An Insight into the Enantioselective Hydrolyses of Cyclic Acetates Catalysed by *Pseudomonas fluorescens* Lipase

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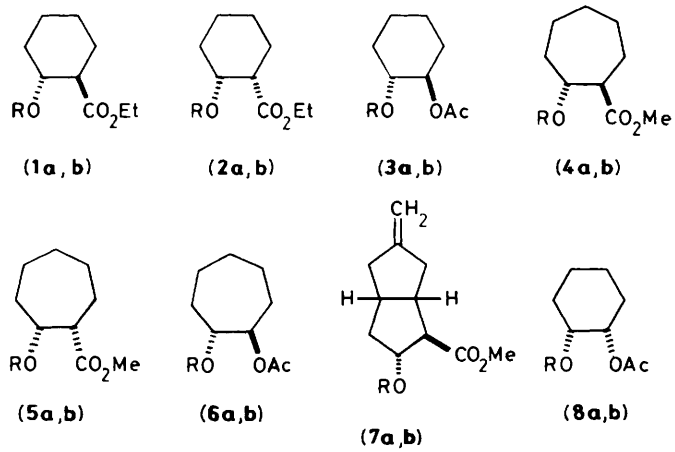
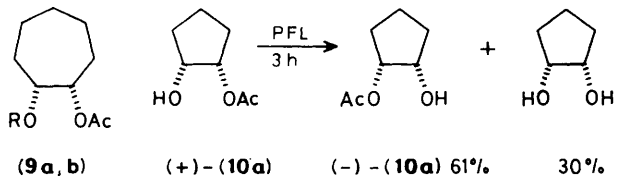
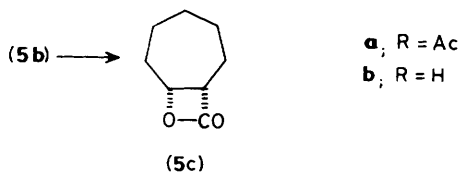
Hydrolysis of racemic acetates with *Pseudomonas fluorescens* lipase (PFL) afforded optically active alcohols with the *R*-configuration, independent of ring size; a three-site model for PFL-catalysed hydrolysis is proposed.

Elucidating the scope of substrate variation[1] in enzyme-catalysed processes is important in the application of enzymatic methods to organic synthesis. If it is possible to design candidate substrates so that enantioselectivity may be predicted, tedious screening procedures may be avoided.[2] We have already reported[3] that the hydrolysis of five-membered ring acetates using *Pseudomonas fluorescens* lipase (PFL) afforded optically active alcohols with high optical purities. To examine the influence of ring size[4] on hydrolysis with PFL, we have studied the hydrolysis of six- and seven-membered ring acetates. The six-membered ring (±)-*trans*- and *cis*-monoacetate (**1a**) and (**2a**) as well as the *trans*-diacetate (**3a**) were hydrolysed with PFL to afford (−)-(**1b**), (−)-(**2b**), and (−)-(**3b**) in enantiomerically pure form, respectively.[5] Similarly, the hydrolysis of the seven-membered ring acetates (±)-(**4a**), (±)-(**5a**), and (±)-(**6a**) also afforded exclusively (−)-(**4b**), (−)-(**5b**), and (−)-(**6b**), respectively (>99% enantiomeric excess, e.e.).[6] This finding attests to the generality of







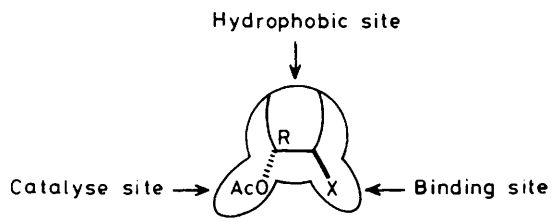


Figure 1

PFL-catalysed hydrolysis,[7] the acetate with the *R*-configuration being hydrolysed preferentially with high enantioselectivity, independent of ring size. Previously, we reported[3] that the hydrolysis of bicyclo[3.3.0]octan-3-yl acetate with a carbonyl function (possibly protected as the ethylene acetal) in one ring and the acetate group in the other ring resulted in low enantioselectivity. In contrast, the highly enantioselective hydrolysis of bicyclo[4.3.0]non-3-en-8-yl acetate[3] suggested that the presence of a hydrophobic function in one ring and the acetate group in the other plays an important role in hydrolysis. With this assumption in mind, the modified acetate (**7a**)[8] was hydrolysed with PFL. As expected, this hydrolysis showed a marked increase in both enantiomeric excess (>99% e.e) and hydrolysis rate (4 h).

