

Biocatalyzed Generation of Molecular Diversity: Selective Modification of the Saponin Asiaticoside

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Abstract: An array of different derivatives of the complex ursane-type triterpene glycoside asiaticoside (**1**), a saponin component isolated from the perennial herb *Centella asiatica*, was generated by exploiting the stereo-, regio- and site-selectivity of four groups of enzymes (glycosidases, glycosyltransferases, lipases and laccases). A library of extracellular α -L-rhamnosidases (31 different items) was obtained by screening 16 different fungal strains under different cultivation conditions, and several of these preparations catalyzed the derhamnosylation of asiaticoside. Specifically, the enzymes produced by *Fusarium oxysporum* were selected for the synthesis of derhamno-degluco-asiaticoside (**2**) and also of derhamno-asiaticoside

(**3**), by *in situ* glucose-inhibition of contaminating β -D-glucosidases. β -1,4-Galactosyltransferase from bovine milk was subsequently used for the galactosylation of the two asiaticoside derivatives **2** and **3**, using 20% v/v DMSO as a cosolvent in order to increase substrate solubility. Finally, new acylated and oxidized derivatives of asiaticoside were also prepared by exploiting the lipase from *Candida antarctica* (Novozym 435) and the laccase from *Trametes pubescens*, respectively.

Keywords: asiaticoside; biotransformations; combinatorial biocatalysis; enzyme catalysis; glycosidases; glycosides

Introduction

Natural products constitute a relevant economic resource for the pharmaceutical, cosmetic and food industries as they are an unsurpassed source of bioactive compounds, useful to improve the well-being and the quality of life of humans. Many biologically active compounds are glycosides,^[1] and clear correlations between the presence of specific sugar residues and the biological activity of these molecules have been shown in many cases.^[2] For example, asiaticoside (**1**), a saponin component isolated from *Centella asiatica* with an ursane-type triterpene structure carrying a trisaccharide unit (Scheme 1), has been shown to have anti-inflammatory properties by stimulating wound-healing processes *via* fibroblast proliferation and collagen and glycosaminoglycan synthesis.^[3–5] Moreover, some asiaticoside derivatives possess strong neuroprotective effects against

beta-amyloid-induced neurotoxicity by antiapoptotic and antioxidative injury mechanisms.^[6]

In the last years it has been shown that biocatalysis offers a number of key advantages over chemical synthesis when working on complex molecules, advantages based on the chemo-, regio- and stereoselectivity of enzymes and on the possibility to carry out reactions under mild conditions.^[7] Even in the cases of simple transformations, for example, an acylation reaction, enzymatic chemo- and regioselectivity often allow one to avoid the protection-deprotection steps embodied in the usual chemical routes, once both the biocatalyst and the reaction conditions have been carefully selected.

Here we report on the use of four groups of enzymes (glycosidases, glycosyltransferases, lipases and laccases) for the preparation of a series of specific derivatives of asiaticoside by modification of its glycosidic moiety. The selected enzymes furnished derivatives with differ-

ent characteristics, for instance, more hydrophilic in the case of laccases and glycosyltransferases and more lipophilic in the case of glycosidases and lipases, thus allowing the generation of molecular diversity that can be exploited to modulate the bioactivity and bioavailability of the starting substrate.

