Journal of Molecular Catalysis B: Enzymatic 67 (2010) 41–44

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Highly regioselective synthesis of novel aromatic esters of arbutin catalyzed by immobilized lipase from *Penicillium expansum*

Rong-Ling Yang[a], Ning Li[a], Min Ye[b], Min-Hua Zong[a,*]

[a] State Key Laboratory of Pulp and Paper Engineering, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] School of Biosciences and Bioengineering, South China University of Technology, Guangzhou 510006, China

ARTICLE INFO

Article history:
Received 18 March 2010
Received in revised form 17 June 2010
Accepted 4 July 2010
Available online 15 July 2010

Keywords:
Enzyme catalysis
Lipase from *Penicillium expansum*
Arbutin
Regioselective acylation
Aromatic esters

ABSTRACT

Ester derivatives of phenolic glycosides have attracted more attention in food, cosmetic and pharmaceutical industries, due to their potent biological activities. In the present study, a group of novel aromatic esters of arbutin were successfully synthesized by using immobilized lipase from *Penicillium expansum* with excellent 6′-regioselectivities (>99%) and moderate to excellent isolated yields (68–93%), except for 6′-*O*-vanilloyl-arbutin and 6′-*O*-(*p*-hydroxycinnamoyl)-arbutin with 28% and 34% yields, respectively. Among all the acyl donors tested, the lipase was most active towards vinyl 3-phenylpropionate, while remarkable decrease in the activity was recorded when the phenyl of acyl donors carried the hydroxyl and/or methoxyl.

1. Introduction

Arbutin, *p*-hydroxylphenyl β-D-glucopyranoside, is attracting increasing attention due to a variety of biological activities such as antioxidative activity, lowing blood glucose, protecting the membrane from the freeze–thaw damage [1,2]. In particular, this phenolic glycoside acts as a well-known inhibitor against tyrosinase which catalyzes two distinct reactions of melanin synthesis and has been used as a skin whitening agent in cosmetics [3]. For centuries, arbutin-containing plant extracts have been used widely as the diuretic and urinary anti-infective agent in folk medicine. It was reported that fatty acid esters of arbutin displayed promoting biological activities compared with the parent compound [4–7]. For example, Tokiwa et al. reported that arbutin undecylenic acid ester was able to inhibit the activity of tyrosinase more efficiently than arbutin [4]. Arbutin laurate was more effective than the unmodified phenolic glycoside in preventing the oxidation of linoleic acid [6].

Arylaliphatic acids, distributed widely in nature, possess various interesting biological properties such as antioxidative, free radical scavenger, and antimicrobial activities, as well as antitumor activities. For example, benzoic acid has a long history of use as an effective preservative in the food industry. In addition, cinnamic acid and its derivatives have antioxidative activities [8] and tyrosinase inhibitory activities [9]. Huang et al. reported that methyl *trans*-cinnamate had more potent tyrosinase inhibitory effect than cinnamic acid [10]. Recent study showed that chemical or enzymatic modification of phenolic acids could result in multifunctional amphiphilic antioxidants, which displayed higher biological activities than the precursors [11]. For example, arbutin ferulate inhibited the oxidation of low-density lipoproteins more efficiently than arbutin and exhibited a higher antiradical activity than ferulic acid, a natural antioxidant [12]. Hence, it is expected that the mutual derivatives of arbutin and aromatic acids, dual prodrugs, have more potent biological activities than the corresponding precursors.

Enzymes have been widely applied in the modification of polyhydroxylated compounds [13–16], due to the mild reaction conditions, excellent selectivity, simplicity and being environmentally friendly. Nevertheless, there are only several reports on enzymatic synthesis of ester derivatives of arbutin [4–7,12,17]. In addition, the product yields were unsatisfactory. For example, arbutin cinnamate was formed in a yield of 28% via an enzymatic route [17].

Lipase from *Penicillium expansum* is a commercial enzyme with molecular mass, optimal pH and temperature being 28 kDa, 9.5 and 34 °C, respectively. It was reported that the enzyme had a low protein sequence homology compared with other typical lipases and had a high percentage of hydrophobic residues (51.4%) in the *N*-terminal region [18]. As a result, it is expected that the lipase will display unique catalytic properties. Previously, we successfully synthesized biodiesel and a series of fatty acid esters of arbutin by using

* Corresponding author. Tel.: +86 20 8711 1452; fax: +86 20 2223 6669.
E-mail address: btmhzong@scut.edu.cn (M.-H. Zong).