In contrast to the case of the five-membered ring *meso*-diacetate,[3] which was hydrolysed to the monoacetate with the *S*-configuration (>99% e.e.), the six- and seven-membered ring diacetates (**8a**) and (**9a**) were hydrolysed to (+)-(**8b**) (70% e.e.) and (−)-(**9b**) (2% e.e.)[9] with the *R*-configuration, respectively. This unusual reverse in absolute configuration

Table 1. Enantioselective hydrolysis of (**1a**)—(**9a**) catalysed by PFL.

Substrate	Reaction time/h	Product		Recovered acetate	
		% Yield[a]	% E.e.	% Yield[a]	% E.e.
(**1a**)	24	41	>99	59	55
(**2a**)	35	32	>99	63	70
(**3a**)	6	33	>99	51	48
(**4a**)	24	45	>99	55	55
(**5a**)	30	38	>99	58	68
(**6a**)	4	36	>99	64	45
(**7a**)	4.5	13	>99	80	30
(**8a**)	2	35	70	54	—
(**9a**)	5	23	2	67	—

[a] Isolated.

may be attributable to acetyl-migration catalysed by PFL, because (+)-(**10a**) (68% e.e.) with the *R*-configuration was converted to the corresponding enantiomer (−)-(**10a**) (16% e.e.) with the *S*-configuration by PFL.

These results allow us to suggest tentatively a three-site model (Figure 1) for PFL-catalysed hydrolysis. The substrate requirements are as follows: (i) the acetates are hydrolysed to afford alcohols with the *R*-configuration (catalyse site); (ii) alkoxycarbonyl or carboxylate groups adjacent to the acetoxy function are required (binding site); (iii) in bicyclic systems, the ring not containing acetate should be hydrophobic (hydrophobic site).

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3 Z.-F. Xie, H. Suemune, and K. Sakai, *J. Chem. Soc., Chem. Commun.*, 1987, 838.
4 P. Mohr, N. W. Sarcevic, C. Tamm, K. Gawaronska, and J. C. Gawaronska, *Helv. Chim. Acta*, 1983, **66**, 2501.
5 For compounds (**1**) and (**2**), see D. Buisson and R. Azerad, *Tetrahedron Lett.*, 1986, **27**, 2631. For compound (**3**), see Th. Posternak, D. Reymond, and H. Friedli, *Helv. Chim. Acta*, 1955, **38**, 205. Optical purities were determined from the 270 MHz ^{1}H n.m.r. spectra of the (+)-α-methoxy-α-trifluoromethylphenylacetic acid esters (see ref. 6, Mosher's method).
6 Reduction of methyl 2-oxocycloheptanecarboxylate with $NaBH_4$ afforded two isomers. The less polar fraction was determined to consist of the *cis*-isomer (**5b**), because it could be easily converted to the β-lactone (**5c**) by Frater's method (see G. Frater, *Helv. Chim. Acta*, 1979, **62**, 2825). In addition, a difference nuclear Overhauser enhancement was also observed between the two angular protons in (**5c**). The absolute configuration of (−)-(**4b**), (−)-(**5b**), and (−)-(**6b**) was determined by Mosher's method (see J. A. Dale, D. L. Dull, and H. S. Mosher, *J. Org. Chem.*, 1969, **34**, 2534).
7 For reports related to the PFL-catalysed hydrolysis of acetates, see Z.-F. Xie, H. Suemune, I. Nakamura, and K. Sakai, *Chem. Pharm. Bull.*, 1987, **35**, 4454; H. Suemune, Y. Mizuhara, H. Akita, T. Oishi, and K. Sakai, *ibid.*, 1987, **35**, 3112; F. Franco, C. Pietro, C. Walter, B. Daniele, M. Tiziano, and F. Marco, *J. Org. Chem.*, 1987, **52**, 5079; S. Yamaguchi, M. Asada, H. Yamamura, and K. Watanabe, Eur. Pat. Appl. Ep., 101,076, 1984.
8 (±)-(**7a**) was synthesized from 3-acetoxy-2-methoxycarbonyl-7-oxobicyclo[3.3.0]octane and triphenylphosphonium methylide (see K. Kojima, S. Amemiya, K. Koyama, and K. Sakai, *Chem. Pharm. Bull.*, 1985, **33**, 2688).
9 The absolute configuration of (+)-(**8b**) and (−)-(**9b**) was determined by Mosher's method (see ref. 6).