ENZYMATIC HYDROLYSIS IN ORGANIC SOLVENTS FOR KINETIC RESOLUTION OF WATER-INSOLUBLE α -ACYLOXY ESTERS WITH IMMOBILIZED LIPASES

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Asymmetric hydrolysis of water-insoluble α -acyloxy esters, $(\pm)$ -**5** and $(\pm)$ -**6**, was carried out using lipases immobilized with celite or synthetic prepolymer (ENTP-4000 or ENT-4000) in water-saturated organic solvent to produce chiral intermediates, (2S,3R)-**3** and (2S,3S)-**4**, for the synthesis of (-)-indolmycin **1** and diltiazem hydrochloride **2**, respectively.

KEYWORDS asymmetric hydrolysis; water-insoluble substrate; α -acyloxy ester; immobilized lipase; (-)-indolmycin; diltiazem hydrochloride;

When substrates are highly lipophilic or essentially insoluble in water, enzymatic reactions often may not proceed at all. This is the main drawback of the enzymatic process. This difficulty may be overcome if the aqueous medium can be replaced by an organic solvent. However, in such cases, the enzymes should be protected from denaturation by the organic solvent. Immobilization would serve effectively for this purpose. We now report the asymmetric hydrolysis of the water-insoluble α -acyloxy esters $(\pm)$ -**5** and $(\pm)$ -**6** with immobilized lipase in water-saturated organic solvents giving (2S,3R)-indolmycenic ester **3** and (2S,3S)- α -hydroxy ester **4**, intermediates for the synthesis of medicinally active (-)-indolmycin **1** or diltiazem hydrochloride **2**, respectively.

It is advantageous to synthesize (2S,3R)-**3**¹⁾ by the direct asymmetric hydrolysis of the readily obtainable $(\pm)$ -**5**.²⁾ Initially, asymmetric hydrolysis of $(\pm)$ -**5** with several lipases was attempted in a phosphate buffer, but the reaction did not proceed at all, which may be attributable to $(\pm)$ -**5** being a water immiscible hard oil. Thus, $(\pm)$ -**5** was dissolved in a mixture of water-saturated isooctane-benzene (5:1)³⁾ and the solution was exposed to the enzymatic reaction using lipases immobilized with celite⁴⁾ or photo-crosslinkable resin prepolymer (ENTP-4000).⁵⁾ The following six lipases were used: "Amano A" and "Amano A-6" (from *Aspergillus niger*), "MY-30", "C. C. Sigma", and "OF-360" (from *Candida cylindracea*), and Saiken "Lilipase" (from *Rhizopus japonicus*). The reaction did yield the desired (2S,3R)-**3**.⁶⁾ The results were shown in Table I. In every case, the hydrolyzed product was found to be (2S,3R)-**3**.⁷⁾ Among six lipases used, "OF-360" gave the best results producing (2S,3R)-**3** with reasonably high optical purity (91-93% ee). When celite was replaced by ENTP-4000, the chemical yield of (2S,3R)-**3** was increased appreciably (from 28% to 37-40%). It should be noted that the preliminary data in entry 7 showed that lipases immobilized with ENTP-4000 fully retained its activity after the reaction had been achieved.

Table I

Entry	Lipase	Time	Product (%)	Optical purity (% ee)
1	"Amano A-6"/celite	46 h	(2S,3R)- <u>3</u> (41) (2R,3S)- <u>5</u> (56), (2S,3R)- <u>3</u> (45) (2R,3S)- <u>5</u> (36)	
2	"MY-30"/celite	75 h	" - <u>3</u> (36) " - <u>5</u> (54), " - <u>3</u> (34) " - <u>5</u> (32)	
3	"Lilipase"/celite	216 h	" - <u>3</u> (10) " - <u>5</u> (81), " - <u>3</u> (84) " - <u>5</u> (17)	
4	"Amano A"/celite	48 h	" - <u>3</u> (21) " - <u>5</u> (72), " - <u>3</u> (35) " - <u>5</u> (19)	
5	"C. C. Sigma"/celite	48 h	" - <u>3</u> (29) " - <u>5</u> (68), " - <u>3</u> (38) " - <u>5</u> (19)	
6	"OF-360"/celite	24 h	" - <u>3</u> (28) " - <u>5</u> (61), " - <u>3</u> (93) " - <u>5</u> (48)	
7*	"OF-360"/ENTP-4000	72 h	" - <u>3</u> (37) " - <u>5</u> (62), " - <u>3</u> (93) " - <u>5</u> (54)	
8	"OF-360"/ENTP-4000	240 h	" - <u>3</u> (40) " - <u>5</u> (51), " - <u>3</u> (91) " - <u>5</u> (72)	