Results and Discussion

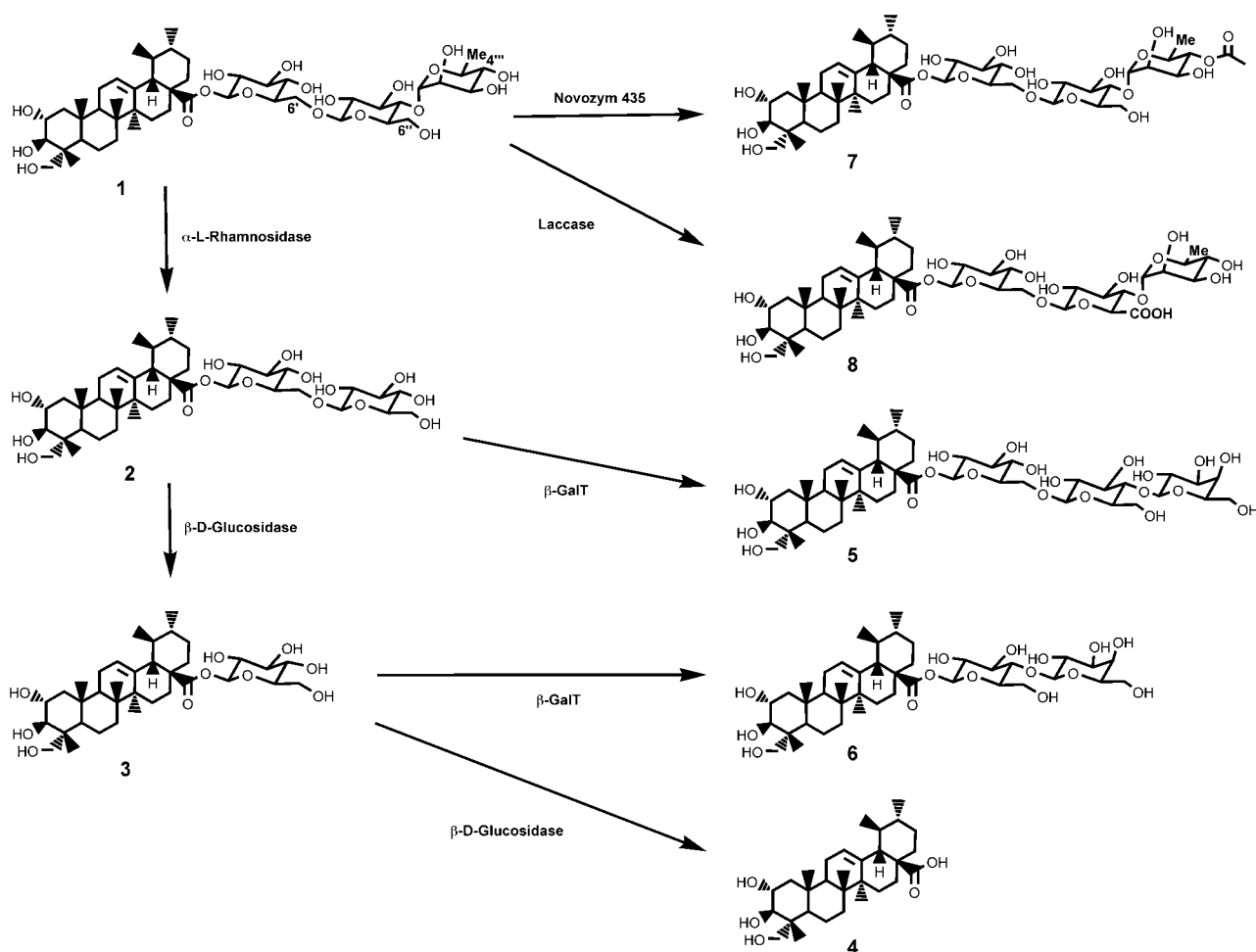
The sugar moiety of asiaticoside is a trisaccharide containing an α -L-rhamnopyranoside linked to the disaccharide gentiobiose. The selective modification of this carbohydrate unit was initially attempted by a combined use of glycosidases and glycosyltransferases (see Scheme 1).

We have recently described the preparation of a library of extracellular α -L-rhamnosidases by growing several fungi in the presence of different inducers (Figure 1), and we have exploited this biocatalysts collection for the selective derhamnosylation of natural glycosides.^[8]

A new screening of these 31 different enzymatic samples allowed the identification of 10 preparations, originating from 4 different fungi, that were able to hydrolyze asiaticoside. As expected, the contaminating β -D-glucosidases present in these crude samples were also active on the derhamnosylated intermediate derivative **2** (DeRha-Asia) and therefore, in all cases, the final products detected by TLC were either the fully deglycosylated aglycone (asiatic acid, **4**) or the monosaccharidic derivative derhamno-degluco-asiaticoside **3** (DeRha-DeGlc-Asia, Figure 2 and Table 1).

As the preparation from *Fusarium oxysporum* CCF 906 induced with rutin did not show the formation of asiatic acid, this sample was chosen for a preparative-scale reaction, whose HPLC chromatogram is reported in Figure 3A.

