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ENZYMATIC PREPARATION OF (3S,6R) AND (3R,6S)-3-HYDROXY-6-ACETOXYCYCLOHEX-1-ENE

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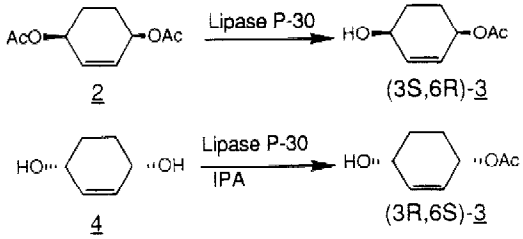
Summary: *Pseudomonas cepacia* lipase-catalyzed enantioselective hydrolysis of **2** in water afforded (3S,6R)-**3**. The antipode (3R,6S)-**3** was prepared by enantioselective acylation of **4** using the same enzyme.

The development of methods for the preparation of 4(S)-acetoxycyclohex-2-en-1-one (**1**) has been the topic of several recent publications.[1] Derivatives of **1** are useful intermediates in the synthesis of the HMG-CoA reductase inhibitors, Mevinolin and Compactin[2] as well as the immunosuppressant FK506[3].

As the use of hydrolytic enzymes to catalyze the stereoselective cleavage of meso-diesters has become a powerful tool in organic synthesis[4], we envisaged that enzymatic hydrolysis of cis-3,6-diacetoxycyclohex-1-ene (**2**) could readily provide us with either of the enantiomerically-pure forms of 3-hydroxy-6-acetoxycyclohex-1-ene (**3**) for transformation into **1**. Moreover, **3** could serve as a valuable precursor to optically-active cyclohex-1-en-3-ol, a chiral building block not readily accessible by conventional enzymic or non-enzymic means.[5] In this letter, we describe our results pertaining to the preparation of both enantiomers of **3** via lipase-catalyzed enantioselective hydrolysis of **2** in water and esterification of **4** in isopropenyl acetate (IPA).



A survey of the literature reveals that the enzyme acetylcholinesterase from the electric eel (EEA) was highly stereoselective in catalyzing the hydrolysis of cis-3,5-diacetoxycyclopent-1-ene (**5**)[6] and cis-3,7-diacetoxy-cyclohept-1-ene (**6**)[7]. This enzyme preferentially cleaved the pro-S acetoxy group of **5** whereas the pro-R acetoxy group of **6** was cleaved with a high degree of enantioselectivity. However, we noted that EEA-catalyzed hydrolysis of **2**[8] proceeded with no stereoselectivity. After 60% conversion, the resulting monoacetate, **3**, was found to be racemic (Table 1). This unexpected finding compelled us to investigate the actions of other enzymes on the meso-diester **2**, for several lipases were reported to be highly enantioselective towards **5**[9]. Of the enzymes examined, the most suitable one was that of *Pseudomonas cepacia* (P-30) lipase, which afforded 64% of the monoacetate, **3**, after 4 hours with an optical purity of 79% ee[10] (Table 1). The absolute configuration

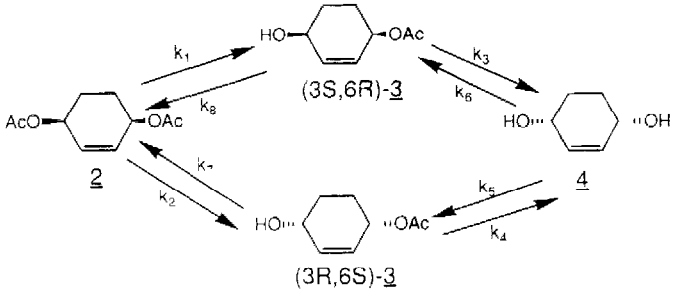
of the resulting monoacetate (+)-**3** was assigned to be (+)-(3S,6R) by its chemical conversion to the enantiomer of a known bicyclic lactone[11] via a three-step reaction sequence.[12] This indicates that this enzyme preferentially cleaved the pro-S acetoxy group of **2**.

