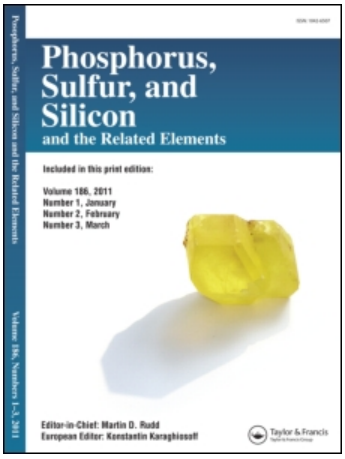

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ENZYMATIC RESOLUTION OF ETHYL α-HYDROXYPHOSPHINATES IN A MODIFIED REACTION ENVIRONMENT

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The enzymatic resolutions of two racemic ethyl hydroxyalkane(*P*-phenyl)phosphinates were performed by both esterification and hydrolysis approaches. The first reaction was performed in anhydrous diisopropyl ether with triethylamine or pyridine as additives by using lipases from three different sources (*Candida cylindracea*, *Aspergillus niger*, and *Mucor javanicus*). The increase in enantioselectivity was observed when NEt_3 was applied. The second reaction—lipase-catalysed hydrolysis of ethyl butyryloxyalkane(*P*-phenyl)phosphinates—was carried out by *Candida cylindracea *lipase in diisopropyl ether saturated with water or in aqueous solutions containing $MgCl_2$, LiCl, or Triton X-100. The usefulness of biphasic systems consisting of diisopropyl ether and water or aqueous solution of $MgCl_2$, LiCl, or Triton X-100 also were tested. The use of biphasic system in the presence of Triton X-100 resulted in the higher conversion of the substrates.

Keywords Biocatalysis; enantioselectivity; hydroxyphosphinates; hydroxyphosphonates; lipases

INTRODUCTION

Chiral hydroxyphosphonates are considered as useful precursors of structurally variable organophosphonic compounds.[1–4] Hydroxyalkanephosphonic acids also exhibit promising biological activity. For example, some of these compounds are interesting antibacterial, antiviral, or anticancer agents acting as inhibitors of enzymes important for development of these pathogens.[5] It is well acknowledged that the biological activity of organic compounds is strongly dependent on their three-dimensional structure, and thus configuration and optical purity. That is one of the reasons why studies on the procedures leading to chiral hydroxyphosphonates are so intensive.[1]

A range of methods is available for the preparation of pure enantiomers of these compounds based on lipase-catalyzed reactions. It is well acknowledged that some additives have an influence on the environment of the enzymes and hence on their selectivity and/or activity.[6] For example, Okamoto and Ueji[7] improved the enantioselectivity

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of lipase-catalysed hydrolysis of racemic butyl 2-(4-substituted phenoxy)propionates by using diisopropyl ether saturated with an aqueous solution of $MgCl_2$ and LiCl instead of using the same solvent saturated with water. The same system, with the use of $MgCl_2$ as an additive, was used for lipase-catalysed hydrolysis of hydroxyphosphonates.[8] Bashkar Rao et al.[9] used 2% solution of nonionic surfactant Triton X-100, which might be considered as an open-chain crown ether analogue, as an additive to increase enantioselectivity of the *Candida cylindracea* lipase. It is also known that basic additives such as triethylamine have influenced enantioselectivity and/or activity of these enzymes in transesterification reactions.[6]

In this article, we report a method to engineer the environment of the lipase-catalysed reactions in order to improve enantioselectivity and conversion of the applied substrates containing two centers of chirality—one located at the phosphorus atom and the second at α-carbon atom of the molecules. The goal was reached by an application of two approaches. The first approach was the esterification in diisopropyl ether containing NEt_3 or pyridine as basic additives. The second approach was hydrolysis of ethyl butyryloxyalkane(*P*-phenyl)phosphinates either in diisopropyl ether saturated with water or with aqueous solutions of $MgCl_2$, LiCl, or Triton X-100 or using biphasic diisopropyl ether/aqueous system.

