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Kinetic resolution of a diltiazem intermediate by lipase-catalyzed enantioselective alcoholysis

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Abstract

A novel enzymatic resolution of an important alcohol intermediate in the Diltiazem process was developed. The enzymatic reaction involved alcoholysis of the alcohol acetate with butanol, thus obtaining the (*R,R*)-alcohol and the remaining (*S,S*)-acetoxy-alcohol in $>95\%$ enantiomeric excess. This resolution may serve as the key step in a possible recycling procedure for the waste streams of the Diltiazem process, which will allow a significant increase in the overall process yield. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Diltiazem; Lipases; Porcine liver acetone powder; Alcoholysis; Resolution

1. Introduction

The utilization of enzymes for the preparation of optically active compounds has greatly increased over the last two decades [1–7]. In particular, enzymatic catalysis is performed either in aqueous media for hydrolysis reactions or in organic solvents mainly for esterification and transesterification reactions [8–13].

Diltiazem, (+)-*cis*-(2*S*,3*S*)-acetoxy-5-[2-(dimethylamino)-ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5*H*)-one (Fig. 1), is one of the most potent calcium channel blockers [14]. The drug was originally developed by Tanabe Seiyaku and was first launched in Japan in 1974. Since then, it has been widely used in more than 100 countries for treatment of angina and hypertension.

Diltiazem has two asymmetric carbon atoms at the C2 and C3 positions. Among the possible diastereomers, only the *cis*-(2*S*,3*S*) isomer exhibits strong coronary vasodilating activity; therefore, the stereoselective synthesis of this stereoisomer has attracted great attention, particularly in view of the commercial significance of the molecule [15].

Approaches to optically active Diltiazem usually involve either an asymmetric synthesis [16] or a resolution of one of the key intermediates **1** or **2** (Fig. 2) [17–30]. Enzymatic resolution of **1** [17–24] and chemical [25,26] or enzymatic [27–29] resolution of **2** are the most common approaches used today. For example, the Tanabe process (Fig. 2) utilizes classical diastereomeric salt crystallization for resolving the acid **2** [30].

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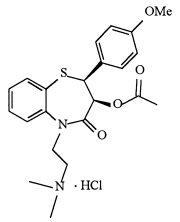

Fig. 1. Diltiazem hydrochloride.

The main disadvantage of these resolution processes is the low theoretical maximum yield of the desired (*S,S*)-lactam **3** — only 40%, due to the necessity of discarding both the erythro [(*R,S*) and (*S,R*)] isomers of **2** (approx. 20%) and the (*R,R*)-threo isomer of **2** (approx. 40%). Consequently, the aim of this work was to find a possible route for recycling the waste streams of the process containing the discarded (*R,R*)-**2** isomer, in order to improve the process yield. The proposed recycling scheme is shown in Fig. 3.

First, the discarded (*R,R*)-**2** isomer is closed to the *R,R*-lactam **3**, according to the process procedure. Then, racemization of the *R,R*-lactam **3** to the *R,R* + *S,S* lactam can be performed according to reported literature procedures using an oxidation–reduction system. The oxidation of the *R,R*-lactam **3** followed by basic hydrolysis to obtain a keto-lactam intermediate was reported by Yamada et al. [16] using a $DMSO/Ac_2O$ system containing pyridine. The keto-lactam derivative can be chemically reduced with $NaBH_4$, according to Morimoto et al. [36] giving only *cis* stereochemistry of the resulting racemic lactam **3**. The racemic lactam is then submitted to enzymatic kinetic resolution.

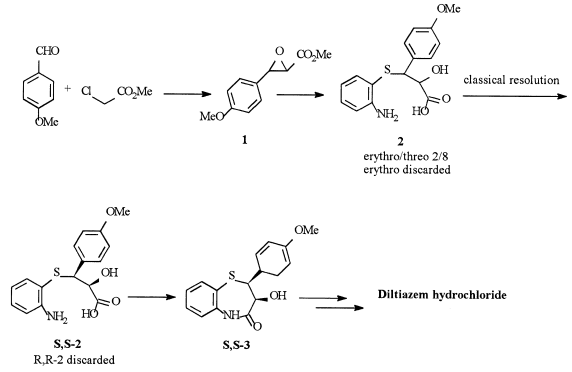

Fig. 2. The Tanebe Seiyaku route to Diltiazem.

