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Regioselective and efficient enzymatic synthesis of antimicrobial andrographolide derivatives

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ABSTRACT

Labdane diterpene andrographolide (**1**) is a major constituent of *Andrographis paniculata* and known to exhibit wide spectrum of biological activities. In this study, regioselective monoesters of (**1**) have been synthesized by using Amano lipase AK (*Pseudomonas fluorescens*) as a biocatalyst. Amano lipase AK was able to execute highly efficient esterification of hydroxyl group attached to C-14 carbon of (**1**) in presence of acyl donors. Among the various synthesized derivatives including two novel compounds such as andrographolide-14-propionate (**3**) and andrographolide-14-caproate (**5**) displayed antimicrobial activity against *Staphylococcus aureus* with low minimal inhibitory concentration (MIC) 4 μg/mL and 16 μg/mL respectively. Furthermore, they have shown low hemolysis activity at their respective MIC and increase in the permeability of the bacterial cell membrane as delineated by FITC uptake and SEM imaging studies.

Introduction

Andrographis paniculata is an medicinal herb, ethanobotanically used to treat various diseases in Asian countries including India and China.[1] Andrographolide (**1**), is the major active constituent isolated from *A. paniculata*.[2,3](**1**) has been reported to exhibit a wide range of pharmacological activities such as anti-cancer,[4,5] anti-diabetic,[6] anti-inflammatory,[7] anti-viral[8] and anti-allergic.[9]-Owing to its promising biological activities, efforts being made to synthesize several derivatives of (**1**) and studied for their activity. Detailed structure-activity-relationship (SAR) studies of (**1**) have been carried out for finding synthetic analogues with improved biological activities.[10,11] Several reports are available on synthesis of andrographolide analogues using traditional chemical synthesis methods to improve bioactivities.[12] Andrographolide monoester derivatives have been reported to demonstrate enhanced anti-cancer,[13] and anti-bacterial[14] activities. However, achievement of regioselective monoesterification of (**1**) by means of conventional reactions is a difficult task due to the presence of similar reactive hydroxyl groups and α, β unsaturated lactone.

Therefore, conventional synthetic methods involve multiple steps with protection-deprotection strategy, which often deliver low yield further, demands the use of hazardous chemical reagents, and solvents.[13,15]

On the other hand, biocatalysis offer an excellent alternative route for conventional synthesis.[16,17] Lipases are known to efficiently catalyze wide variety of organic reactions such as esterification, chiral resolution with enhanced regio and stereoselectivity in non-aqueous media.[18,19] Due to their environmentally benign nature, lipase catalyzed *trans*-esterification for the synthesis of drug intermediates, surfactants, and natural product analogues has attracted increasing attention in recent times.[20]

Although, the biological activity of (**1**) is well studied, very little is known on biocatalyst mediated synthesis of monoesters of (**1**)[21,22] and their anti-microbial activities with their mode of action. In our continued interest in the biocatalyst mediated transformation of natural products and their derivatives,[23–26] we have screened several lipases for the regioselective esterification of one of the hydroxyl group of andrographolide (**1**) in presence of various acyl donors (Scheme 1).

Kinetics of esterification reaction including the effect of solvents, temperature and chain length of acyl donors on initial

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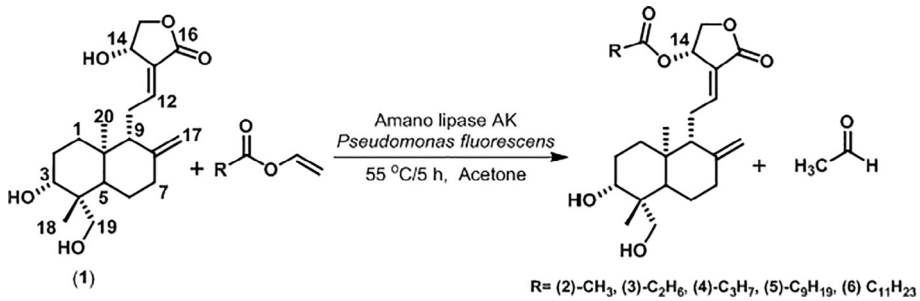

Scheme 1. Amano lipase AK (*P. fluorescens*) lipase catalyzed regioselective acylation of (**1**) with acyl donors at 55 °C/5h; 100 rpm.

Table 1
Effect of acyl donor chain length on initial rate of Amano lipase AK (*P. fluorescens*) catalyzed acylation of (**1**)[a].

