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Candida antarctica Lipase B Catalysed Kinetic Resolutions: Substrate Structure Requirements for the Preparation of Enantiomerically Enriched Secondary Alcanols

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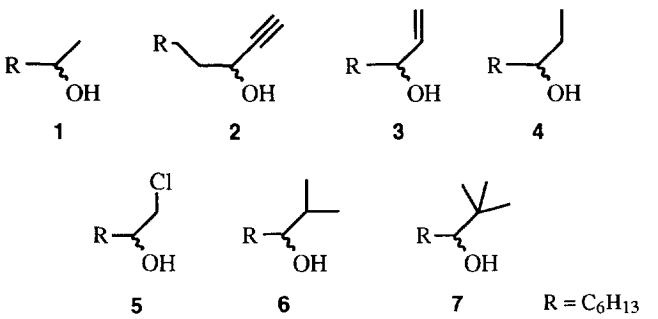
Abstract: The lipase B of the *Candida antarctica* yeast displays high enantioselectivity in transesterification reactions with chiral secondary alcohols in non-aqueous media. This was exploited to resolve a series of racemates structurally related to 2-octanol, namely 3-hydroxy-1-undecyne, 3-hydroxy-1-nonene, 3-nonanol, 1-chloro-2-octanol, 2-methyl-3-nonanol, 2,2-dimethyl-3-nonanol. The substrates were designed to probe the alcohol binding part of the active site of the lipase. The first four racemates could be resolved to produce compounds of high enantiomeric purity. A lipase catalysed transesterification of 1-chloro-2-octanol was observed. 2-Methyl-3-nonanol and 2,2-dimethyl-3-nonanol did not form any detectable amounts of product ester. The kinetic resolutions of the alcohols were performed with S-ethyl thiooctanoate as the acyl donor.

INTRODUCTION

Several studies on *Candida antarctica* lipase B (CAB) catalysed reactions (hydrolyses, esterifications, and transesterifications) show impressive results in stereochemical applications.[1-5] An explanation on a molecular level of this high selectivity of CAB in the discrimination of enantiomers will become available since the crystal structure of the enzyme has been solved.[6] In order to probe some of the substrate structure requirements of CAB, we have extended previous work on transesterification reactions with secondary aliphatic alcohols as nucleophilic substrates in kinetic resolutions for the preparation of enantiomerically enriched chiral synthons. Included here are 2-octanol[7] (**1**), 3-hydroxy-1-undecyne (**2**), 3-hydroxy-1-nonene (**3**), 3-nonanol (**4**), 1-chloro-2-octanol (**5**), 2-methyl-3-nonanol (**6**), and 2,2-dimethyl-3-nonanol (**7**) (Scheme 1). These substrate candidates represent a series of structurally related alcohols with some variation in the "medium size group" according to the model of Kazlauskas *et al.* for lipase substrates.[8] Racemates of all alcohols except **2** (a gift from Prof. Kurt Faber) were synthesized (**3**-**7**) or commercially available (**1**). Substrate candidates **3**, **4**, and **6** were easily prepared through corresponding Grignard reagents reacting with 1-heptanal under dry conditions. 1-Chloro-2-octanol (**5**) was prepared according to Damin *et al.* [9] *t* -Butyllithium reacting with 1-heptanal at -78 °C afforded (**7**). Kinetic resolutions were performed with S-ethyl thiooctanoate acylating the enzyme as previously described.[7]



Scheme 1. Secondary alcohols employed in CAB catalysed kinetic resolution, with different structure of the medium sized substituent.[8]

RESULTS

In our investigations of possible applications with CAB, alcohols with a *t*-Bu group as the medium sized group have been found to be non-reactive.[3] These findings were confirmed with **7** (Table 1). In addition, the alcohol **6**, with its *i*-Pr group, was found not to react with the acyl enzyme at a reasonable rate. The transesterification of 2-octanol (**1**) is known to be highly selective.[7] In order to establish a limiting substituent size, reactions with **2-5** were studied. Remaining alcohol and produced ester of **5** showed optical activity of opposite sign indicating a CAB catalysed reaction. However, in our hands, chiral separation on chromatographic columns (GLC and HPLC) has not yet been achieved with this compound. 3-Nonanol (**4**) was resolved with a selectivity higher than or equal to that of 2-octanol (**1**). In the remaining alcohol fraction of **4** only one enantiomer could be detected (denoted >99.5% ee, 99.5% used for conversion and apparent E calculation). Also **2** and **3** could be kinetically resolved to yield compounds of high enantiomeric purity with the help of CAB.

