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Stereochemical Observation on the Enantioselective Hydrolysis Using *Pseudomonas fluorescens* Lipase

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Abstract: Three-site model concerning the hydrolysis of acetate on the mono-cyclic or bicyclic ring using Pseudomonas fluoresence lipase (PFL) could be simplified to a two site model, and the R-preference of hydrolysed alcohol could be predicted by the analysis of a proposed stereomodel. Optically pure (+)-(3) was readily converted to (-)-(**17**), which is an important precursor for the synthesis of isocarbacyclin.

Introduction

Pseudomonas fluoresence lipase (PFL) is an enzyme which releases fatty acids regiospecifically from the outer 1- and 3- positions of acylglycerols. It has been proven to be synthetically useful enzyme for the creation of chiral compounds.[1] In connection with the PFL-catalysed highly enantioselective hydrolysis of mono-cyclic or bicyclic ring acetates, we previously described the concept of three-site model,2 which consists of i) a catalytic site to afford the alcohol with R-configuration, ii) a binding site for groups such as alkoxycarbonyl or acetate groups and, iii) a hydrophobic site for the bicyclic ring system (Fig. 1). During further studies on PFL-catalysed hydrolysis, it occurred to us that the catalytic site might also serve as the binding site, based on the fact that the ester or acetate adjacent to the hydrolysed acetoxy function has no influence on the stereochemistry of resulting alcohols.

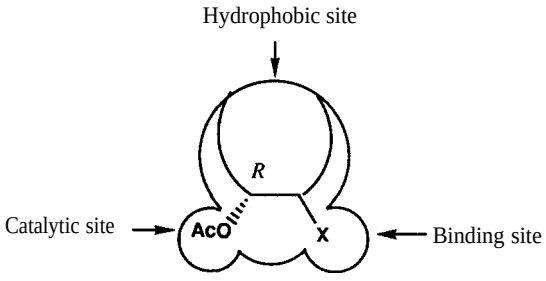
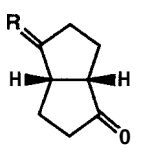


Fig. 1 three-site model

On the basis of this assumption, we examined the PFL-catalysed hydrolysis of the acetate on the bicyclic ring without any ester adjacent to the acetoxy function.

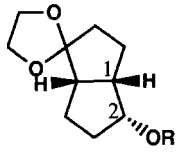
Results and Discussion

Monoketalisation of bicyclo[3.3.0] octane-2,6-dione3 followed by reduction with $NaBH_4$ afforded a mixture of the endo-alcohol (3) and the exo-alcohol (**5**) in a ratio of 10 to 1, which could be seperated by silica gel column chromatography. (4) and (6) were obtained by usual acetylation. The assignments of exo- and endo- alcohols were made by the observation of a difference nuclear Overhauser enhancement between the Cl-H (2.43 ppm) and the C_2-H (5.09 ppm) in (4). Deketalisation of (4) and (6) yielded the corresponding ketones (8) and (**10**), respectively. Wittig reaction of (2) with triphenylphosphonium methylide afforded (**11**), which was converted to (14) and (16) via deprotection, reduction, and acetylation.



