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Journal of Molecular Catalysis B: Enzymatic 11 (2001) 315–321

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Lipase catalysed acylation of hydroxylamine and hydrazine derivatives

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Abstract

The lipase catalysed acylation of hydroxylamine-and hydrazine as well as their derivatives by octanoic acid is very efficient. Cross-linked crystals of *Candida rugosa* lipase (ChiroCLEC-CR) mediated the conversion of racemic ibuprofen into (*S*)-ibuproxam. A number of lipases also catalysed the condensation of hydrazine with an excess of octanoic acid giving *N*,*N*′-dioctanoylhydrazine. The hydrazide of 2-(4-isobutylphenyl)propanoic acid (ibuprofen), prepared by non-enzymatic reaction of ibuprofen methyl ester with hydrazine, acted as nucleophile towards several lipases that do not accept ibuprofen derivatives as acyl donor.

Keywords: Lipase; Acyl transfer; Hydroxylamine; Hydrazine; Ibuprofen

1. Introduction

It is now well established that in organic media, lipases (E.C.3.1.1.3) readily catalyse a variety of acylation reactions which they do not perform in nature [1]. The lipase catalysed aminolysis of carboxylic esters, which was discovered more than 10 years ago by Zaks and Klibanov [2], has been widely investigated [1,3]. The use of hydroxylamine and hydrazine and their derivatives in the acyl acceptor role has received rather less attention [4–7], although these compounds are, due to the α-effect, highly active nucleophiles. Hydroxamic acids (*N*-hydroxy carboxylic amides) and hydrazides are versatile intermediates, which prompted us to study their synthesis in detail.

2. Materials and methods

2.1. Chemicals

All solvents were of analytical purity and were dried over activated Uetikon CaA zeolite prior to use. Accurel EP100 (a macroporous polypropylene) was kindly donated by Akzo Nobel Faser. All other reagents were purchased from Aldrich or Acros and used as received unless they had to be synthesised.

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1381-1177/01/$ - see front matter
PII: S1381-1177(00)00144-2

2.2. Lipases

Novozym 435 (*Candida antarctica* lipase on Lewatit E), SP 525 (pure *C. antarctica* lipase B), SP 526 (*C. antarctica* lipase A) and SP 523 (*Thermomyces lanuginosus* lipase[2]) were kindly donated by Novo Nordisk. *Pseudomonas alcaligenes* lipase was obtained from Gist-brocades as a gift. *Pseudomonas* lipoprotein lipase was kindly donated by Roche Diagnostics. CLEC-CR (cross-linked crystals of *C. rugosa* lipase) was received from Altus Biologics as a gift. Immobilised enzymes (Novozym 435 and CLEC-CR) were used as received; SP 525, SP 526, SP523, *P. alcaligenes* lipase and *Pseudomonas* lipoprotein lipase were immobilised on Accurel EP 100 according to a published procedure [8]. The hydrolytic activities of the immobilised enzymes are given in Table 1.

2.3. Analysis

The progress of the reactions of octanoic acid-and ibuprofen derivatives was monitored with HPLC on a Waters 8×100 mm 4μ Novapak C_{18} reversed phase RCM column with a Waters 510 pump, a Shimadzu SPD-6A UV detector, a Shodex RI SE-61 RI detector and a Spectra-Physics SP 4270 integrator. The eluent was methanol–water (65:35, v/v) containing 0.05 M acetate buffer pH 4.3 at a flowrate of 1.0 ml/min.

Chiral HPLC of ibuprofen was performed on a Baker 4.6×250 mm 5μ Chiralcel OD column with a Waters 510 pump and a Waters 486 UV detector operating at 254 nm, eluent isopropyl alcohol-hexane-formic acid (98:2:0.1, v/v) at a flowrate of 0.5 ml/min. Chiral HPLC of *N*-octanoyl-*N′*-2-(4-isobutylphenyl)-propanoylhydrazine was performed similarly except that the eluant did not contain formic acid.

