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LIPASE-CATALYZED IRREVERSIBLE TRANSESTERIFICATION USING ENOL ESTERS: XAD-8 IMMOBILIZED LIPOPROTEIN LIPASE-CATALYZED RESOLUTION OF SECONDARY ALCOHOLS

Shu-Hui Hsu, Shihn-Sheng Wu, and Yi-Fong Wang *
School of Pharmacy, Kaohsiung Medical College,
Kaohsiung city 80708 Taiwan, R. O. C.

Chi-Huey Wong
Department of Chemistry, The Research Institute of
Scripps Clinic, La Jolla, CA 92037

Summary: Procedures for the preparation of XAD-8 immobilized lipoprotein lipase and the resolution of secondary alcohols of synthetic value in organic solvents using this immobilized enzyme have been developed.

Lipase-catalyzed reaction in organic solvents is becoming increasingly important in enantioselective synthetic chemistry[1]. Certain reactions which are sensitive to water can be effectively carried out in organic media. Recently, we have used this procedure to prepare several chiral compounds which are not optically stable in water[2]. There are several advantages of performing biocatalytic reaction in organic media: 1) Most organic substrates have better solubility in organic solvents than in water, making the reaction proceed smoothly; 2) Compounds which are sensitive to water may be prepared via this process; 3) Undesired hydrolysis reaction of substrates containing ester group(s) or other groups sensitive to enzymatic hydrolysis can be reduced[3] ; 4) Recovery of products is easier; 5) Enzymes can be easily recovered for reuse, and 6) The enantioselectivity in organic solvents is often higher than that of the corresponding hydrolytic reaction in water[1a] .

In spite of the advantages of enzymatic reaction in organic solvents, there are some disadvantages. Two of the potential drawbacks in these processes are the slow reaction rate and the decrease of optical purity of the desired product due to the reversible nature of the reaction. To overcome the latter problem, we have developed an irreversible procedure for the resolution of alcohol compounds using enol esters as acylating reagents and lipase as catalyst[2a] . This process has proven to be more effective and more enantioselective than other transesterification processes. On the other hand, the former problem remains unsolved. In our previous experiment, we found that the ratio of reaction rate in organic solvent to that in water was about 1 : >100 [2b] . Furthermore, the reaction rate of the transesterification of seconary alcohols in organic solvets was extremely slow . For example, the conversions of alcohols **1a**, **4a**, **5a**, and **6a**, were only 2%, 3%, 7%, and <1% , respectively, after a 5-day reaction in organic solvents using vinyl acetate as acylating reagent and lipoprotein lipase as catalyst (substrate (mmol) : lipoprotein lipase (mg) = 1 : 25). This slow reaction rate limited the application of this process for the resolution of seconary alcohols.

Recently, we reported that the reaction rate of XAD-8 immobilized enzyme catalyzed hydrolysis of **6b** was 2.4 time faster than that of soluble enzyme in aqueous media[4]. We then used this XAD-8 immobilized enzyme

as catalyst to examine the resolution of bulky secondary alcohol **6a** using vinyl acetate as acylating reagent in tert-butyl methyl ether. Surprisingly, the reaction rate was found to be >200 times faster than that of unimmobilized enzyme. This immobilized enzyme was then used as catalyst to perform the resolution of several secondary alcohols of synthetic value in organic media using vinyl acetate as acylating reagent. Here we report the results.

The XAD-8 immobilized enzyme was prepared as following: 1 g of lipoprotein lipase from Pseudomonas species (LPL, from Amano company) was dissolved in 100 mL of 0.05M, pH 7.0, phosphate buffer. 100 g of XAD-8 (from Sigma) was then added and the resulting suspension was stirred for 16 h at 8 °C. Most of the buffer was removed by pipet, the residue was dried over vacuum pump (rt 24 h) to give 40.4 g of dried XAD-8 immobilized enzyme.