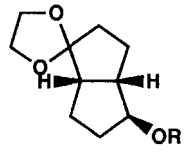
(1) R=O

(2) R=OCH_2CH_2



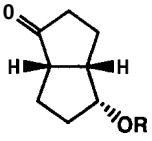
(3) R=H

(4) R=$COCH_3$



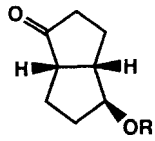
(5) R=H

(6) R=$COCH_3$



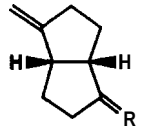
(7) R=H

(8) R=$COCH_3$



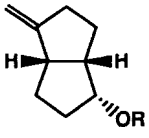
(9) R=H

(10) R=$COCH_3$



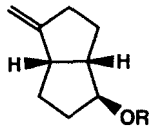
(11) R=OCH_2CH_2

(12) R=O



(13) R=H

(14) R=$COCH_3$



(15) R=H

(16) R=$COCH_3$

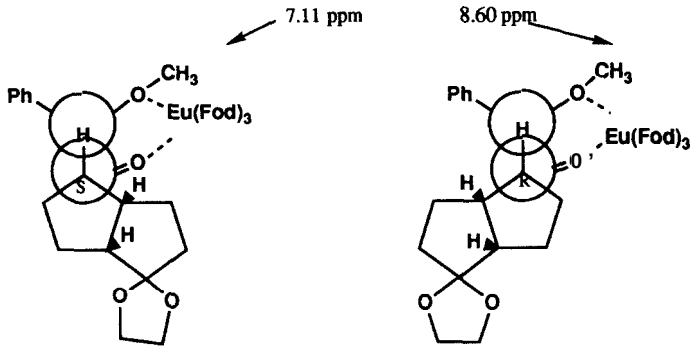


Fig. 2 assignment of absolute configuration

The PFL-catalysed hydrolyses of **(4),(8),** and (14) were performed in 0.1 M phosphate buffer solution **(pH** 7.0) at **30°C** for a given time as shown in table 1. The enantiomeric excesses4 of the resulting alcohol and recovered acetate were determined by comparing the 270 MHz ^{1}**H-NMR** spectra after convertion to (+)-MTPA esters with that of racemic (4). The signals due to the two types of acetoxy methyl protons appeared in distinctly different fields **(3.54, 3.47** ppm) in the ^{1}**H** NMR spectra. The absolute configuration of the resulting alcohols was assigned according to Lightner's method.5 For example, addition of **Eu(fod)3** shift reagent to the (+)-MTPA ester of R-alcohol (13) showed the larger shift value (8.60 ppm) of the methoxy signal than that of S-alcohol (7.11 ppm) (15) (Fig. 2).

Table 1. PFL-catalysed Hydrolysis of **(4),(8),** and (14).

Substrate	Reaction Time (h)	Product		Recovered Acetate	
		Yield (%)	E.e (%)	Yield (%)	E.e (%)
4	24	43	99	49	99
	2	4	99	92	--
8	24	20	99	80	15
14	2	23	99	54	32

The results are summarized in table 1. As expected, PFL-catalysed hydrolyses of the racemic **(4),(8),** and (14) proceeded in highly enantioselective fashion **(>99%** ee) to afford the corresponding alcohols with R-configuration, **(+)-(3),(+)-(7),** and (+)-(13). A controlled experiment was undertaken to investigate the influence of the substituent in the ring not

containing acetate on the PFL-catalysed hydrolysis. After 24 hours of hydrolysis, the (+)-(4) containing the ethylenedioxy function was isolated in 43% yield, while the (+)-(8) with the ketone function was isolated in 20% yield. Thus, the substrate with the more hydrophobic function showed a faster rate of hydrolysis. It is clear that the catalytic site also serves as binding site in our model.

It is noteworthy that the PFL-catalysed hydrolyses of the exo-acetates **(6)**, **(10)**, and (16) were much more resistant than that of the endo-acetates. Even after 10 h, PFL-catalysed hydrolysis of the exo-acetate resulted in the complete recovery of substrate. This dramatic difference for hydrolysis is quite contrary to the general prediction for a chemical reaction, which may be caused by the fact that either substrate has matching shape with the active site of enzymes. We propose a stereochemical analysis of the substrate for PFL-catalysed hydrolysis to explain this difference. When the substrate is drawn in Newman projection with the acetate up, putting the Cl-C2 bond perpendicular to an axis which is formed by two planes perpendicular to one another, the bicyclic skeleton of the endo acetate (4) (1S,2R,5S) occupies the right hand segments (Figure 3), while for the enantiomer (1R,2S,5R) it occupies the left hand segments. on the other hand both the left and right hand segments are occupied in the case of the exo acetate (1S,2S,5S) (Figure 4) and its enantiomer (1R,2R, 5R). Taking the results of PFL-catalysed hydrolysis of the endo- and the exo-acetates into the consideration, the above analysis allows us to conclude that active substrates bind to the active centre in PFL only via the right hand volume as in (4). Substrates, which occupy the left site such as the enantiomer of **(4)**, and both sites such as (6) and its enantiomer, are unacceptable to the enzyme.