RESULTS AND DISCUSSION

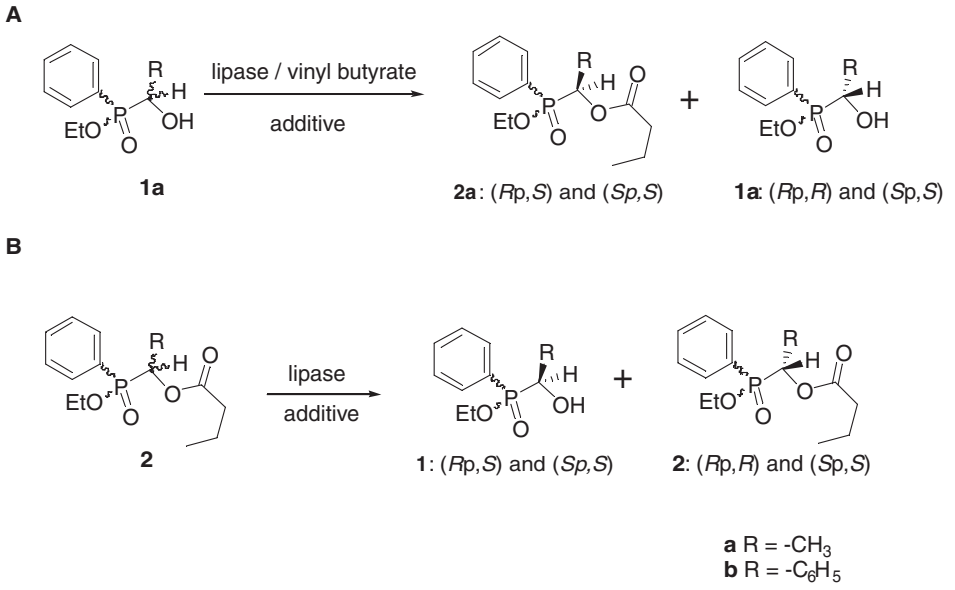
Enzymatic Transesterifications

Racemic hydroxyphosphinate (compound **1a**) was obtained by addition of ethyl phenylphosphinite to the appropriate aldehyde.[10] The kinetic resolution of this compound by transesterification with lipases from *Candida cylindracea*, *Aspergillus niger*, and *Mucor javanicus* in organic solvents has been already described.[11] The observed enantioselectivity and degree of conversion was quite good but not excellent. In this report, we have studied how the application of additives such as pyridine and triethylamine affect the course of this reaction (Scheme 1A). As seen in Table I, addition of triethylamine results in enhancement of enantioselectivity of the reaction catalyzed by all three lipases, with the addition of pyridine having a far less pronounced effect.

Enzymatic Hydrolysis

In order to study the hydrolytic procedure, racemic hydroxyphosphinates **1** were converted into their *O*-butyryl derivatives **2** by simple acylation with butyryl chloride.[11] The esters **2** were then hydrolyzed using *Candida cylindracea* lipase. The kinetic resolutions of compounds **2** (Scheme 1B) were carried out in two solvent systems—either in diisopropyl ether saturated with water or aqueous solutions of $MgCl_2$, LiCl, or Triton X-100 or in analogous biphasic system. The hydrolysis was stopped when chemical yield reached a value close to 50% (which is typical for kinetic resolution). As shown in Table II, all the reactions carried out in a homogenous system resulted in low enatioselectivity of the reaction, which disqualifies this procedure.

Therefore we decided to use a biphasic system composed of the same organic and water fractions, namely water or aqueous solution of $MgCl_2$, LiCl, or Triton X-100 and diisopropyl ether in a volume ratio of 1:2. As seen from Table III, this approach resulted in increasing enantioselectivities of the hydrolysis of compound **2b** and did not affect either



Scheme 1

the rate of hydrolysis or enantioselectivity of hydrolysis of **2a**. The application of Triton X-100 also resulted in significant increase of the conversion of compound **2b** without harmful effect on the enantioselectivity of the process, which still is low.

