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Effective Enzymatic Caffeoylation of Natural Glucopyranosides

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ABSTRACT

Reaction system was developed for enzymatic caffeoylation of model saccharidic acceptor methyl β-D-glucopyranoside to obtain exclusively methyl 6-*O*-caffeoyl-β-D-glucopyranoside. Reaction with starting concentration of acceptor 0.2 M provided 73% yield of purified product within 17 days. Reactions with low acceptor concentrations (0.04 and 0.08 M) run to the completion within 7 days. Such highly effective and regioselective reaction was promoted by Lipozyme TL IM in *tert*-butanol, using vinyl caffeate as acylation donor. The optimized reaction conditions were used in preparative caffeoylation of natural substances – arbutin and salidroside, giving 75% of 6-*O*-caffeoylated arbutin (robustaside B) and 74% of 6-O-caffeoylated salidroside as the only products after 12 and 16 days, respectively.

Keywords:
Regioselective caffeoylation
Lipozyme TL IM
Robustaside B
6-*O*-Caffeoyl-salidroside
Phenylpropanoid glycosides

Caffeic acid occurs in nature as a constituent of more complex molecules widely distributed in medicinal plants, fruits, coffee grains, propolis etc. These substances possess a broad spectrum of health-beneficial biological activities,[1-4] their extraction from natural sources and purification use to be however laborious and costly and there is a growing demand for their effective chemical synthesis. Starting with caffeic acid phenethyl ester (CAPE)[5] through chicoric and chlorogenic acid[6] to phenylpropanoid glycosides,[7-9] their synthesis appears more and more complicated in view of complexity of their chemical structure.

Robustaside B (6-*O*-caffeoyl-arbutin, *p*-hydroxyphenyl 6-*O*-caffeoyl-β-D-glucopyranoside) isolated from *Viburnum dilatatum*[10] and 6-*O*-caffeoyl-salidroside (p-hydroxyphenetyl 6-*O*-caffeoyl-β-D-glucopyranoside) isolated from *Prunus grayana*[11] belong to natural substances with remarkable biological activities. Robustaside B expressed a stronger anti-melanin activity and lower toxicity than

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arbutin,[12] and its antioxidant[13] and antibacterial activities were documented.[14] 6-*O*-Caffeoyl-salidroside exhibited antibacterial activities against *S. aureus* and cytotoxic activities against human tumor Hela and Siha cell lines.[15]

Direct selective enzymatic acylation of a respective acceptor with caffeic acid provides therefore simple and straightforward alternative to chemical procedures. Given the complexity of acceptor, the appropriate biocatalyst may provide the necessary chemo- or regioselectivity in one reaction step. Different phenylalkyl esters of caffeic acid have been recently synthetized with use of enzymes. The esterification of caffeic acid by lipophilic acyl acceptor - phenethyl alcohol was catalyzed by action of *Candida antarctica* lipase B (CAL B, Novozym 435) in high yields.[5,16,17] The enzymatic synthesis of 3-cyclohexylpropyl caffeate, a mimetic of CAPE, was carried out by a two-step reaction starting from 5-caffeoylquinic acid and catalyzed by chlorogenate hydrolase from *Aspergillus japonicus* and CAL B.[18]