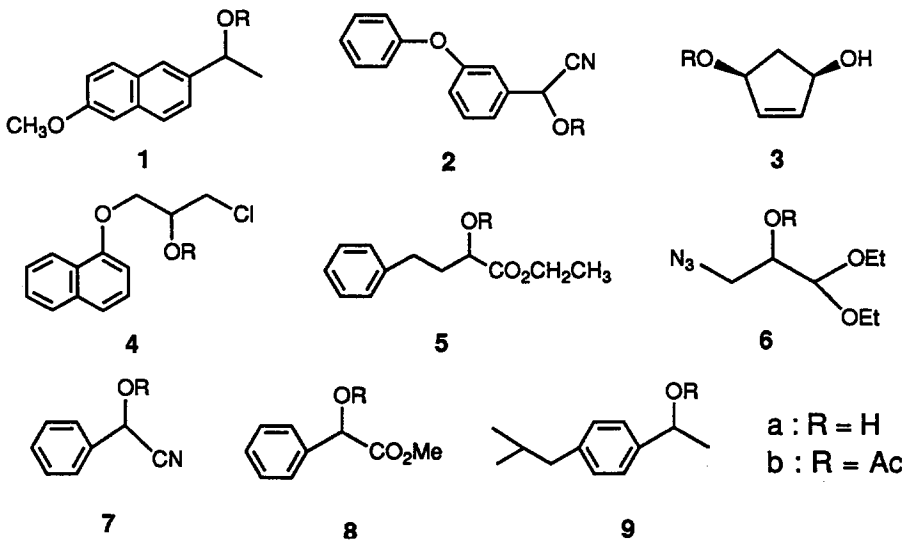


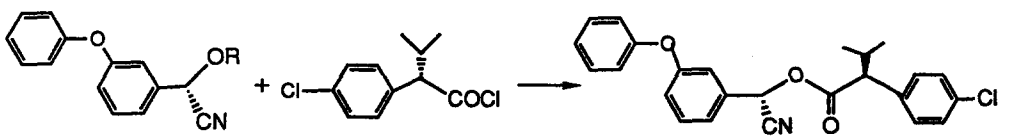
Table 1 Eantioselective transesterification of secondary alcohols **1a-9a** catalyzed by XAD-8 immobilized lipoprotein lipase from Pseudomonas species

Compound	Reaction Time, h	Extent of [a] Conversion, %	Optical Purity (%) [b] Acetate	Alcohol	Stereochemical Preference	E [c]	Ref.
1a	8	49	93	88	R	78	5
2a	57	41	90	63	S	37	6
3a	0.7	68	98		S		7
4a	50	29	94		S	45	
4a	144	56		>98	S		
5a	40	48	>98	89	S	188	2c
5a	52	51	94	>98	S		2c
6a	106	52	90	>98	R	77	4
7a	7	56	76	95	S	27	
8a	129	53	82	93	S	31	
9a	14	52	89	96	R	73	

a : The ratio of reaction rate of immobilized and unimmobilized enzyme was determined to be 1 : 20->200.
b : For the determination of optical purity see ref. 13.
c : E is the ratio of the specificity constants of the two enantiomers [14].

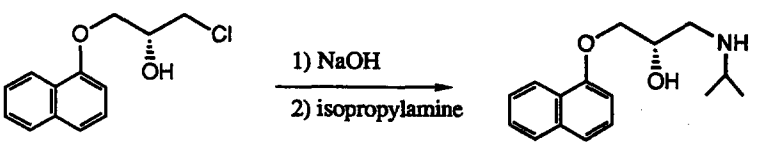
To perform the resolution of alcohols **1a~9a,** the solution of substrate (4 mmol) and vinyl acetate (16 mmol) in 16 mL of tert-butyl methyl ether or dichloromethane was mixed with 4.0 g of XAD-8 immobilized enzyme. The resulting suspension was stirred gently at 28 °C. The immobilized enzyme was then filtered off and the products were purified and analyzed. The results were summarized in Table 1. As shown in table 1, this enzyme showed high enantioselectivity to compounds **1a-9a** and the reaction rate of XAD-8 immobilized enzyme was 20~>200 times faster than that of the unimmobilized. The reasons for higher reaction rate of the XAD-8 immobilized enzyme may be the following : 1)The enzyme participated in the transesterification reaction was much more than that in the unimmobilized condition due to its homogeneous distribution on the surface of XAD-8 ; 2) XAD-8 itself may absorb the substrate to increase the contact with enzyme, thereby increase the reaction rate.

