Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Molecular Catalysis B: Enzymatic 27 (2004) 97–102
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Enzyme-catalyzed regioselective synthesis of lipophilic guaifenesin ester derivatives

Na Wang [a,b], Qi Wu [a], Bo Kai Liu [a], Ying Cai [a], Xian Fu Lin [a,*]

[a] *Department of Chemistry, Zhejiang University, Hangzhou 310027, PR China*
[b] *College of Chemistry and Biochemistry, Guizhou University, Guiyang 550025, PR China*

Received 7 July 2003; received in revised form 9 October 2003; accepted 14 October 2003

Abstract

Enzyme-catalyzed synthesis of fatty acid guaifenesin esters through transesterification was performed in acetone. Guaifenesin and vinyl fatty acid esters were used as substrates and the reactions were catalyzed by lipase from *Mucor miehei* (Lipozyme®). Guaifenesin was regioselectively acylated at the primary hydroxyl groups and guaifenesin derivatives with long chain acyl group were prepared in good yields. Various reaction conditions were examined in detail. The results showed that immobilized lipase from *M. miehei* had the highest activity and a maximum yield of 88% was obtained in acetone, which was accepted by the EEC for use in the manufacture of food products and pharmacology.

Keywords: Biocatalysis; Regioselectivity; Immobilized lipase; Guaifenesin esters; Transesterification

1. Introduction

The modification of drug or prodrug is one approach that can lead both to prolong pharmacological activity and reduce adverse effects. Additionally, this increases water solubility or lipophilicity, improves site-specificity [1].

Esterification is one of the useful methods for drugs modification. However, conventional chemical acylation methods show an almost lack of regioselectivity and lead invariably to a mixture of the possible mono-, di- and poly-esters [2–4]. Enzymatic acylation was used in the synthesis of pharmaceutically compounds due to its high regioselectivity and mild conditions [5]. For example, chloramphenicol ester derivatives were regioselectively synthesized by lipase catalyst [6], while esters derived from swainsonine were obtained in a similar way [7]. Ferrero and Gotor reviewed the utility of biocatalysis for the modification of nucleosides and steroids [8]. In our previous work, regioselectively transesterifications of acyclovir and ascorbic acid were achieved by alkaline protease from *Bacillus subtilis* and Lipozyme®, respectively [9,10].

Guaifenesin (I, 3-(*o*-methoxyphenoxy)-1,2-propanediol) has been extensively used as an expectorant and presented in a variety of pharmaceutical formations [11]. In spite of its beneficial activity, it has some adverse effects such as nausea, drowsiness and gastroenteric impairment. The increased lipid solubility of some esters of guaifenesin would be expected to render them more suitable for diffusion across the lipophilic membranes of the intestinal cells, thus improving intestinal and cellular absorption. Therefore, the modification of guaifenesin via esterification or transesterification can be used as a tool to improve curative effects.

Here, we wish to report an effective way to guaifenesin fatty acid esters by biocatalysis transesterification. Immobilized lipase from *Mucor miehei* was selected as enzyme and acetone was chosen as solvent. As a result, a series of guaifenesin esters with long chain alkyl group were regioselectivity prepared in good yields.

2. Experimental

2.1. Materials

Lipozyme® immobilized lipase from *M. miehei*, lipase from *Candida cylindracea* and lipase from hog pancreas

* Corresponding author. Tel.: +86-571-87951588; fax: +86-571-87952618.
E-mail address: llc123@css.zju.edu.cn (X.F. Lin).

1381-1177/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2003.10.002

were purchased from Fluka. Lipase from porcine pancreas was purchased from Sigma. Alkaline protease from *B. subtilis* was purchased from Wuxi Enzyme Co. Ltd. (Wuxi, PR China). Solvents were dried over 3 Å molecular sieves for 24 h prior to use. Analytical grade guaifenesin, fatty acid and all other chemicals were used without further purification.