1381-1177/$ – see front matter © 2010 Published by Elsevier B.V.
doi:10.1016/j.molcatb.2010.07.003

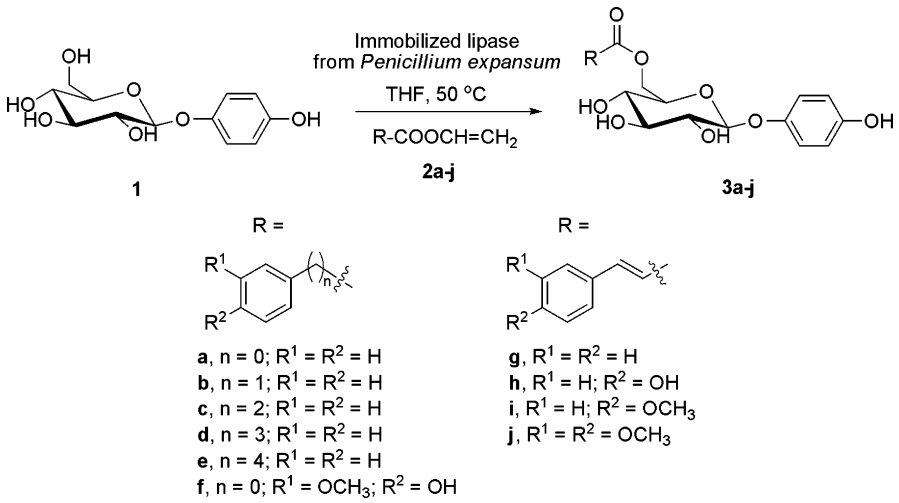

Fig. 1. Lipase-catalyzed synthesis of ester derivatives of arbutin.

immobilized lipase from *P. expansum* [19–21]. In the present work, we continued to extend its application potential to the synthesis of a group of novel aromatic esters of arbutin **3a–j** with highly potent interests (Fig. 1).

2. Materials and methods

2.1. Biological and chemical materials

Crude powder of lipase from *P. expansum* (3000 U/g of hydrolytic activity) was kindly donated by Leveking Bioengineering Co., Ltd., Shenzhen, China. The macroporous adsorbent resin D4020 with the specific surface area of 540–580 m^2/g and average pore diameter of 100–105 Å, which was composed of the crosslinked-polystyrene, was from Chemical Co. of Nankai University, Tianjin, China. The enzyme was immobilized on resin D4020 according to the method described by Li et al. [19]. The immobilization method and determination of lipase transesterification activity are available as supplementary information. The immobilized lipase showed a transesterification activity of 32 U/g resin (one unit of lipase activity was defined as the amount of enzyme required for producing 1 μmol 6′-*O*-butyryl-arbutin from arbutin and vinyl butyrate in THF per minute at 35 °C). Arbutin was from Sigma–Aldrich, USA. Phenylacetic acid, 3-phenylpropionic acid, 4-phenylbutyric acid, 5-phenylvaleric acid, vanillic acid, *p*-coumaric acid, *p*-methoxycinnamic acid and 3,4-dimethoxycinnamic acid were from Darui Chemicals Co., Ltd., Shanghai, China. Vinyl cinnamate and vinyl benzoate were purchased from Alfa Aesar, USA. All other chemicals were also from commercial sources and were of high purity available.

2.2. Synthesis of vinyl esters

Vinyl esters were synthesized from aromatic acid as described by Swern and Jordan [22]. Upon completion of the reaction, the reaction mixture was filtered. The solvent was removed by vacuum distillation, and the crude product was purified through silica gel column chromatography using petroleum ether/ethyl acetate as the eluent. The vinyl esters of phenylacetic acid (**2b**), 3-phenylpropionic acid (**2c**), 4-phenylbutyric acid (**2d**), 5-phenylvaleric acid (**2e**) are colorless oil. The vinyl esters of vanillic acid (**2f**), *p*-coumaric acid (**2h**), *p*-methoxycinnamic acid (**2i**) and 3,4-dimethoxycinnamic acid (**2j**) are white powder. The NMR data of the vinyl esters **2b–e** is the same as that reported previously [23]. Isolated yields for vinyl esters were as follows: vinyl vanillate **2f**, 45%; vinyl *p*-coumarate **2h**, 53%; vinyl *p*-methoxycinnamate **2i**, 61%; vinyl 3,4-dimethoxycinnamate **2j**, 56%.

The NMR data of the vinyl esters **2f–j** is available as supplementary information.