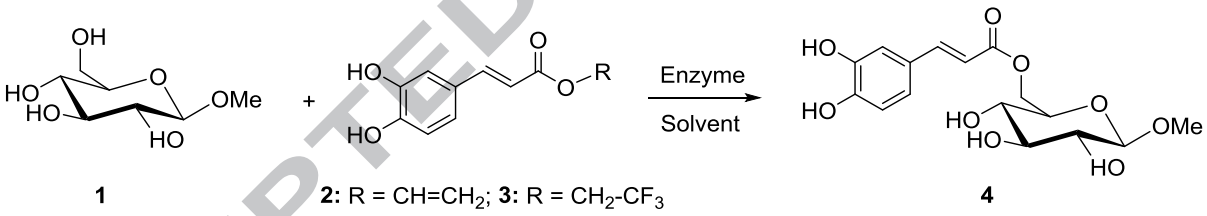
On the other side, caffeoylation of hydrophilic polyhydroxylated acceptors represents a more complex problem. The enzyme should be active in polar organic solvents, which are necessary for dissolving of such acceptor while lipase-catalyzed esterifications preferably proceed in non-polar hydrophobic environment. Esterification and transesterification of caffeic acid or its methyl ester with glycerol using feruloyl esterase from *Aspergillus niger* was not successful.[19] The first direct syntheses of 4-O-hydroxycinnamoyl derivatives of quinic and shikimic acids were accomplished by regioselective esterification with lipase A from *Candida antarctica*,[20] but no successful enzymatic caffeoylation of quinic acid derivatives was reported so far.

Previous studies of enzymatic acylation of glycosides by derivatives of hydroxycinnamic acids with lipases or esterases were mainly focused on feruloylations.[21-24] Only a few reports of enzymatic caffeoylations of saccharides have appeared, usually with very low or zero yields of products. Otto et al.[25] used variety of non-activated arylaliphatic acids for acylation of glucose and salicin catalyzed by immobilized CAL B. They failed to obtain esters of aromatic carboxylic acids or unsaturated arylaliphatic acids, like cinnamic acid and its derivatives. López-Munguía et al.[26] used successfully the same enzyme to acylate analogues of salidroside with saturated and unsaturated hydroxycinnamic acids. Chemical yields of the resulting esters were moderate or very low, reaching 40% of ferulate and only less than 5% of the respective caffeate. Cultured cells from sweet potato (*Ipomoea batatas)* caffeoylated isoquercitrin in 38% yield to produce the corresponding 6-*O*-caffeate using caffeic acid, coenzyme A and ATP.[27] The enzyme system catalyzing this synthesis is however based on plant transferase and not lipase or esterase. Several previous reports[28,29] have demonstrated relative feasibility of the enzymatic feruloylation of arbutin and another phenolic glycosides[30] with low to moderate yields. Formation of caffeoylated salidroside catalyzed by Novozyme 435 (CAL B) was recently reported, the proof of the product synthesis is however demonstrated only by TLC, without its

isolation and structural characterization.[31] To our best knowledge, the preparative enzyme-catalyzed caffeoylation of arbutin or salidroside has not been so far described.

In our previous work,[32] we have prepared a large scale of vinyl esters of phenolic acids and their analogues for studies of substrate specificity of Lipolase 100T (lipase from *Thermomyces lanuginosus*) in acylations of methyl α-D-glucopyranoside as a model acceptor. The synthetic reactivity of Lipolase corresponds to the hydrolytic activity of feruloyl esterase type A proposed by Crepin et al.[33] For example the acylation of the acceptor with vinyl ferulate yielded 68% of 6-*O*-feruloylated product while use of vinyl caffeate afforded only 16% of 6-*O*-caffeoylated glucoside.

To select a more specific candidate for enzymatic caffeoylations of saccharides, we have recently developed a spectrophotometric method for assay of caffeoyl esterase and used it for screening this activity within a scale of lipases, esterases and crude industrial glycanases.[34] This paper presents our study of enzymatic caffeoylation of methyl β-D-glucopyranoside (**1**) as an inexpensive model acceptor manifesting reactivity of hydroxyls comparable to natural β-D-glucopyranosides (Scheme 1). Vinyl caffeate (**2**)[32] and 2,2,2-trifluoroethyl caffeate (**3**) were prepared and used as activated donors. The latter donor was synthesized as a new substance. The conditions optimized for acylation of **1** were applied in preparative caffeoylations of arbutin (**5**) and salidroside (**6**).



Scheme 1. Enzymatic caffeoylation of methyl-β-D-glucopyranoside.

