ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 21 (2003) 55–58

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Enantioselective enzymatic preparation of chiral glutaric monocarboxylic acids and amides

Elisabeth Egholm Jacobsen [a], Bård Helge Hoff [b], Anders Riise Moen [a], Thorleif Anthonsen [a,*]

[a] *Department of Chemistry, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*
[b] *Borregaard Synthesis, P.O. Box 162, N-1701 Sarpsborg, Norway*

Abstract

Enantioselective hydrolyses and ammonolyses of diethyl-3-hydroxyglutarate (**1**) and dimethyl-3-hydroxyglutarate (**2**) gave a maximum of 91 and 98% enantiomeric excess (ee), respectively, using immobilized lipase B from *Candida antarctica*. The ees were determined using chiral GLC of the monoamides and achiral GLC of diasteromeric derivatives of the monocarboxylic acids. The catalyst was re-used more than 10 times with retention of high activity and selectivity.

Keywords: Monoamides; Monocarboxylic acids; Enantioselective hydrolyses; Ammonolysis; Chiral GLC

1. Introduction

Enantiopure ethyl- and methyl-3-hydroxyglutaric monocarboxylic acids are precursors for synthesis of molecules of biological interest such as pimaricin, L-carnitine, carbapenem and compactin. These chiral building blocks have been obtained by hydrolysis of prochiral diethyl- and dimethyl-3-hydroxy glutarates catalyzed by various proteases and esterases [1–7].

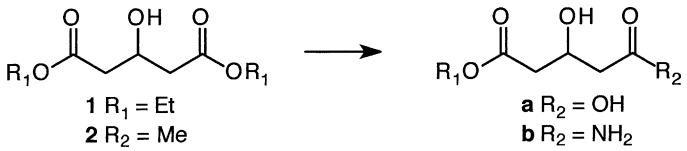
2. Results and discussion

Hydrolysis of prochiral diethyl-3-hydroxyglutarate (**1**) and dimethyl-3-hydroxyglutarate (**2**) with immobilized lipase B from *Candida antarctica*, CALB, Novozyme 435, as catalyst gave **1a** and **2a** with enantiomeric excess, ee, of 91 and 90%, respectively (Scheme 1 and Table 1). The (*S*)-configuration was predominant which is the opposite of the hydrolysis product of **1** and **2** catalyzed by α-chymotrypsin. Other enzymes gave lower ee's and slower reactions or no reaction (LPS). The immobilized catalyst CALB has been re-used more than 10 times for hydrolysis with retention of high activity and selectivity. Ammonolysis of **1** with CALB as catalyst showed an ee of the product ethyl (*S*)-4-carbamoyl-3-hydroxybutanoate (**1b**) of 98%. Attempted ammonolysis of **1** and **2** using PLE or α-chymotrypsin were unsuccessful.

During our work with enzyme catalysis, efficient and accurate chiral analyses have been crucial [8]. Also, in the present work, we have used GLC to determine ee. Previous workers have, to a large extent, relied on optical rotation for this purpose with well-known problems and inaccuracies [9–11]. We have developed GLC methods that are simple and

* Corresponding author. Tel.: +47-73596206; fax: +47-73550877.
E-mail address: thorleif.anthonsen@chembio.ntnu.no (T. Anthonsen).
URL: http://bendik.chembio.ntnu.no

1381-1177/02/$ – see front matter
PII: S1381-1177(02)00138-8



Scheme 1.

Table 1
Asymmetrization by hydrolysis and ammonolysis of **1** and **2**

Product	Enzyme	Activity	ee (%)	Yield (%)	$[\alpha]_D^{20}$	Configuration
1a	CALB	7 PLU/mg	91	80	+1.8 (c 11.5, acetone)	(*S*)
1a	CALA		91	77	+1.8 (c 11.5, acetone)	(*S*)
1a	CLEC-CALB	17 U/mg	86	80		(*S*)
1a	HLL		72	89		(*S*)
1a	RML	60 U/g	74	89		(*S*)
1a	PLE	15 U/mg	35	76	+0.2 (c 11.5, acetone)	(*S*)
1a	α-Chymotrypsin	70 U/mg	50	65		(*R*)
2a	CALB	7 PLU/mg	90	70	+0.8 (c 11.5, acetone)	(*S*)
2a	PLE		22	75		(*S*)
2a	α-Chymotrypsin		45	59		(*R*)
1b	CALB	7 PLU/mg	98	95	−6.9 (c 10.0, dioxane), −6.5 (c l.3, $CHCl_3$)	(*S*)
2b	CALB	7 PLU/mg	98	95	−2.0 (c 3.5, dioxane)	(*S*)

