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BIOCATALYTIC RESOLUTION OF (±)-HYDROXYALKANOIC ESTERS. A STRATEGY FOR ENHANCING THE ENANTIOMERIC SPECIFICITY OF LIPASE-CATALYZED ESTER HYDROLYSIS.

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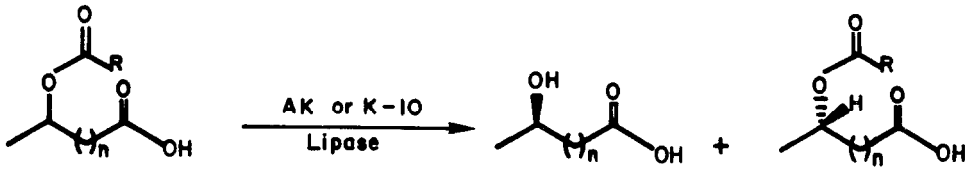
Summary. A general biocatalytic resolution procedure for the preparation of a variety of R- and S-hydroxyalkanoic esters of high optical purity has been developed. The noteworthy feature of this methodology resides in the selection of a non-hydrolyzable ester at the carboxyl terminus to improve the enantiospecificity in the lipase-catalyzed hydrolysis of the acyloxy ester.

The carbon framework of many bioactive natural products are made up of chiral hydroxyalkanoic acid and derivatives thereof.[1] Further, enantiomerically-pure β-hydroxybutanoates have been used as building blocks for the synthesis of a wide variety of compounds.[2] Therefore, the ready accessibility of optically-active hydroxyalkanoic esters could greatly facilitate the asymmetric synthesis of several classes of natural products[3] and the biologically-active enantiomers of many therapeutic agents[4].

Although Noyori[5] has recently made notable advances in the asymmetric reduction of β-keto esters, there is a lack of a general simple method for the preparation of optically-pure R and S hydroxyalkanoic esters. Yeast-mediated reduction of keto esters has gained widespread popularity as a method of obtaining optically-active hydroxy esters, but the chemical and optical yields of the resulting products are frequently variable.[6] Moreover, the stereochemical outcome (R or S) often cannot be controlled because the corresponding antipodal enzyme is not available.

Here, we disclose a general biocatalytic resolution procedure for the preparation of a variety of R and S hydroxyalkanoic esters of high optical purity. The noteworthy feature of our methodology resides in the introduction of a non-hydrolyzable carboxylic ester group to enhance the enantiomeric specificity of the enzymatic hydrolysis of the acyloxy ester.

Our continued interest in the application of commercial microbial lipases for the separation of enantiomers led us to evaluate a number of these enzymes for their capabilities in catalyzing the enantiospecific hydrolysis of (±)acetoxyalkanoic acids (**1**, **2a**, **3**) of varying chain lengths.



1 n = 1; R = CH_3 **2c** n = 5; R = n-C_5H_{11}

2a n = 5; R = CH_3 **3** n = 10; R = CH_3

2b n = 5; R = n-C_3H_7

Contrary to our expectations, all of these lipases exhibited rather low enantiomeric specificities[7] towards these substrates. Results for the *Pseudomonas sp.* (AK and K-10) lipases[8] are shown in Table 1, which are representative for the other commercial lipases examined. They have R-stereochemical preference[7] with enantiospecificities (expressed as the E[9] value) ranging from 1-7 (Table 1). As the E value can often be enhanced by modifying the acyloxy ester group on the chiral center, and the microbial lipases have a general proclivity towards longer chain esters, the n-butyl (**2b**) and n-hexyl (**2c**) esters were synthesized. Although no improvement was noted with the AK lipase, a moderate increase in enantiomeric specificity was observed with the K-10 lipase. In the latter, the E value for the n-hexyl and n-butyl esters was improved from 3 to 9 and 13, respectively (Table 1).

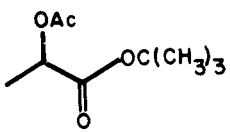
Table 1. Lipase-catalyzed hydrolysis of (±)-acyloxyalkanoic acids.

				ee		
Substrate	Lipase (mg)	Time (h)	c	R-Alcohol	S-Ester	E
1	AK (100)	14	0.41	0.10	0.07	1
1	K-10 (180)	24	0.17	0.50	0.10	3
2a	AK (50)	100	0.42	0.57	0.42	6
2a	K-10 (440)	16	0.26	0.45	0.16	3
2b	AK (100)	12	0.32	0.65	0.31	7
2b	K-10 (100)	13	0.17	0.86	0.17	13
2c	AK (100)	3	0.50	0.53	0.54	6
2c	K-10 (100)	4	0.50	0.64	0.65	9
3	AK (150)	60	0.88	0.10	0.73	2
3	K-10 (270)	16	0.16	0.64	0.12	3

The reaction mixture contained 100 mg of the substrate and varying amounts of lipases suspended in 5 ml of 0.2 M phosphate buffer, pH 6.9 at 25°C.