Screening of enzymes displaying caffeoyl esterase activity was realized by the method published recently.[34] The protocol was modified to assay also immobilized enzymes insoluble in water. The screening selected five enzyme candidates for more detailed study, namely Lipex 100T, Lipolase 100T, Lipozyme TL IM, Pentopan 500BG, Liquanase 2.5L. The results are summarized in Table 1.

These six enzymes were tested by model transcaffeoylations monitored by HPLC. It is interesting, that their performance in synthetic reaction did not correspond to their hydrolytic caffeoyl esterase activity. The far most active enzyme, Pentopan 500 BG, produced less than 5% of the caffeoylated **1** after 10 days, while conversion of the same reaction catalyzed by Lipozyme TL IM, a lipase from *Thermomyces lanuginosus*, reached over 54% in 8 days (Table 1). The hydrolytic assay method did not allow us to reliably estimate caffeoyl esterase activity of Novozyme 435 due to its physical properties. Anyway, this enzyme is known to catalyze acylations of several acceptors with various

phenolic acids[25] and therefore we tested its performance in the studied acylation, in which was however Novozyme 435 only moderately effective catalyst (Table 1).

Table 1

Caffeoyl esterase activity in selected enzyme preparations and reaction conversions in caffeoylation of **1** with vinyl caffeate after 8 days in acetonitrile

Enzyme	Microbial origin	Enzyme class	Caffeoyl esterase (U/g)	Conversion of **1** to **4** (%)
Lipex 100T	*Thermomyces lanuginosus*	Lipase	1.9	18.5
Pentopan 500BG	*Humicola insolens*	Xylanase	360	3.3
Lipozyme TL IM	*Thermomyces lanuginosus*	Lipase	2.3	54.3
Novozym 435	*Candida antarctica*	Lipase	n.d.	19.3
Lipolase 100T	*Thermomyces lanuginosus*	Lipase	0.9	8.9
Liquanase 2.5L[a]	*Bacillus licheniformis*	Protease	28.0	22.6

[a]Ultrafiltered and lyophilized enzyme

Lipozyme TL IM was our obvious choice of biocatalyst for further studies. Its catalytic performance was significantly influenced by the nature of reaction media. While only half of glucoside **1** reacted after 6 days in acetonitrile, its complete caffeoylation in less toxic *tert*-butanol was observed within the same time interval (Fig. 1). Other solvents allowed moderate to low conversions. The reactivity of Lipozyme TL IM in *tert.*-butanol was assessed also for use of 2,2,2-trifluoroethyl caffeate **3** as caffeoyl donor. The conversion within 8 days was only 78 % (Fig. 1), which makes vinyl caffeate **2** the more favorable donor for enzymatic caffeoylations.

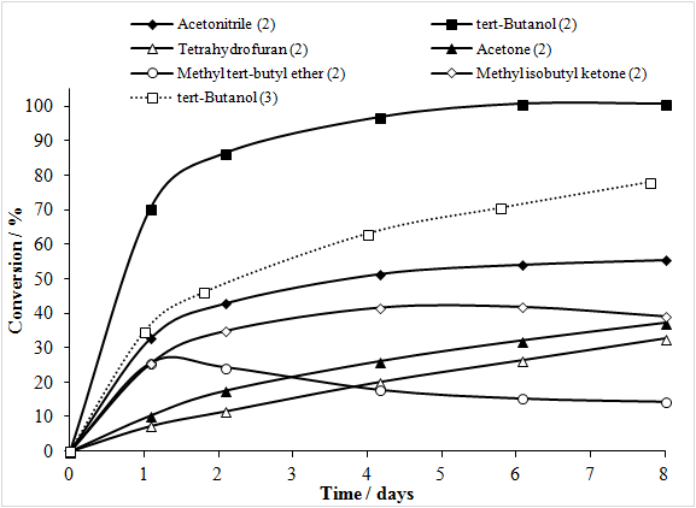


Figure 1. Effect of reaction media on caffeoylation of methyl-β-D-glucopyranoside (**1**) by Lipozyme TL IM with vinyl caffeate (**2**) or 2,2,2-trifluoroethyl caffeate (**3**) as time course of its conversion to **4**.