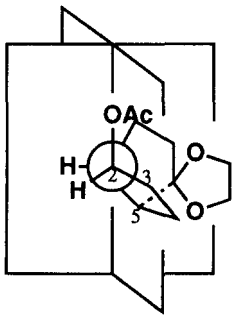


Fig. 3 Endo-Acetate (4)

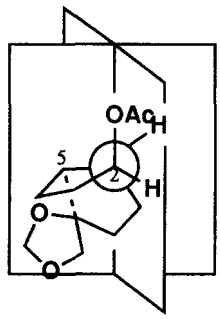


Fig. 4 Exo-Acetate (6)

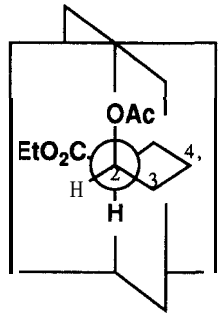


Fig. 5 Cis-1-Ethoxycarbonyl-2-acetoxycyclohexane

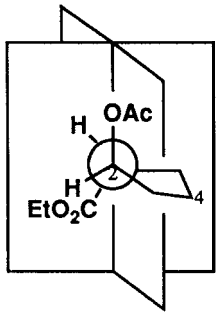


Fig. 6 Trans-I-Ethoxycarbonyl-2-acetoxycyclopentane

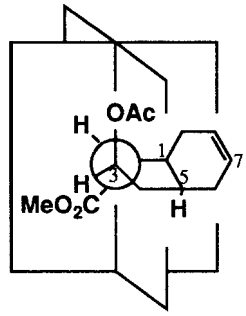
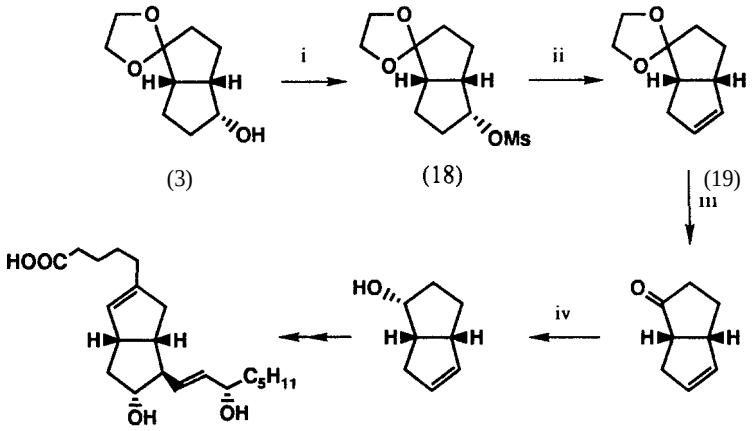


Fig. 7 3β–Acetoxy-2α-methoxy-carbonyl-1αH, 5αH-bicyclo-[4.3.0]non-7-ene

The proposed stereochemical analysis of substrates for PFL-catalysed hydrolysis could be also applied to the preference of the acetate with the R-configuration in the hydrolysis of the acetoxy function adjacent to the ester in 5, 6, 7-membered rings and bicyclic rings. For example, the R-preference of hydrolysed acetates of cis-1-ethoxycarbonyl-2-acetoxycyclohexane, trans-1-ethoxycarbonyl-2-acetoxycyclopentane, and 3β-acetoxy-2α-methoxycarbonyl-1αH,5αH-bicyclo[4.3.0]non-7-ene[6] are understandable from our stereomodel, in which the six-, five-membered ring, and bicyclic ring occupy the right hand segments (Fig. 5, 6, and 7).



Reagent: i) MsCl, Et_3N, CH_2Cl_2, RT, 4 h, 75%; ii) t-BuOK, DMF, RT, 2 h, 69%; iii) H^+, THF, RT, 2 h, 92%; iv) $NaBH_4$, MeOH, -78° C, 30 min.