2.2. Analytical methods

All reactions were monitored by TLC on silica gel plates eluted with petroleum ether/ethyl acetate (2/1, v/v). ^{1}H and ^{13}C spectra were obtained on a Bruker AMX-500 MHz spectrometer. ^{13}C NMR spectra were obtained with broad band proton decoupling. Spectra were run in $CDCl_3$. ^{1}H and ^{13}C NMR spectra were referenced to an internal TMS standard. Infrared spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. The degrees of conversion were calculated by Shimadzu SPD-10Avp HPLC with a UV-Vis detector and a reversed-phase Shim-Pack VP-ODS column (150 mm × 4.6 mm). Elution was performed with a mixture of methanol/water (95/5, v/v) at 0.8 ml/min.

2.3. Synthesis of vinyl fatty acid esters

Vinyl esters were synthesized and purified as described by Yang et al. [12]. Carboxylic acid (0.05 mol) and $Hg(OAc)_2$ (0.006 mol) were dissolved in 150 ml vinyl acetate. After stirring the mixture for 30 min at room temperature, 0.5 ml of concentrated H_2SO_4 was added dropwise and the solution was refluxed for 6 h. Then the mixture was cooled to room temperature and 5 g NaOAc was added to quench the catalyst. The resulting mixture was filtered and the filtrate was concentrated under reduced pressure. The crude products were purified by silica gel column chromatography (petroleum ether/ethyl ether 20/1, v/v).

2.4. Synthesis of 1-O-capryloylguaifenesin

The reaction was initiated by adding 10 mg/ml Lipozyme® to 25 ml acetone containing guaifenesin (2.5 mmol), vinyl caprylate (10 mmol). The suspension was kept at 50 °C and stirred at 250 rpm for 12 h. The reaction was terminated by filtering of the enzyme, and acetone was evaporated. Formation of guaifenesin ester was confirmed by TLC. The product was separated by silica gel chromatography with an eluent consisting of petroleum ether/ethyl acetate (5/2, v/v). The product had a yield of 88%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.95 (m, 4H, $H_{7–10}$), 4.24 (m, 3H, –$CHCH_2OCO$–), 4.08 (dd, 1H, J = 9.8, 3.8 Hz, –CH_2O–), 4.02 (dd, 1H, J = 9.8, 6.2 Hz, –CH_2O–), 3.83 (s, 3H, –OCH_3), 3.17 (s, 1H, –OH), 2.33 (t, 2H, –CH_2CO), 1.61 (m, 2H, –CH_2–), 1.27 (m, 8H, 4–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3472 ($\nu_{–OH}$), 3066 ($\nu_{=C–H}$), 2928, 2856 ($\nu_{C–H}$), 1738 ($\nu_{C=O}$), 1594, 1506 (ν_{arom}), 1254, 1225, 1179, 1125 ($\nu_{C–O–C}$), 1030 ($\nu_{C–OH}$), 743 ($\delta_{C–H}$).

2.5. Synthesis of 1-O-caprinoylguaifenesin

1-*O*-Caprinoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-capryloylguaifenesin. Here the reaction time was 24 h and the yield of the product is 84%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.95 (m, 4H, $H_{7–10}$), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.06 (dd, 1H, J = 9.8, 3.5Hz, –CH_2O–), 4.02 (dd, 1H, J = 9.8, 6.3 Hz, –CH_2O–), 3.85 (s, 3H, –OCH_3), 2.34 (t, 2H, –CH_2CO), 1.62 (m, 2H, –CH_2–), 1.27 (m, 12H, 6–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3475 ($\nu_{–OH}$), 3071 ($\nu_{=C–H}$), 2926, 2855 ($\nu_{C–H}$), 1738 ($\nu_{C=O}$), 1593, 1504 (ν_{arom}), 1254, 1224, 1176, 1120 ($\nu_{C–O–C}$), 1030 ($\nu_{C–OH}$), 744 ($\delta_{C–H}$).