Reaction conditions: 0.04 M methyl-β-D-glucopyranoside, 0.06 M vinyl caffeate, 0.4 g molecular sieves, 0.4 g Lipozyme TL IM, 5 mL of solvent, 40°C, 300 rpm.

Higher acceptor levels influenced negatively the rate of the reaction, anyway, its starting concentrations up to 0.2 M allowed to achieve conversion on the level of 90 % in 9 days (Fig. 2).

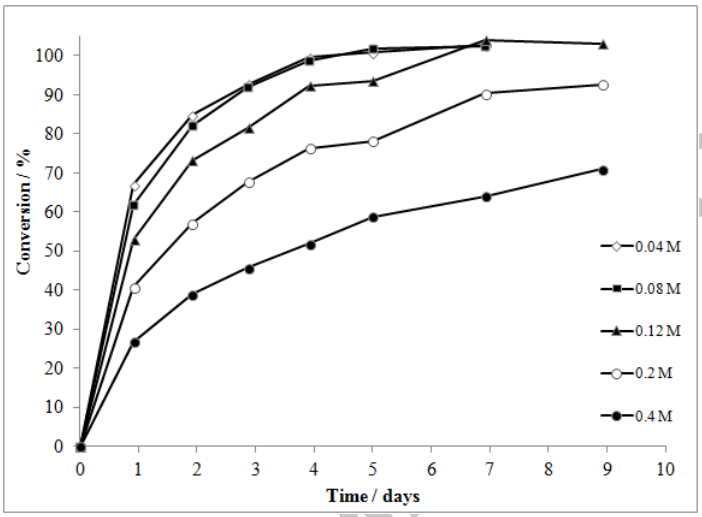
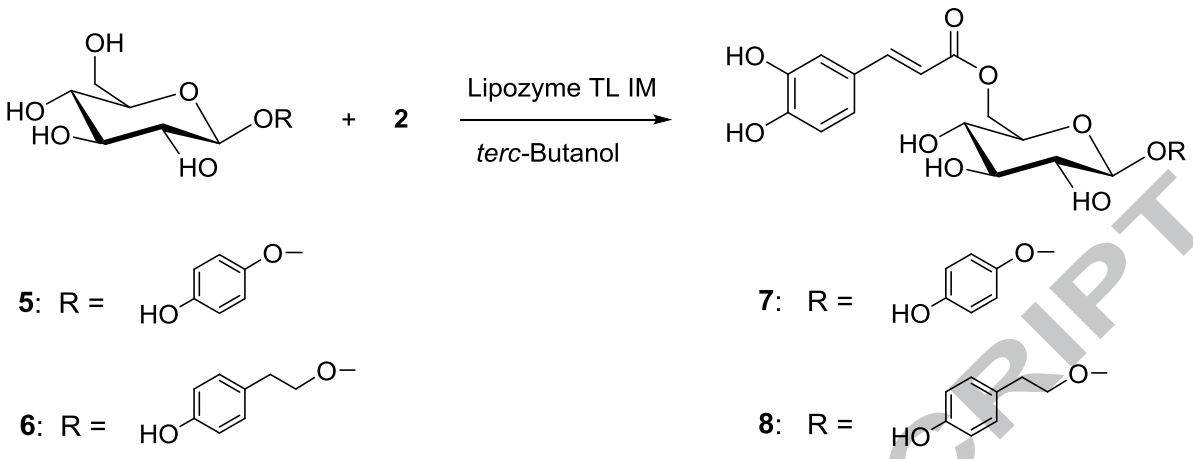


Figure 2. Effect of methyl-β-D-glucopyranoside (**1**) concentration on its caffeoylation by Lipozyme TL IM (expressed as time course of conversion **1** to **4**). Reaction conditions: 1.5 eq. vinyl caffeate, 0.4 g molecular sieves, 0.4 g Lipozyme TL IM, 5 mL *tert*-butanol, 40°C, 300 rpm.

