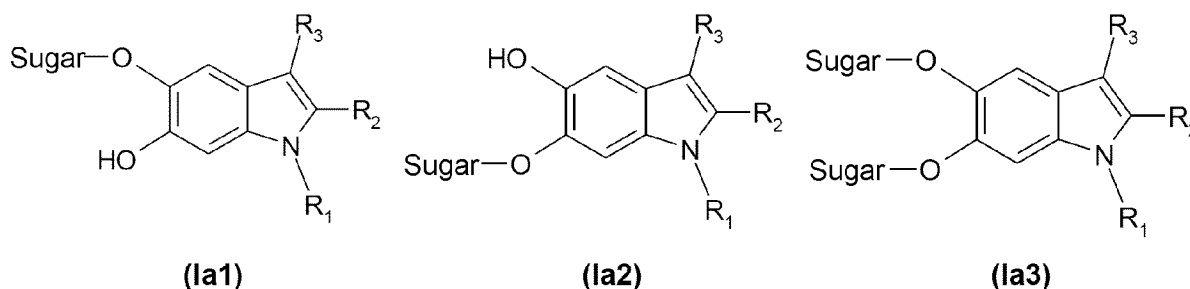
5 • **p** is equal to 1 or 2; and

- **q** is equal to 0 or 1;

it being understood that the sum $p+q$ is equal to 2;

particularly, p is equal to 1 and q is equal to 1, and Sugar-O- is in position 5 or p is equal to 1 and q is equal to 1, and Sugar-O- is in position 6 or else p is equal to 2, and Sugar-O- is in position 5 and 6 and q is equal to 0.

4. Use according to any one of the preceding claims, in which the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from those of formulae **(Ia1)**, **(Ia2)** and **(Ia3)**:



and also the the enantiomers, diastereoisomers and tautomers thereof, and the organic or mineral acid or base salts thereof, and/or the solvates thereof;

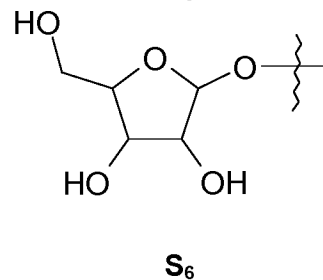
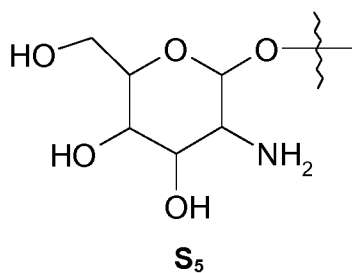
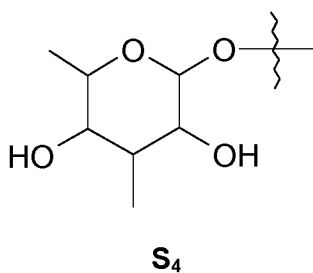
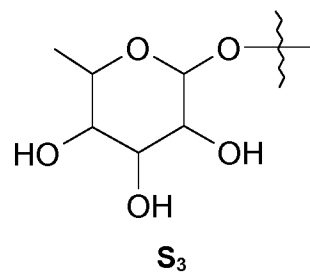
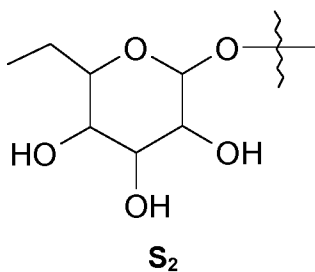
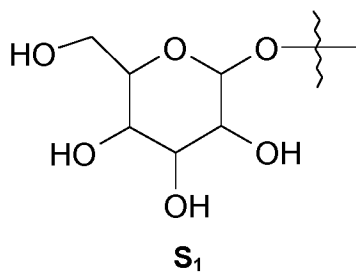
20 in which formulae **(Ia1)**, **(Ia2)** and **(Ia3)**,

R₁, **R₂** and **R₃** and **Sugar** are as defined for **(Ia)**, in Claim 3, in particular R_1 , R_2 and R_3 represent a hydrogen atom or (C_1-C_4) alkyl, preferably hydrogen; in particular, the hydroxyindol(in)e derivative(s) glycosylated on at least one hydroxyl group i) is (are) chosen from the hydroxyindole derivatives(s) glycosylated on at least one hydroxyl group and in particular those of formulae **(Ia1)** and **(Ia2)** as defined above.