^{1}H-and ^{13}C NMR spectra were recorded on a Varian VXR-400S spectrometer. Mass spectra were recorded on a VG 70 SE spectrometer with the EI method.

[2] *T. lanuginosus* was formerly named *Humicola lanuginosa*.

Table 1
Activity of immobilised lipase preparations[a]

Lipase preparation	Activity (kLU/g)
C. antarctica A on Accurel EP100	n.d.
C. antarctica B on Lewatit E (Novozym 435)	4.1
C. antarctica B (SP525) on Accurel EP100	5.1
P. alcaligenes on Accurel EP100	4.7
Pseudomonas lipoprotein lipase	4.8
T. lanuginosus on Accurel EP100	18.1

[a] Measured in the hydrolysis of tributyrin by automatic titration at pH 7.2. 1 Unit (U) will liberate 1 μmol of butyric acid per minute.

2.4. Synthesis of ibuprofen derivatives

Ibuprofen hydrazide 2-(4-isobutyl-phenyl)-propanohydrazide)was synthesised from 10 g racemic or (*S*)-ibuprofen methyl ester (45.5 mmol) and 10 ml hydrazine (206 mmol) hydrate in 25 ml methanol. After 48 h at 40°C, the solvent was evaporated in vacuo and the residue was recrystallised from hexane/isopropyl alcohol (20:1 v/v). Yield 7.31 g (73%), m.p. 75°C. 2-(4-Isobutyl-phenyl)-propano-hydrazide, ^{1}H NMR (DMSO-d_6): (propanohydrazide moiety) δ 9.98 (1H,N*H*,d), 3.60 (1H,C*H*,q), δ 3.37 (2H,NH_2,br.s), δ 1.32 (3H,CH_3,d), (ring) δ 7.3–7.0 (4H,C*H*2,3,5,6,m), (isobutyl moiety) δ 2.39 (2H,CH_2,d) δ 1.78 (1H,C*H*,m), δ 0.93 (6H,CH_3,d). ^{13}C NMR: (propanoyl moiety) δ 172.067 (C=O), δ 44.140 (C2), δ 22.077 (C3), (ring) δ 139.261 (C1), δ 138.686 (C4), δ 128.852, 128.627, 126.960 (C2,C3,C5,C6), (isobutyl moiety) δ 42.429 (C1), δ 29.509(C2), δ 18.205, 18.103 (C3,C4). MS: *m/z* 220 (27%), 188 (23%), 161 (100%), 145 (9%) 119 (30%), 105 (11%), 91 (21%), 57 (25%).

Racemic- and *N*-octanoyl-*N′*-(2,*S*)-2-(4-isobutylphenyl)propanoylhydrazine were synthesised as reference compounds from 1.0 g racemic or (*2,S*)-2-(4-isobutyl-phenyl)propionyl chloride (4.45 mmol), 750 mg octanohydrazide (4.75 mmol) and 1 ml pyridine in 50 ml ice-cold *tert*-butyl methyl ether. After washing three times with 50 ml 0.1 M HCl, three times with 50 ml 0.1 M $NaHCO_3$ and three times with 50 ml water, the organic layer was dried on Na_2SO_4 and the solvent was evaporated in vacuo. The residue was recrystallised from hexane; yield