The preparative caffeoylation of **1** was carried out under the conditions selected in previous experiments. The glucoside (0.2 M) was treated with 1.5 equiv. of vinyl caffeate in *tert*-butanol under catalysis of Lipozyme TL IM. The reaction time was extended to 17 days until all glucoside was consumed (HPLC). The yield of isolated product was 73%. Its structural analysis by NMR spectroscopy confirmed regioselective caffeoylation of **1** at the primary hydroxyl on C-6 of the glucopyranoside ring. No formation of dicaffeates or positional isomers was observed. Such strict regioselectivity in acylation of primary hydroxyl of acceptor is consistent with our previous results from feruloylation of methyl α-D-glucopyranoside catalyzed by Lipolase 100T.[30])

The enzymatic reaction system was then applied in preparation of two bioactive plant metabolites harboring the caffeate moiety: arbutin **5** and salidroside **6** were caffeoylated under the conditions. The reaction proceeded again with absolute regioselectivity, 6-*O*-caffeoylated arbutin - robustazide B (*p*-hydroxyphenyl 6-*O*-caffeoyl-β-D-glucopyranoside, **7**) was isolated after 12 days of reaction in 75 % and 6-*O*-caffeoylated salidroside (p-hydroxyphenetyl 6-*O*-caffeoyl-β-D-glucopyranoside, **8)** after 16 days in 74 % yield (Scheme 2).



Scheme 2. Enzymatic caffeoylation of arbutin and salidroside by Lipozyme TL IM.

In conclusion, we have optimized enzymatic caffeoylation of methyl β-D-glucopyranoside as a model glycoside for syntheses of natural glycophenolics. Vinyl and 2,2,2-trifluoroethyl caffeates were studied as donors. Lipozyme TL IM in *tert*-butanol using vinyl caffeate catalyzed regioselective formation of methyl 6-O-caffeoyl-β-D-glucopyranoside with 100% substrate conversion. The optimized caffeoylation conditions were verified on reaction with arbutin and salidroside as acceptors. Robustaside B and 6-O-caffeoylated salidroside as the only caffeoylated products were isolated in 75% and 74 % yield. The results give rise to a green enzymatic access to caffeoylated glycosides as medicinally important plant metabolites.

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Supplementary data

Supplementary data (analytical methods, experimental procedures, NMR data for compounds **3**–**6**) associated with this article can be found, in the online version, at…