Table I. Enantiomeric Purities of Resolved *sec*-Alcohol Racemates

Substrate	Conversion[a]	r.t.[b]	Products						E[c]
racemate of			Remaining alcohol			Reacted alcohol			
			ee	rot.	abs. conf.	ee	rot.	abs. conf.	
	%	hrs	%			%			
1	50 (52)	1	98.2	(+)	*S*	97.3	(-)	*R*	>300
2	50 (57)	3.7	95.8	(+)	*R*	95.2	(-)	*S*	>150
3	49 (46)	4	93.2	(-)	*R*	95.9	(+)	*S*	>150
4	51 (53)	4	>99.5	(+)	*S*	96.5	(-)	*R*	>300
5	-[d] (45)	24	[d]	(+)	*R*	[d]	(-)	*S*	
6	no reaction	170							
7	no reaction	170							

[a] Value calculated from ee of remaining and reacted alcohol[10]; within brackets conversion based on the disapperance of the substrate alcohol. [b] Reaction time in hours. [c] Enantiomeric ratio calculated according to Rakels *et al.* [10] [d] Not determined.

DISCUSSION

In this investigation, some substrate structure requirements for stereoselective catalysis by CAB have been established. The methyl group adjacent to the hydroxyl group of long chain secondary alcohols substituted in the 2-position, can be extended one carbon atom without loss of stereoselectivity in the kinetic resolution. The reaction rate is however significantly lower for 3-nonanol, **4**, with the ethyl substituent, than for 2-octanol, **1**. Evidently, the enzyme provides space large enough to accomodate a methyl or an ethyl group as the medium sized substituent of the substrate. This is supported by our observation that the stereoselectivity of CAB for 2-butanol is low.[11]

A rigid medium sized substituent, such as in 3-hydroxy-1-undecyne, **2**, and 3-hydroxy-1-nonen, **3**, affords an enantiomeric ratio lower than that observed for substrates with a methyl or ethyl group in the same position (Table 1).

The separation of 1-chloro-2-octanol enantiomers is desirable since such substrates would be of interest to differentiate between steric and electronic properties of substituent atoms and those compounds are also useful synthons for chiral 1,2-epoxyalkanes.

EXPERIMENTAL

General Procedure
In an open reaction vessel, alcohols (4.0 mmol) were added to a mixture of S-ethyl thiooctanoate (4.0 mmol) and enzyme preparation (50 mg). The reaction proceeded at 39 °C and was quenched through removal of the enzyme by filtration. Work-up by liquid column chromatography[12] with silica gel afforded separation of reactants and products.[7]

Enzyme
The lipase (component B) Novozym435™ derived from *Candida antarctica* is a product of Novo Nordisk A/S, Denmark. The enzyme used was an immobilized preparation on a macroporous polypropylic resin, containing 1% (w/w) enzyme, with a catalytic activity of approximately 25 000 LU/g preparation.

Gas Chromatography
Instrumentation: Varian 3500 and 3300 and Carlo Erba Fractovap. Columns: DB1 (15 m, widebore 0.32 mm i.d., 0.25 μm film) for conversion determination (hexadecane as internal standard); Chrompack Cp-cyclodextrin-B-2,3,6-M-19 (50 m, 0.25 mm i.d., 0.25 μm film) and Astec Chiraldex™ G-TA (10 m, 0.25 mm i.d., 0.25 μm film) for enantiomeric excess determinations.

Absolute Configuration.
Assignment of absolute configuration was done by optical rotation measurements (Perkin Elmer 241 Polarimeter) and literature data comparisons.

Hydrolysis of Ester Products, General Procedure
Ester was added to a solution of 0.5 g KOH in methanol (20 ml, HPLC grade) and stirred overnight. Formic acid (1 ml) neutralized the reaction mixture and the solvent was evaporated under reduced pressure. Ethyl acetate (100 ml) was used in the filtration and washing of solid residue. The product-containing solvent was evaporated in the presence of silica gel and liquid chromatography (hexane/ethyl acetate/ethanol gradient) isolated the alcohol. Alternatively (**2**), the hydrolysis mixture was evaporated and the alcohol retrieved by extraction with dichloromethane (100 ml, dist.). The organic layer was washed with water (3x50 ml).