Optically pure (+)-(3) was converted to (-)-(**17**), a key intermediate for the synthesis of isocarbacyclin7 by following a conventional sequence. The mesylation of (+)-(3) gave (**18**), which was treated with t-BuOK in DMF to afford **oletin (19)** in 61% yield. Facile deketalisation of (19) gave the ketone (**20**), which underwent a stereospecific reduction with $NaBH_4$ at -78°C to afford the desired **(21),** an important precursor in the synthesis of isocarbacyclin.

Conclusion

We have successfully proposed a novel stereomodel for R-preference in PFL-catalysed hydrolysis. This stereochemical analysis is in good agreement with the experimental results among the tested 25 substrates. The obtained optically pure bicyclic compounds are important building blocks of synthetic interest.

Experimental

^1H NMR spectra were obtained in CDC13 solution at 270 MHz. Each reaction was carried out under an N2 atmosphere and monitored by TLC (silica gel 60F-254 plates). All organic solvent extracts were washed with brine, dried over MgS04, and evaporated under reduced pressure on a rotary evaporator. Each product was purified by flash chromatography (230-400 mesh silica gel) and obtained as oily substances. For enzymatic hydrolysis, PFL(Pseudomonas fluorescens lipase, Amano Pharmaceutical Co., Amano P) was used.H_2O

Synthesis of substrates

(1SR, 2RS, 5SR)-6,6-Ethylenedioxybicyclo[3.3.0]octan-2-ol (3).

NaBH4 reduction of (2) at -78°C gave the endo- and exo-alcohols ((3) and (5)) in a ratio of 10:1. These could be easily seperated by column chromatography using hexane/ethyl acetate 3: 1 as an eluent. Usual acetylation of (3) and (5) gave the corresponding (4) and (6), respectively. Treatment of (3) and (5) with AcOH/THF/H_2O 3:1:1 at room temperature led to (8) and (10). Wittig reaction of (2) with triphenylphosnium methylide followed by NaBH4 reduction gave (13) and (15) in a ratio of 10:1.

(1SR,2RS,5SR)-2-Acetoxy-6,6-ethylenedioxybicyclo[3.3.0]octane(4). ^1H NMR δ 1.50-1.85 (8H, m). 2.04(3H, s), 2.35-2.44(1H, m), 2.72-2.81(1H, m), 3.91(4H, s), 4.99-5.18(1H, m).

(1SR, 2RS, 5SR)-2-Acetoxy-6-oxobicyclo[3.3.0]octane (8). ^1H NMR δ 1.67-2.03(6H, m), 2.04(3H, m), 2.13-2.41(2H, m), 2.61-2.79(1H, m), 2.85, 3.07(1H, m), 5.15-5.30(1H, m).

(1SR, 2RS, 5SR)-2-Acetoxy-6-methylenebicyclo[3.3.0]octane (14). 1H NMR δ1.56-1.76(4H, m), 1.80-1.91(2H, m), 2.04(3H, s), 2.19-2.39(2H, m), 4.76-4.78(1H, m), 4.86-4.88(1H, m), 5.09(1H, q, J=7.2Hz).

General procedure of PFL-catalysed hydrolysis.

A suspension of (4) (200 mg) and PFL (100 mg) in 0.1 M phosphate buffer (40 ml) was stirred for 24 hours at 30°C. The hydrolysis was terminated by extracting with AcOEt (50 ml X 3). The extract was washed and dried, then concentrated in vacuo to leave an oily residue, which was

purified by flash column chromatography on silica gel (5 g). The fraction eluted with hexane/AcOEt 1: 1 gave (+)-(3) (69 mg, 43%) and recovered (-)-(4) (98 mg, 49%).

(1S, 2R, 5S)-2-Hydroxy-6,6-ethylenedioxybicyclo[3.3.0]octane (3). $[\alpha]_D^{21}$ + 29.7 (c=1.0, CHC13);

^1H NMR δ1.67-1.82(8H, m), 2.20-2.43(1H, m), 2.43-2.66(2H, m), 3.93(4H, s), 3.97-4.16(1H, m). ν_{max} 3420, 2950, 1450, 1350, 1300 cm^{-1}; m/z 184(M^+), 166, 99.

(1S, 2R, 5S)-2 Hydroxy-6-oxobicyclo[3.3.0]octane (7). $[\alpha]_D^{20}$+ 103.8 (c=1.38, CHC13).

