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Lipase–Catalyzed Enantioselective Esterification of 2–Methylalkanoic Acids

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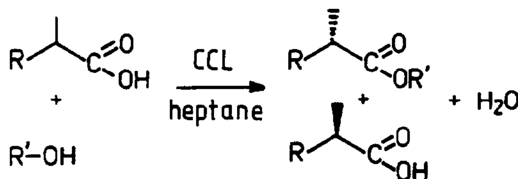
Abstract: A preference for (S)-enantiomers has been observed in the course of the esterification of racemic 2-methylalkanoic acids catalyzed by lipase from *Candida cylindracea* in heptane.

Optically pure 2-alkylalkanoic acids are useful building blocks for syntheses of biologically active compounds with branched-chain structures, e.g. pheromones[1]. Short chain 2-methylalkanoic acids and their esters occur naturally in various foods[2] and contribute significantly to their aroma; an almost exclusive presence of the (S)-enantiomers has been demonstrated[3]. The need for 2-methylalkanoic acids of high enantiomeric purity is reflected by the various chemical approaches to their asymmetric synthesis[4]. Kinetic resolution of the racemic acids and esters, respectively, by means of enantioselective lipase-catalyzed reactions, an area of increasing importance[5], could be a useful alternative to chemical procedures. For structurally related 2-substituted acids, such as 2-hydroxyalkanoic acids[6], 2-chloro- and 2-bromoalkanoic acids[7], 2-arylpropionic acids[8], 2-phenoxypropionic acids[9] and 3-aroylthio-2-methylpropionic acids[10] this strategy has been successfully applied. 2-Methylalkanoic acids, on the other hand, have been reported as inhibitors of some lipases[7,11]. For the esterifications described[12,13,14] a possible stereodifferentiation has only been indicated by an increased reaction rate of one enantiomer compared to the racemate[14]. However, detailed investigations of a potential kinetic resolution of 2-methylalkanoic acid enantiomers by means of lipase-catalyzed esterification have been lacking.

The present study demonstrated that lipase from *Candida cylindracea* (CCL) preferentially catalyzes the esterification of (S)-configurated 2-methylalkanoic acids according to Scheme 1.



Scheme 1

In a typical experiment 500 mg of the commercially available enzyme preparation (Sigma L 1754, 750 olive oil units per mg of solid), 0.5 mMol 2-methylalkanoic acid and alcohol, respectively, and 10μl hexadecane as internal standard were added to 5 ml heptane. The mixture was shaken at room temperature; the conversion rate was monitored by means of gas chromatographic analysis of aliquote parts. Products and remaining substrate were separated by means of liquid-solid chromatography on silica gel. Their optical purities were determined via capillary GC separations of diastereomeric (R)-1-phenylethylamides and (S)-2-octylesters, respectively. The data obtained are summarized in Table 1.

Table 1: CCL-catalyzed esterification of racemic 2-methylalkanoic acids via Scheme 1

substrates R	R'-OH	t (h)	conversion[a] (%)	(R)-acid[b,c] e.e. (%)[d]	(S)-ester[b] e.e. (%)[e,f]	E[g]
1a C_2H_5	methanol	4	48	34.2	37.3	3
1b C_2H_5	ethanol	5	50	34.4	34.4	3
1c C_2H_5	cyclohexanol	8	54	45.4	38.5	3
1d C_2H_5	octanol	7	53	48.9	43.4	4
1e C_2H_5	octadecanol	3	51	51.9	50.6	5
2a C_3H_7	3-methyl-2-buten-1-ol	9	38	52.0	84.5	20
2b C_3H_7	octanol	10	46	79.5	93.3	70
2c C_3H_7	octadecanol	12	45	72.3	88.3	35
3a C_4H_9	ethanol	24	32	6.3	13.2	1.4
3b C_4H_9	butanol	18	55	70.1	58.4	8
3c C_4H_9	cyclohexanol	20	49	67.9	71.3	12
3d C_4H_9	cyclohexylmethanol	12	44	60.6	77.1	14
3e C_4H_9	octanol	22	49	76.2	79.6	20
3f C_4H_9	(R)-(-)-octan-2-ol	50	23	27.1	89.3	23
3g C_4H_9	(S)-(+)-octan-2-ol	50	10	10.4	93.8	35
3h C_4H_9	decanol	19	48	75.2	83.2	25
3i C_4H_9	octadecanol	18	50	84.0	84.0	30