S-Ethyl Thiooctanoate
Ethanethiol (49.7 g, 0.80 mol) and pyridine (77.6 ml, 0.96 mol) were dissolved in dry diethyl ether (295 ml) at 0 °C. A solution of octanoic acid chloride (65.1 g, 0.40 mol) in diethyl ether (95 ml) was added dropwise. Following the addition, the reaction mixture was stirred at room temperature for 24 hours. After filtration, the reaction mixture was washed with water twice and subsequently dried over $MgSO_4$. Distillation (b.p 65 °C at 2 mm Hg) yielded the crude product (75 g). Pure product (74.4 g, GC purity of 99.5%) was obtained after flash chromatography on silica gel 60 (Merck; hexane/ethyl acetate, 90:10, v/v). ^{1}H-NMR ($CDCl_3$), Bruker 250 MHz: 0.88 (t, 3H), 1.21-1.4 (m, 8H), 1.25 (t, 3H), 1.59-1.68 (m, 2H), 2.49-2.55 (t, 2H), 2.72-2.91 (m, 2H).

3-Hydroxy-1-nonene (3), 3-Nonanol (4), and 2-Methyl-3-nonanol (6)
All reagents are commercially available and were bought from ALDRICH. **3**, **4** and **6** were prepared through standard procedure Grignard reactions with vinylmagnesium bromide solution (1 M in anhydrous THF, 52 mmol, 52 ml), 1-bromo ethane (6 mmol, 0.5 ml), and 2-bromo propane (60 mmol, 8.5 ml), respectively. The Grignard reagent was reacted with 1-heptanal (1 eq.) at 0 or -5 °C and pure products were obtained after addition of saturated ammonium chloride solution, diethyl ether extraction (3x50 ml), and liquid column chromatography (hexane/ethyl acetate).

1-Chloro-2-octanol (5)[9]
In a three necked flask equipped with a condenser containing chloramine T (50 mmol, 11.38 g) in acetone/water, 1-octene (50 mmol, 5.6 g) was added dropwise during 40 minutes. The mixture was then heated under reflux until chloramine T was completely consumed (24 hours). The aqueous layer was extracted (diethyl ether, 3x100 ml) and the organic fraction was subsequently dried over $MgSO_4$. Crude product mixture, 1-chloro-2-octanol and 2-chloro-1-octanol (4:1) was subjected to liquid column chromatography as above and pure product was isolated in low yield (16%).

2,2-Dimethyl-3-nonanol (7)
To a round bottom flask containing 1.7 M t-butyllithium solution in pentane (30 mmol, 18 ml) at -78 °C 1-heptanal (30 mmol, 4.2 ml) in 30 ml anhydrous THF was added dropwise. The reaction mixture slowly equilibrated to room temperature and K_2CO_3 (aq) was added. The organic layer was dried over $MgSO_4$ and subsequent liquid column chromatography afforded pure product (53% yield).

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REFERENCES

1. Waagen, V.; Hollingsæter, I.; Partali, V.; Thorstad, O.; Anthonsen, T. *Tetrahedron: Asymmetry* **1993**, *4*, 961
2. Öhrner, N.; Martinelle, M.; Mattson, A.; Norin, T.; Hult, K. *Biocatalysis* **1994**, *9*, 105
3. Orrenius, C.; Mattson, A.; Öhrner, N.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1994**, *7*, 1363
4. Mattson, A.; Öhrner, N.; Hult, K.; Norin, T. *Tetrahedron: Asymmetry* **1993**, *4*, 925
5. Öhrner, N.; Martinelle, M.; Mattson, A.; Norin, T.; Hult, K. *Biotechnol. Lett.* **1993**, *4*, 263
6. Uppenberg, J.; Trier Hansen, M.; Patkar, S.; Jones, T.A. *Structure* **1994**, *2*, 293
7. Frykman, H.; Öhrner, N.; Norin, T.; Hult, K. *Tetrahedron Lett.* **1993**, *8*, 1367
8. Kazlauskas, R.J.; Weissfloch, A.N.; Rappaport, A.T.; Cuccia, L.A. *J. Org. Chem* **1991**, *56*, 2656
9. Damin, B.; Garapon, J.; Sillion, B. *Synthesis* **1981**, *5*, 362
10. Rakels, J.L.L.; Straathof, A.J.J.; Heijnen, J.J. *Enzyme Microb. Technol.* **1993**, *15*, 1051
11. Öhrner, N. *Unpublished results*
12. Bäckström, P.; Stridh, K.; Li, L.; Norin, T. *Acta Chem. Scand.* **1987**, *41*, 1705

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