2.6. Synthesis of 1-O-lauroylguaifenesin

1-*O*-Lauroylguaifenesin was synthesized by the same synthesis method as for 1-*O*-caprinoylguaifenesin. The yield of product is 75%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.96 (m, 4H, $H_{7–10}$), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.06 (dd, 1H, J = 9.8, 3.8 Hz, –CH_2O–), 4.03 (dd, 1H, J = 9.8, 6.2 Hz, –CH_2O–), 3.85 (s, 3H, –OCH_3), 2.34 (t, 2H, –CH_2CO), 1.62 (m, 2H, –CH_2–), 1.27 (m, 16H, 8–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3469 ($\nu_{–OH}$), 3066 ($\nu_{=C–H}$), 2925, 2854 ($\nu_{C–H}$), 1739 ($\nu_{C=O}$), 1594, 1506 (ν_{arom}), 1254, 1225, 1179, 1125 ($\nu_{C–O–C}$), 1029 ($\nu_{C–OH}$), 741 ($\delta_{C–H}$).

2.7. Synthesis of 1-O-myristoylguaifenesin

1-*O*-Myristoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-caprinoylguaifenesin. The yield of product is 62%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.97 (m, 4H, $H_{7–10}$), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.09 (dd, 1H, J = 9.9, 3.8 Hz, –CH_2O–), 4.02 (dd, 1H, J = 9.9, 6.5 Hz, –CH_2O–), 3.84 (s, 3H, –OCH_3), 2.62 (s, 1H, –OH), 2.34 (t, 2H, –CH_2CO), 1.62 (m, 2H, –CH_2–), 1.27 (m, 20H, 10–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3479 ($\nu_{–OH}$), 3066 ($\nu_{=C–H}$), 2925, 2854 ($\nu_{C–H}$), 1738 ($\nu_{C=O}$), 1594, 1503 (ν_{arom}), 1254, 1225, 1176, 1120 ($\nu_{C–O–C}$), 1031 ($\nu_{C–OH}$), 743 ($\delta_{C–H}$).

2.8. Synthesis of 1-O-palmitoylguaifenesin

1-*O*-Palmitoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-capryloylguaifenesin. The reaction time was 48 h. The yield of product is 60%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.94 (m, 4H, $H_{7–10}$), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.08 (dd, 1H, J = 9.8, 3.8 Hz, –CH_2O–), 4.02 (dd, 1H, J = 9.8, 6.3 Hz, –CH_2O–), 3.86 (s, 3H, –OCH_3), 2.35 (t, 2H, –CH_2CO), 1.61 (t, 2H, –CH_2–), 1.28 (m, 24H, 12–CH_2–), 0.88 (t, 3H, –CH_3). IR (cm^{-1}): 3479 ($\nu_{–OH}$), 3059 ($\nu_{=C–H}$), 2920, 2850 ($\nu_{C–H}$), 1738 ($\nu_{C=O}$), 1593, 1509 (ν_{arom}), 1259, 1226, 1175, 1127 ($\nu_{C–O–C}$), 1030 ($\nu_{C–OH}$), 744 ($\delta_{C–H}$).

2.9. Synthesis of 1-O-stearoylguaifenesin

1-*O*-Stearoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-palmitoylguaifenesin. The yield of product is 65%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.95 (m, 4H, $H_{7–10}$), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.08 (dd, 1H, $J = 9.8$, 3.8Hz, –CH_2O–), 4.02 (dd, 1H, $J = 9.8$, 6.2 Hz, –CH_2O–), 3.85 (s, 3H, –OCH_3), 2.34 (t, 2H, –CH_2CO), 1.62 (t, 2H, –CH_2–), 1.27 (m, 28H, 14–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3477 ($\nu_{–OH}$), 3064 ($\nu_{=C–H}$), 2925, 2854 ($\nu_{C–H}$), 1739 ($\nu_{C=O}$), 1593, 1504 (ν_{arom}), 1255, 1224, 1176, 1121 ($\nu_{C–O–C}$), 1031 ($\nu_{C–OH}$), 743 ($\delta_{C–H}$).