Acyl donor	*Vo* (mM h^{-1})	Yield (%)[b]	Regioselectivity (%)
Vinyl acetate	37.1 ± 1.4	98.2	100
Vinyl propionate	33.4 ± 1.5	98.5	100
Vinyl butyrate	29.5 ± 0.5	96.3	100
Vinyl decanoate	25.2 ± 1.8	95.6	100
Vinyl laurate	20.2 ± 2.5	94.5	100

[a] The reaction condition: 5 mg of lipase; 0.1 mmol of **1**; 1.0 mmol of acyl donor; 3 mL of acetone and incubated at 55 °C on a rotary shaker (100 rpm) for 5 h.
[b] The yield was determined by HPLC analysis.

rate of reaction was studied (Table 1, Fig. 1). Furthermore, (**1**) and its monoesters have been screened for their antimicrobial and hemolytic activities. Bacterial cell permeability assay was carried out using fluorescent staining assay and SEM imaging.

Screening of lipases for andrographolide acylation

Several commercially available lipases were screened for regioselective acylation of (**1**) in presence of acyl donors with varied chain length. Screening results indicated that among all the lipases studied, Amano lipase AK (*P. fluorescens*) was able to carry out the esterification of hydroxyl group at C-14 of (**1**) in very efficient manner compared to the other lipases studied. Among the screened lipases, Amano lipase PS (*B. cepacia*), *CRL* (*C. cylindracea*), Lipase Type II PPL (*P. pancreas*) and Lipase type VII (*C. rugosa*) did not show esterification with any acyl donors. Lipases from *P. camemberti* and *R. niveus* accepted vinyl acetate as a acyl donor and Amano lipase A (*A. niger*) accepted only vinyl butyrate as acyl donor to carry out the esterification of C-14 hydroxyl moiety of (**1**). CAL-A lipase showed reaction with vinyl propionate and vinyl caproate whereas CAL-B lipase gave ester product only with acyl esters such as acetate, butyrate and laurate. All these lipases did not show acylation activity with range of acyl donors with desired yield. On the other hand, Amano lipase AK (*P. fluorescens*) catalyzed acylation reactions in presence of vinyl acyl donors such as vinyl acetate, vinyl propionate, vinyl butyrate, vinyl caproate and vinyl laurate with good to excellent regioselectivity, and hence we further optimized the reaction conditions using Amano lipase AK. In control, which is devoid of lipase, no acylated product formation was observed.

Effect of solvents on andrographolide acetylation

Solvent is known to play an important role in lipase catalyzed esterification with respect to the reaction time and yield.[27] Amano lipase AK catalyzed acetylation of (**1**) was examined in various solvents at standardized reaction conditions. These results suggested that, acetone seems to be better solvent for carrying out acetylation of (**1**) (Fig. 1A). The reason of good yield with acetone in 6 h, may be due to greater solubility of (**1**) in acetone and also inactivation of enzyme by other solvents.[28]

Effect of temperature on andrographolide acetylation

In lipase mediated reactions, temperature has an important role on the enzyme activity, stability and thermodynamic equilibrium.[29] Thermal stability of Amano lipase AK (*P. fluorescens*) during acetylation of (**1**) was examined. Substrate conversion was increased with increase in temperature from 30 °C to 55 °C (Fig. 1B). Further increase in temperature, the rate of product formation was decreased. These results suggested that 50 °C–55 °C was optimal temperature for Amano lipase AK mediated acetylation of (**1**). However, there was no change in the acylation position on (**1**) was observed with varying the temperature.

Time course study of andrographolide acetylation

Time dependent progress of (**1**) acetylation under optimized reaction conditions was studied (Fig. 1C) by monitoring the substrate and product ratios using HPLC attached UV–Visible detector at 235 nm. These results suggested that the reaction rate was high at initial incubation period and reached maximum at 5 h, at the end of 5 h incubation period, >98.0% of (**1**) was converted into andrographolide-14-acetate.

Operational stability of Amano lipase AK (*P. fluorescens*) in andrographolide acetylation

Operational stability of lipase was studied by performing acylation of (**1**) in batch at optimized reaction conditions. Amano lipase AK was recovered after each batch of incubation by ultrafiltration (3KD cutoff membrane) and washed with acetone. This recovered protein was again incubated with (**1**) in presence of vinyl acetate in acetone. The HPLC analysis of the each batch extracts indicated that, there was reduce in lipase activity and after fifth batch, the lipase activity was completely lost (Fig. 1D). These results indicated that Amano lipase AK can be reused for the production of andrographolide-14-acetate using (**1**) as a substrate.

Effect of alkyl substituent of the acyl donor on acylation of andrographolide

Initial rate of the Amano lipase AK mediated acylation of **1** was varied with the chain length of the acyl donor. In all cases, the Amano lipase efficiently carried out hydroxy at C-14 acylation of **1** to form respective acylated products. Initial rate of acylation increased as the chain length increased from C-2 to C-4 in the acyl donor. Further increase in the chain length of the side chain of vinyl donors (**5** and **6**) marginally decreased in the level of respective acylated product of (**1**) (Table 1). These results sug-