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Lipase-catalyzed Stereoselective Hydrolysis of Thiolacetate

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Racemic ethyl α-acetylthiopropionate, 1-phenylethanethiol acetate and 2-octylthiol acetate were subjected to lipase-catalyzed hydrolysis and *R*-form of the three thiols were found to be hydrolyzed preferentially in enantiomeric excess ranging over 6～99% e.e.

KEY WORDS: Lipase/ Stereoselective hydrolysis/ Thiol acetate/ Optically active thiols/

INTRODUCTION

It has been noted that a number of enzymes can catalyze very unnatural substrates just by altering the reaction conditions such as reaction medium[1] and by making the reaction non-equilibrium[2]. As reported already by us, 1-phenylethylhydroperoxide (**1**) in organic solvent were found to be a good substrate of lipase-catalyzed acylation in spite of the fact that hydroperoxy-group is much more acidic than hydroxy-group and has strong oxidative function. It should also be noted that the process was highly stereoselective[3].

From this view, thiols or their esters may likely be a substrate of lipase and other hydrolytic enzymes. Here, we describe stereoselective hydrolysis of three thiol acetates by use of lipases in a two phase medium involving phosphate buffer (pH 7.0) and cyclohexane or hexane.

RESULTS AND DISCUSSION

We first examined lipase-catalyzed acylation of racemic thiols and in organic solvent using vinyl or isopropenyl aceate in excess as an activated acetylation reagent. No reaction, however, occured at all.

Ester exchange reactioin was also examined for the same thiols in an excess of esters. In these cases, too, no enzymatic reaction occured even at 50°C and with large excess of the enzyme. The same was the case with a thiol (**9**).

Generally, it is known that an equilibrium between thiol and thiol ester favors the left side (eq. 1)[4]. Therefore, if such situation is responsible for the innertness of thiols to the lipase-catalyzed acylation described already, the reverse mode, i.e., thiol ester hydrolysis must be very easy. Thus, thiol acetate (**2**) was subjected to lipase-catalyzed

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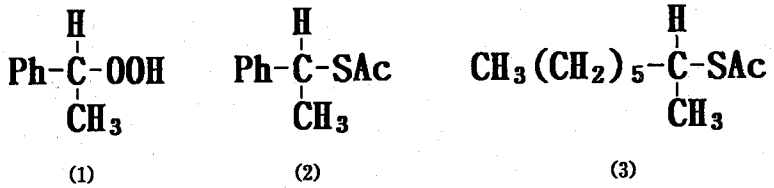
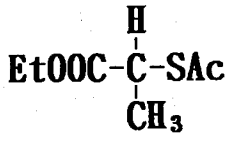


(1) (2) (3)



(4)

Fig. 1.

hydrolysis in a mixture of phosphate buffer (pH 7) and cyclohexane and the reaction was quenched at 55% conversion yield simply by separating organic and aqueous phases. Here, the thiol acetate was in the organic phase and the lipase in the aqueous phase. The organic phase was washed with water two times and concentrated. Vpc analysis of the extract revealed that the hydrolysis proceeded very rapidly as expected from the above consideration. The product thiol and unreacted thiol acetate were isolated pure by preparative vpc. As shown in the Table, their optical rotation showed that the product thiol (5) had 76% optical purity and the ester (6), 94% optical purity. Thus, the lipase-catalyzed hydrolytic process found to be stereoselective.

eq. 1

These results encouraged us to examine further the hydrolysis of a thiol ester carboxylate (4). Here, we presumed that carboxylate ester might not be hydrolyzed since esters of carboxylic acid was resist to the hydrolysis when α-carbon atom to carboxyl groups had alkyl substituents[5].