2.10. Synthesis of 1-O-oleoylguaifenesin

1-*O*-Oleoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-palmitoylguaifenesin. The yield of product is 52%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.95 (m, 4H, $H_{7–10}$), 5.34 (m, 2H, –CH=CH–), 4.26 (m, 3H, –$CHCH_2OCO$–), 4.09 (dd, 1H, $J = 9.9$, 3.6 Hz, –CH_2O–), 4.02 (dd, 1H, $J = 9.9$, 6.5 Hz, –CH_2O–), 3.83 (s, 3H, –OCH_3), 3.30 (s, 1H, –OH), 2.34 (t, 2H, –CH_2CO), 2.04 (m, –2H, –CH_2–), 1.61 (t, 2H, –CH_2–), 1.27 (m, 20H, 10–CH_2–), 0.88 (t, 3H, –CH_3). IR (cm^{-1}): 3470 ($\nu_{–OH}$), 3065, 3004 ($\nu_{=C–H}$), 2925, 2854 ($\nu_{C–H}$), 1739 ($\nu_{C=O}$), 1594, 1506 (ν_{arom}), 1254, 1226, 1178, 1125 ($\nu_{C–O–C}$), 1030 ($\nu_{C–OH}$), 742 ($\delta_{C–H}$).

2.11. Synthesis of 1-O-linoleoylguaifenesin

1-*O*-Linoleoylguaifenesin was synthesized by the same synthesis method as for 1-*O*-palmitoylguaifenesin. The yield of product is 73%. ^{1}H NMR ($CDCl_3$), δ (ppm): 6.95 (m, 4H, $H_{7–10}$), 5.36 (m, 4H, 2–CH=CH–), 4.25 (m, 3H, –$CHCH_2OCO$–), 4.09 (dd, 1H, $J = 9.8$, 2.88 Hz, –CH_2O–), 4.02 (dd, 1H, $J = 9.7$, 6.5 Hz, –CH_2O–), 3.86 (s, 3H, –OCH_3), 3.30 (s, 1H, –OH), 2.77 (t, 2H, =CH–CH_2–CH=), 2.34 (t, 2H, –CH_2CO), 2.04 (m, 4H, 2–CH_2), 1.62 (m, 2H, –CH_2–), 1.30 (m, 14H, 7–CH_2–), 0.87 (t, 3H, –CH_3). IR (cm^{-1}): 3479 ($\nu_{–OH}$), 3066, 3009 ($\nu_{=C–H}$), 2926, 2855 ($\nu_{C–H}$), 1738 ($\nu_{C=O}$), 1594, 1504 (ν_{arom}), 1254, 1225, 1178, 1125 ($\nu_{C–O–C}$), 1029 ($\nu_{C–OH}$), 742 ($\delta_{C–H}$).

3. Results and discussion

3.1. Enzymatic synthesis of 1-O-fatty acid guaifeneisn esters

So far, there has been no report about the synthesis of guaifenesin esters by enzyme. The aim of this study was to develop enzymatic methods for the regioselective synthesis of esters derivatives of guaifenesin. Transesterification of guaifenesin with fatty acid vinyl esters (C8–18) catalyzed by immobilized lipase from *M. miehei* was shown in Fig. 1. The products were purified by silica gel chromatography and analyzed by ^{13}C NMR spectrometry to identify the position esterified, as shown in Table 1. Based on the general strategy described by Yoshimoto [13], acylation of a hydroxyl group will lead the *O*-acylated carbon ($^*CH_2OCOR$) downfield, while the adjacent carbon ($^*CCH_2OCOR$) upfield in ^{13}C NMR. Analysis ^{13}C NMR spectra of all the products revealed that esterification of guaifenesin occurred in the primary alcohol position. As listed in Table 1, the chemical shift for carbon number 1 was downfield from 63.3 to 65.2 ppm, and the chemical shift for carbon number 2 was upfield from 70.2 to 68.7 ppm. Therefore, the esterification of guaifenesin catalyzed by the Lipozyme® inevitability occurred in primary alcohol without any question.

3.2. Influence of enzyme

One of the most important parameters for enzyme-catalyzed reactions is the selection of enzyme sources. Enzymes derived from a variety of sources including bacteria, yeast and molds exhibit different properties, including stability, activity and specificity. To choose the more efficient enzyme on the synthesis of guaifenesin fatty acid esters, five