The cyanohydrin acetate (S)-**2b** has the appropriate stereochemistry for the synthesis of fenvalerate Aα, **10,** which is an insecticide with outstanding insecticidal activity, moderate mammalian toxicity and adequate stability in the field [8] . (S)-**2b** (ee=90%) was transformed to **10** by hydrolysis with the same enzyme first (conversion=82%), the resulting alcohol (ee=96%) was then reacted with (S)-2-(4-chlorophenyl)-3-methyl - butanoyl chloride[9] in the presence of pyridine. Chiral (R)-**4a** is useful for the synthesis of β -adrenergic blocking agents[10] such as (S)-propranolol ,**11.** Chiral (R)-**5a** is useful for the synthesis of Angiotensin-converting enzyme inhibitors such as enalepril ,**12,** for the treatment of hypertension and congestine heart failure.[11] The synthesis of (S)-propranolol was achieved by treatment of R-**4a**(ee >98%) with sodium



(S)-**2b** R = Ac
(S)-**2a** R = H

10



(R)-**4a**

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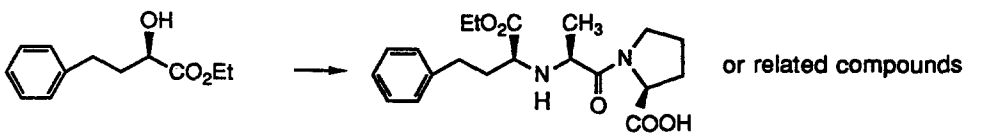

(R)-**5a**

12

hydroxide in ethanol followed by reaction of the resulted epoxide with excess isopropylamine. A single recrystallization of (S)-propranolol as its hydrochloride from methanol-ethyl acetate gave optically pure (S)-

propranolol hydrochloride; $[\alpha]_D^{25}$ -25.8° (c=1.0, EtOH). [lit[10] $[\alpha]_D^{21}$ -25.5° (c=1.01, EtOH)].The antipodes (S)-**4b** and (S)-**5b** were also obtained in high optical purity by terminating the reaction in 29% and 48% conversion, respectively. (S)-**4b** and (S)-**5b** were inverted to the desired (R)-**4a** and (R)-**5a**, respectively, by reaction with a catalytic amount of sodium ethoxide in ethanol followed by treatment of the resulting alcohols with diethyl azodicarboxylate, triphenylphosphine, and formic acid [12] and hydrolysis of the formate ester intermediate.

In summary, the high reaction rate makes this XAD-8 immobilized enzyme as a useful catalyst for the preparation of chiral secondary alcohols or esters which are difficult to prepare based on the unimmobilized enzyme.

