Tetrahedron Letters, Vol. 33, No. 47, pp. 7157-7160, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

DRASTIC SOLVENT EFFECT ON LIPASE-CATALYZED ENANTIOSELECTIVE HYDROLYSIS OF PROCHIRAL 1,4-DIHYDROPYRIDINES

Yoshihiko Hirose*, Kinya Kariya, Ikuharu Sasaki, Yoshiaki Kurono, Hirosato Ebiike† and Kazuo Achiwa†*

Central Research Laboratory, Amano Pharmaceutical Co., Ltd.
51 Nishishiroyashiki , Kunotsubo, Nishiharu, Nishikasugai, Aichi 481, Japan

† School of Pharmaceutical Sciences, University of Shizuoka
395 Yada, Shizuoka 422, Japan

Abstract: Two enantiomers of 1,4-dihydropyridine compounds have been obtained with high enantiomeric purity by lipase-catalyzed hydrolysis in organic solvent saturated with water. Enantioselectivity of this reaction is dependent on the solvent used.

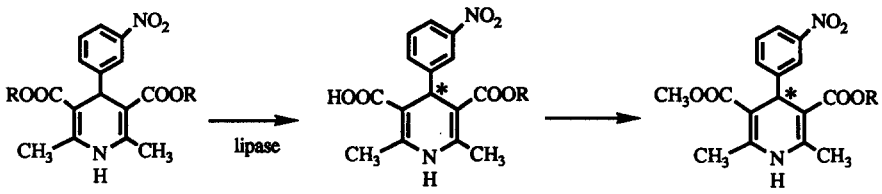
Lipases have been widely used for the synthesis of optically active alcohols and carboxylic acids via enantioselective hydrolysis of the corresponding esters in aqueous solution or via enantioselective esterification and transesterification in organic solvents. There are many advantages to employ enzymes in organic solvent rather than in aqueous media to catalyze these organic reactions. Therefore, many enzymatic reactions have been carried out in organic solvents.[1] It has been reported that the substrate specificity and the enantioselectivity of enzymes were affected by the solvent used,[2] but this effect is not so clear at present. Klibanov *et al* reported that enantioselectivity was correlated with basic physiochemical characteristics of the solvents such as hydrophobicity, dipole moment and dielectric constant.[2a] Two other groups reported that enantioselectivity was affected by the content of water in the solvent.[2d,e] Nakamura *et al* showed that enantioselectivity of lipase-catalyzed transesterification was affected by the solvent structure such as cyclic and acyclic forms.[2g] Recently, Klibanov *et al* reported that a complete reversal of enzyme enantioselectivity was observed in transesterification catalysed by *Aspergillus oryzae* protease upon transition from hydrophilic to hydrophobic solvents.[3]

In order to investigate the solvent effect, we carried out the enzymatic hydrolyses of prochiral substrates by two lipases in various organic solvents containing some water. We used bis (acyloxymethyl)-1,4-dihydro-3,5-pyridine dicarboxylates as prochiral substrates.[4] Diisopropyl ether and cyclohexane saturated with water were used as typical organic solvents.

4-Aryl-1,4-dihydro-2,6-dimethyl-3,5-pyridine dicarboxylates have been widely used for the treatment of cardiovascular diseases since 1975. There are more than 50 derivatives under pharmacological and clinical development and some of them have already been employed therapeutically. Different substituents in these compounds lead to the chiral derivatives possessing an asymmetric carbon at 4-position and the two enantiomers

have been reported to show different biological activities.[5] Although most of these compounds have been developed and employed as racemates, mepirodipine[5b] was recently employed as a novel chiral cardiovascular drug.