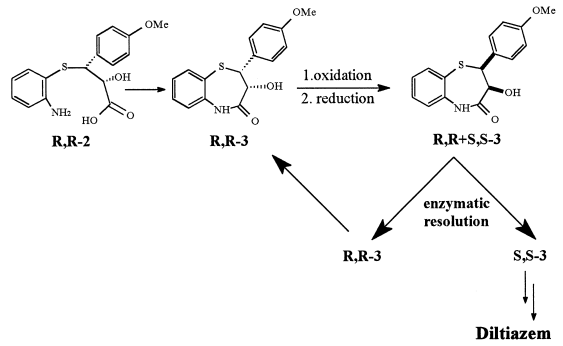

Fig. 3. Proposed route for recycling waste streams in the Diltiazem process.

This work describes the resolution step in the proposed recycling procedure (Fig. 3). We present a novel enzymatic resolution of the racemic lactam **3**, as its acetoxy derivative, by using lipase-mediated catalysis in organic solvents.

2. Experimental

2.1. General

HPLC analyses were performed on a Hewlett-Packard 1050 series instrument equipped with a Jasco 975 UV detector. ^1H NMR spectra were recorded on a Varian 500 MHz spectrometer in $CDCl_3$. The centrifuge used was an Eppendorf 5403 equipped with a swing-bucket rotor. The shaker used for the enzymatic experiments was Haake SWB 20. Porcine (PLAP) and bovine (BLAP) acetone powders, porcine pancreas lipase and pig liver esterase were purchased from Sigma. Horse liver esterase was purchased from Fluka. *Candida rugosa* lipase was purchased from Altus. The *Pseudomonas sp*. lipase was obtained from Amano Pharmaceutical. The racemic amino acid **2** was kindly supplied by Teva Pharmaceutical. Neutral alumina (90 active neutral) was pur-

chased from Merck. The solvents used for the enzymatic reactions were of analytical grade. Unless otherwise stated, materials were obtained from commercial suppliers and were used without further purification.

2.2. Determination of conversion and enantiomeric excess (ee)

The progress of the reaction was followed by HPLC using a 5 μm Kromasil 5 C8 column (25 cm × 4.6 mm) from Phenomenex or ISI. Eluent composition: Acetonitrile:H_2O 50% (0), 50% (10), 100% acetonitrile (20), 100% (25), 50% (30); flow rate: 1 ml/min; detection: 254 nm. The conversion was calculated from the relative areas beneath the two peaks, using a response factor obtained from calibration of the substrate and product (the factor was found to be ~1). The retention times were: $R_f = 7.6$ min for lactam **3** and $R_f = 10.8$ min for the acetoxy lactam **4**.

The % ee of the reaction product and substrate (**3** and **4**, respectively) was determined by HPLC using a Chiralcel OJ column from Daicel, (5 μm, 4 × 250 mm). Eluent composition: Ethanol:Hexane 70:30, 40°C, 0.7 ml/min, λ = 254 nm. The retention times were: $R_f = 7.2$ min for (*R,R*)-**4**, $R_f = 8.0$ min for (*S,S*)-**4**, $R_f = 12.3$ min for (*R,R*)-**3** and $R_f = 24.9$ min for (*S,S*)-**3**.

2.3. Synthesis of racemic [(R,R) + (S,S)]-acetoxy lactam 4

We used a direct synthesis of this acetoxy derivative from the racemic amino acid **2**, which was available to us. The starting material for this reaction was given to us by Teva Pharmaceutical.

In a 250-ml round bottom flask were placed 10 g of racemic-threo (*R,R* + *S,S*)-amino acid **2** and 54 ml of DMF. After dissolving the starting material, 3 ml of pyridine was added dropwise over 20 min and the mixture was magnetically stirred at r.t. for another 40 min. Then 7 ml of acetic anhydride was added over a period of 15 min. The stirring was continued overnight at r.t. The mixture was then transferred to a 500 ml beaker, and 210 ml of ice water were added. An off-white solid was obtained and was stirred at 0–5°C for 1 h. The solid was filtered and washed with water. Reslurrying with the minimum amount of methanol, filtering and washing with cold methanol and ether afforded 7.5 g of racemic-acetoxy lactam **4** (70% yield), 99.5% by HPLC, ^{1}H NMR δ 8.11 (1 H, s), δ 7.68–7.70 (1 H, m), δ 7.40–7.47 (3 H, m), δ 7.18–7.26 (2 H, m), δ 6.86–6.88 (2 H, m), δ 5.33 (1 H, d, J = 7.0 Hz), δ 5.16 (1 H, d, J = 6.5 Hz), δ 3.81 (3 H, s), δ 1.92 (3 H, s), m.p. (non-calibrated) = 183°C, MS (Instrument: VG Prospec, magnetic section; interphase: plasma spray) = 344 $[MH]^+$, 284.