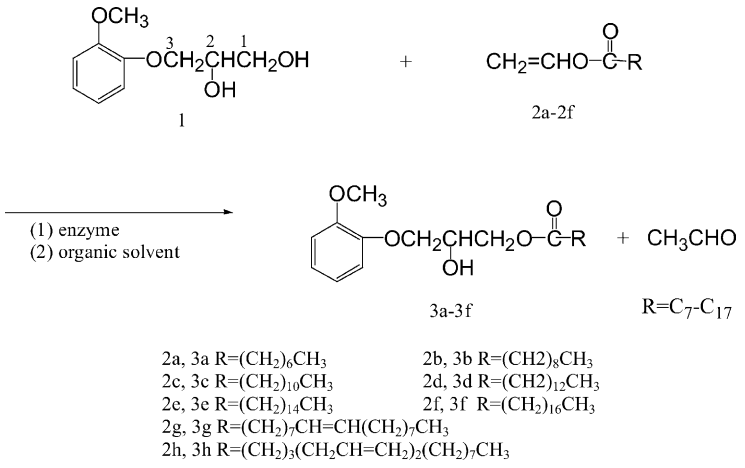

Fig. 1. Enzyme-catalyzed synthesis of guaifenesin esters in organic solvents.

Table 1
Chemical shifts of ^{13}C NMR ($CDCl_3$) of guaifenesin and guaifenesin esters

Carbon	1	3a	3b	3c	3d	3e	3f	3g	3h
1	63.3	65.2	65.2	65.2	65.2	65.2	65.2	65.2	65.2
2	70.2	68.7	68.7	68.7	68.8	68.8	68.6	68.6	68.6
3	70.6	71.6	71.6	71.7	71.8	71.8	71.7	71.6	71.6
4	55.6	56.1	56.1	56.1	56.1	56.1	56.0	56.0	56.0
5	148.5	148.3	148.2	148.2	148.2	148.2	148.2	148.2	148.2
6	149.4	150.2	150.1	150.2	150.4	150.3	150.2	150.1	150.1
7	112.4	112.4	112.3	112.3	112.3	112.2	112.2	112.2	112.2
8	121.1	122.6	122.7	122.8	122.9	122.9	122.6	122.6	122.7
9	120.9	121.3	121.3	121.3	121.3	121.3	121.3	121.3	121.3
10	113.8	115.7	115.6	115.9	116.1	116.0	115.9	115.6	115.7
C=O		174.1	174.2	174.2	174.2	174.2	174.2	174.1	174.2
$-CH_2$		34.4	34.4	34.4	34.4	34.4	34.4	34.3	34.4
		31.9	32.1	32.2	32.2	32.2	32.1	32.1	31.8
		29.3	29.7	29.9	29.9	29.9	29.9	30.0	29.9
		29.1	29.5	29.7	29.9	29.9	29.9	29.9	29.8
		25.1	29.4	29.6	29.8	29.9	29.8	29.7	29.6
		22.8	25.1	29.5	29.7	29.7	29.6	29.5	29.6
			22.9	29.4	29.6	29.6	29.5	29.4	29.4
				25.2	29.5	29.5	29.3	29.3	29.3
				22.9	29.4	29.4	29.4	27.4	27.4
					25.2	25.2	25.1	27.4	25.9
					23.0	23.0	22.9	25.1	25.1
								22.9	22.8
$-CH_3$		14.3	14.3	14.4	14.4	14.4	14.3	14.3	14.3
–CH=CH								130.2	130.5
								130.0	130.2
									128.3
									128.1

commercially available enzymes were tested for the transesterification of guaifenesin with capric acid vinyl ester in acetone at 50 °C. The results were compared and presented in Table 2. From Table 2, it clearly shows that no transesterification occurs without enzyme participation. Moreover, Lipozyme® exhibits its unique advantage to catalyze the reaction, while the alkaline protease from *B. subtilis* reveals the lowest catalysis activity.