quick to perform. The mixtures of enantiomers of amides **1b** and **2b** were separated directly on a chiral column as TFA-esters, while the monocarboxylic acids **1a** and **2a** were derivatized to diastereomeric mixtures using (*R*)-α-phenylethylamine and analyzed on an achiral column. The optical rotation values of the products were compared with known values in order to determine the configuration of **1a** and **2a** [1,9], and **2b** [6].

The ee of the hydrolysis product of **1** with CALB as catalyst, was optimized by lowering the substrate concentration (Table 2). However, the reaction time increased with decreasing substrate concentration.

Table 2
Variation of concentration of **1** hydrolyzed by CALB

Substrate concentration (g/ml)	ee (%)	Reaction time (min)
0.75	85	20
0.23	87	25
0.11	91	30
0.06	90	35
0.03	91	40

(The amount of enzyme was the same in all the experiments.)

3. Experimental

3.1. General

Immobilized lipase B from *C. antarctica* (CALB, Novozyme 435) from Novozymes had a water content of 1–2% (w/w), lipase A from *C. antarctica* (CALA), *Humicola lanuginosa* lipase (HLL), *Rhizomucor miehei* lipase (RML) from Novozymes were immobilized on Accurel, lipase from *Pseudomonas cepacia* (LPS) was purchased from Amano Pharmaceutical Co. Ltd. ChiroCLEC-CALB was a gift from Altus Biologics Inc., Cambridge, MA, USA and α-chymotrypsin and porcine liver esterase (PLE) were purchased from Fluka and Sigma, respectively. Other chemicals were purchased from Fluka. For enzyme activities, see Table 1 (PLU: palm oil lipase units). The hydrolyses were performed in phosphate buffer (pH 7.0, 0.1 M) using a Metrohm pH-Stat 718 Titrino. For the ammonolyses, a G24 environmental

incubator shaker from New Brunswick Co. Inc., Edison, NJ, USA, was used. Optical rotation and NMR spectra were measured as described earlier [12].

3.2. Hydrolysis

Diethyl-3-hydroxyglutarate (**1**) (3.0 g, 14.7 mmol) was suspended in phosphate buffer (20 ml, pH 7.0, 0.1 M), CALB (0.5 g) was added, and the reaction mixture was stirred for approximately 0.5 h. The reactions were stopped after addition of 14.5 ml 1.0 M NaOH. The enzyme was filtered off and washed with CH_2Cl_2 for re-use. The water phase was extracted with Et_2O (5 × 30 ml). The organic phase was dried over $MgSO_4$ and the solvent was removed to give the product. Similar reactions of **1** (1.0 g, 4.9 mmol) were performed using α-chymotrypsin (0.4 g, reaction time 48 h), PLE (0.15 g, reaction time 6 h), ChiroCLEC-CALB (50 μl, 100 mg enzyme/ml susp.), HML (0.2 g, reaction time 5 h), RML (0.1 g, reaction time 5 h) and CALA (0.1 g, reaction time 4 h). Hydrolyses of dimethyl-3-hydroxyglutarate (**2**) (1.00 g, 5.67 mmol) were performed as for **1**.

3.3. Ammonolysis

NH_3 was bubbled through 1,4-dioxane (7 ml) at 0 °C for 10 min, after which **1** and **2** (0.4 g, 1.96 and 2.3 mmol, respectively) and CALB (20 mg) were added. The mixture was shaken at 30 °C and 20 rpm over night. The enzyme was filtered off and washed with CH_2Cl_2 for re-use. The organic solvents were evaporated to obtain the products.