* The data show the average value after the reaction was repeated two times.

Diltiazem hydrochloride 2 is a representative calcium antagonist which is widely used to treat ischemic heart diseases.⁸⁾ Its important intermediate (2S,3S)-4 has been produced industrially by the optical resolution of the corresponding racemic α -hydroxy acid. In the present study, we examined the lipase-catalyzed kinetic resolution of ($\pm$)-6a, b. The reaction in phosphate buffer did not proceed at all in this case either, which again could be ascribed to the compound's highly water-insoluble nature. From preliminary screening experiments, immobilized "Amano P" from *Pseudomonas* sp. appeared to be most suitable for our purpose and thus the asymmetric hydrolysis was carried out for both ($\pm$)-6a and 6b using variously entrapped "Amano P" in a water-saturated mixture of isooctane and benzene (10:3)(Table II). The desired (2S,3S)-4 and 6b were obtained with high optical purity¹⁰⁾ when Amano P immobilized with ENTP-4000 or ENT-4000 was used. Here again, the used enzymes kept their enzymatic activity without

Table II

Entry	Substrate	Lipase	Time	Product (%)	Optical purity (% ee)
1	($\pm$)- <u>6a</u>	"Amano P"/celite	68 h	(2R,3R)- <u>4</u> (29) (2S,3S)- <u>6a</u> (67), (2R,3R)- <u>4</u> (>99) (2S,3S)- <u>6a</u> (48)	
2	"	"Amano P"/celite/ENTP-4000	16 d	" - <u>4</u> (44) " - <u>6a</u> (52), " - <u>4</u> (98) " - <u>6a</u> (81)	
3	"	"Amano P"/ENTP-4000	21 d	" - <u>4</u> (24) " - <u>6a</u> (75), " - <u>4</u> (>99) " - <u>6a</u> (31)	
4	"	"Amano P"/ENT-4000	21 d	" - <u>4</u> (49) " - <u>6a</u> (50), " - <u>4</u> (97) " - <u>6a</u> (94)	
5	($\pm$)- <u>6b</u>	"Amano P"	7 d	" - <u>4</u> (14) (2S,3S)- <u>6b</u> (82), " - <u>4</u> (70) (2S,3S)- <u>6b</u> (8)	
6	"	"Amano P"/celite	38 h	" - <u>4</u> (44) " - <u>6b</u> (49), " - <u>4</u> (91) " - <u>6b</u> (69)	
7	"	"Amano P"/ENTP-4000	19 d	" - <u>4</u> (49) " - <u>6b</u> (50), " - <u>4</u> (91) " - <u>6b</u> (90)	
8*1)	"	"	21 d	" - <u>4</u> (49) " - <u>6b</u> (48), " - <u>4</u> (95) " - <u>6b</u> (94)	
9	"	"Amano P"/ENT-4000	21 d	" - <u>4</u> (56) " - <u>6b</u> (40), " - <u>4</u> (74) " - <u>6b</u> (95)	
10*2)	"	"	12 d	" - <u>4</u> (51) " - <u>6b</u> (48), " - <u>4</u> (88) " - <u>6b</u> (94)	

*1) The same enzyme used in Entry 7 was employed.