Thus, under the same conditions, the thiol ester (4) was submitted to lipase-catalyzed hydrolysis, and the results are listed in Table 1. With this substrate, it was found that (*R*)-form was preferentially hydrolyzed and the optical purity of the product was 4～10% at the reaction conversions. On the other hand, those of remained substrates showed 80～99% e.e. Here, the optical purity of the product thiol (9) appeared to be too small at the conversion yields comparing with the results for substrates (2) and (3). This situation may be caused in two cases: (i) Stereoselective disulfide bond formation being attendant with a sort of kinetic resolution and resulting in the decrease of the optical yield. (ii) The partially optical active thiol in the reaction mixture was subjected to further enzymatic hydrolysis at carboxylic acid ethyl ester having *(R)*-configuration preferentially resulting in decrease of optical purity. The first possibilty was excluded

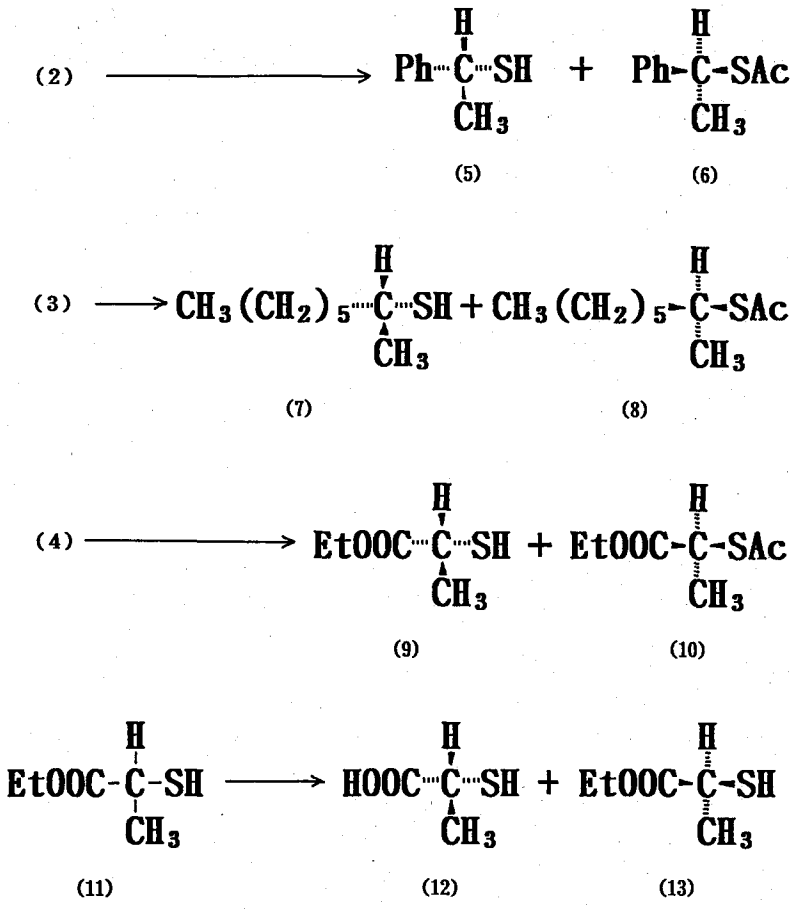


Fig. 2.

Table 1. Lipase-catalyzed Kinetic Resolution of Racemic Thiol Acetate.

substrate	lipase	cosolvent	rect. period	react. temp.	% conversion	RSH			RSAc		
						$[\alpha]_D$	% e.e.	configuration	$[\alpha]_D$	% e.e.	configuration
2	A	cyclohexane	40 hr	30°C	55	+153.5°[a]	76	*R*	−659.9°[a]	94	*S*
3	A	—	46	30	62	−10.3	35	*R*	−4.2	61	*S*
4	A	cyclohexane	60 min	25	64	+9.9	16	*R*	−110.8	81	*S*
4	A	cyclohexane	90	25	75	+3.8	6	*R*	−127.8	93	*S*
4	A	hexane	260	0	77	+6.8	11	*R*	−136.7	99	*S*
4	A	hexane	120	28	75	+3.6	6	*R*	−124.6	91	*S*
4	B	cyclohexane	1000	25	66	+7.5	12	*R*	−125.2	91	*S*
4	C	cyclohexane	860	ambient	42	+9.6	16	*R*	−100.1	73	*S*