2.3. Enzymatic acylation of arbutin

In a typical experiment, a reaction mixture of arbutin (0.04 mmol), 5 equivalents of vinyl ester in anhydrous THF (2 ml) was added to a sealed-cap vial (10 ml) containing immobilized enzyme (1.7 U) and incubated at 50 °C, 200 rpm. Aliquots were withdrawn from the reaction mixture at specified time intervals, and then diluted by 25-fold with the corresponding mobile phase prior to HPLC analysis. All the experiments were conducted in triplicate.

2.4. HPLC analysis

HPLC analysis was carried out on an Agilent 1100 chromatograph (Agilent Technologies Co., Ltd., USA) with a UV detector at 282 nm using Zorbax SB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent Technologies Co., Ltd., USA). A gradient elution at 1.0 ml/min with water/methanol (40:60, v/v) from 0 to 3.0 min, and then water/methanol (20:80 v/v) from 5.0 min was used. The retention times of the parent compound and the esters were 2.35 min (arbutin **1**), 3.48 min (6′-*O*-benzoyl-arbutin, **3a**), 3.50 min (6′-*O*-phenylacetyl-arbutin, **3b**), 4.35 min [6′-*O*-(3-phenylpropionyl)-arbutin, **3c**], 5.59 min [6′-*O*-(4-phenylbutyryl)-arbutin, **3d**], 7.10 min [6′-*O*-(5-phenylvaleryl)-arbutin, **3e**], 2.80 min (6′-*O*-vanilloyl-arbutin, **3f**), 4.58 min (6′-*O*-cinnamoyl-arbutin, **3 g**), 2.94 min (6′-*O*-*p*-hydroxycinnamoyl-arbutin, **3h**), 4.65 min (6′-*O*-*p*-methoxycinnamoyl-arbutin, **3i**), 4.80 min [6′-*O*-(3,4-dimethoxycinnamoyl)-arbutin, **3j**], respectively.

2.5. Synthesis, purification and structure characterization of the desired ester derivatives

THF (20 ml) containing arbutin (0.4 mmol, 989 mg), 5 equivalents of vinyl ester and immobilized enzyme (16.7 U) was incubated in a 100 ml Erlenmeyer shaking flask capped with a septum at 200 rpm and 50 °C. Upon completion of the reaction, the enzyme was filtered off, and the filtrate was concentrated *in vacuo*. The residue was separated and purified through silica gel column chromatography using petroleum ether/ethyl acetate as the eluent.

Table 1
Enzymatic synthesis of aromatic esters of arbutin.

Entry	Acyl donor	Time (h)	Conversion (%)	6′-Regioselectivity (%)
1	**2a**	1	13	>99
2		72	97	>99
3	**2b**	1	13	>99
4		72	99	>99
5	**2c**	1	81	>99
6		4	99	>99
7	**2d**	1	77	>99
8		5	99	>99
9	**2e**	1	70	>99
10		5.5	99	>99
11	**2f**	96	30	>99
12	**2g**	1	22	>99
13		68	99	>99
14	**2h**	96	36	>99
15	**2i**	1	10	>99
16		80	80	>99
17	**2j**	1	9	>99
18		80	70	>99

The reaction conditions: arbutin (0.04 mmol), 5 equivalents of vinyl ester, immobilized enzyme (1.7 U) in anhydrous THF (2 ml) at 50 °C, 200 rpm.

The structures of aromatic esters of arbutin were characterized by ^{13}C and ^{1}H NMR (Bruker AVANCE Digital 400 MHz NMR spectrometer, Germany) at 100 and 400 MHz, respectively. All ester derivatives of arbutin are white powder. Isolated yields of aromatic esters of arbutin were as follows: 6′-*O*-benzoyl-arbutin **3a**, 86%; 6′-*O*-phenylacetyl-arbutin **3b**, 87%; 6′-*O*-(3-phenylpropionyl)-arbutin **3c**, 93%; 6′-*O*-(4-phenylbutyryl)-arbutin **3d**, 91%; 6′-*O*-(5-phenylvaleryl)-arbutin **3e**, 90%; 6′-*O*-vanilloyl-arbutin **3f**, 28%; 6′-*O*-cinnamoyl-arbutin **3g**, 88%; 6′-*O*-(*p*-hydroxycinnamoyl)-arbutin **3h**, 34%; 6′-*O*-(*p*-methoxycinnamoyl)-arbutin **3i**, 78%; 6′-*O*-(3,4-dimethoxycinnamoyl)-arbutin **3j**, 68%.

For NMR data, see supplementary information.