(1S, 2R, 5S)-2-Hydroxy-6-methylenebicyclo[3.3.0]octane (13). $[\alpha]_D^{25}$ +67.6 (c=0.34, CHC13).

Formal synthesis of isocarbacyclin

(IS, 2R, 5S)-6,6-Ethylenedioxy-2-mesyloxybicyclo[3.3.0]octane (18). Et_3N (92 mg, 0.91 mmol) and MsCl (70 ul, 0.91 mmol) were added dropwise to a solution of (+)-(3) (112 mg, 0.61 mmol) in CH_2Cl_2 (10 ml) at 0°C. The whole was stirred for 4 h and diluted with CH_2Cl_2. The mixture was washed with aq. NaHC03, and brine. The resulting crude oil was purified by flash column chromatography on silica gel (elution with hexane/EtOAc 3: 1) to afford 74 mg of (18) as a colorless oil. $[\alpha]_D^{22}$ +54.7 (c=1.0, $CHCl_3$); ^1H NMR δ1.55-2.04(8H, m), 2.31-2.53(1H, m), 2.75-2.91(1H, m), 3.01(3H, s), 3.91(4H, s), 5.03(1H, q, J=6.3Hz); ν_{max} 2960, 1450, 1340, 1170,960 cm^{-1}; m/z 262(M^+), 183, 167, 99.

(1S, 2R, 5S)-6,6-Ethylenedioxybicyclo[3.3.0]octan-2-ene (19). t-BuOK (72 mg, 0.64 mmol) was added to the solution of (+)-(18) (84 mg, 0.32 mmol) in DMF (5 ml) at 0°C. After 2.5 h at room temperature, the mixture was diluted with ether. The whole was washed with 5% aq. NaHC03 and brine. The crude product was purified by flash column chromatography (elution with hexane/EtOAc 6: 1) to give 38 mg of (+)-(19) (69% yield) as a colorless oil. $[\alpha]_D^{21}$ -39.7 (c=1.27, CHC13); ^1H NMR δ1.18-1.96(4H, m). 2.15-2.31(1H, m). 2.41-2.67(2H, m), 3.05-3.37(1H, m), 3.92(4H, s), 5.30-5.68(2H, m); vmax 3050, 2930, 1440, 1330, 1200 cm-; m/z 166(M^+), 138, 122, 99.

(1S, 2R, 5S)-6-Oxobicyclo[3.3.0]octan-2-ene (20). A mixture of (-)-(19) (35 mg, 0.21 mmol), 5% HCI (0.1 ml) and THF (2 ml) was stirred for 2 h at room temperature. The mixture was diluted with ether. The whole was washed with 5% aq. NaHC03 and brine. The crude product was purified by flash column chromatography (elution with hexane/EtOAc 5: 1) to afford 27 mg of (20) as a colorless oil. $[\alpha]_D^{25}$ + 259.2 (C=1.3, CHC13); ^1H NMR δ1.90-2.20(4H, m), 2.52-2.77(3H, m), 5.51-5.76(2H, m); vmax 3050, 1730, 1635, 1440, 1400, 1260 cm^{-1}; m/z 122(M^+), 94, 80, 66.

(1S, 2R, 5R)-2-Hydroxybicyclo[3.3.0]oct-6-ene (17). $NaBH_4$ (8.4 mg, 23 mmol) was added to a solution of (20) (23 mg, 0.19 mmol) in MeOH (1 ml) at -78°C. The whole was stirred for 30 min. The reaction mixture was diluted with brine and then extracted with EtOAc. After usual workup, the resulting residue was purified by a flash column chromatography (elution with hexane/EtOAc 1:1) to afford 22 mg of (17) (93%). $[\alpha]_D^{20}$ -67.5 (c=1.28, CHC13); ^1H NMR δ

1.40-1.81(4H, m), 2.26-2.80(3H, m), 3.18(1H, m), 4.19(1H, m), 5.59(1H, m), 5.68(1H, m); ν_{max} 3050, 1630, 1440, 1370 cm^{-1}; m/z 124(M^+), 106, 91, 78.

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