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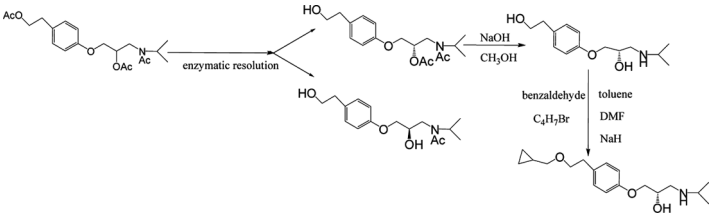
CHEMOENZYMATIC ROUTE TO S-BETAXOLOL

Yong-Hong Li,[1,2] Li-Hua Huang,[1,2] and Hong-Min Liu[1,3]
[1]*New Drug Research and Development Center, Zhengzhou University, Zhengzhou, China*
[2]*Department of Chemistry, Zhengzhou University, Zhengzhou, China*
[3]*School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou, China*

GRAPHICAL ABSTRACT



Abstract *An efficient chemoenzymatic route to S-betaxolol is reported. A strain (*Rhodotorula mucilaginosa *DQ832198) screened from soil was used as biocatalyst for the kinetic resolution of the key acetylated intermediates. Excellent enantiomeric excess (ee > 99%) was obtained under very mild conditions. The biocatalyst is quite stable and could be used several times with little decrease of the resolving ability.*

Keywords Chemoenzymatic route; kinetic resolution; *Rhodotorula mucilaginosa*; S-betaxolol

INTRODUCTION

Pharmacological studies have shown that an organism often reacts in a different way when it interacts with each enantiomer of the same molecule, and this promoted chirality studies in pharmaceutical research. Recently one of the most studied classes of drug has been the β-blockers, because of the increase in cardiovascular diseases. Studies on these drugs showed that for compounds such as 1-alkylamino-3-aryloxypropan-2-ols, the cardiovascular activity resides mainly in their (**S**)-isomer, whereas the (**R**)-isomer is usually responsible for the side effects.[1] Our studies are related to betaxolol, a very interesting β_1-selective blocker, whose (**S**)-isomer has much stronger activity in reducing intraocular pressure and is the most widely used antiglaucoma agent.[2]

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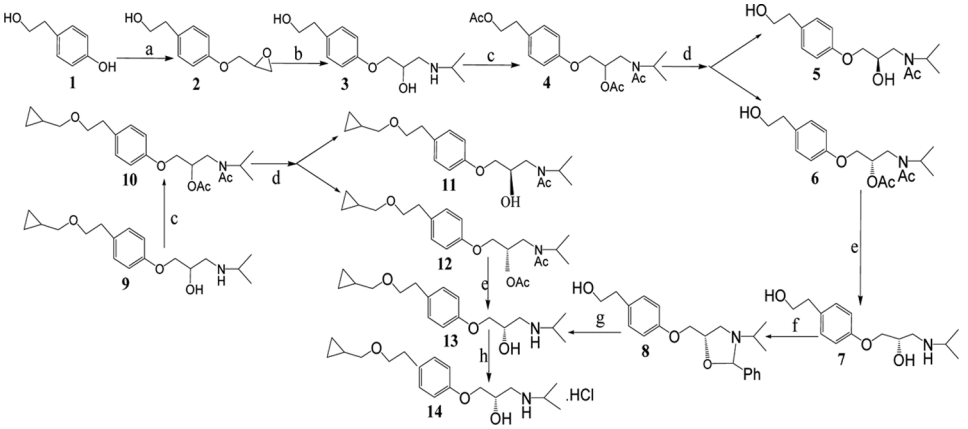
Address correspondence to Hong-Min Liu, New Drug Research and Development Center, Zhengzhou University, Zhengzhou 450001, China. E-mail: liuhm@zzu.edu.cn



Scheme 1. Synthetic outlines of S-betaxolol. (a) epichlorohydrin, K_2CO_3, CH_3CN, reflux, 6 h; (b) i-$PrNH_2$, CH_3OH, rt, 7 h; (c) Ac_2O, pyridine, Et_3N, rt; (d) *Rhodotorula mucilaginosa*, 28 °C, 12 h; (e) NaOH, CH_3OH, rt; (f) benzaldehyde, toluene, paratoluenesulfonic acid, reflux; (g) C_4H_7Br, DMF, NaH, −15 °C 3 h, rt, 2 h; and (h) HCl, rt.

The synthesis of drugs in their enantiomerically pure form becomes very important for the pharmaceutical industries, because of increased demand for more effective and safer single isomers. Both asymmetric synthesis and chemoenzymatic approaches have been described in the literature[3–7] for the preparation of (**S**)-betaxolol. The asymmetric synthetic methods need rigorous reaction conditions, expensive chiral catalyst, or optical voidness material. The growing need to find new strategies with lower output cost pushes the research toward the biotransformation field, in which the ability of microorganisms, and their cheap enzymes (especially lipases), as enantioselective catalysts could be used for such a purpose. Lipases, with their capacity to work in organic solvents unaffectedly and their high enantioselectivity, offer an attractive route for industrial exploitation, because they could lower output costs in a significant way. In the chemoenzymatic method reported by Di Bono et al.[1] the enantiomeric excess of (**S**)-betaxolol was 82%, which was further improved to 91% after crystallizing its hydrochloride salt. However, the catalyst used in the method is pure enzyme, which is quite expensive, especially when a large quantity is needed.