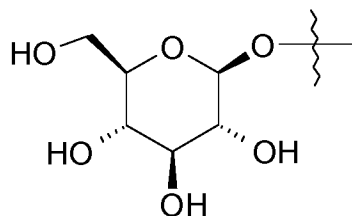
5. Use according to any one of the preceding claims, in which the sugar is attached to the oxygen atom of the $-\text{O}-\text{sugar}$ by the C_1 carbon atom of the sugar, which sugar is chosen from the monosaccharides or polysaccharides composed of the following sugars: pentoses; hexoses, hexosamines and deoxyhexoses; these saccharides can be in D or L configuration, preferentially D configuration; preferentially, said "sugar" represents a group chosen from glucose, glucosamine, rhamnose and xylose, preferentially D-glucose, D-glucosamine, D-rhamnose and D-xylose and more preferentially D-glucose.

6. Use according to any one of Claims 2 to 5, in which the Sugar-O-

represents a monosaccharide chosen from **S**₁ to **S**₆:

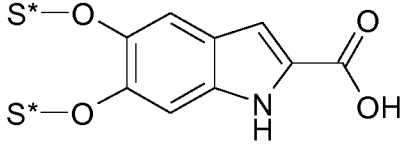
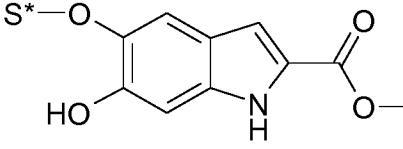
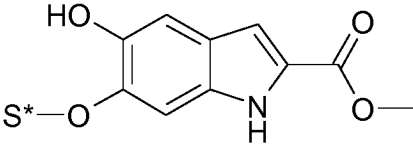
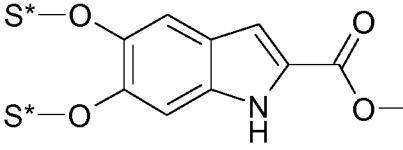
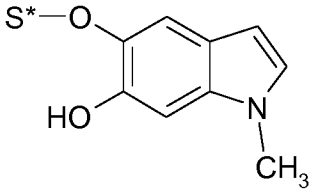
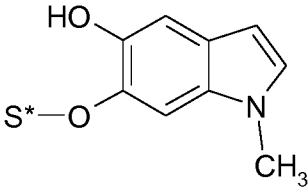
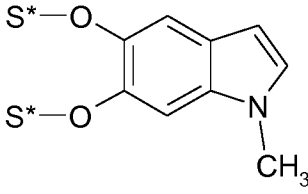


particularly **S**₁ and preferentially –O-Sugar represents:

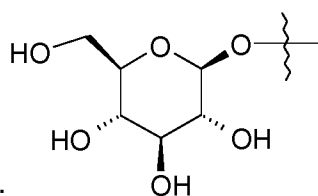


- 5 7. Use according to any one of the preceding claims, in which the hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups is (are) chosen from the following compounds:

(1)	(2)
(3)	(4)
(5)	(6)

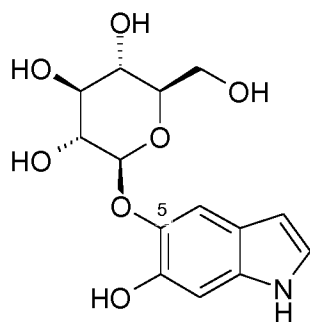
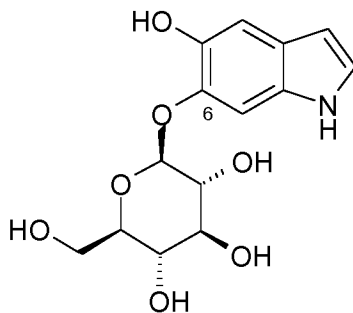
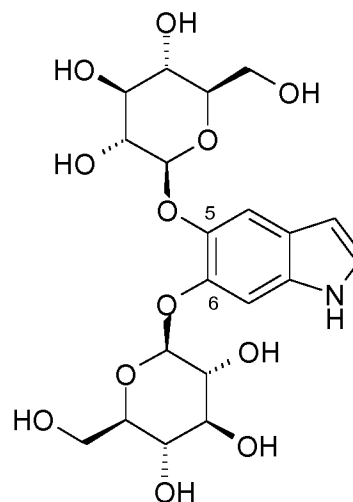
(7) 	(8) 
(9) 	(10) 
(11) 	(12) 
(13)	(14)
	
(15)	

and the organic or mineral acid or base salts thereof, and/or the solvates thereof, with S*-O- representing a monosaccharide chosen from **S₁** to **S₆** as defined in the preceding claim, and particularly S*-O- represents a monosaccharide chosen from **S₁**, **S₃**, **S₅**, **S₆**, more particularly