Enzyme: A, lipase from *Pseudomonas fluorescens* (Amano Pharm. Co., Ltd.); B, from *Pseudomonas sp.* (Kurita Kogyou Co., Ltd.); C, from *Pseudomonas sp.* (Toyobo Co., Ltd.). a) optical rotation at 436 nm.

since the thiol was not converted to the disulfide at all against exposures to air or oxygen gas at ambient temperature for several hours. To examine the second posibility, a racemic mixture of ethyl 2-Mercaptopropionate was subjected to enzymatic hydrolysis using lipase Amano P in a mixture of phosphate buffer (pH 7.0) and cyclohexane as a cosolvent. Indeed, it was found that the reaction proceeded under this condition although it was considerably slow comparing with that of the thiol acetate, and when the reaction was quenched at 58% conversion, the product (**12**) showed 6% e.e. with *(R)*-configuration in slight excess and the remained substrate (**13**), 5% e.e. with (*S*)-configuration in excess. This outcome strongly suggested that the second possibility was responsible for the low optical purities observed for the hydrolysis of (**4**). However, we could not evaluate the extent quantitatively because of technical difficulties.

The present study demonstrated for the first time that thiol ester could be a substrate for lipase in the hydrolytic reaction and the process was moderatory stereoselective.

EXPERIMENTAL

Optical rotations were measured with Perkin-Elmer 241 polarimeter. Percent conversion of the enzymatic reactions were determined by vpc analysis on Shimadzu GC-4CM equipped with glass column (3 m, 10%-DC-QF-1, 180°C). Preparative vpc was carried out using Varian Aerograph Model 920 for isolation of enzymatic reaction products and unreacted substrates.

Racemic phenylethanethiol acetate was prepared by nucleophilic substitution of 1-phenylethyl bromide with potassium thioacetate in DMF at 40°C for 4 h. bp. 124–126°C/9 mm. 68.7% yield. Racemic 2-octanethiol acetate was prepared likewise. bp. 77–79°C/6 mm. 80% yield. Racemic ethyl 2-(acetylthio)propionate was prepared by the same method. bp. 58–60°C. 66% yield.

E.e.'s were calculated from reported maximum rotations. (*R*)-(+)-2-Mercaptpropionic acid: $[\alpha]_D+60.5°$ ($CHCl_3$)[6]; (*S*)-(−)-Ethyl 2-mercaptpropionate: $[\alpha]_{578}$-137.5° ($CHCl_3$)[6]; (*R*)-(−)-2-Octanethiol: $[\alpha]_D$-29.3° (ethanol)[7]; (*S*)-(−)-Octanethiol acetate: $[\alpha]_D$-6.9° (ethanol)[8]; (*S*)-(−)-α-Phenylethanthiol: $[\alpha]_{436}+202.4°$ (abs. ethanol)[9]; Maxium optical rotation of (*S*)-(−)-α-phenylethanethiol acetate was calculated as {$[\alpha]_{436}$-659.9° (CH_2Cl_2)} from the corresponding (*R*)-(+)-α-phenylethanethiol which was obtained by a lithiumalminum hydride reduction of the acetate and known to have {$[\alpha]_{436}$ +202.4° (c 6.19, abs. EtOH)}

General procedure for lipase-catalyzed hydrolysis.

A solution of racemic acetate (1.00 mmol) and lipase (0.2～0.3 g) in a mixture of phosphate buffer (pH 7.0, 0.1 M, 40 ml) and organic solvent (cyclohexane or hexane, 20～30 ml) was stirred at an ambient temperature. The reaction was quenched at a % conversion shown in the Table and the product was extracted with hexane four times in the presence of small amount of methanol. The extract was dried over anhydrous sodium sulfate and concentrated. The product thiol and remained acetate was isolated pure by preparative vpc and their optical rotations were measured.

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