The monoglycosidic compound **3** was isolated in 47% yield and its structure confirmed by the respective mass and NMR data. The ESI-MS showed a molecular mass of 673 a.m.u., corresponding to the sodium salt of a monoglucosylated asiaticoside. In comparison to the starting substrate asiaticoside, the NMR spectra of **3** were lack-



Scheme 1. Enzymatic modification of the natural glycoside asiaticoside (**1**).

Strains	
<i>Ascremonium persicinum</i>	CCF 1830
<i>Aspergillus aculeatus</i>	CCF 108
<i>Aspergillus aculeatus</i>	CCF 3134
<i>Aspergillus aculeatus</i>	CCF 3138
<i>Aspergillus niger</i>	CCIM K2
<i>Aspergillus terreus</i>	CCF 3039
<i>Circinella muscae</i>	CCF 2417
<i>Emericella nidulans</i>	CCF 2912
<i>Eurotium amstelodami</i>	CCF 2723
<i>Fusarium oxysporum</i>	CCF 906
<i>Mortierella alpina</i>	CCF 2514
<i>Mucor circinelloides griseo-cyanus</i>	CCIM
<i>Penicillium oxalicum</i>	CCF 2430
<i>Rhizopus arrhizus</i>	CCF 100
<i>Talaromyces flavus</i>	CCF 2686
<i>Trichoderma harzianum</i>	CCF 2687

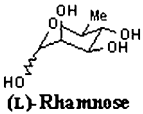
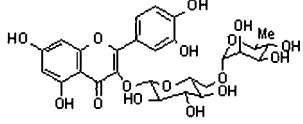
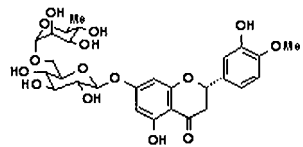
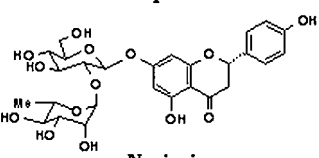
Inducers	
	(1)-Rhamnose
	Rutin
	Hesperidin
	Naringin

Figure 1. Fungal strains and inducers used for the generation of the α -L-rhamnosidase library. CCF: Culture Collection of Fungi, Department of Botany, Charles University, Prague, Czech Republic; CCIM: Culture Collection of the Institute of Microbiology, Prague, Czech Republic.

ing of the signals of the L-rhamnopyranosyl and one of the two D-glucopyranosyl units. Selected diagnostic ^1H -NMR signals of the only glucopyranosyl moiety linked to the aglycone were a doublet at 5.37 ppm ($J = 8.0$ Hz) due to the anomeric proton H-1' and two dd at 3.81 ($J_1 = 12.0$ and $J_2 = 2.3$ Hz) and 3.70 ($J_1 = 12.0$ and $J_2 = 4.4$ Hz) ppm due to H-6_a' and H-6_b'. Similarly, in the ^{13}C -NMR spectrum, the signals of a single saccharidic unit were detected at 94.4 (C-1'), 72.6 (C-2'), 70.0 (C-4') and 61.3 ppm (C-6'), together with two of the three

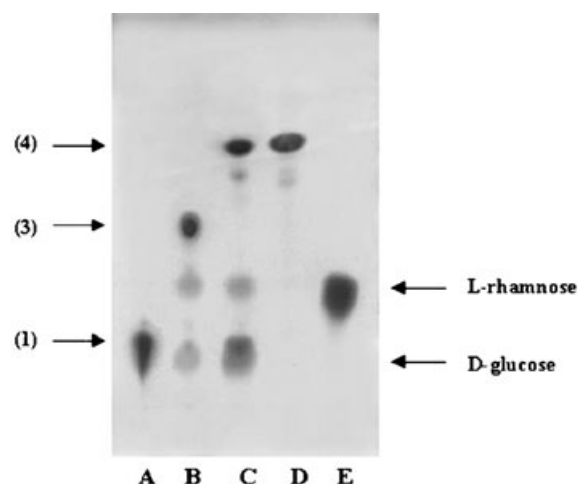


Figure 2. TLC analysis of selected reactions from the screening of the α -L-rhamnosidase library. A) Asiaticoside; B) reaction with α -L-rhamnosidase from *Aspergillus terreus* CCF 3039 induced with rhamnose; C) reaction with α -L-rhamnosidase from *Emericella nidulans* CCF 2912 induced with hesperidin; D) asiatic acid; E) L-rhamnose.

signals at 77.3, 77.2 and 77.1 ppm (C-3' and C-5'; the third signal was due to the C-3 of asiatic acid).

