Lipase-catalyzed enantioselective hydrolysis of β-acetyloxymethyl-β-valerolactone

Hyun-Joon Ha*, Young-Soo Park, and Gha-Seung Park

Department of Chemistry, Hankuk University of Foreign Studies Yongin, Kyunggi-Do 449-791, Korea

E-mail: hjha@maincc.hufs.ac.kr

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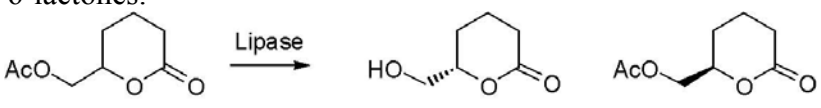
Abstract

The enantioselective hydrolysis of δ-acetyloxymethyl-δ-valerolactone was studied with various lipases. Among all of the lipases which were evaluated, PPL (porcine pancreatic lipase) in a phosphate buffer yielded the hydrolytic product (*S*)-δ-hydroxymethyl-δ-valerolactone and the unreacted substrate (*R*)-δ-acetyloxymethyl-δ-valerolactone with the highest optical purities.

Keywords: Lipase, δ-acetyloxymethyl-δ-valerolactone, hydrolysis

Introduction

Enzymes as biocatalysts find considerable utility for the preparation of enantiomerically pure compounds.[1] Among them, lipases (triacylgycerol hydrolases, EC 3.1.1.3) are the most widely employed enzymes not only because they are cheap and readily available from many different sources but because they possess high enantioselectivity for a broad range of substrates and high stability in organic solvents. The utility of Lipases has been demonstrated by successful kinetic resolution of diverse substrates including some δ-lactones.[2] Recently we have succeeded in the resolution of β-substituted γ-acetyloxymethyl-γ-butyrolactones in optically active form via enantioselective hydrolysis of acetyl by use of lipase PS (*Pseudomonas cepacia*).[3] They were used for the synthesis of natural products bearing γ-butyrolactones.[3,4] In this paper is described the enantioselective hydrolysis of δ-acetyloxymethyl-δ-valerolactone to yield the hydrolytic product δ-hydroxymethyl-δ-valerolactone and the unreacted substrate δ-acetyloxymethyl-δ-valerolactone with high optical purity as valuable chiral starting materials to build natural products bearing δ-lactones.[5]



(±)-**1** **2** (−)-**1**

Scheme 1

Table 1. Hydrolysis of δ-acetyloxymethyl-δ-valerolactone (**±1**) using various lipases[a]

Entry	Lipase[b]	Time (h)	Convn[c] (%)	% ee_p[d]	E[e]
1	PPL	9.2	48	95	51
2	PSL	1.8	38	32	3
3	CCL	17.8	14	66 4	
4	ANL	0.5	21	88	11
5	RNL	2.4	48	52	5
6	RAL	3.8	46	15	2
7	WGL	0.1	16	76	5

[a] The reaction of substrate (86 mg, 0.5 mmol) was carried out in 12 ml of 0.1 M phosphate buffer at pH 7.2. [b] Enzyme 258 mg (300 wt %) was used for each reaction. PPL: Porcine Pancreatic lipase, PSL: *Pseudomonas cepacia*, CRL: *Candida cylindracea*, ANL: *Aspergillus niger*, RNL: *Rhizopus niveus*, RAL: *Rhizopus arrhizus*, WGL: Wheat germ. [c] Conversion rate was deduced by NaOH consumption. [d] Ee values were determined by capillary GC analysis using Rt-βDEXsa (φ 0.25 mm x 30 M, N_2, 200-200 °C). [d]E values = $In[1-convn(1+ee_p)]/ In[1-convn(1-ee_p)]$.

The substrate δ-acetyloxymethyl-δ-valerolactone **±1** was prepared from acetylation of δ-iodomethyl-δ-valerolactone originated from iodolactonization[6]of 5-hexenoic acid in quantitative yield.[3] At first we tried to find the best lipase from all possible enzyme sources for enantioselective hydrolysis of δ-acetyloxymethyl-δ-valerolactone as shown in the **Scheme 1**, including PPL(Porcine Pancreatic lipase), PSL(*Pseudomonas cepacia*), CRL(Candida cylindracea), ANL(*Aspergillus niger*), RNL(*Rhizopus niveus*), RAL(*Rhizopus arrhizus*), and WGL(Wheat germ).