3. Results and discussion

The immobilized enzyme-mediated acylation of arbutin was conducted with aromatic acid vinyl esters as acyl donors (Table 1). Interestingly, in all cases, only the 6′-ester of arbutin was detected by NMR and HPLC, indicating that the immobilized enzyme displayed almost absolute 6′-regioselectivity (>99%). This is in agreement with our recent report [21].

As shown in Table 1, the reaction rate was greatly dependent on the chain length of acyl donors **2a**–**e** (entries 1–10). For example, the lipase displayed the lowest activity in the benzoylation (entries 1 and 2), which might stem from the unfavorable resonance effect and the steric hindrance of the phenyl ring present in the acyl moiety [24,25]. According to the classical valence–bond theories, delocalization of the electrons occurs in the conjugated system where the C=O of the carboxyl is conjugated with the aromatic phenyl, thus resulting in the increase of the electron density of the carbon of the C=O [25]. Consequently, it is unfavorable for the nucleophilic attack of the alcohol on the acyl–enzyme intermediate. In addition, the bulky and rigid phenyl ring would result in a serious steric strain in enzymatic benzoylation [24]. The reaction rate and the maximum substrate conversion of enzymatic phenylacetylation (entries 3 and 4) were similar to those of benzoylation. Interestingly, the reaction was markedly accelerated when vinyl phenylacetate **2b** was replaced by vinyl 3-phenylpropionate **2c**, and the enzymatic acylation afforded a substrate conversion of 99% in 4 h (entries 5 and 6). This could be accounted for by the significantly reduced steric strain of the acyl group. However, the reaction rate decreased slightly with further increment of the chain length (entries 7–10). For example, a 99% conversion was achieved with 4-phenylbutanoylation after 5 h (entries 7 and 8) and 5-phenyvalerylation after 5.5 h (entries 9 and 10). It might derive from the larger steric hindrance of the longer acyl chain. These results demonstrate that the lipase is more specific toward vinyl 3-phenylpropionate **2c** and differs in substrate specificity from lipase from *Burkholderia cepacia*, which is more specific toward vinyl 4-phenybutyrate **2d** in the synthesis of aromatic esters of floxuridine [23].

It was worth noting that the enzymatic cinnamoylation of arbutin was much slower than 3-phenylpropionation (entries 6 and 13) in spite of the fact that cinnamoyl is similar in chain length to the 3-phenylpropionyl. It could come from the unfavorable resonance effect caused by the conjugated system between the C=C and C=O double bonds. In addition, as compared to 3-phenylpropionate, cinnamate is more rigid due to the presence of unsaturated C=C, thus resulting in steric strain. Similar results were also reported by other researchers with different enzymes [26,27]. Encouragingly, the isolated yield of 6′-*O*-cinnamoyl-arbutin **3g** achieved in this work was much higher than that of the previous report (88% vs. 28%), demonstrating the potential of the enzyme in the preparation of arbutin esters.

It was clear from Table 1 that substituents present in the phenyl moiety of the acyl donor exerted a negative impact on the reaction. For example, the replacement of vinyl benzoate **2a** with vinyl vanillate **2f** as the acyl donor substantially lowered the reaction rate of the enzymatic acylation, as indicated by the low conversion (30%) after a reaction time of 96 h (entry 11). The unfavorable steric hindrance of the substituent(s) might contribute to this. Additionally, different substituents affected the enzymatic reaction quite differently (entries 14–18). The enzyme was slightly more active in the *p*-methoxylcinnamoylation (entry 16, 80% after 80 h) than that in the 3,4-dimethoxycinnamoylation (entry 18, 80% after 70 h). It might be attributed to the steric strain from extra 3-methoxy in 3,4-dimethoxycinnamoyl compared with the *p*-methoxylcinnamoyl. And the reaction rate and conversion were much higher in the *p*-methoxylcinnamoylation (entries 15 and 16, 80% after 80 h) than those in the *p*-hydroxylcinnamoylation (entry 14, 36% after 96 h). Although the hydroxyl is less steric than the methoxyl, the former is more polar than the later and thus may have more significant negative effect on enzyme performances owing to the non-polar environment of the acyl-binding pocket in the enzyme active site [24,26–28].

4. Conclusions

Immobilized lipase from *P. expansum* is an excellent biocatalyst for the synthesis of the mutual derivatives of arbutin and aromatic acids. Some interesting information about the interactions between the enzyme and acyl groups was obtained via the study of enzyme acyl recognition. These findings may help to tailor the catalytic performances of the synthetically useful enzyme by chemical modification and protein engineering approaches.

Acknowledgments

We thank the National Natural Science Foundation of China (20876059 and 20906032), and Major State Basic Research Development Program '973' (2010CB732201) for financial support.