In order to isolate the gentiobiose derivative **2**, it was necessary to overcome the limitation related to the non-homogeneous nature of our enzyme preparations. In a usual approach, the more promising catalysts should have been purified using classical protein chromatography. However, in this specific case, we reasoned that the contaminating β -D-glucosidases could be inactivated by product inhibition. Accordingly, enzymatic hydrolysis was performed in the presence of 15% w/v glucose and we were pleased to find that a different product was formed with an intermediate polarity between **1** and **3** (Figure 3B). This compound was isolated in 46% yield and its structure confirmed by spectroscopic analysis. ESI-MS analysis gave the value of 835 a.m.u. for the molecular peak, corresponding to the expected value for the sodium salt of DeRha-Asia (**2**). The NMR spectra confirmed the presence of a gentiobiose unit and the lack of the L-rhamnopyranosyl moiety. Selected diagnostic ^1H -NMR signals were two doublets at 5.33 and 4.36 ppm, due to the two anomeric protons H-1' and H-1'', and four dd at 4.12 and 3.76, 3.87 and 3.67, due to CH₂-6' and CH₂-6'', respectively, while the signals due to the gentiobiose unit could be identified and assigned in the ^{13}C -NMR spectrum (see Experimental Section).

Having selectively “trimmed” asiaticoside, we decided to attempt some enzymatic “grafting” exploiting the nowadays commercially available β -1,4-galactosyl-transferase from bovine milk (β -GalT). This enzyme is able to transfer regio- and stereospecifically a sugar unit, usually a galactopyranoside, from an activated UDP donor to the C-4-OH of an unprotected glucopyr-

Table 1. Screening of the library of α -L-rhamnosidases for asiaticoside modification.

Strain	Inducer	Main Product	
		DeRha-DeGlc Asiaticoside (3)	Asiatic Acid (4)
<i>Aspergillus terreus</i> CCF 3059	L-Rhamnose	X	
	Rutin	X	
	Hesperidin	X	
	Naringin	X	
<i>Emericella nidulans</i> CCF 2912	L-Rhamnose	X	
	Hesperidin		X
	Naringin		X
<i>Fusarium oxysporum</i> CCF 906	Rutin	X	
	Naringin		X
<i>Talaromyces flavus</i> CCF 2686	Naringin	X	

anosyl acceptor to give the corresponding lactosyl derivative and UDP.^[9] Natural acceptors of this enzyme are either glucose itself or *N*-acetylglucosamine linked to a protein chain. However, in previous papers, we have shown that this enzyme is quite versatile concerning the aglycone moiety and therefore different natural glucopyranosides (i.e., colchicoside and ginsenosides) could be selectively galactosylated.^[10] As compounds **2** and **3** possess a free C-4-OH on their external glucopyranosyl moiety, both of them could be submitted to the action of β -1,4-GalT. Due to the low solubility of these substrates, the reactions were performed in the presence of 20% v/v DMSO, a cosolvent that is well compatible with this enzyme and does not affect its activity and stability when used in this concentration.^[11] Both these biotransformations were successful and, as shown in Scheme 1, the corresponding galactosylated derivatives **5** and **6** could be isolated and characterized in the usual way. Both ESI-MS and NMR analysis confirmed the addition of a galactopyranosyl unit to **2** and **3** (isolated yields 47 and 9%, respectively. For details see Experimental Section).

As a final step we considered the possibility to modify regio- and stereospecifically asiaticoside with enzymes different from the previous ones, that are naturally devoted to hydrolyze or to synthesize oligosaccharides. From our previous experiences in the modification of other natural compounds, the choice fell upon hydrolytic and oxidative enzymes.