Solvent engineering belongs to the classic tools for the improvement of the course of biocatalytic processes. Esterification of 1-hydroxyethane(*P*-phenyl)phosphinate **1a** in diisopropyl ether under molecular sieves (3Å mesh) proceeds with satisfactory enantioselectivity.[11] Using the same system enriched with triethylamine resulted in improved enatioselectivity of this reaction. It is worth mentioning that this biocatalytic process

Table I Enzymatic esterification of ethyl 1-hydroxyethane(*P*-phenyl)phosphinate **1a** by lipases from different sources

Lipase	Additive	Enzyme amount (mg)	Time (h)	Conversion (%)		ee of ester (%)	
				(S_P,*R*) (R_P,*S*)	(R_P,*R*) (S_P,*S*)	(R_P,*S*)	(S_P,*S*)
CCL	— *	200	72	44	42	91	79
	NEt_3	**200**	**72**	**40**	**46**	**95**	**90**
	pyridine	200	72	39	41	79	85
ANL	— *	200	168	44	44	91	>98
	NEt_3	**200**	**72**	**43**	**43**	**93**	**>98**
	pyridine	200	72	53	50	89	>98
MJL	— *	20	96	48	46	38	51
	NEt_3	**20**	**72**	**45**	**50**	**79**	**>98**
	pyridine	20	72	43	43	37	47

Reactions were carried out in diisopropyl ether with addition of triethylamine or pyridine (0.023 mmol of substrate, 0.023 mmol of additive) and compared with esterification carried out without additive as described previously.[11]

*Data described previously in publication.[11]

Table II Enzymatic hydrolysis of ethyl 1-butyryloxyethane(*P*-phenyl)phosphinate **2a** and 1-butyryloxyphenyl methane(*P*-phenyl)phosphinate **2b** in organic solvent

			Conversion (%)		ee of alcohol (%)	
Substrate	Additive	Time (h)	(R_P,R); (S_P,S)	(R_P,S); (S_P,R)	(S_P,S)	(R_P,S)
2a	—	2	37	63	18	50
	$MgCl_2$	2	37	65	21	46
	LiCl	2	33	61	16	48
	Triton X-100	2	37	63	15	45
2b	—	168	48	34	28	18
	$MgCl_2$	168	24	20	11	11
	LiCl	168	21	20	18	11
	Triton X-100	168	29	23	25	14

Reactions were carried out in diisopropyl ether saturated with water or aqueous solution of LiCl, $MgCl_2$, or Triton X-100 (100 mg of *Candida cylindracea* lipase, 0.036 mmol of substrate, 15 μL water or aqueous solution, 3 mL diisopropyl ether).

did not recognize chirality at the phosphorus atom. The esterification of the second substrate—hydroxy(phenyl)methane(*P*-phenyl)phosphinate **1b**—did not proceed despite of the solvent used. Hydrolysis of its acylated derivative is an alternative approach.

Hydrolysis of butyryloxyphosphinates **2** was carried out using *Candida cylindracea* lipase, and two solvent systems indicated that the biphasic system, rather than the organic solvent saturated with water, gives more reliable results. The biphasic system supplemented with surfactant such as Triton X-100 appeared to be the most effective. Quite interestingly, in this case better resolutions were obtained for compound **2b**, which shows that the two reactions are complementary. However, also in this case, chirality of the phosphorus atom has no influence on the discrimination by the enzyme.

Table III Enzymatic hydrolysis of ethyl 1-butyryloxyethane(*P*-phenyl)phosphinate **2a** and 1-butyryloxyphenyl methane(*P*-phenyl)phosphinate **2a** in biphasic system

			Conversion (%)		ee of alcohol (%)	
Substrate	Additive	Time (h)	(R_P,R); (S_P,S)	(R_P,S); (S_P,R)	(S_P,S)	(R_P,S)
2a	—	1	44	60	13	44
	$MgCl_2$	1	39	67	16	46
	LiCl	1	40	64	20	28
	Triton X-100	1	47	61	29	58
2b	—	48	59	59	64	49
	$MgCl_2$	48	35	30	67	38
	LiCl	48	40	37	70	46
	Triton X-100	**48**	**71**	**67**	30	34

Reactions were carried out in a modified biphasic system consisting of diisopropyl ether and water or aqueous solutions of LiCl, $MgCl_2$, or Triton X-100 (20 mg of *Candida cylindracea* lipase, 0.036 mmol of substrate, 1 mL water or aqueous solution, 2 mL diisopropyl ether).