Appendix A. Supplementary data

The methods of preparing immobilized enzyme, assaying lipase transesterification activity, NMR data and spectra of vinyl esters (**2f**–**j**) and aromatic esters of arbutin (**3a**–**j**) are available as supplementary information.

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2010.07.003.

References

[1] H. Takii, K. Matsumoto, T. Kometani, S. Okada, T. Fushiki, Biosci. Biotechnol. Biochem. 61 (1997) 1531–1535.
[2] D.K. Hincha, A.E. Oliver, J.H. Crowe, Biophys. J. 77 (1999) 2024–2034.
[3] I. Hori, K. Nihei, I. Kubo, Phytother. Res. 18 (2004) 475–479.
[4] Y. Tokiwa, M. Kitagawa, T. Raku, Biotechnol. Lett. 29 (2007) 481–486.
[5] Y. Tokiwa, M. Kitagawa, T. Raku, S. Yanagitani, K. Yoshino, Bioorg. Med. Chem. Lett. 17 (2007) 3105–3108.
[6] Y. Watanabe, M. Nagai, K. Yamanaka, K. Jose, M. Nomura, Biochem. Eng. J. 43 (2009) 261–265.
[7] M. Nagai, Y. Watanabe, M. Nomura, Biosci. Biotechnol. Biochem. 73 (2009) 2501–2505.
[8] F. Natella, M. Nardini, M. Di Felice, C. Scaccini, J. Agric. Food Chem. 47 (1999) 1453–1459.
[9] K. Iwai, N. Kishimoto, Y. Kakino, K. Mochida, T. Fujita, J. Agric. Food Chem. 52 (2004) 4893–4898.
[10] Q.S. Huang, Y.J. Zhu, H.L. Li, J.X. Zhuang, J.J. Zhou, W.G. Li, Q.X. Chen, J. Agric. Food Chem. 57 (2009) 2565–2569.
[11] M.C. Figueroa-Espinoza, P. Villeneuve, J. Agric. Food Chem. 53 (2005) 2779–2787.
[12] N.T.L. Chigorimbo-Murefu, S. Riva, S.G. Burton, J. Mol. Catal. B: Enzyme 56 (2009) 277–282.
[13] N. Li, D. Ma, M.H. Zong, J. Biotechnol. 133 (2008) 103–109.
[14] N. Li, M.H. Zong, D. Ma, Eur. J. Org. Chem. (2008) 5375–5378.
[15] N. Li, M.H. Zong, D. Ma, Tetrahedron 65 (2009) 1063–1068.
[16] S. Riva, J. Mol. Catal. B: Enzyme 19–20 (2002) 43–54.
[17] N. Nakajima, K. Ishihara, S. Matsumura, H. Hamada, K. Nakamura, T. Furuya, Biosci. Biotechnol. Biochem. 61 (1997) 1926–1928.
[18] C.B. Bian, C. Yuan, L. Lin, J.H. Lin, X.L. Shi, X.M. Ye, Z.X. Huang, M.D. Huang, Biochim. Biophys. Acta, Proteins Proteomics 1752 (2005) 99–102.
[19] N.W. Li, H. Wu, M.H. Zong, W.Y. Lou, Chin. J. Catal. 28 (2007) 333–338.
[20] N.W. Li, M.H. Zong, H. Wu, Process Biochem. 44 (2009) 685–688.
[21] R.L. Yang, N. Li, R.F. Li, T.J. Smith, M.H. Zong, Bioresour. Technol. 101 (2010) 1–5.
[22] D. Swern, E.F. Jordan, Org. Synth. 30 (1963) 106–107.
[23] N. Li, Q.M. Zeng, M.H. Zong, J. Biotechnol. 142 (2009) 267–270.
[24] R.T. Otto, H. Scheib, U.T. Bornscheuer, J. Pleiss, C. Syldatk, R.D. Schmid, J. Mol. Catal. B: Enzyme 8 (2000) 201–211.
[25] M.B. Smith, J. March, March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure, John Wiley & Sons, Inc., Hoboken, New Jersey, 2007, pp. 32–105.
[26] N. Armesto, M. Ferrero, S. Fernandez, V. Gotor, J. Org. Chem. 68 (2003) 5784–5787.
[27] P. Weitkamp, K. Vosmann, N. Weber, J. Agric. Food Chem. 54 (2006) 7062–7068.
[28] M. Ardhaoui, A. Falcimaigne, J.M. Engasser, P. Moussou, G. Pauly, M. Ghoul, Biocatal. Biotransform. 22 (2004) 253–259.