The regioselective acylation of polyhydroxylated compounds catalyzed by lipases and proteases in organic solvents is a quite established methodology^[12] and previous studies have shown that enzymatic acylation of natural glycosides usually takes places on their sugar moieties even in the presence of reactive hydroxyl groups on the respective aglycones.^[13] Therefore, it was not surprising that the monoacylated derivatives isolated by reacting **1** (dissolved in THF) with vinyl acetate in the presence of different lipases or proteases were all characterized as sugar esters. The best results in terms

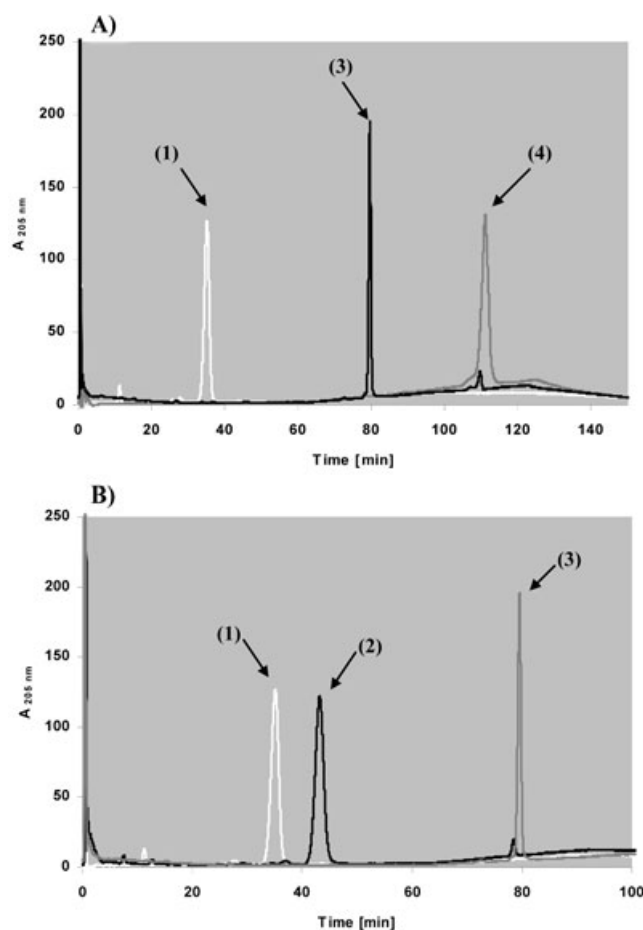


Figure 3. HPLC analysis of the reactions catalyzed by *Fusarium oxysporum* CCF 906 preparation induced by rutin. **A)** Hydrolysis of **1** to give **3** as the main product: black line, chromatogram of the reaction; white line and dark grey line, chromatograms of the standard asiaticoside (**1**) and asiatic acid (**4**), respectively. **B)** Hydrolysis of **1** to give **2** as the main product: black line, chromatogram of the reaction; white line and dark grey line, chromatograms of the standard asiaticoside (**1**) and DeRha-DeGlu-Asia (**3**), respectively.

of selectivity were obtained with the lipase B from *Candida antarctica* (Novozym 435) and the single product (**7**) was isolated in 59% yield. Its mass spectrum (1023 a.m.u.) indicated that **7** was a monoacetyl derivative. We have previously shown^[14] that Novozym 435 is able to acylate regioselectively the C-4-OH of L-rhamnopyranosyl derivatives, and this was also the case of asiaticoside. The ¹H-NMR spectrum of **7** showed a triplet due to a proton shifted downfield at 4.94 ppm. Analysis of the bidimensional COSY spectrum of **7** clearly indicated that this signal was correlated to the signal (dq) at 4.31 ppm due to H-5'''. Additionally, the ¹³C-NMR spectrum of **7** showed the diagnostic^[15] downfield shift of the signal due to the acylated C-4''' and the concomitant upfield shift of the signals due to the adjacent C-3''' and C-5'''.