3.3. Influence of organic solvents

To establish appropriate conditions for transesterification by the lipase, different solvents, listed in Table 3, were used for the transesterification of guaifenesin with capric acid vinyl ester. In our study, conversion degrees varied with solvents. No relationship between the conversion degrees and solvents could be clearly generated. Ideally, a good solvent for biocatalysis should not only maintain the enzyme activity, but also dissolve starting materials as well. Hydrophobic solvents were normally used in enzyme-catalyzed reaction [15,16], such as, hexane and cyclohexane. To this transesterification reaction, non-polar solvents were unfavorable

Table 2
Enzyme screen for guaifenesin esters synthesis

Enzyme	Yield of ester (%)[a]
Control, no enzyme	0
Alkaline protease from *B. subtilis*	3
Lipozyme® immobilized lipase from *M. miehei*	83
Lipase from porcine pancreas	72
Lipase from hog pancreas	69
Lipase from *C. cylindracea*	32

[a] Experimental conditions: 0.1 mmol guaifenesin; 0.4 mmol vinyl caprate; 20 mg enzyme; 2 ml acetone; 50 °C; 24 h. Ester yield was determined by HPLC.

Table 3
Effect of solvents on lipase catalyzed 1-*O*-capryloylguaifenesin

Solvent	Log *P*[a]	Yield of ester (%)[b]
DMF	−1.0	22
Dioxane	−0.5	31
Acetonitrile	−0.39	54
Acetone	−0.23	67
THF	0.46	39
Dichloromethane	0.6	32
Pyridine	0.65	66
2-Methyl-2-propanol	0.79	41
Chloroform	2.0	36
Toluene	2.6	34
Cyclohexane	3.4	27
Hexane	3.9	26

[a] From [14].

[b] Experimental conditions: 0.1 mmol guaifenesin; 0.4 mmol vinyl caprate; 10 mg/ml Lipozyme®; 2 ml solvent; 50 °C; 12 h.

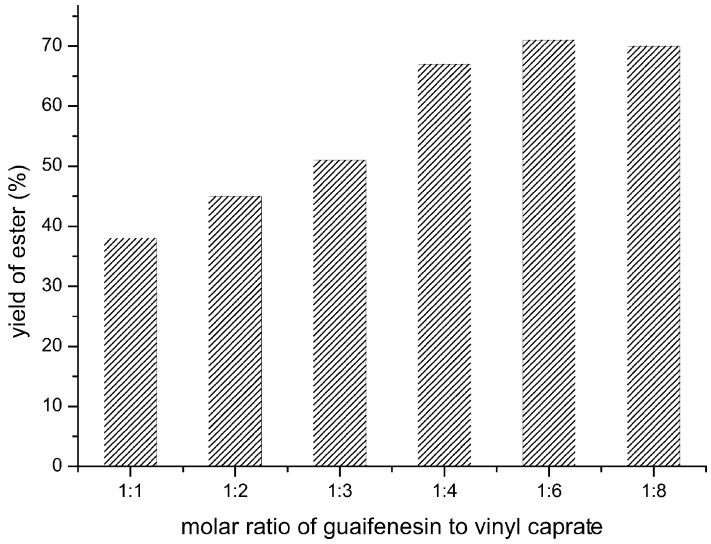

Fig. 2. Reaction between guaifenesin and vinyl caprate, at different molar ratios of substrates. Experimental conditions: 20 mg guaifenesin; 10 mg/ml Lipozyme®; 1 ml acetone; 50 °C; 12 h.

due to guaifenesin solubility. On the other hand, when DMF or dioxane was used in this reaction, low conversion degrees were also observed because of enzyme activity. As a result, acetone and pyridine exhibited the highest conversion yields. Concerning the lower toxicity and easier processing, acetone was chosen as reaction media for this transesterification.

3.4. Influence of substrates molar ratio

Different molar ratios of guaifenesin to vinyl caprate were examined under Lipozyme® at 50 °C. Results were listed in Fig. 2. From the diagram, the yields of 1-*O*-capryloylguaifene considerably increased from 38 to 70% when the molar ratio of guaifenesin to vinyl ester was varied from 1:1 to 1:8, and the plateau was reached at a ratio of 1:6. It was obviously that the excess amount of vinyl caprate would benefit the transesterification equilibrium.

4. Conclusion

In conclusion, a facile route to prepare guaifenesin esters bearing with a long chain acyl group on the primary alcohol position was developed in this paper. Different reaction conditions were systematically examined. To make summarize, this transesterification between guaifenesin and fatty acid vinyl ester should be carried out in acetone at 50 °C under Lipozyme® catalyst. The molar ratio of guaifenesin to vinyl caprate should be 1:6. The 1-*O*-acyl derivatives of guaifenesin obtained are more lipophilic than the parent guaifenesin and potential usage of guaifenesin esters are under investigation.