3.4. Analyses

Chiral analyses were performed using Varian 3800 and 3400 gas chromatographs equipped with a chiral CP-Chirasil-DEX G-TA column from Chrompack (10 m, 0.25 mm i.d., and 0.25 μm film density) and an achiral DBWAX-N30 (25 m, 0.25 mm i.d., and 0.25 μm film density) from J&W Scientific, respectively. *GLC* (*amides*): Ethyl and methyl 1-4-carbamoyl-3-hydroxybutanoate as trifluoroacetic anhydride derivatives (**1b** and **2b**) were separated on Chirasil-DEX G-TA at 90(1)-105/1(0)-150/15(2), column pressure 6.0 psi and splitflow 60 ml/min. **1b**: RT_1 = 10.40, RT_2 = 10.90, R_s = 1.8. **2b**: RT_1 = 9.10, RT_2 = 9.40, R_s = 1.50.

GLC (*monocarboxylic acids*): To **1a** and **2a** (20 μl) was added $SOCl_2$ (20 μl) and dimethylformamide (20 μl), dissolved in Et_2O (2 ml) and the mixture was shaken for 5 min. (*R*)-α-Phenylethylamine (40 μl) was added and the mixture shaken for another 5 min during which HCl gas was formed. The Et_2O was evaporated and the remaining mixture dissolved in CH_2Cl_2 (2 ml). The solution was washed with water and saturated Na_2CO_3, dried over $MgSO_4$ and separated on DBWAX-N30 at 235 °C isothermic 35 min, column pressure 12 psi, splitflow 60 ml/min. **1a**: RT_1 = 31.4, RT_2 = 32.1, R_s = 2.9. **2a**: RT_1 = 31.6, RT_2 = 32.2, R_s = 2.5. ^{1}H NMR **1a**: 1.27 (3H, t), 2.59 (4H, dd), 4.17 (2H, q), 4.49 (1H, m), 6.97 (2H, br. s). ^{13}C **1a**: 14.2, 40.6, 40.7, 61.2, 64.8, 172.2, 176.3. ^{1}H NMR **2a**: 2.60 (4H, dd), 3.70 (3H, s), 4.50 (1H, m), 6.80 (2H, br. s). **1b**: 1.27 (3H, t), 2.42–2.57 (4H, dd), 3.70 (1H, OH), 4.16 (2H, q), 4.49 (1H, m), 6.05 (1H, NH), 6.45 (1H, NH). **2b**: 2.43–2.59 (4H, dd), 3.72 (3H, s), 4.16 (1H, br. s), 4.45 (1H, m), 6.05 (1H, NH), 6.45 (1H, NH).

4. Conclusion

Lipase B from *C. antarctica* is a suitable catalyst for asymmetrization of prochiral diesters, both in hydrolysis and ammonolysis. Lowering of substrate concentration in the hydrolysis of **1** gave higher ee of the product. The catalyst can be re-used more than 10 times without loss of activity. Cross-linked enzyme crystals (ChiroCLEC-CALB) increased the rate of the hydrolysis, but with apparent reduction of ee.

Acknowledgements

We thank Novozymes, Denmark and Altus Biologics Inc., USA for gift of enzymes.

References

[1] S.G. Cohen, E. Khedouri, J. Am. Chem. Soc. 83 (1961) 4228.
[2] J. Monteiro, J. Braun, F. Le Goffic, Synth. Comm. 20 (1990) 315.
[3] D.W. Brooks, J.T. Palmer, Tetrahedron Lett. 24 (1983) 3059.

[4] M.A. Morrison, M.J. Miller, J. Org. Chem. 48 (1983) 4421.
[5] A.S. Gopalan, C.J. Sih, Tetrahedron Lett. 25 (1984) 5235.
[6] S. Puertas, F. Rebolledo, V. Gotor, J. Org. Chem. 61 (1996) 6024.
[7] F.-C. Huang, L.F.H. Lee, R.S.D. Mittal, P.R. Ravikumar, C.J. Sih, J.A. Chan, J. Am. Chem. Soc. 97 (1975) 4144.
[8] B.H. Hoff, T. Anthonsen, Chirality 11 (1999) 760.
[9] T. Rosen, M. Watanabe, C.H. Heathcock, J. Org. Chem. 49 (1984) 3657.
[10] R. Ozegowski, A. Kunath, H. Schick, Liebigs Ann. Chem. (1993) 805.
[11] E. Santaniello, M. Chira, P. Ferraboschi, S. Trave, J. Org. Chem. 53 (1988) 1567.
[12] E.E. Jacobsen, B.H. Hoff, T. Anthonsen, Chirality 12 (2000) 654.