EXPERIMENTAL

All materials were purchased from commercial suppliers: Sigma, Aldrich, Fluka, POCh, and Serva, and were used without purification. Details for lipase purchases are as follows: *Candida cylindracea*—CLA (Sigma), *Aspergillus niger*—ANL (Fluka), and *Mucor javanicus*—MJL (Fluka). All compounds were purified by gradient column chromatography using Merck Silica Gel 60 (63–230 mesh) or by HPLC (Varian, Dynamax HPLC Column 250 × 21.4 mm; MICROSORB 300–10 C18). The enantiomeric excess (ee) was measured by ^{31}P NMR with quinine used as a chiral solvating agent or by HPLC with chiral column CHIRALPAK AD. Absolute configuration of hydroxyphosphinates **1** was determined and described previously.[11,12]

Synthesis of Ethyl Hydroxyalkane(*P*-phenyl)phosphinates 1 and Ethyl Butyryloxy Alkane(*P*-phenyl)phosphinates 2

Compounds **1** and **2** were synthesized and characterized previously.[11,12]

Enzymatic Transesterification in Modified Organic Solvent

Enzymatic esterification of ethyl 1-hydroxyethane(*P*-phenyl)phosphinate **1a** was carried out in diisopropyl ether (2 mL) with the addition of powdered molecular sieves (20 mg, 3Å mesh). Triethylamine or pyridine (0.023 mmol), substrate (0.023 mmol), suitable lipase (20 or 200 mg), and vinyl butyrate (0.165 mmol) were added into the reaction mixture (see Table III). The reaction was carried out at 36°C in a shaker (150 rpm) and stopped when conversion was close to 50% by filtration of biocatalyst followed by evaporation of organic layer. The resulting product was purified by HPLC (C-18 column, gradient: 40% acetonitrile in water to 70% acetonitrile in water, retention time of **2a**: 11.2 min) and analyzed by HPLC (CHIRALPAK AD, Diacel, 10% 2-propanol in n-hexane, retention time: (S_P,*S*): 7.6 min; (R_P,*R*): 8.2 min; (R_P,*S*): 8.5 min, and (S_P,*R*): 9.7 min).

Enzymatic Hydrolysis Reactions in Diisopropyl Ether Saturated with Aqueous Solutions

Distilled water (15 μL) or aqueous solution (15 μL) of LiCl (2.4 M), $MgCl_2$ (1.2 M), or Triton X-100 (2%) was added to diisopropyl ether (2 mL) and mixed. After addition of substrate **2** (0.2 mmol) and *Candida cylindracea* lipase (100 mg), the enzymatic reaction was carried out at room temperature with shaking (150 rpm). The reaction was stopped when the conversion was close to 50% or after 168 h by filtration of catalyst and addition of ethyl acetate (15 mL). Drying of the organic solvent over anhydrous magnesium sulfate, filtration, and removal of the solvent by evaporation yielded the desired hydroxyphosphinate, which was analyzed with ^{31}P NMR using quinine as a chiral discriminator.

Enzymatic Hydrolysis Reactions in Modified Biphasic Medium

The biphasic system (3 mL) was composed of diisopropyl ether (2 mL) and water or aqueous solution (1 mL) of LiCl (2.4 M), MgCl2 (1.2 M), or Triton X-100 (2%). After addition of substrate (0.2 mmol) and *Candida cylindracea* lipase (100 mg), reactions were carried out at room temperature with shaking (150 rpm). The reaction was stopped after

certain intervals of time, and the product was extracted twice with ethyl acetate (15 mL), and the organic phase was dried over anhydrous magnesium sulfate. After filtration, the organic solvent was removed by evaporation, and the obtained hydroxyphosphinate was analyzed with ^{31}P NMR using quinine as a chiral discriminator.

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