Herein we report a process for the chemoenzymatic synthesis of (**S**)-betaxolol (Scheme 1). A strain (*Rhodotorula mucilaginosa* DQ832198) screened from soil was used as biocatalyst for the kinetic resolution of the key acetylated intermediates. This approach provides high enantioselectivity, and the catalyst is very cheap compared with the approach reported before.

RESULTS AND DISCUSSION

Because of the interesting results reported[1] in the lipase-catalyzed resolution of β-amino alcohols, we decided to try an enantioselective hydrolysis of the N,O-diacetyl derivative **10** of betaxolol (**9**). With this target in mind, we projected

the synthesis of the betaxolol in racemic form, according to the patented methodology.[8] After purification, betaxolol was N,O-bisacetylated in quantitative yield by acetic anhydride in pyridine, and microbe screening was carried out on this acetylated product **10** to differentiate which strains were able to catalyze the hydrolysis of either the ester or amide functionality. In this screening, 52 strains that can live on the substrate as the only carbon source and have the ability to produce transparent circles were used. The screening was analyzed by thin-layer chromatography (TLC) and optical rotations, and the results showed that only two strains, **1** and **2** were able to catalyze the hydrolysis of the substrate.

The scale-up of these reactions allowed the determination of the final products R- and S-betaxolol via high-performance liquid chromatography (HPLC) using a Chiralcel OJ column. Results showed that both strains exhibit poor selectivity with E values of 1.5 and 3.5 respectively.

Thus, a new substrate compound **4** was synthesized.[1,8,9] Compound **4** (14.86 g, 0.041 mol) was prepared from the reaction of compound **3** 3-[4-(2-hydroxyethyl-pheoxy-1-(N-isopropyl) amino-propan-2-ol (12.19 g, 0.051 mol) and Ac_2O[1] with the yield of 92%, and compound **3** was prepared via two steps from compound **1** according to the patented methodology.[8]

According to a previous screening, it was noticed that only **1** and **2** strains led to significant amount of kinetic resolution after 24 h; hence a scale-up of these reactions allowed the determination of enantiomeric excesses. After 24 h, **1** strain gave intermediate **6** with ee_p of 45% and an enantioselectivity factor E of 5.3, while **2** strain gave intermediate **6** with ee_p of 95% and E of 10.6.

These results show that strain **2** is more suitable for use in kinetic resolution. It has been identified as *Rhodotorula mucilaginosa* DQ832198 and was deposited in China General Microbiological Culture Collection Center (GGMCC2257). It was used as a biocatalyst for kinetic resolution in further investigations.

The steroselectivity of lipase in the strain is not perfect. A product with better optical purity was obtained with lower yield: the better the optical purity, the lower the yield. So a two-resolution procedure was adopted. Product with lower *ee* value was obtained with greater yield first, and the product obtained was acetylated and used as resolution substrate again. Product with greater *ee* value can be obtained with greater yield after the substrate resolved twice.

After kinetic resolution twice catalyzed by **2** strain for 12 h, substrate **10** completely transferred into compound **11** with the molar ratio 65% and compound **12** with the molar ratio 35%. Compound **12** was the S-isomer, the one we need, and 0.96 g compound **12** was obtained from 3 g substrate **10** with the yield of 31%. Substrate **4** completely transferred into compound **5** with the molar ratio 52%, and compound **6** with the molar ratio 48%. Compound **6** was the S-isomer, the one we need, and 0.59 g compound **5** (*ee* 95%) was obtained from the same substrate, with the yield of 51.2%, and 0.68 g compound **6** was obtained from 4 g substrate **4**, with the yield of 47.5%.

Optically active intermediates (−)-**6** and (−)-**12** were easily converted in good yields into S-betaxolol following the known procedure.[8] Compound **12** (1 g, 0.0026 mol) could be transferred into S-betaxolol (**13**) (0.71 g, 0.0023 mol) directly, with the yield of 91% and *ee* value of 95%. Compound **6** (5 g, 0.018 mol) was transferred into compound **7** (4.22 g, 0.017 mol) with the yield of 98% first, and then

turned into S-betaxolol (95%) with the yield of 98.1% according to the reported methodology.[8,9]

S-Betaxolol was transferred into its hydrochloride (**14**) (*ee* 95%), according to the reported methodology,[8,9] and its *ee* value was improved to 99% after recrystallization from diethyl ether (Et_2O).