With a view to obtaining optically active compounds effectively, we focused on the chemoenzymatic method shown below.[4] First, we carried out the enzymatic hydrolysis of prochiral substrate (**1a**) with lipase AH (*Pseudomonas sp.*) in diisopropyl ether saturated with water. The product (**2a**) was obtained in a good yield (87%) and its enantiomeric excess was more than 99%. The absolute configuration of the monoester (**2a**) was determined to be (S)-form.[6]



1a-e

a.R=CH_2OCOt-Bu (POM)
b.R=CH_2OCOi-Pr (IBOM)
c.R=CH_2OCOPr (BOM)
d.R=$CH_2OCOC_2H_5$ (PROM)
e.R=CH_2OCOCH_3 (ACOM)

(S) and (R)-2a-e

a.R=POM
b.R=IBOM
c.R=BOM
d.R=PROM
e.R=ACOM

(R) and (S)-3a-f

a.R=POM
b.R=IBOM
c.R=BOM
d.R=PROM
e.R=ACOM
f.R=H

Scheme 1

Next, the reaction was carried out in cyclohexane saturated with water by using the same lipase to afford the hydrolyzed product in a good yield (88%). Surprisingly, the absolute configuration of the monoester (**2a**) was (R), its ee being as high as 89%.

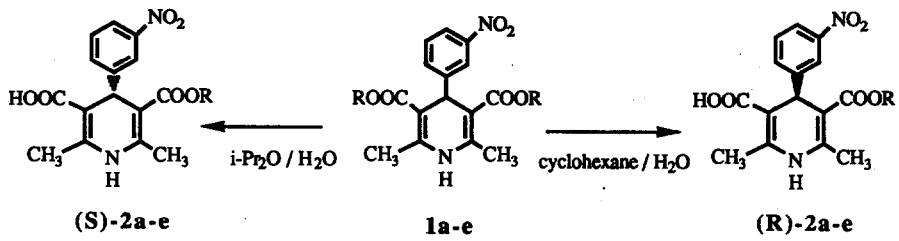
Table 1 Lipase AH-Catalyzed Enantioselective Hydrolyses [7]

Substrate	i-Pr_2O saturated with H_2O				Cyclohexane saturated with H_2O			
	reac.time (hr)	yield (%)	ee (%)	config.of 2	reac.time (hr)	yield (%)	ee (%)	config. of 2
1a	48	87	>99.0	S	48	88	88.8	R
1b	24	83	89.0	S	48	71	91.0	R
1c	24	71	88.9	S	48	62	91.1	R
1d	1	83	68.1	S	17	57	91.4	R
1e	5	77	42.3	S	17	32	88.2	R

This means that different enantiomers can be obtained by simply changing the solvent. As mentioned before, Klibanov *et al* recently reported a complete reversal of enzyme enantioselectivity by changing the solvent for the resolution of a racemic substrate.[3] Herein we report, for the first time, the same phenomena using

prochiral compounds as the substrate for asymmetric synthesis. This is a practical method of the enzymatic reaction.

In order to clarify the generality of this striking solvent effect, we applied this reaction to other prochiral acyloxymethylesters (**1b-e**). In the case of diisopropyl ether, lipase AH gave (S)-form monoesters ((**S**)-**2b-e**) in good yield (about 80%), with enantiomeric excess of 89% to 42%. On the contrary, when cyclohexane was used as solvent, lipase AH gave (R)-monoesters ((**R**)-**2b-e**) of about 90%ee in moderate yields (71% to 32%). Results are shown in Scheme 2 and Table 1.



(S)-2a-e　　1a-e　　(R)-2a-e

Scheme 2 Lipase AH-Catalyzed Enantioselective Hydrolyses in Organic Solvents Saturated with Water

Our second trials were carried out in the same organic solvents used in the first experiments by using lipase PS (*Pseudomonas cepacia)* to observe no inversion. In both media, the absolute configurations of the monoesters (**2a-e**) were uniformly (R) with about 95%ee. Results are shown in Table 2.