Another selective modification of **1** was achieved by laccase-mediated oxidation. Laccases are copper-containing oxidases which, in a catalytic cycle, reduce one molecule of oxygen to two molecules of water with the concomitant oxidation of four substrate molecules to give four radicals.^[16] Typical substrates of laccases are phenols and aliphatic or aromatic amines, but oxidation of non-phenolic groups like primary alcohols or aromatic methyl groups, is also possible thanks to the auxiliary action of the so-called "mediators" (i.e., TEMPO, HBT, ABTS).^[17] The oxidation step is performed by the oxidized form of a suitable mediator, generated by its interaction with the enzyme. Specifically, we have recently shown that a laccase from *Trametes pubescens* coupled with the chemical mediator TEMPO can catalyze the regioselective oxidation of the primary hydroxy groups of a series of alkyl sugar derivatives to the corresponding glycopyranosiduronates.^[18] Asiaticoside carries two primary OH's, the first one on the C-23 of the aglycone moiety and the second one on the internal glucopyranosyl moiety (C-6''). We were pleased to find that the laccase-TEMPO-mediated oxidation of **1** (performed in a water-acetone mixture) was quite selective and a single product (**8**) could be isolated. Mass analysis indicated that **8** was a monooxidized product (1017 a.m.u., corresponding to the molecular peak of the disodium salt of **8**: M – H + 2 Na⁺). The ¹³C-NMR spectrum of **8** showed that the signal at 61.9 ppm, originally due to C-6'', was lacking, while in the ¹H-NMR spectrum showed, in addition to a doublet at 3.22 ppm due to H-5'', the disappearance of the protons due to CH₂-6'', resonating between 3.90 and 3.65 ppm in the ¹H-NMR spectrum of **1**, and a single signal remaining in that region, a broad doublet of doublets at 3.88 due to H-2''' (easily assigned by COSY correlation).

Conclusion

It has been shown that enzymes from different classes (specifically, oxidoreductases, transferases and hydrolas-

es) used in different reaction media (water solutions, water solutions containing water-miscible organic solvents, organic solvents) can selectively modify asiaticoside, a complex natural compound carrying several reactive functionalities. An array of different derivatives, with defined chemical structures, was generated from this lead compound exploiting the stereo-, regio- and site-selectivity of these biocatalysts. Worthy of note are the results obtained with the library of α -L-rhamnosidases, as they are an additional example of the exploitation of a group of enzymes that are easily generated and isolated and whose synthetic performances can be tuned in situ without need of purification steps.

Experimental Section

Yeast extract was from OXOID. Casamino acids, *p*-nitrophenyl α -L-rhamnopyranoside (*p*NP- α -L-Rha), *p*-nitrophenyl β -D-glucopyranoside (*p*NP- β -D-Glc), L-rhamnose, rutin, hesperidin, naringin, α -lactalbumin, porcine pancreas lipase (PPL), subtilisin A from *Bacillus* sp. (subtilisin Calsberg) were from Sigma. *Pseudomonas cepacia* lipase (PSL) was from Amano. *Chromobacterium viscosum* lipase (CVL) was from FINNSUGAR. Lipase B from *Candida antarctica* (Novozym 435) was from Novo Nordisk. Bovine milk β -1,4-galactosyltransferase (β GalT), UDP-galactose (UDP-Gal) and bovine intestine alkaline phosphatase were from Fluka (β -1,4-galactosyltransferase KIT, no. 59505). Asiaticoside (**1**) and asiatic acid (**4**) were a gift from Indena.

Thin-layer chromatography (TLC): precoated silica gel 60 F₂₅₄ plates (Merck); flash chromatography: silica gel 60 (70–230 mesh, Merck). HPLC analyses were carried out using a Jasco 880-PU pump equipped with a Jasco 870-UV detector and a Hewlett-Packard HP-3395 integrator. Enzymatic activities were monitored using a Jasco V-530 UV/VIS spectrophotometer. ¹H and ¹³C NMR spectra at 300 MHz and 75.2 MHz were recorded in CD₃OD on a Bruker AC-300. Electrospray mass spectrometry (ESI-MS) was recorded with an LCQTM quadrupole ion-trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with a Nano-ESI source (Protana, Odense, Denmark). Due to the unavoidable presence of Na⁺ ions during sample preparation and the high alkali-cation affinity of oligosaccharides, all positively charged ions produced were ionized with Na⁺.