*2) The same enzyme used in Entry 9 was employed.

appreciable denaturation (entry 8, 10). This, coupled with the data in Table I, entry 7, shows the particular feature of the photo-crosslinkable resin prepolymer method for greatly increasing the stability of the native enzymes. "Amano P" itself is claimed to be fairly stable in various organic solvents.¹¹⁾ However, the use of "Amano P" in the same organic solvent system, as noted above, gave only poor results (entry 5). This clearly demonstrates the effectiveness of enzyme-immobilization. From these experiments it became apparent that even water-insoluble compounds can be a substrate for enzymatic reactions provided the lipases are immobilized properly and suitable organic solvents were used. The only drawback in the present method is that the reactions proceed extremely slowly. This should be improved by developing new immobilization techniques and finding a more effective solvent system.

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REFERENCES AND NOTES

- 1) We have already succeeded in synthesizing of (2S,3R)-**3**, via (2R,3R)-**7**, which was prepared by the lipase-catalyzed kinetic resolution of ($\pm$)- α -acetoxy ester **7**.¹²⁾ In this case, although ($\pm$)-**7** was subjected to the enzymatic reaction suspended in a phosphate buffer solution, kinetic hydrolysis proceeded smoothly giving (2S,3S)-**8** (42%, 70% ee) and the unreacted (2R,3R)-**7** (43%, 95% ee). However, when hydrolysis was achieved with lipase immobilized with ENTP-4000 in water saturated isooctane, the optical purity of the hydrolyzed product (2S,3S)-**8** greatly increased (98% ee).

- 2) M. Schach von Wittenau and H. Els, J. Am Chem. Soc., **85**, 3425 (1963).
- 3) Benzene was added to increase the solubility of ($\pm$)-**5**.
- 4) O. Hoshino, K. Itoh, B. Umezawa, H. Akita and T. Oishi, Tetrahedron Lett., **29**, 567 (1988).
- 5) S. Fukui and A. Tanaka, Advances in Biochemical Engineering/Biotechnology, **29**, 1 (1984).
- 6) To determine the absolute structure of the reaction products, (2S,3R)-**3** and (2R,3S)-**5** were converted into the corresponding (2S,3R)-**3**-(+)-MTPA¹³⁾ ester and (2R,3S)-**5**-(+)-MTPA ester, respectively, and the absolute structures of both (+)-MTPA esters were determined by the direct comparison of the NMR (400 MHz) spectra with those of authentic samples⁷⁾ [(2S,3R)-**3**-(+)-MTPA; δ 3.806 (COOMe), (2R,3S)-**5**-(+)-MTPA; δ 3.763 (COOMe)]
- 7) H. Akita, H. Matsukura and T. Oishi, Tetrahedron Lett., **27**, 5397 (1986).
- 8) K. Abe, H. Inoue and T. Nagao, Yakugaku Zasshi, **108**, 716 (1988).
- 9) Here again, the hydrolyzed product was found to be (2R,3R)-**4**.
- 10) The absolute structure and the optical purity of the reaction products were determined by correlating the NMR (400 MHz) spectra [(2R,3R)-**4**-(+)-MTPA; δ 3.792 (aromatic OMe), (2S,3S)-**4**-(+)-MTPA; δ 3.750 (aromatic OMe) of the corresponding (+)-MTPA esters with those of the authentic samples.¹⁴⁾
- 11) For example, see Y. Terao, K. Tsuji, M. Murata, K. Achiwa, T. Nishino, N. Watanabe, and K. Seto, Chem. Pharm. Bull., **37**, 1653 (1989).
- 12) H. Akita, T. Kawaguchi, Y. Enoki and T. Oishi, Chem. Pharm. Bull., in press.
- 13) J. A. Dale and H. S. Mosher, J. Am. Chem. Soc., **95**, 512 (1973).
- 14) The authentic (2S,3S)-**4**-(+)-MTPA ester was derived from (2S,3S)-**4**, which was obtained by CH_2N_2 treatment of the corresponding (2S,3S)- α -hydroxy acid.⁸⁾

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