TABLE 1. Enzymatic hydrolysis of **2**.[13]

Enzyme[14]	Time (hrs)	Yield (%)	($CHCl_3$) $[\alpha]_D^{25}$	ee[10] (%)
Aspergillus niger lipase AP	2	27	+16.0	
Chromobacterium viscosum lipase CV	2	51	+42.8	47
Pig liver esterase (PLE)	7	59	-43.5	49
Pseudomonas sp. lipase K-10	7	47	+37.3	
Alcaligenes sp. lipase PL	27	43	-23.6	
Porcine pancreatic lipase (PPL)	27	37	-19.2	
Electric eel acetylcholinestrase	2	60	-0.6	
Candida cylindracea lipase type VII S	4	27	+47.1	
Pseudomonas sp. lipase AK	4	19	+36.5	
Candida cylindracea lipase MY	4	60	+35.1	
Pseudomonas cepacia lipase P-30	4	64	+70.0	79
Subtilisin type XXIV	6	38	-34.6	

Since the monoacetate, **3**, is concomitantly hydrolyzed by the lipase to the meso-diol, **4**, the kinetics of the reaction was carefully monitored (Table 2). To optimize chemical and optical yields, the relative second order rate constants for the first asymmetric hydrolytic reaction and the subsequent kinetic resolution step were determined.[15]

Scheme 1



Hydrolysis

$k_1 = 4.9$; $k_2 = 1$; $k_3 = 1.3$; $k_4 = 8.6$

Esterification

$k_5 = 1$; $k_6 = 0.25$; $k_7 = 0.19$; $k_8 = 1.8$

Although the ratio of the relative rate constants for the first enantioselective hydrolytic step ($k_1/k_2 = 4.9/1$) is not large, highly enantiomerically-enriched (3S,6R)-**3** (36%, 92% ee) can still be obtained, for the same enzyme preferentially hydrolyzes the minor antipode (3R,6S)-**3** with moderate enantioselectivity ($k_4/k_3 = 8.6/1.3$). Hence, longer reaction times lead to the accumulation of (3S,6R)-**3** of higher enantiomeric purities but at lower chemical yields and vice versa.

TABLE 2. The kinetics of lipase P-30-catalyzed hydrolysis of **2**.[13]

Time (hr)	Yield (%)			$(CHCl_3)$ $[\alpha]_D^{25}$	ee (%)
	2	**3**	**4**		
8	27	56	18	+72.4°	82
22	2	55	43	+78.3°	88
33	2	44	54	+80.0°	90
46	1	36	63	+81.9°	92

Although both pig liver esterase (PLE) and subtilisin preferentially cleaved the pro-R-acetoxy group of **2**, the enantioselectivity was comparatively low (Table 1). Since lipase-catalyzed esterification reactions in organic solvents are often more enantioselective than the corresponding hydrolytic reactions in water[16], we turned our attention to the transesterification of the meso-diol **4** in non-aqueous systems using the *Pseudomonas cepacia* lipase P-30. Further, because the sense of preferred chirality for the forward and reverse reactions is generally retained[17], this approach provides a direct route to (3R,6S)-**3**.

While most lipase-catalyzed enantioselective transesterification reactions are conducted in non-polar organic solvents[16], the most suitable condition for this system was that of using neat isopropenyl acetate[19] as solvent and acyl donor. Under these conditions, a 51% yield of (3R,6S)-**3** (ee = 95%) was obtained (Table 3). The ratio of the relative second order rate constants for the first enantioselective acylation reaction and the subsequent kinetic resolution step was determined to be k_5/k_6 = 1/0.25 and k_8/k_7 = 1.8/0.19 respectively (Scheme 1).

TABLE 3. Lipase P-30-catalyzed enantioselective transesterification of **4**.[18]

Solvent	Time (hr)	Yield (%)			$(CHCl_3)$ $[\alpha]_D^{25}$	ee (%)
		2	**3**	**4**		
Isooctane	66	13	68	11	-16.4°	19
Benzene	47	6	53	30	-19.4°	23
THF	23	8	72	8	-52.5°	61
Dioxane (40°C)	45	--	64	--	-39.9°	46
Isopropenyl acetate	8	46	46	5	-48.5°	54
Isopropenyl acetate	14	27	55	15	-64.7°	75
Isopropenyl acetate	29	4	56	33	-85.8°	92
Isopropenyl acetate	40	--	51	44	-90.1°	95
Isopropenyl acetate	55	--	12	88	-91.2°	98

In conclusion, the synergistic coupling of the asymmetric biocatalytic reaction with the subsequent kinetic resolution step has allowed the successful preparation of both antipodes of **3** in highly enantiomerically-pure forms. This strategy of extending the usefulness of enzymes of modest enantioselectivity appears to be of general applicability in asymmetric catalysis.