1.31 g (85%), m.p. 115°C (racemate), 138°C ((*S*)-enantiomer). ^{1}H NMR ($CDCl_3$): (propanohydrazide moiety) δ 9.02 (2H,N*H*,d), 3.66 (1H,C*H*,q), δ 1.57 (3H,CH_3,d), (ring) δ 7.21, 7.08 (4H,C*H*2,3,5,6,dd), (isobutyl moiety) δ 2.42 (2H,CH_2,d), δ 1.83 (1H,C*H*,m), δ 0.88 (6H,CH_3,m), (octanoyl moiety) δ 2.21 (2H,CH_2,t), δ 1.60 (2H,CH_2,t), δ 1.13 (8H,CH_2,m), δ 0.88 (3H,CH_3,s). ^{13}C NMR: δ 170.765, 169.586 (C=O), (propanoyl moiety), δ 45.042 (C2), δ 22.390 (C3), (ring) δ 140.980 (C1), δ 137.304 (C4), δ 129.603, 127.288 (C2,C3,C5,C6), (isobutyl moiety) δ 44.409 (C1), δ 29.174(C2), δ 18.350 (C3, C4), (octanoyl moiety) δ 34.051, 31.635, 30.150, 28.970, 25.331, 22.594 (C2–C7), δ 14.041 (C8). MS: 328 (6%), 285 (11%), 244 (12%), 220 (13%), 161 (100%), 149 (17%), 119 (26%), 91 (21%), 57 (43%).

2.5. Reactions

Reactions were performed in 30 ml glass vessels with a teflon coated cap in a stirred thermostated oil bath at 40°C or at room temperature. Samples of 50 μl were taken at regular intervals with a cut-off pipet from the stirred reaction mixtures, diluted with 200 μl isopropyl alcohol and analysed by HPLC. In some cases, when heavy precipitation prevented unbiased sampling, separate reactions were performed for each data point.

2.6. Isolation, identification and characterisation of the products

Ibuproxam crystallised from the reaction mixture. The enantiomeric ratio *E* was calculated from the conversion and the *ee* of the reactant, because separation of the product enantiomers could not be accomplished on the Chiralcel OD or Chiralcel OB columns. The crystals were isolated by filtration, washed with a small amount of water and dissolved in acetone. After removal of the catalyst by filtration, the solvent was removed in vacuo to yield the 36.8 mg of the pure product, m.p. 126°C, $[\alpha]_D^{22} = +44.8°$ (c 0.30; ethanol abs.); Literature [9]: m.p. 119-121°C, $[\alpha]_D^{23} = +44.4°$ c 0.30; ethanol abs.). We conclude on account of the optical rotation that enrichment of *S*-ibuproxam had occurred during the workup.

Acylation products of hydroxylamine derivatives were isolated for analytical purposes. After prolonged reaction times to reach full conversion, the solvent was evaporated in vacuo and the residue was recrystallized from petroleum ether (bp 40-60°C). *O*-benzyl-*N*-octanoylhydroxylamine, which remained liquid, was taken up in petroleum ether and washed successively with 0.1 M Na_2CO_3 solution, 0.1 M HCl solution and water and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo yielded the pure product. The products were identified by comparison with samples that had been prepared chemically using standard procedures.

Octanohydroxamic acid, m.p. 77°C. ^{1}H NMR: δ 7.23 (2H,N*H*,O*H*,s), δ 2.15 (2H,CH_2-2,t), δ 1.64 (2H,CH_2-3,qi), δ 1.29 (8H,C$H_2$4–7,m), δ 0.88 (3H,CH_3,t). ^{13}C NMR: δ 171.81 (C=O), δ 33.53 (C2), δ 31.65 (C3), δ 29.09, 28.93 (C4,5), δ 25.38 (C6), δ 22.59 (C7), δ 14.04 (C8). MS: $m/z = 160$ (3%,m + 1), 159 (2%), 127 (86%), 109 (17%), 84 (8%), 75 (31%), 67 (10%), 57 (100%).

O-phenyloctanohydroxamic acid, m.p. 130°C. ^{1}H NMR: δ 8.47, 8.20 (1H,N*H*,br.d), 7.4–7.0 (5H,*ring H*,m), 2.30 (2H,CH_2,br.s), 1.67 (2H,CH_2,br.s), 1.3 (8H,CH_2, m) 0.88 (3H,CH_3). ^{13}C NMR: δ 159.503, 129.603, 123.169 113.146 (ring), δ 31.638 (C4), δ 29.188, 28.930 (C5,6), δ 22.587 (C7), δ 14.051 (C8). MS: $m/z = 235$ (39%), 151 (8%), 142 (9%), 127 (73%), 109 (84%), 94 (95%), 65 (49%), 57 (100%).