Most lipases except CCL showed good activity to hydrolyze acetate with quite difference enantioselectivity. PPL was the best with an E value[7] of 51 to yield δ-hydroxymethyl-δ-valerolactone (**2**) with 95% ee at 48% conversion after 9.2 hr of the reaction time. ANL was the second best with an E value of 11. PSL and RNL were quite active enzymes for deacetylation while their enantioselectivities were not satisfactory (Table 1).

Table 2. PPL-catalyzed hydrolysis of δ-acetyloxymethyl-δ-valerolactones (**±1**) in aqueous media in the presence of organic co-solvents[a]

Entry	Cosolvent	Time (h)	Convn (%)[b]	% ee_s[c]	% ee_p[c]
1	*n*-Hexane	1.5	41	59	87
2	Acetone	2.5	54	84	73
3	Dioxane	2.5	51	83	78
4	THF	3.5	62	90	56
5	t-BME	0.7	48	82	89

[a] The reaction of substrate (86 mg, 0.5 mmol) was carried out in the mixed solvent of 11.64 ml of 0.1 M phosphate buffer at pH 7.2 and 0.36 ml of co-solvent. Enzyme PPL (258 mg, 300 wt %) was used for each reaction. [b] Conversion rate was deduced by ee_s and ee_p. [c] Ee values were determined by capillary GC analysis using Rt-βDEXsa (φ 0.25 mm x 30 M, N_2, 200-200 °C).

Once we found that PPL was the best for enantioselective hydrolysis of the substrate δ-acetyloxymethyl-δ-valerolactone, the effect of organic co-solvent in the reaction media for the hydrolytic reaction by PPL (Table 2) was studied. Addition of any organic co-solvent accelerated the hydrolytic reactions but with less enantioselectivity, possibly due to improvement of the solubility of the organic substrate. Even though the reaction takes a little longer time to carry out, the hydrolysis in a buffer without addition of any co-solvent led to products and unreacted substrate with higher enantioselectivity.

From the separate experiments with 3.12 g of substrate was obtained δ-hydroxymethyl-δ-valerolactone with >98% *ee* at 36% conversion. In the same manner, unreacted δ-acetyloxymethyl-δ-valerolactone was also obtained with 86% ee at 75% conversion with $[\alpha]_D^{25}$ –25.4 (c 1.5, $CHCl_3$), which indicates the absolute stereochemistry of δ-position is (*R*).[8] The configuration of the favorable substrate for the hydrolytic reaction catalyzed by PPL was deduced as (*S*). Both of the hydrolytic product δ-hydroxymethyl-δ-valerolactone and the unreacted substrate δ-acetyloxymethyl-δ-valerolactone were reduced by $LiAlH_4$ followed by the treatment with acetone with catalytic amount of *p*-TsOH to yield (5*S*)- and (5*R*)-5,6-isopropylidenedioxyhexaol **3** respectively whose configuration were confirmed again by comparison of the optical rotation values in the literature.[9] The same stereochemical outcome as (*S*) for the favorable substrate toward the hydrolysis of δ-acetyloxymethyl-δ-valerolactone by PPL was the same as we observed from the hydrolysis of γ-acetyloxymethyl-γ-valerolactone as a substrate.[3] However better enantioselectivity was observed with δ-acetyloxymethyl-δ-valerolactone rather than γ-acetyloxymethyl-γ-valerolactone.[3] Though there are some reports[10] getting chiral intermediates for δ-substituted-δ-lactones with lipases the enzymatic resolution of

racemic δ-acetyloxymethyl-δ-valerolactone is proved to be a good way for chiral δ-lactone compounds. (*S*)-δ-Hydroxymethyl-δ-valerolactone (**2**) with >98% ee could be transformed to elaborate some natural products including Fostriecin (CI-920) following the literature.[5b]