[a]GC: DB Wax; [b]LSC: silica gel, ester: pentane/CH_2Cl_2 (1:2), acid: ether; [c]assignment of configurations: (1) 2-methylbutanoic acid: GC of reference compound (S)-2-methylbutanoic acid (98%, Aldrich); (2) 2-methylpentanoic acid: entry 2b (2.5 mMol substrates, 53% conversion), e.e.$_{(GC)}$=98%, $[\alpha]_D^{20}$= -20.1 (c=5.5, Et_2O), ref.[15] $[\alpha]_D^{20}$= -18.4 (neat); (3) 2-methylhexanoic acid: entry 3i (2.5 mMol substrates, 56% conversion), e.e.$_{(GC)}$=97%, $[\alpha]_D^{20}$= -20.2 (c=5.3, Et_2O), ref[4d] $[\alpha]_D^{22}$= -21.9 (c=5.5, Et_2O); [d]2-methylbutanoic acid: + (R)-(+)-1-phenylethylisocyanate/toluene, 100°C, 12 h; GC: DB 210; 2-methylpentanoic and 2-methylhexanoic acid: + (S)-(+)-octan-2-ol/acetylchloride (5:1), 80°C, 15 h; GC: DB 210; [e]alkaline hydrolysis prior to derivatization; [f]d.e.(%) for entries 3f and g; [g]cf. ref.[16]

The present results confirm the formerly described capability of crude[12] and polyethylene glycol-modified[13] lipase from *Candida cylindracea* to catalyze esterifications of 2-methylalkanoic acids. They demonstrate that in contrary to previous conclusions[7] the acceptance of 2-substituted acids as substrates by this enzyme does not necessarily require an electron-withdrawing group in C_2-position.

Two aspects are noteworthy from a mechanistical standpoint of view: (1) the stereochemical course of the esterification of 2-methylalkanoic acids is opposite to enantiodiscriminations observed for analogous acids with a halogen substituent[7]; (2) the enantioselectivity (E) of the reaction, expressed as ratio of the specificity constants for the two enantiomers[16], is markedly influenced by the structures of the substrates. The effect of the alcohol chain length on the discrimination of 2-methylhexanoic acid enantiomers (3a-3i) is similar to results obtained for the CCL-catalyzed esterification of 2-(4-chlorophenoxy)propanoic acid[9b]. On the other hand, the poor enantioselectivity determined for reactions of 2-methylbutanoic acid (1a-1e) cannot be significantly improved by alterations of the alcohol structure.

A methyl substituent in C_2-position of the acid substrates causes a sharp decrease of the esterification rate compared to the unbranched acid (Table 2). A similar effect has been observed for reactions catalyzed by immobilized lipase from *Mucor miehei*[14]. Introduction of additional branching in the alcohol moiety (3f,g) leads to increased enantioselection. The attractive potential for kinetic resolution of two racemic substrates in one enzyme-catalyzed step deserves further investigations, although the additionally reduced reaction rates (Table 2) seem unfeasible for preparative applications.

Table 2: Structural influences on the reaction rate of CCL-catalyzed esterifications

substrates acid	alcohol	relative reaction rate (%)
hexanoic acid	octanol	100[a]
2-methylbutanoic acid	octanol	50
2-methylpentanoic acid	octanol	30
2-methylhexanoic acid	octanol	14
2-methylhexanoic acid	(R)-(-)-octan-2-ol	3
2-methylhexanoic acid	(S)-(+)-octan-2-ol	1

[a] $v = 0.15\ \mu Mol \cdot h^{-1} \cdot mg^{-1}$ CCL

The use of octanol (2b) and octadecanol (3i) as acyl acceptors leads to sufficient enantiodiscrimination of 2-methylpentanoic and 2-methylhexanoic acid, respectively. Both enantiomers can be obtained in highly pure form, especially if optically enriched mate-

rial is subjected to a repetitive esterification[16]. Possible limitations of the procedure caused by decreasing optical purities at higher conversion rates due to reversible reactions[17] are currently under detailed investigation.

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