O-benzyloctanohydroxamic acid, ^{1}H NMR: δ 8.39 (1H,N*H*,s), δ 7.4–7.2 (5H,ring,m), δ 4.90 (2H,O-CH_2,s), δ 2.02 (2H,CH_2-2,t), δ 1.60 (2H,CH_2-3,qi), δ 1.26 (8H,C$H_2$4–7,m), δ 0.87 (3H,CH_3,t). ^{13}C NMR: δ 171.10 (C=O), δ 129.22, 129.03, 128.61, 128.22 (ring), δ 78.12 (O-CH_2), δ 33.31 (CH_2-2), δ 31.67 (CH_2-3), δ 29.17, 28.96 (CH_2-4,5), δ 25.40 (CH_2-6), δ 22.60 (CH_2-7), δ 14.07 (CH_2-8). MS: $m/z = 250$ (34%, m + 1), 214 (32%), 181 (11%), 127 (12%), 91 (100%), 57 (23%).

Octanohydrazide, m.p. 87°C. ^{1}H NMR: δ 6.80 (1H,N*H*,s), δ 3.60 (2H,NH_2,s), (δ 2.15 (2H,CH_2-2,t), δ 1.64 (2H,CH_2-3,qi), δ 1.29 (8H,C$H_2$4–7,m), δ 0.88 (3H,CH_3,t). ^{13}C NMR: δ 174.01 (C=O), δ 34.62 (CH_2-2), δ 31.66 (CH_2-3), δ 29.24, 28.96 (CH_2-4,5), δ 25.51 (CH_2-6), δ 22.59 (CH_2-7), δ

14.05 (CH_2-8). MS: $m/z = 158$ (14%), 127 (54%), 109 (10%), 83 (6%), 74 (33%), 57 (100%).

N,N′-dioctanoylhydrazine m.p. 154°C. ^{1}H NMR: δ 8.25 (2H,N*H*,s), δ 2.23 (4H,CH_2-2,t), δ 1.65 (4H,CH_2-3,qi), δ 1.28 (16H,C$H_2$4–7,m), δ 0.88 (6H,CH_3,t). ^{13}C NMR: δ 170.91 (C=O), δ 33.04 (CH_2-2), δ 31.06 (CH_2-3), δ 28.39, 28.32 (CH_2-4,5), δ 24.94 (CH_2-6), δ 21.94 (CH_2-7), δ 13.84 (CH_2-8). MS: $m/z = 284$ (3%), 158 (71%), 127 (66%), 74 (29%), 57 (100%).

3. Results

3.1. Hydroxylamine

The acyl transfer from ethyl octanoate to hydroxylamine was efficiently catalysed by a number of lipases in *tert*-butyl alcohol medium (see Table 2), provided that a small amount of water was present to stabilise hydroxylamine. With *C. antarctica* lipase B and 0.5 M hydroxylamine, the initial synthesis rate increased with the water concentration and peaked at 1.75 M water (data not shown). Due to the presence of water, the reaction was accompanied by some hydrolysis to octanoic acid, which was subsequently also transformed into octanohydroxamic acid (Fig. 1) as as has also been reported by Servat et al. [4,5].

The direct condensation of a carboxylic acid is an attractive option because it would save a reaction step. Hence, we next investigated the lipase catalysed condensation of octanoic acid and a small excess of hydroxylamine using *C. antarctica* lipase. Octanoic acid reacted much slower than the ester, but nearly quantitative conversions were eventually achieved (Table 3). A number of reaction media were compared and it became apparent that the reaction could even be performed in water. In an aqueous medium the reaction became very slow at approx. 70% conversion because the reaction mixture solidified, which is a distinct disadvantage. Ap-