A logical extension of this chemical modification approach led us to inquire as to whether the introduction of a suitable carboxy ester might be used as a means of improving the enantiospecificity in the lipase-catalyzed hydrolysis of the acyloxy group. However, when (±)-methyl-7-acetoxyoctanoate or (±)-benzyl-7-acetoxyoctanoate was exposed to the lipases (AK or K-10), they were very readily cleaved to 7-acetoxyoctanoic acid with low enantiomeric specificity (E = 2).

Since t-butyl esters are generally not attacked by lipases, (±)-t-butyl-7-acetoxyoctanoate (5b) was prepared with a view to blocking the esteratic action at the carboxyl terminus. In accord with our expectations, both lipases retained the R-stereochemical preference, but more importantly the enantiospecificity with respect to the cleavage of the acetoxy ester was markedly elevated (E = >100). The distance relationship between the t-butyloxycarbonyl function and the chiral catalytic center, does not appear to be critical to the lipases, for high enantiomeric specificities were observed for all the substrates (4, 5a-5c) examined (Table 2). Hence, this concept may serve as a general useful means of optimizing the lipase-catalyzed enantiospecific hydrolysis of acetoxyalkanoic esters.



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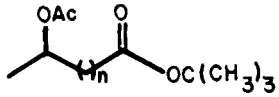
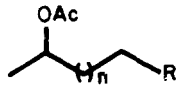


5a n = 1
5b n = 5
5c n = 10



6 R = H
7 R = $OC(CH_3)_3$

On the other hand, the acetoxy ester in substrates **6** and **7** were cleaved with only modest degrees of enantiospecificities (E = 6-11), which suggests that a t-butoxycarbonyl or a non-hydrolyzable ester may be required for attaining maximal enantiospecificity.

Table 2. Lipase-catalyzed hydrolysis of (±)-acetoxyalkanoic t-butyl ester and other substrates.

				ee		
Substrate	Lipase	Time (h)	c	R-Alcohol	S-Ester	E
4	K-10	30	0.63	>0.99	0.96	>100
5a	AK	15	0.41	>0.99	0.68	>100
5a	K-10	16	0.38	>0.99	0.63	>100
5b	AK	3	0.31	>0.99	0.44	>100
5b	K-10	5	0.28	>0.99	0.33	>100
5c	AK	6	0.21	>0.99	0.27	>100
5c	K-10	6	0.28	>0.99	0.39	>100
6	AK	2	0.25	0.71	0.24	8
6	K-10	3	0.23	0.80	0.23	10
7	AK	10	0.35	0.60	0.33	6
7	K-10	10	0.30	0.76	0.33	11

The reaction mixture contained 200 mg of various substrates and 200 mg lipases suspended in 10 ml of 0.2 M phosphate buffer, pH 6.9 at 25°C.

This study provides another strategy that extends the usefulness of microbial lipases in organic synthesis by simple substrate optimization to improve enantiomeric specificity. We are currently investigating the influence of the recalcitrant carboxy esters on the biocatalytic kinetic parameters (K_m and V_{max}) and the

further application of this methodology to the kinetic resolution of even more highly functionalized substrates.

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7. Enantiomeric excess (ee) was determined by PMR measurements of the methyl esters and/or the acetoxy esters using $Eu(hfc)_3$. The absolute configuration of the hydroxyalkanoic acids was made on the basis of the sign of optical rotation. See: Kinoshita, M.; Ishii, K.; Umezawa, S. Bull. Chem. Soc. (Japan) (1971) 44, 3395.
8. The lipases AK and K-10 (*Pseudomonas sp.*) were products of Amano.
9. The enantiomeric ratio (E value) is calculated from:

$$E = \frac{\ln[(1-c)(1-ee_s)]}{\ln[(1-c)(1+ee_s)]} \qquad \text{where } c = ee_s/(ee_s + ee_p)$$

See: Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. J. Am. Chem. Soc. (1982) 104, 7294.

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