We also have tried to utilize δ-acetyloxymethyl-δ-valerolactone with high optical purity obtained as unreacted substrate by PPL after removal of acetyl by chemical methods. If deacetylation was successful to yield δ-hydroxymethyl-δ-valerolactone, enantioselective esterification catalyzed by lipases also could be tried to obtain both optically active δ-acyloxymethyl-δ-valerolactone as a reaction product and δ-hydroxymethyl-δ-valerolactone as an unreacted substrate. All trials under the commonly applied methods[11] with bases such as Na_2CO_3, K_2CO_3 or KCN in MeOH were unsuccessful to afford δ-hydroxymethyl-δ-valerolactone from δ-acetyloxymethyl-δ-valerolactone, instead methyl 5,6-dihydroxyhexanote was obtained. Lewis acid catalyzed reaction[12] with 10 mol % of $Sc(OTf)_3$ in MeOH yielded 24% of δ-hydroxymethyl-δ-valerolactone with ring opened products for the best result. Another Lewis acid $Yb(OTf)_3$ afforded the expected product in 12% yield monitored by GC. Considering no chemical method is available for deacetylation of δ-acetyloxymethyl-δ-valerolactone active lipase enzymes such as PPL and PSL could be good reagents yielding δ-hydroxymethyl-δ-valerolactone without breaking the ring. When we carried the hydrolytic reaction in DME with 3.0 mass equivalent of PSL at 35 ºC for 20 hours δ-hydroxymethyl-δ-valerolactone could be obtained in 95% yield (by GC).

In this report we have described the enantioselective hydrolysis of δ-acetyloxymethyl-δ-valerolactone to yield the hydrolytic product (*S*)-δ-hydroxymethyl-δ-valerolactone and the unreacted substrate (*R*)-δ-acetyloxymethyl-δ-valerolactone with high optical purity.

Experimental Section

General Procedures. ^{1}H NMR and ^{13}C-NMR spectra were recorded on a Varian Gemini 200 (200 MHz for ^{1}H and 50.3 MHz for ^{13}C). Chemical shifts were given in ppm using TMS as internal standard. Elemental analysis was taken on a Perkin-Elmer 240 DS elemental analyzer. Optical rotation was measured with Rudolph Research Autopole 3 polarimeter. The silica gel used for column chromatography was Merck 200-230 mesh. TLC was carried out with Merck 60F-254 plates with 0.25 mm thickness. E.e. values were determined by capillary GC analysis using Rt-βDEXsa (φ 0.25 mm x 30 M, He_2, 150 °C for 5min, 4 °C/min, 210 °C) purchased from Altech Co., Ltd. PPL(Porcine Pancreatic lipase) and WGL(Wheet germ) were purchased from Sigma and ANL(*Aspergillus niger*) RNL(*Rhizopus niveus*) and RAL(*Rhizopus arrhizus*) from Fluka. PSL(*Pseudomonas cepacia*) and CRL (*Candida cylindracea*) were obtained from Amano Pharmaceutical Co., Ltd.

δ-Acetyloxymethyl-δ-valerolactone (±1). Into the solution of δ-iodomethyl-δ-valerolactone (10.4 g) in 100 mL of acetic acid was added AgOAc (14.5 g) at room temperature. The resultant

solution was refluxed for 12 hr for completion. The reaction mixture was filtered through Celite and concentrated to remove all volatiles, and then EtOAc (200 mL) and water (100 mL) were added. The organic layer was separated and the aqueous layer was washed again with EtOAc (100 mL). The combined organic layers were washed with brine and dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. After Kugelrohr distillation the title compound 6.4 g was obtained in 86% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.55-2.10 (4H, m), 2.01 (s, 3H), 2.34-2.53 (2H, m), 4.05-4.17 (2H, m), 4.21-4.47 (1H, m); ^{13}C-NMR ($CDCl_3$) δ: 17.9, 20.5, 23.9, 29.1, 65.4, 77.3, 170.3, 170.6. Anal. Calcd for $C_8H_{12}O_4$: C, 55.8; H, 7.02. Found: C, 55.6; H, 7.11.