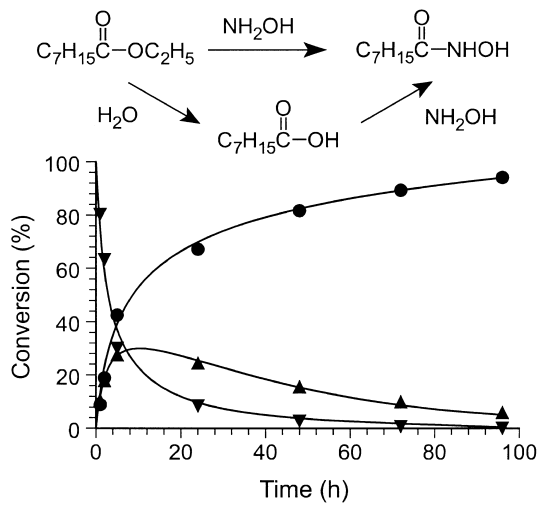


Fig. 1. Hydroxylaminolysis and competing hydrolysis of ethyl octanoate catalyzed by *T. lanuginosus* lipase. Legend: ▼, ethyl octanoate; ▲, octanoic acid; ●, octanohydroxamic acid. Reaction conditions: 688 mg (4 mmol) ethyl octanoate, 0.5 M NH_2OH, 1.75 M H_2O, 10 mg immobilised *T. lanuginosus* lipase in 10 ml *tert*-butyl alcohol at 40°C.

Table 2
Lipase catalyzed hydroxylaminolysis of ethyl octanoate

Lipase (on Accurel EP 100)	Initial S/H[a]	Initial synthesis rate[b] (μmol g^{-1} min^{-1})	Yield[c] (%)
C. antarctica B (SP 525)	2.3	1270	89
T. lanuginosus (SP 523)	0.9	630	93
Pseudomonas lipoprotein	0.9	320	89
P. alcaligenes	2.4	170	79

[a] S/H: ratio of octanohydroxamic acid and octanoic acid formed.

[b] Reaction conditions: 688 mg (4 mmol) ethyl octanoate, 0.5 M NH_2OH, 10 mg immobilized lipase and 1.75 M H_2O in 10 ml *tert*-butyl alcohol at 40°C.

[c] Reaction conditions: 344 mg (2 mmol) ethyl octanoate, 0.5 M NH_2OH, 50 mg immobilized lipase and 1.75 M water in 5 ml *tert*-butyl alcohol, 24 h at 40°C.

Table 3
Effect of the solvent on the *C. antarctica* lipase catalyzed condensation of hydroxylamine and octanoic acid

Solvent	Initial rate[a] (μmol g^{-1} min^{-1})	Yield[b] (%)
Water[c]	4780	66
tert-Butyl alcohol	160	95
Dioxane	450	91
tert-Butyl methyl ether	470	87
1,2-Dimethoxyethane	260	89
Toluene	1680	84

[a]Reaction conditions: 576 mg (4 mmol) octanoic acid, 0.5 M NH_2OH, 10 mg Novozym 435 and 0.92 M water in 10 ml solvent at 40°C.
[b]Reaction conditions: 288 mg (2 mmol) octanoic acid, 0.5 M NH_2OH, 50 mg Novozym 435 and 0.92 M water in 5 ml solvent at 40°C for 24 h.
[c]1.7 mg *C. antarctica* lipase SP525.

parently, the octanoacid is hydrolytically stable under the reaction conditions. We note that it was generally believed that the condensation of a carboxylic acid with an amine was not feasible, because the formation of the unreactive salt was expected to prevail, but more examples of lipase mediated condensations of carboxylic acids and amines have been published in recent years [10–14]. Hydroxylamine is a weak base (pK_a 6.0) but a strong nucleophile, which explains why its condensation with octanoic aicd is so efficient.