2.4. Kinetic measurements of the alcoholysis reaction with free PLAP catalysis

All the reactions were carried out in a batch manner in a 25-ml Erlenmeyer flask. The reaction mixture contained: racemic-**4** (25 mg), butanol/hexane 2/8 (10 ml), PLAP (0.3 g). The reaction mixture was placed in the shaker at 50°C and 130 rpm. Samples were withdrawn periodically from the reaction mixture and analyzed by HPLC. Integration of the HPLC chromatograms afforded quantitative determination of the reaction progress. The R_f value of the obtained lactam **3** was compared to an authentic sample of the lactam, obtained from Teva Pharmaceutical.

2.5. Kinetic measurements of the alcoholysis reaction with PLAP / alumina catalysis

The procedure as described in the previous paragraph was used, but the enzyme was mixed with neutral alumina before addition to the reaction mixture. The ratio of PLAP/alumina was

generally 1/2 or a different ratio as mentioned in the text.

2.6. Recycling experiments

All the experiments were carried out in a batch manner in a 100-ml centrifuge tube. The reaction mixture contained: racemic-**4** (50 mg), butanol/hexane 2/8 (10 ml), PLAP/alumina 0.6 g/1.2 g. The reaction mixture was placed in the shaker at 50°C and 130 rpm for 24 h. After each run, 10 ml of solvent (BuOH/hex 2/8) was added, the tubes were centrifuged (5000 rpm, rt, 25 min) and the solvent decanted. Two more washings with 20 ml solvent were conducted and centrifuged under the same conditions. Following this step, a new batch of racemic-4 and solvents was added to the washed enzyme and another cycle was conducted.

3. Results and discussion

Three options were considered as possible routes for the resolution of the lactam **3**, and are shown in Fig. 4.

Initially, the hydrolysis alternative was tested. Thus, the acetoxy lactam derivative was submitted to the action of several commercial enzyme preparations and progress of the hydrolysis was

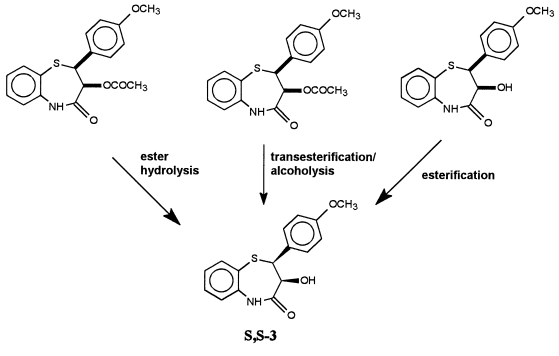

Fig. 4. Alternative routes to the enzymatic resolution of the lactam intermediate.

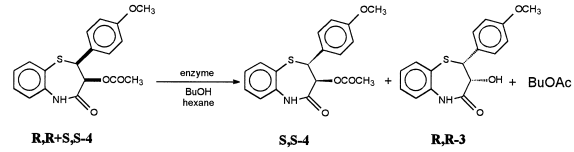

Fig. 5. Enzymatic transesterification of racemic acetoxy lactam **4** with butanol in hexane.

followed by HPLC. The hydrolysis was performed in phosphate buffer at neutral pH, with the addition of 10% of DMF as a co-solvent. These preliminary screening experiments revealed that only two enzymes — the porcine and bovine acetone powders — catalyzed the reaction. Out of these two enzymes, the porcine liver acetone powder (PLAP) was more active and was therefore further studied. Chiral HPLC analysis of the reaction mixture showed that the PLAP hydrolyzed the (*R*,*R*)-acetoxy lactam **4** to give mainly the (*R*,*R*)-lactam **3** and the remaining enriched (*S*,*S*)-acetoxy lactam **4**. However, the stereoselectivity of the reaction was not very high — about 85% ee for the product (*R*,*R*)-**3** at 35–40% conversion. Consequently, it was decided to test the other possible routes — esterification and transesterification (or alcoholysis) which are performed in organic solvents and, hence, a significant effect on enantioselectivity may be observed [31,32].