Table 2 Lipase PS-Catalyzed Enantioselective Hydrolyses[7]

Substrate	i-Pr_2O saturated with H_2O				Cyclohexane saturated with H_2O			
	reac.time (hr)	yield (%)	ee (%)	config.of 2	reac.time (hr)	yield (%)	ee (%)	config. of 2
1a	200	8	73.5	R	200	23	97.0	R
1b	20	34	86.0	R	48	32	99.0	R
1c	20	91	96.0	R	48	29	99.0	R
1d	24	86	99.0	R	72	31	92.0	R
1e	24	87	>99.0	R	72	28	>99.0	R

The reversal of enantioselectivity was only observed in lipase AH-catalyzed hydrolysis in organic solvent saturated with water. This phenomenon can not be explained on the basis of the hydrophobicity of the solvent and might be the interaction between this enzyme and the solvent saturated with water.[8]

REFERENCES AND NOTES

1 a) Jones, J. B. *Tetrahedron* **1986**, 42, 3351. b) Whitesides, G. M.; Wong, C-H. *Angew. Chem. Int. Ed. Engl.* **1985**, 24, 617. c) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, 11, 194. d) Klibanov, A. M. *Acc. Chem. Res.* **1990**, 23, 114. e) Chen, C-S.; Sih, C. J. *Angew. Chem. Int. Ed. Engl.* **1989**, 28, 695. f) Ohno, M.; Otsuka, M. *Organic Reactions* ; Wiley : New York, **1989**, 37, 1. g) Boland, W.; Frößl, C.; Lorenz, M. *Synthesis* **1991**, 1049.

2. a) Fitzpatrick, P. A.; Klibanov, A.M. *J. Am. Chem. Soc.* **1991**, 113, 3166. b) Sakurai, T.; Margolin, A. L.; Russell, A. J.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, 110, 7236. c) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, 111, 3094. d)Stokes, T. M.; Oehlschlager, A. C. *Tetrahedron Lett.* **1987**, 28, 2091. e) Kitaguchi, H.; Itoh, I.; Ono, M. *Chem. Lett.* **1990**, 1203. f) Kanerva, L. T.; Vihanto, J.; Halme, M. H.; Loponen, J. M.; Euranto, E. K. *Acta Chem. Scand.* **1990**, 44, 1032. g) Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, 32, 4941.

3. Tawaki, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1992**, 114, 1882.

4. a) Ebiike, H.; Terao, Y.; Achiwa, K. *Tetrahedron Lett.* **1991**, 32, 5805. b) Ebiike, H.; Maruyama, K; Achiwa, K. *Chem. Pharm. Bull.* **1992**, 40, 1083.

5. a) Goldmann, S.; Stoltefuss, J. *Angew. Chem. Int. Ed. Engl.* **1991**, 30, 1559. and references cited therein. b) Tamazawa, K.; Arima, H.; Kojima, T.; Isomura, Y.; Okada, M.; Fujita, S.; Furuya, T.; Takenaka, T.; Inagaki, O.; Terai, M. *J. Med. Chem.* **1986**, 29, 2504. c) Holdgrün, X. K.; Sih, C. J. *Tetrahedron Lett.* **1991**, 32, 3465.

6. The absolute configuration of (**2a**) was determined based on the sign of optical rotation of (**3f**) according to reference 4.

7. a) All reactions were carried out at 20°C by stirring a mixture of a substrate (25mg), lipase (25mg) and organic solvent (1ml) saturated with water. b) Organic solvents were saturated with water at 20°C and lipases were lyophilized for 2 days and kept in a refrigerator. c) The chemical yields were measured by HPLC on an ODS column (CH_3CN/H_2O) and the optical yields were determined by HPLC on a Chiralcel AD (Daicel) column (EtOH/ hexane) after conversion to (**3a-e**).

8. Lipase AH showed no enantioselectivity for this hydrolysis in various alcohols containing some water. For instance, the hydrolysis carried out in BuOH-H_2O (10:1) gave the monoester (**2a**) of 7%ee in a moderate yield.

(Received in Japan 10 July 1992)