References

[1] C.G. Wermuth, J. Gaignault, C. Marchandeau, Designing prodrugs and bioprecursors, in: C.G. Wermuth (Ed.), The Practice of Medicinal Chemistry, Academic Press, London, 1996, 674 pp.

[2] A. Kontogianni, V. Skouridou, V. Sereti, H. Stamatis, F.N. Kolisis, Regioselective acylation of flavonoids catalyzed by lipase in low toxicity media, Eur. J. Lipid Sci. Technol. 103 (2001) 655–660.

[3] V.V. Mozhaev, C.L. Budde, J.O. Rich, A.Y.A. Usyatinsky, J.S. Dordick, et al., Regioselective enzymatic acylation as a tool for producing solution-phase combinatorial libraries, Tetrahedron 54 (1998) 3971–3982.

[4] B. Danieli, A. Bertario, G. Carrea, B. Redigolo, F. Secundo, S. Riva, Chem-enzymatic synthesis of 6″-*O*-(3-arylprop-2-enoyl) derivatives of the flavonerl glucoside isoquevcitrim, Helv. Chem. Acta 76 (1993) 2981–2991.

[5] E. Keinan, S.C. Sinha, A. Sinha-Bagch, Thermostable enzymes in organic synthesis. Part 6. Total synthesis of (*S*)-(−)-zearalenone using a TBADH-generated trifunctional chiron, J. Chem. Soc., Perkins Trans 1 (12) (1991) 3333–3339.

[6] G. Ottolina, G. Carrea, S. Riva, Synthesis of esters derivatives of chloramphenicol by lipase-catalyzed transesterification in organic solvents, J. Org. Chem. 55 (1990) 2366–2369.

[7] G.G. Perrone, K.D. Barrow, I.J. Mcfarlane, The selective enzymatic synthesis of lipophilic esters of swainsonine, Bioorg. Med. Chem. 7 (1999) 831–835.

[8] M. Ferrero, V. Gotor, Biocatalytic selective modification of conventional nucleosides, carbocyclic nucleosides, and C-nucleosides, Chem. Rev. 100 (2000) 4319–4347.

[9] X.T. Xue, D.S. Lv, Z.C. Chen, Q. Wu, Y. Cai, X.F. Lin, Enzyme-catalyzed transesterification of unusual substrate: synthesis of acyclovir and L-ascorbic acid (Vitamin C) vinyl esters, Chin. Chem. Lett. 14 (2003) 163–166.

[10] D.S. Lv, X.T. Xue, N. Wang, Q. Wu, X.F. Lin, Enzyme-catalyzed synthesis of some vinyl drug esters in organic medium, Prep. Biochem. Biotechnol., in press.

[11] A.O. Chairman, S.C. Harvey, G.D. Chase, et al., Remington's Pharmaceutical Science, 16th ed., Meck Publishing Company, Philadelphia, USA, 1980, 806 pp.

[12] H. Yang, E. Henke, U.T. Bronscheuer, The use of vinyl esters significantly enhanced enantioselectivities and reaction rates in lipase-catalyzed resolutions of arylaliphatic carboxylic acids, J. Org. Chem. 64 (1999) 1709–1712.
[13] K. Yoshimoto, Y. Itatani, Y. Tsuda, Utilization of sugars in organic-synthesis for C-13-nuclear magnetic-resonance (NMR) spectra of *O*-acylglucoses—additivity of shift parameters and its application to structure elucidations, Chem. Pharm. Bull. 28 (1980) 2065–2076.
[14] C. Laane, S. Boeren, K. Vos, C. Veeger, Rules for optimization of biocatalysis in organic solvents, Biotechnol. Bioeng. 30 (1987) 81–87.
[15] S. Parida, J.S. Dordick, Substrate structure and solvent hydrophobicity control lipase catalysis and enantioselecitivity in organic media, J. Am. Chem. Soc. 113 (1991) 2253–2259.
[16] A.M. Klibanov, Improving enzymes by using them in organic solvents, Nature 409 (2001) 241–246.