The esterification of the racemic lactam **3** with various acyl donors in organic solvents was examined. PLAP, which gave the best results in the hydrolysis reaction, and several other commercial enzyme preparations were tested. The acyl donors used were vinyl acetate, acetic anhydride and butyl acetate. In all cases, no reaction occurred at all. This route was therefore abandoned and the third option — alcoholysis — was examined (Fig. 5).

Butanol was chosen as the transesterification agent because it is known to work well in enzymatic reactions [33]. The solvent of choice was hexane for the same reason. The screening results for several commercially available enzyme preparations in the alcoholysis reaction are shown in Table 1.

Table 1
Enzymatic alcoholysis of racemic acetoxy lactam **4** with butanol in hexane[a]

Entry	Enzyme	Reaction time (h)	% Conversion to (*R*,*R*)-**3**[b]	% ee of (*R*,*R*)-**3**[c]
1	PLAP	24	23.4	96.9
2	bovine liver acetone powder	24	no reaction	–
3	pig liver esterase	96	no reaction	–
4	horse liver esterase	96	no reaction	–
5	*Candida rugosa* lipase[34]	72	14.5	97.3
6	porcine pancreatic lipase	120	no reaction	–
7	*Pseudomonas sp*. lipase	120	no reaction	–

[a]The experimental protocol is described in the Experimental Section. No reaction took place in the absence of the enzyme under the conditions used.
[b]Reaction progress was monitored by integration of HPLC chromatograms, which enabled quantitative determination of the remaining acetoxy lactam **4** and the product lactam **3**.
[c]Determined by HPLC on chiral column (Chiralcel OJ, Daicel) with a 70:30 mixture of ethanol and hexane.

As can be seen from Table 1, two enzymes showed positive results, PLAP and the lipase from *Candida rugosa* (formerly termed *Candida cylindracea*). [1] Both enzymes showed the same stereoselectivity — transesterifying the (*R*,*R*)-acetoxy lactam **4** to give (*R*,*R*)-lactam **3** and the remaining enriched (*S*,*S*)-**4**. Nevertheless, in contrast to the hydrolysis reaction, the stereoselectivity of the alcoholysis was very high, giving (*R*,*R*)-**3** in $>95\%$ ee. Since the activity of the PLAP was higher than the *Candida rugosa* lipase, further work was conducted using PLAP only.

The reaction was examined in different mixtures of butanol/hexane in order to obtain the optimal butanol content. The optimum was found to be 10–30% of butanol (by volume). The stereoselectivity was not affected by the butanol content and was always very high. Therefore, subsequent work was conducted with 20% butanol in hexane.

As the solubility of the substrate and product was low in the butanol/hexane system, several alternative solvents were checked. We proposed that enhanced solubility might increase the reaction rate. Screening of 18 solvents, from nonpolar ones like toluene and hexane to polar solvents like THF, revealed that the alcoholysis worked best in the hydrophobic solvents hexane and cyclohexane, despite the low solubility. Hence, hexane was chosen for further work.

Although the reaction was highly stereospecific with regard to the optical purity of the lactam (*R*,*R*)-**3**, it was very slow, and after 3 days only 30% conversion was obtained. Furthermore, it was practically impossible to reach complete conversion (50%), and thus obtain the desired (*S*,*S*)-isomer in high enantiomeric excess. As a result, the possibility of immobilizing the enzyme on a solid support in order to increase its activity and stability was considered. Several immobilization methods were tested including physical and covalent attachments. It was found that by simply mixing the crude enzyme powder with neutral alumina, a considerable increase in the reaction rate was achieved, enabling an almost complete conversion within 24 h (Fig. 6). PLAP was also mixed with silica

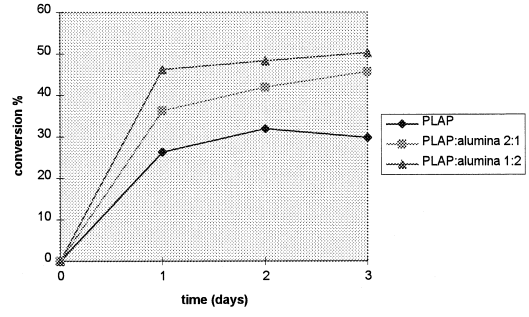

Fig. 6. Reaction progress using different ratios of PLAP:alumina.

[1] The enzyme was purchased from Altus in a crystallized ChiroCLEC form.