3.2. (*S*)-ibuproxam

(*S*)-Ibuproxam, the hydroxamic acid derived from ibuprofen, is a prodrug which makes it an attractive target compound. *C. rugosa* lipase, which is known to possess a preference for (*S*)-ibuprofen contrary to most other lipases, was not active in hydroxylaminolysis when adsorbed on Accurel EP100. When stabilised as cross-linked crystals, *C. rugosa* lipase (ChiroCLEC CR) maintained its activity in the presence of hydroxylamine and mediated a slow condensation of ibuprofen and hydroxylamine which caused enantiopure (*S*)-ibuproxam to precipitate from the reaction mixture (Fig. 2).

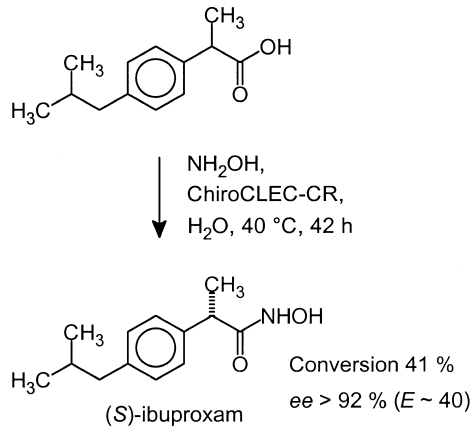


Fig. 2. Synthesis of (*S*)-ibuproxam. Reaction conditions: 103 mg (0.5 mmol) (*R,S*)-ibuprofen, 0.5 M NH_2OH, 25 mg ChiroCLEC-CR in 5 ml water at 40°C.

3.3. Derivatives of hydroxylamine

A number of substituted hydroxylamine derivatives were also smoothly acylated by octanoic acid in the presence of *C. antarctica* lipase (see Fig. 3).

3.4. Hydrazine

Ethyloctanoate as well as octanoic acid were quantitatively transformed into octanohydrazide by

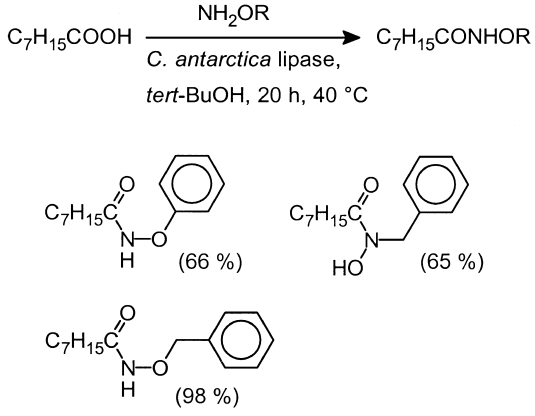
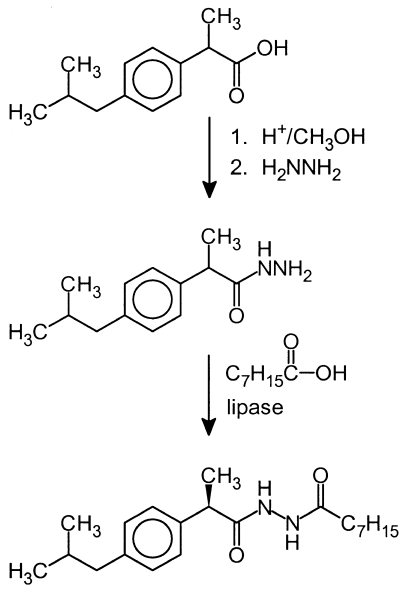


Fig. 3. *C. antarctica* lipase mediated acylation of substituted hydroxylamines; yields are given in parenthesis. Reaction conditions: 58 mg (0.4 mmol) octanoic acid, 1.0 mmol amine and 50 mg Novozym 435 in 1.0 ml *tert*-butyl alcohol, 20 h at 40°C.

an excess of hydrazine in the presence of *C. antarctica* lipase. The hydrazinolysis of ethyl octanoate took place at approx. half the rate of hydroxylaminolysis, but contrary to the latter reaction hydrolysis was almost absent and accounted for only a few % of the conversion. When we used an excess of octanoic acid, we found, much to our surprise, that the initially formed octanohydrazide was slowly but steadily transformed into *N,N′*-dioctanoyl-hydrazine. Many lipases mediated this reaction, the one from *T. lanuginosus* (Fig. 4) being the most active.