Acknowledgment

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References and Notes

1. a) S. J. Danishefsky and B. Simoneau, *Pure Appl. Chem.*, **60** (1988) 1555 b) J. E. Audia, L. Boisvert, A. D Patten, A Villalobos and S. J. Danishefsky, *J. Org. Chem.*, **54** (1989) 3738. c) M. Carmen Carreno, J. L. Garcia Ruano, M. Garrido, M P Ruiz and G. Solladié, *Tetrahedron Lett.*, **31** (1990) 6653.
2 S. J. Danishefsky and B. Simoneau, *J Am. Chem. Soc.*, **111** (1989) 2599.
3. A. B. Jones, M. Yamaguchi, A. Patten, S. J Danishefsky, J A Ragan, D B. Smith and S L. Schreiber, *J. Org. Chem.*, **54** (1989) 17.
4 D. Seebach, *Angew. Chem Int. Ed. Engl.*, **29** (1990) 1320
5. a) R. K. Hill and J. W. Morgan, *J Org. Chem.*, **33** (1968) 927 b) L Otzet, J. Pascual and J. Sistare, *Anales Real Soc Espan. Fis. Quim Ser. B*, **62** (1966) 965
6. D. R. Deardorff, A. J Matthews, D. S. McMeekin and C L. Craney, *Tetrahedron Lett*, **27** (1986) 1255.
7 A J. Pearson, H. S. Bansal and Y S. Lai, *J Chem. Soc. Chem. Commun.* (1987) 519. See also C. R. Johnson and C. H. Senanayake, *J. Org. Chem.*, **54** (1989) 736.
8 J. E. Backvall, S E. Bystrom and R. E. Nordberg, *J. Org. Chem*, **49** (1984) 4619.
9 K. Laumen and M P. Schneider, *J. Chem. Soc. Chem. Commun.* (1986) 1298.
10. The enantiomeric excess (ee) of **3** was determined by ^{1}H NMR ($CDCl_3$, 300 MHz) in the presence of the chiral shift agent, Eu(hfc)$_3$ Under these conditions, the acetate singlet of (±)-**3** was split into two singlets which could be successfully integrated
11. E. J. Corey and B. B Snider, *Tetrahedron Lett*, **13** (1973) 3091.
12. S Terashima, M. Nara and S. I. Yamada, *Tetrahedron Lett.*, **17** (1978) 1487.
13. The enzymatic hydrolysis of **2** was conducted as follows: A suspension consisting of **2** (50 mg) and enzyme (20 mg) in 2 ml of 0.2M phosphate buffer, pH 7.0, was stirred vigorously at 23°C. The progress of the reaction was followed by TLC [ethyl acetate-diethyl ether (1:1)] The R_f values of **2**, **3** and **4** were: 0.8, 0.4 and 0.15 respectively. Enzymatic incubations that produced predominantly **3** were worked up and the mixture was separated by silica gel flash chromatography. Elution of the column with ethyl acetate-hexane (1:10) afforded **2**; (1.5) gave **3** and finally ethyl acetate yielded **4**
14. All the enzymes were purchased from Sigma Chemical Co except lipases P-30, AP, K-10 and AK were products of Amano. *C. cylindracea* lipase MY and lipase PL were obtained from Meito Sangyo Ltd. *Chromobacterium viscosum* lipase CV was a product of U S Biochemical Corp.
15 Y. F Wang, C. S Chen, G. Girdaukas and C. J. Sih, in Ciba Foundation Symposium, *Enzymes in Organic Synthesis*, **111** (1985) p. 128, Pitman, London.
16. C. S. Chen and C. J. Sih, *Angew. Chem. Int Ed Engl.*, **28** (1989) 695.
17 C. S. Chen, S. H. Wu, G. Girdaukas and C. J. Sih, *J Am. Chem. Soc*, **109** (1987) 2812.
18 The reaction mixture contained: **4** (1 mmmol), isopropenyl acetate (3 mmol) and 100 mg of P-30 lipase, suspended in 4 ml of organic solvent (dried over molecular sieve, 3 Å). The mixture was incubated on a rotary shaker (200 rpm) at 32°C.
19. M. Degueil-Custaing, B DeJeso, S Drouillard and B. Maillard, *Tetrahedron Lett*, **28** (1987) 953; K Laumen, D Breitgoff and M. Schneider, *J. Chem. Soc., Chem Comm*, (1988) 1459, Y.F. Wang, J.J Lalonde, M. Momongan, D.E. Bergbreiter and C.H Wong, *J. Am Chem Soc*, **110** (1988) 7200; Y. Terao, M. Murata, K. Achiwa, T Nishio, M. Akamtsu and M. Kamimura, *Tetrahedron Lett.*, **29** (1988) 5173, K.B Babiak, J.S. Ng, J.H. Dygos, C.L. Weyker, Y.F. Wang and C.H. Wong, *J Org. Chem.*, **55** (1990) 3377.

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