Production of Glycosidases^[18]

The strains used in this study originated from the Culture Collection of Fungi (CCF), Department of Botany, Charles University, Prague, and from the Culture Collection of the Institute of Microbiology (CCIM), Prague, Czech Republic. The cultivation medium contained [g/L]: KCl, 0.5; KH₂PO₄, 15; NH₄Cl, 4; yeast extract, 5; casamino acids, 1. Before sterilization, the medium was supplemented by the specific inducer (L-rhamnose, rutin, hesperidin and naringin, 5 g/L) and 1 mL/L of trace element Vishniac solution,^[20] the pH was adjusted to 6.0 and after sterilization MgSO₄·7 H₂O sterile solution (10% w/v, 5 mL/L) was added. Conical flasks (500 mL) with 100 mL of medium were inoculated with 0.5 mL of a spore sus-

pension in 0.1% (v/v) Tween 80 solution and cultivated on a rotary shaker at 200 rpm and 28 °C. To test constitutive production of α -L-rhamnosidases, each strain was cultured in the same medium, but replacing the inducer with respective amounts of casamino acids.

Clear filtrate samples for each culture were collected daily and submitted to the α -L-rhamnosidase activity assay. Cultures were filtered when the maximum activity of α -L-rhamnosidase was reached, typically after 5–10 days of growth. Crude α -L-rhamnosidase preparations were obtained as ammonium sulfate precipitates (80% saturation) and stored in saturated ammonium sulfate solution at 4 °C.

Glycosidase Activity Assays

α -L-Rhamnosidase and β -D-glucosidase activities were assayed spectrophotometrically using *p*NP- α -L-Rha and *p*NP- β -D-Glc as substrates, respectively. One unit of the respective glycosidase activity was defined as the amount of enzyme releasing 1 μ mol of *p*-nitrophenol per minute at pH 6.0 and 35 °C.

Screening of the Library for Asiaticoside Hydrolysis

The screening of the α -L-rhamnosidase library for asiaticoside hydrolysis was performed on a 2 mg/mL asiaticoside solution in 50 mM potassium phosphate buffer, pH 6.0. A sample of each preparation of the enzyme library (5 μ L, minimum 6 Units) was added to 1 mL of substrate solution and the mixtures were incubated at 35 °C for 48 h. The reactions were monitored by TLC [AcOEt-MeOH-H₂O = 8:3:0.5; R_f: asiaticoside (**1**), 0.10; L-rhamnose, 0.21; D-glucose, 0.07; asiatic acid (**4**), 0.37] with the molybdate reagent [(NH₄)₆Mo₇O₂₄·4 H₂O, 42 g; Ce(SO₄)₂, 2 g; H₂SO₄ conc., 62 mL; made up to 1 L with deionized water].

Preparative Synthesis of Derhamno-Deglucosyl Asiaticoside (**3**)

The synthesis of **3** was accomplished by dissolving asiaticoside (**1**, 75 mg) in 25 mL of 50 mM potassium phosphate buffer, pH 6.0 and adding 10 U (200 μ L of a 50 U/mL suspension) of the α -L-rhamnosidase from *Fusarium oxysporum* CCF 906 (inducer: rutin). The reaction was incubated at 35 °C and monitored by TLC (AcOEt-MeOH-H₂O = 8:3:0.5) and HPLC analyses [reverse-phase analytical column Lichrospher® RP-18, 5 μ m, 4 × 125 mm, Merck; mobile phase: H₂O (A), CH₃CN (B): 0'–60', 80% v/v A; 120', 60% v/v A; 150', 80% v/v A; flow rate: 1 mL/min, UV detector: 205 nm; samples: 100 μ L of the reaction mixture were lyophilized and dissolved in 20 μ L of H₂O: EtOH = 1:1, injection volume: 10 μ L, retention times: **1**, 35 min; **3**, 79 min; asiatic acid (**4**), 110 min]. After 48 h, the product was recovered by extraction with AcOEt and the solvent was evaporated. The crude residue (170 mg) was purified by flash chromatography (eluent: CHCl₃-MeOH-H₂O = 8:2:0.1) to give **3**; yield: 25 mg (47%).

Preparative Synthesis of Derhamno-Asiaticoside (**2**)

Synthesis of **2** was accomplished as described for **3**, but in the presence of 15% w/v glucose in order to inhibit contaminating β -D-glucosidase activities *in situ*. The reaction was monitored by TLC and HPLC analyses (same conditions used for **3**, retention times: **1**, 35 min; **2**, 43 min; **3**, 79 min). After 48 h, 4.25 g of NaCl were added to the reaction mixture and the product was extracted with AcOEt and the solvent was evaporated. The crude residue (343 mg) was submitted to two subsequent flash chromatography columns (eluent: CHCl₃-MeOH-H₂O = 8:2:0.1 and CHCl₃-MeOH-H₂O = 8:3:0.4) to give **2**; yield: 28 mg (46%).