Enzymatic resolution of δ-acetyloxymethyl-δ-valerolactone. Enzyme lipase (258 mg, 3.0 mass equiv.) was added to a stirred solution of δ-acyloxymethyl-δ-valerolactone (86 mg, 0.5 mmol) in 12 mL of 0.1 M phosphate buffer at pH 7.2. The resulting solution was stirred well at 35 ºC while pH of the solution was maintained to 7.2 by adding NaOH solution (0.1 M). After the conversion of the reaction reached at certain percentage according to NaOH consumption, the reaction was quenched by adding celite and ice. The cake was filtered through Celite with water and EtOAc. The combined organic layer was washed with water twice, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Hydrolyzed product and starting substrate were isolated and Ee values were determined by capillary GC analysis using Rt-βDEXsa (φ 0.25 mm x 30 M, N_2, 200-200 °C). A large scale resolution with PPL was also carried out. PPL (10.3 g) was added to a stirred solution of racemic δ-acyloxymethyl-δ-valerolactone (3.12 g, 18 mmol) in the mixed solvent of 1N phosphate buffer at pH 7.2 (480 ml). The resulting solution was stirred well at 35 ºC while pH of the solution was maintained to 7.2 by adding 0.5 N NaOH solution. After the reaction to be reached at certain conversion rate according to NaOH consumption, the reaction was quenched by adding celite and ice. The cake was filtered through Celite with water and EtOAc (100 mL). The first EtOAc solution contained most of unreacted δ-acyloxymethyl-δ-valerolactone while hydrolyzed product still remained in aqueous layer. Continous extraction with CH_2Cl_2 (400 mL) of the aqueous layer over night gave δ-hydroxymethyl-δ-valerolactone (**2**). Both of the hydrolytic products (*S*)-δ-hydroxymethyl-δ-valerolactone and the unreacted substrate (*R*)-δ-acetyloxymethyl-δ-valerolactone was purified by flash chromatography on silica gel. (*R*)-δ-Acetyloxymethyl-δ-valerolactone (86% ee, by GC) $[\alpha]_D^{25}$ –25.4 (c 1.5, $CHCl_3$) lit,[8] $[\alpha]_D^{25}$ +29.4 (c 0.48, $CHCl_3$) for (*S*)-δ-acetyloxymethyl-δ-valerolactone. (*S*)-δ-Hydroxymethyl-δ-valerolactone (≥98% ee, by chiral GC). $[\alpha]_D^{20}$ +27.1 (c 0.7, $CHCl_3$). . ^{1}H-NMR ($CDCl_3$) δ: 1.51-1.96 (4H, m), 2.15-2.44 (2H, m), 3.46-3.64 (2H, m), 3.94 (1H, brs), 4.22-4.34 (1H, m); ^{13}C-NMR ($CDCl_3$) δ: 17.7, 23.2, 29.1, 64.0, 80.9, 172.0. Anal. Calcd for $C_6H_{10}O_3$: C, 55.4; H, 7.74. Found: C, 55.5; H, 7.68.

(5*R*)-5,6-Isopropylidenedioxyhexaol ((5R)-3). To the solution of unreacted substrate (*R*)-δ-acetyloxymethyl-δ-valerolactone (1.2 g, 7.0 mmol) in THF (50 mL) which was being cooled in an ice-water bath, was added $LiAlH_4$ (0.57 g, 15 mmol) in small portions. The reaction mixture was stirred at room temperature for 12 hr before adding water (20 mL). The reaction product was extracted with THF (30 mL) three times. The combined organic layer was washed with NaOH

solution (1N, 50 mL), water (50 mL) and brine (50 mL) successively. The organic layer was then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. This crude product was dissolved in acetone (30 mL). Into the solution of this crude product in acetone (50 mL) was added *p*-TsOH (80 mg). The resultant solution was stirred at room temperature for 18 h. After the reaction was completed according to TLC the reaction mixture was neutralized by $NaHCO_3$ solution and concentrated to dryness. This was then dissolved with EtOAc (50 mL) with small amount of water. The EtOAc solution was washed with water (50 mL) dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. This crude reaction mixture was purified by flash chromatography on silica gel to give 861 mg (5*R*)-5,6-isopropylidenedioxyhexaol (5*R*)-**3** (86% ee, by GC) in 71% yield. $[\alpha]_D^{25}$ –15.3 (c 0.20, $CHCl_3$) lit,[8] $[\alpha]_D^{25}$ +18 for (5*S*)-5,6-isopropylidenedioxyhexaol.[10] The same reaction starting from the hydrolytic product δ-hydroxymethyl-δ-valerolactone produced (5*S*)-5,6-isopropylidenedioxyhexaol (5*S*)-**3.**

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