3.5. Indirect resolution of ibuprofen

The enzyme catalysed acylation of alkanohydrazides could be a useful technique for the indirect resolution of carboxylic acids that are, e.g. for steric reasons, too unreactive for a conventional kinetic resolution. Ibuprofen was selected as a suitable model to explore this methodology. Due to the large distance between the nucleophilic nitrogen atom and the chiral centre, the enantiomeric differentiation of most lipases was disappointlingly low. The best results were obtained with *C. antarctica* lipase A and *Pseudomonas* lipoprotein lipase, which besides being enantioselective, also showed a relatively high rate (Fig. 5).



Lipase	Time (h)	Yield (%)	*ee* (% *R*)	*E*
C. antarctica A	30	43	74	11
Ps. lipoprotein	18	50	82	26

Fig. 5. Resolution of ibuprofen via its hydrazine.

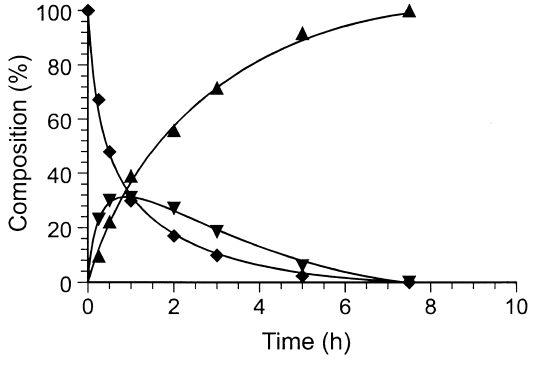


Fig. 4. Time-course of the formation of octanohydrazide (▼) and *N,N′*-dioctanoylhydrazine (▲) from octanoic acid (◆) and hydrazine catalysed by *T. lanuginosus* lipase; 100 % yield of *N,N′*-dioctanoylhydrazine equals 1 mmol. Reaction conditions: 243 mg (2 mmol) octanoic acid, 1 mmol $N_2H_4 \cdot H_2O$, 50 mg immobilised lipase in 5 ml isooctane at 40°C.

4. Conclusion

Hydroxylamine and hydrazine, as well as their derivatives, are readily acylated by carboxylic acids and esters in the presence of lipases. The acylation of hydrazine to the *N,N′*-diacyl derivate is likewise catalysed by a variety of lipases. Several lipases convert the intermediately formed *N*-acyl hydrazine faster than hydrazine itself.

C. antarctica lipase A and *Pseudomonas* lipoprotein lipase mediated the enantioselective acylation of the chiral hydrazide derived from ibuprofen (*N*-octanoyl-*N′*-2-(4-isobutyl-phenyl)propanoylhydrazine). Both enzymes were (*R*)-specific and the latter catalysed the reaction with an enantiomeric ratio of 26 when the reaction was performed at room temperature.

Acknowledgements

Generous donations of enzymes by Novo Nordisk (Bagsvaerd, Denmark), and Roche Diagnostics (Penzberg, Germany) are gratefully acknowledged. The authors wish to thank Gist-brocades (Delft, The Netherlands) for a gift of Lipomax and Akzo Nobel Faser (Obernburg, Germany) for donations of Accurel EP100. ChiroCLEC-CR was kindly donated by Altus Biologics (Cambridge, MA, USA) to whom the authors express their thanks. Thanks are due to Mrs. A.C. Kock-van Dalen and Mr. M.H.A. Janssen for the immobilisation and assay of enzymes. The authors wish to thank Messrs. A. Sinnema and A. van Estrik for recording the NMR spectra and Mrs. A. Knol for the mass spectroscopy measurements.

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