Table 2
Comparison of different ratios of PLAP:alumina[a]

PLAP:alumina ratio	% Conversion to (*R,R*)-**3**[b] 24 h	% ee of (*R,R*)-**3**[c] 24 h	% ee of (*S,S*)-**4**[c] 24 h	% Conversion to (*R,R*)-**3**[b] 72 h	% ee of (*R,R*)-**3**[c] 72 h	% ee of (*S,S*)-**4**[c] 72 h
free PLAP	26.3	98.3	39.1	29.8	94.3	43.1
PLAP:alumina 2:1	36.3	98.7	62.1	45.7	97.8	90.8
PLAP:alumina 1:1	41.0	98.5	75.4	48.0	96.2	98.2
PLAP:alumina 1:2	46.3	89.9	92.1	**50.3**	**87.7**	**99.1**
PLAP:alumina 1:5[d]	20.4	61.7	22.6	–	–	–
PLAP:alumina 1:10[d]	29.0	35.1	25.6	–	–	–

[a]The experimental protocol is described in the Experimental Section. No reaction took place in the absence of the enzyme under the conditions used.
[b]Reaction progress was monitored by integration of HPLC chromatograms, which enabled quantitative determination of the remaining acetoxy lactam **4** and the product lactam **3**.
[c]Determined by HPLC on chiral column (Chiralcel OJ, Daicel) with a 70:30 mixture of ethanol and hexane.
[d]Only a quarter of the amount of enzyme was used, therefore the conversion is lower.

giving similar results. This approach had been previously used by Shkolnik and Gutman [34] in a different enzymatic reaction.

As can be seen from Fig. 6 and Table 2, increasing the ratio of alumina:PLAP significantly increased the reaction rate, but also caused a decrease in enantioselectivity. Thus, in a ratio of 1:5 PLAP:alumina, the % ee of *R,R*-**3** decreased to 62%. Among the values tested, it seems that a ratio of PLAP:alumina 1:2 is the best, giving high yield and high enantiomeric excess of the desired (*S,S*)-**4**.

In order for the enzymatic reaction to be economically feasible, the catalyst has to be recycled. Therefore, the PLAP:alumina 1:2 preparation was further tested in recycling experiments, to check the possibility of reusing the enzyme. Each cycle was run for 24 h, then the enzyme was centrifuged, washed, and reused in the next cycle. The results are presented in Fig. 7.

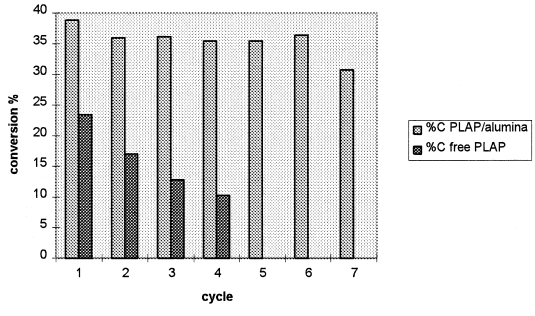

Fig. 7. Comparison of recycling experiment — PLAP/alumina and free PLAP.

It can be seen that for PLAP/alumina 1:2, the activity remained nearly constant during seven alcoholysis cycles. On the other hand, the conversion to product using free PLAP preparation decreased to approximately 50% of its initial value after only three cycles. Thus, the PLAP/alumina system shows a potential for enzyme recycling. It is assumed that the alumina prevents the enzyme from aggregating, thus keeping the surface area of the enzyme very high throughout the reaction, in contrast to the free PLAP. The observed enhancement of enzymatic activity and stability, when adsorbed on alumina, is in accordance with the results of Kim and Lee [35] for silica gel as a solid support, for their enzymatic system.

4. Conclusions

A novel enzymatic resolution of an important Diltiazem intermediate was accomplished, using a crude, inexpensive enzyme powder. Of the three possible resolution routes tested, alcoholysis was the most successful, giving the desired product (*S,S*-**4**) in $>95\%$ enantiomeric excess at approx. 50% conversion. The enzymatic reac-

tion was conducted at 50°C using 20% butanol in hexane. The activity and stability of the enzyme was considerably enhanced when it was pre-mixed with neutral alumina in a ratio of 1:2 enzyme:alumina. This resolution may serve as the key step in a possible recycling procedure for the waste streams of the Diltiazem process, enabling a significant increase in the maximum yield of the process.

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