S₁ and preferentially:

- 5 more preferentially the hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups is (are) chosen from the following compounds:

**Compound 1****Compound 2****Compound 3.**

8. Use according to any one of the preceding claims, in which ii) the enzyme(s) with glycosidase activity are chosen from gentiobiase, cellobiase, emulsin, elaterase, aryl-beta-glucosidase, beta-D-glucosidase, beta-glucoside glucohydrolase, arbutinase, amygdalinase, p-nitrophenyl beta-glucosidase, primeverosidase, amygdalase, linamarase, salicilase, and beta-1,6-glucosidase (EC number 3.2.1.21 - Locus Chr. 4 p15.31), amygdalin beta-glucosidase, prunasin beta-glucosidase, vicianin beta-glucosidase, glucosylceramidase, and the enzymes produced essentially by fungi, bacteria, and protozoa which catalyse cellulose hydrolysis.

9. Use according to any one of the preceding claims, in which the ingredients i) and/or ii) are used at a pH inclusively between 3 and 8, particularly at a pH inclusively between 4 and 7 and more particularly between 4.5 and 6.

10. Process for dyeing keratin fibres comprising the application to the fibres of a composition comprising one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined in any one of Claims 1 to 7.

11. Process according to the preceding claim, which uses i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined in any one of Claims 1 to 7, and ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined in either of Claims 1 and 8.

12. Process according to either one of Claims 10 and 11, comprising the application to the fibres of a composition which comprises:

- i) one or more hydroxyindol(in)e derivative(s) glycosylated on at least one of the hydroxyl groups, as defined in any one of Claims 1 to 8;
- ii) one or more enzyme(s) with glycosidase activity and in particular beta-glucosidase

activity, as defined in either one of Claims 1 and 8;
iii) at a pH of inclusively between 3 and 8 as defined in Claim 9.

13. Process according to Claim 10 in which said fibres are treated with a composition resulting from mixing the two compositions (A) and (B) in which:
the composition A comprises:

i) one or more hydroxyindol(in)e derivative(s) glycosyated on at least one of the hydroxyl groups, as defined in any one of Claims 1 to 7;

and

the composition B comprises:

ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined in either one of Claims 1 and 9; or else

a support bearing ii) one or more enzyme(s) with glycosidase activity and in particular beta-glycosidase activity, as defined in either one of Claims 1 and 9;

it being understood that at least one of the compositions (A) and/or (B) is an aqueous solution and that the pH of the mixture (A) + (B) is of inclusively between 3 and 8, more particularly between 4 and 6, preferably between 4.5 and 6.

14. Process according to the preceding claim, in which the composition resulting from mixing (A) + (B) is left on the keratin fibres for a period ranging from 5 minutes to 1 hour, more particularly from 15 to 45 minutes and preferentially from 20 to 40 minutes.

15. Process according to the preceding claim, comprising a step of separating the enzymes before application to the keratin fibres.

16. Process according to any one of Claims 10 to 15, in which, after the application of the cosmetic composition to said fibres, iv) an alkaline composition at a pH above 7, in particular of inclusively between 8 and 11, preferably of inclusively between 8 and 10, more preferentially between 8.5 and 10, is applied.

17. Multi-compartment dyeing device or kit which comprises from 2 to 5 compartments containing from 2 to 5 compositions in which the ingredients i) to iii) as defined in any one of Claims 1 to 9 are distributed, it being understood that the ingredients i) and ii) are in separate compartments; and optionally in another separate compartment is an alkaline composition iv) which contains one or more alkaline agents as defined in the preceding claim, preferably comprising aqueous ammonia.

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2017/058715

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K8/60 A61Q5/10 A61K8/66
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	DE 196 48 019 C1 (WELLA AG [DE]) 12 February 1998 (1998-02-12) claims; examples page 2, lines 17-26 page 3, lines 10-13 -----	1,9-14, 17 1-17
X Y	US 6 656 229 B1 (TAGUCHI KAZUMI [JP] ET AL) 2 December 2003 (2003-12-02) claims 1, 3; examples column 5, lines 28-42 column 6, line 61 - column 7, line 8 -----	1,8,10, 11,17 1-17
Y	FR 2 769 835 A1 (OREAL [FR]) 23 April 1999 (1999-04-23) claims 1, 12-14; example 1 -----	1-17



Further documents are listed in the continuation of Box C.



See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

3 July 2017

Date of mailing of the international search report

31/07/2017

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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