References and notes

1. Mølgaard, P.; Ravn, H. *Phytochemistry* **1988**, *27*, 2411.
2. Silva, T.; Oliveira, C.; Borges, F. (2009 - 2013). *Expert Opin. Ther. Patents* **2014**, *24*, 1.
3. Magnani, C.; Isaac, V. L. B.; Correa, M. A.; Salgado, H. R. N. Anal. Methods, 2014, 6, 3203.
4. Calis, I. In Biodiversity; Sener B. Ed.; Springer US, 2002, pp 137 149
5. Zhang, P.; Tang, Y.; Li, N. G.; Zhu Y.; Duan J. A. *Molecules* **2014**, *19*, 16458.
6. Clifford, M. N. *J. Sci. Food Agric*. **2000**, *80*, 1033.
7. Kurkin, V. A. *Chem. Nat. Comp*. **2003**, *39*, 123.
8. Pan, J.; Yuan, C.; Lin, C.; Jia, Z.; Zheng, R. *Pharmazie* **2003**, *58*, 767.
9. Fu, G.; Pang, H.; Wong, Y. H. *Curr. Med. Chem*. **2008**, *15*, 2592.
10. Machida, K.; Nakano, Y.; Kikuchi, M. *Phytochemistry* **1991**, *30*, 2013.
11. Shimomura, H.; Sashida, Y.; Adachi, T. *Phytochemistry* **1987**, *26*, 249.
12. Xu, M.; Lao, Q.-C.; Zhao, P.; Zhu, X.-Y.; Zhu, H.-T.; Luo, X.-L.; Yang, C.-R.; He, J.-H.; Li, C.-Q.; Zhang, Y.-J. *Nat. Prod. Res*. **2014**, *28*, 932.
13. Adisa, R. A.; Olorunsogo, O. O. *Mol. Med. Rep*. **2013**, *8*, 1493.
14. Kostadinova, E. P.; Alipieva, K. I.; Kokubun, T.; Taskova, R. M.; Handjieva, N. V. *Phytochemistry* **2007**, *68*, 1321.
15. Zeng, X.; Wang, H.; Gong, Z.; Huang,J.; Pei, W.; Wang, X.; Zhang, J.; Tang, X. *Fitoterapia* **2015**, *101*, 153.
16. Chen, H. C.; Chen, J. H.; Chang, C.; Shieh, C.-J. *Ultrason. Sonochem*. **2011**, *18*, 455.
17. Wang, J.; Gu, S. S.; Cui, H. S.; Wu, X. Y.; Wu, F. A. *Bioresource Technol.* **2014**, *158*, 39.
18. Kurata, A.; Takemoto, S.; Fujita, T.; Iwai, K.; Furusawa, M.; Kishimoto, N. *J. Mol. Catal. B: Enzym*. **2011**, *69*, 161.
19. Tsuchiyama, M.; Sakamoto, T.; Fujita, T.; Murata, S.; Kawasaki, H. *BBA Gen. Subjects*. **2006**, *1760*, 1071
20. Armesto, N.; Ferrero, M.; Fernández, S.; Gotor, V. *J. Org. Chem*. **2003**, *68*, 5784.
21. Topakas, E.; Vafiadi, C.; Christakopoulos, P. *Process Biochem*. **2007**, *42*, 497.
22. Koseki, T.; Fushinobu, S.; Ardiansyah, Shirakawa, H.; Komai, M. *Appl. Microbiol. Biotechnol*. **2009**, *84*, 803.
23. Faulds, C. B. *Phytochem. Rev*. **2010**, *9*, 121.
24. Zeuner, B.; Kontogeorgis, G. M.; Riisager, A.; Meyer, A. S. *New Biotechnol.* **2012**, *29*, 256.
25. Otto, R. T.; Scheib, H.; Bornscheuer, U. T.; Pleiss, J.; Syldatk, C.; Schmid, R. D. *J. Mol. Catal. B: Enzym*. **2000**, *8*, 201.
26. López-Munguía, A.; Hernández-Romero, Y.; Pedraza-Chaverri, J.; Miranda-Molina, A.; Regla, I.; Martínez, A.; Castillo, E. *PLoS ONE* **2011**, *6*, e20115.

27. Nakajima, N.; Ishihara, K.; Hamada, H.; Kawabe, S. I.; Furuya, T. *J. Biosci. Bioeng.* **2000**, *90*, 347.
28. Chigorimbo-Murefu, N. T. L.; Riva, S.; Burton, S. G. *J. Mol. Catal. B: Enzym.* **2009**, *56*, 277.
29. Ishihara, K.; Katsube, Y.; Kumazawa, N.; Kuratani, M.; Masuoka, N.; Nakajima, N. *J. Biosci. Bioeng*. **2010**, *109*, 554.
30. Mastihubová, M.; Mastihuba, V.; Bilaničová, D.; Boreková, M. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 54.
31. Yu, H.-L.; Xu, J.-H.; Su, J.-H.; Lu, W.-Y.; Lin, G.-Q. *J. Biosci. Bioeng*. **2008**, *106*, 65.
32. Mastihubová, M.; Mastihuba, V. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 5389.
33. Crepin, V. F.; Faulds, C. B.; Connerton, I. F. *Appl. Microb. Biotechnol*. **2004**, *63*, 647.
34. Chyba, A.; Mastihubová, M.; Mastihuba, V. *Anal. Biochem.* **2014**, *445*, 49.