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References and Notes

1. For reviews in the field see: a) Klibanov, A. M. *Trends Biochem. Sci.* **14**, 141 (1989); b)Wong, C-H. *Science*, **244**, 1145 (1989); c) Chen, C.-S.; and Sih, C. J. *Angew. Chem. Int. Ed. Engl.*; **28**, 695 (1989).
2. a) Wang, Y.-F.; and Wong, C.-H. *J. Org. Chem.*, **53**, 3127 (1988); b) Wang, Y.-F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; and Wong, C.-H. *J. Am. Chem. Soc.*, **110**, 7200 (1988); c) Wang, Y.-F.; Chen,S.-T.; Liu, K. K.-C.; and Wong, C.-H. *Tetrahedron Lett.*, **30**, 1917 (1989).
3. Babiak, K. A.; Ng, J. S.; Dygos, J. H.; Weyker, C. L.; Wang, Y.-F.; Wong, C.-H. *J. Org. Chem.*, **55**, 3377 (1990).
4. Osten, C. H.; Sinskey, A. J.; Barbas, C. F.; Peterson, R. L.; Wang, Y.-F. and Wong, C.-H. *J. Am. Chem. Soc.*, **111**, 3924 (1989).
5. Hiyama, T.; and Inoue, M. *Synthesis*, 689 (1986).
6. Hirohara, H.; Mitsuda, S.; Ando, E.; and Komaki, R. in "*Biocatalysts in Organic Syntheses* " edited byTramper, J.; Plas, H. C.; and Linko, P., P 119-135, Elsevier, 1986.
7. Theil, F.; Ballschuh, S.; Schick, H.; Haupt, M.; Hafner, B.; and Schwarz, S. *Synthests*, 540 (1988) and references cited therein.
8. Ohno, N.; Fujimoto, K.; Okuno, Y.; Mizutani, T.; Hirano, M.; Itaya, N.; Honda, T.; and Yoshioka, H. *Pestic. Sci.*, **7**, 241 (1976).
9. a) Miyakado, M.; Ohno, N.; Okuno, Y.; Hirano, M.; Fujimoto, K.; and Yoshioka, H. *Agr. Biol.* Chem., **39**, 267 (1975); b) Miyakado, M.; Ohno, N.; Horiuchi, A. *Japan Kokai*, 75 25,544 (1975).
10 a) Howe, R.; and Shanks R. G. *Nature*, **210**, 1336 (1966); b) Howe, R.; and Rao, B. S. *J. Med. Chem.*, **11**, 1118 (1968).
11. a) Urbach, H.; AND Henning, R. *Tetrahedron Lett.*, **25**, 1143 (1984); b) Attwood, M. R.; Hassall,C. H.; Krohn, A.; Lawton, G. T.; and Redshaw, S. *J. Chem. Soc., Perkin Trans.1*, 1011 (1986); c) Flynn, G. A.; Giroux, E. L.; and Dage, R. C. *J. Am. Chem. Soc.*, **109**, 7914 (1987); d) Yanagisawa, H.; Ishihara, S.; Ando, A.; Kanazaki, T.; Miyamota, S.; Koike, H.; Iijima, Y.; Oizumi, K.; Matsushita, Y.; and Hata, T. *J. Med. Chem.*, **30**, 1984 (1987).
12. Mitsunobu, O.; and Eguchi, M. *Bull. Chem. Soc. Japan*, **44**, 3427 (1971).
13.The optical purity of **1-9** was determined as following: a) Compounds **2b, 3b, 6b, 7b, 8b,** and **9b** were directly analyzed with ^{1}H-NMR spectroscopy in the presence of Tris[3-heptafluoropropylhydroxymethylene)-(+)-camphorato]-europium(III) (Eu(hfc)$_3$). **2a** and **7a** were acylated to their corresponding acetates (Ac_2O/pyridine) and analyzed with the same procedures as above. b) **4a, 6a, 8a, and 9a** were transformed to their corresponding (+)-2-methoxy-2-(trifluoromethyl)phenylacetyl (MTPA) derivatives and then analyzed with 1H-NMR spectroscopy. c) **1a, 1b** and **5b** were analyzed with HPLC using Chiralcel OC column (Daicel Chemical Industries) as stationary phase (solvent : n-hexane/isopropanol = 99:1-99:3). **5a** was transformed to **5b** and then analyzed with the same procedure as above.
14. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G; and Sih, C. J. *J. Am. Chem. Soc.*, **104**, 7294 (1982)

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