Galactosylation of **2** and **3**

Compound **2** (28 mg) or **3** (25 mg) was dissolved into 2 mL of DMSO. 7 mL of reaction solution, containing 50 mM TRIS/HCl, pH 7.4, 10 mM MnCl₂, 7.9 mg/mL UDP-Gal and 0.28 mg/mL α -lactalbumin, and 1 mL of enzymatic solution [50 mM TRIS/HCl, pH 7.4, 2 U/mL bovine milk β -1,4-galactosyltransferase (β GalT), 66 U/mL bovine intestine alkaline phosphatase] were then added to the substrate solutions and the reactions were incubated at 35 °C. TLC (CHCl₃-MeOH-H₂O = 8:3:0.5) and HPLC analyses [reverse-phase analytical column Lichrospher® RP-18, 5 μ m, 4 × 125 mm, Merck; mobile phase: H₂O (A), CH₃CN (B): 0'–60', 80% v/v A; 120', 60% v/v A; 150', 80% v/v A; flow rate: 1 mL/min, UV detector: 205 nm; injection volume: 2 μ L, retention times: **2**, 54.2 min; **3**, 80.2 min; **5**, 41.1 min; **6**, 75.9 min] were carried out at scheduled times. After 72 h, the products were purified by flash chromatography (eluent: CHCl₃-MeOH-H₂O = 8:3:0.4) to give **5** (yield: 16 mg, 47%) or **6** (10 mg). As this last product contained traces of uridine (as shown by TLC, eluent AcOEt-MeOH-H₂O = 7:3:0.5), it was further purified by flash chromatography (eluent: CHCl₃-MeOH-H₂O = 8:2:0.3) to give pure **6** (yield: 3 mg, 9%).

Acylation of Asiaticoside by Lipases and Proteases

Five different hydrolases [the protease subtilisin Calsberg and the lipases from porcine pancreas (PPL), *Pseudomonas cepacia* (PSL), *Chromobacterium viscosum* (CVL) and *Candida antarctica* (Novozym 435)] were screened for their ability to acylate **1** in the presence of THF and vinyl acetate. Before use, PPL was adsorbed on Celite and subtilisin Calsberg on K₂HPO₄. The reactions were monitored by TLC (CHCl₃-MeOH-H₂O = 10:5:1). The formation of products was observed only when using Novozym 435 and PSL, therefore the reactions with these enzymes were scaled-up in order to recover and characterize the products.

Two substrate solutions each containing **1** (200 mg) dissolved in 18 mL of dry THF and 2 mL of vinyl acetate as acylating agent were prepared. 150 mg of Novozym 435 or 1 g of PSL on Celite were then added, respectively, and the mixtures were shaken at 250 rpm and 45 °C. The reactions were monitored daily by TLC and, after 3 days, fresh immobilized enzymes (150 mg of Novozym 435 and 400 mg of PSL, respectively) and vinyl acetate (1 mL to each reaction) were added. After 7 days, products were recovered by filtration and purified by flash chromatography (eluent: CHCl₃-MeOH-H₂O =

10:3:0.3). The Novozym 435 reaction gave 4'''-*O*-acetylasiatricoside (**7**; yield: 122 mg, 59%), whereas the PSL reaction gave **7** (47 mg, 23%) and a mixture of 2'''- and 3'''-*O*-acetylasiatricoside (yield: 38 mg, 18%).

Oxidation of Asiaticoside by Laccases

Asiaticoside (**1**; 101 mg, 0.1 mmol) was dissolved in a mixture of 15 mL acetone and 35 mL acetate buffer (20 mM pH 4.5). Following the addition of TEMPO (3.5 mg, 0.2 equivs.) and 2 mL of a 26 U/mL solution of a laccase from *Trametes pubescens*, the reaction mixture was mildly shaken at room temperature for 7 days. The water solution was evaporated and the crude residue purified by silica flash chromatography (eluent: AcOEt-MeOH-H₂O = 10:3:1) to give **8**; yield: 25 mg (26%).

Supporting Information

Characterization data for compounds **1–8** are available in the Supporting Information.

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