CONCLUSION

In summary, we have established a new chemoenzymatic route to S-betaxolol from cheap and easily obtained material 4-(2-hydroxyethyl)-phenol (**1**) with good optical purity ($ee > 99\%$) and moderate chemical yield (32.4% overall), including a lipase-catalyzed kinetic resolution. We envisage that our route could find application in the synthesis of β-amino alcohols with acyl or hydroxy functionality in stereogenic carbon such as some β-adrenergic receptor blockers (propranolol, alptenolol, oxprenalol, pindolol, nadolol, timolol, esmolol, and flestolol), ephedrine, epinephrine, dropropizine, and salbutamol sulfate. Further investigations to improve the stereoselectivity through screening new substrates with longer acyl chains in stereogenic carbon and performing the kinetic resolution in organic–aqueous double systems are under way.

EXPERIMENTAL

Reagent-grade solvents were distilled prior to use. All reported NMR spectra were collected on a Bruker DPX 400 NMR spectrometer with tetramethylsilane (TMS) as the internal reference. Infrared (IR) spectra were recorded on Nicolet IR2001 instrument using KBr disks in the 400- to 4000-cm regions. High-resolution mass spectra (HRMS) were obtained on a TM Waters Micromass Q-Tof Micro instrument using the electrospray ionization (ESI) technique. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. Enantiomeric excess was determined by chiral HPLC at room temperature using Syltech 500 pump equipped with a UV 500 version 4.1 ultraviolet detector with a Chiralcel OJ (4.6 mm × 250 mm) column.

Screening of the Microbes

Microbe screening proceeded according the method reported.[10] A strain with steroselectivity lipase activity (*Rhodotorula mucilaginosa* DQ832198) was screened from soil and used for kinetic resolution of betaxolol intermediates. The culture medium of the strain contained corn paste 1%, peptone 0.5%, and glycerol 2% with an initial pH of 6.0. The culture was carried out in 500-ml shaking flasks containing 110 ml medium at 32 °C and 220 rpm for 16 h.

Enzyme activity of the strains was evaluated by the substrate conversion rate after conversion for the same period catalyzed by different strains.

Preparation and Characterization of Kinetic Resolution Substrates

Preparation and characterization of compound 4. Compound **4** was prepared from the reaction of compound **3** with Ac_2O,[1] and compound **3** was prepared from compound **1** according to the patented methodology.[8]

^{1}H NMR: 7.14, 6.80 (each 2H, d, $J = 8.5$ Hz, Ar-H); 3.80 (2H, t, $J = 6.6$ Hz, H-1); 2.88 (2H, t, $J = 6.6$ Hz, H-2); 3.60 (1H, dd, $J = 1.44$ Hz, $J = 14.0$ Hz, H-31); 3.4 (1H, dd, $J = 7.5$ Hz, $J = 14.0$ Hz, H-32); 4.46 (1H, s, H-4); 4.10 (1H, dd, H-51); 4.03 (1H, dd, H-52); 3.70 (1H, m, H-6); 1.26, 1.25 (each 3H, d, $J = 6.76$ Hz, $J = 6.56$ Hz, CH_3*2); 2.24 (9H, s, -$COCH_3$).

ESI-MS m/z: calcd. for $C_{20}H_{29}NO_6$ 379.1995. Found: 380.2067 $[M + H]^+$; 402.1888 $[M + Na]^+$.

Preparation of compound 10. Compound **10** was prepared from the reaction of racemic betaxolol (**9**) with Ac_2O[1], and racemic betaxolol was prepared according to the patented methodology.[8]

Kinetics Resolution

The culture fluid of *Rhodotorula mucilaginosa* was filtered, and 0.1 M pH 6.0 Na_2HPO_4–citric acid buffer and 3 g/L substrate were added to a proper amount of the obtained cells, and the mixture was incubated at 28 °C for 12 h. The resulting broth was extracted three times with an equal volume of ethyl acetate. The organic layer was first washed three times with saturated $NaHCO_3$, then three times with saturated NaCl, and last with water. The resulting saturated $NaHCO_3$ and NaCl layer was extracted with acetic ether. The organic phase was combined and dried over Na_2SO_4.

The resolution products were separated by flash chromatograph (eluent acetone/petroleum ether 1:2). The intermediates resolved by **2** strain and **1** strain are mostly the same except for the optical rotations, so the date of intermediates resolved by **2** strain are reported as the following.

Data of Compound 5 and Compound 6

Compound 5. ^{1}H NMR: 7.14, 6.80 (each 2H, d, $J = 8.5$ Hz, Ar-H); 3.81 (2H, t, $J = 6.6$ Hz, H-1); 2.81 (2H, t, $J = 6.6$ Hz, H-2); 3.60 (1H, dd, $J = 1.44$ Hz, $J = 14.0$ Hz, H-3a); 3.43 (1H, dd, $J = 7.5$ Hz, $J = 14.0$ Hz, H-3b); 4.46 (1H, Ws, H-4); 4.10 (1H, dd, H-5a); 4.03 (1H, dd, H-5b); 3.60 (1H, m, H-6); 1.26, 1.21 (each 3H, d, $J = 6.76$ Hz, $J = 6.56$ Hz, CH_3^*2); 2.20 (3H, s, -$COCH_3$).

ESI-MS m/z: calcd. for $C_{16}H_{25}NO_4$ 295.1784. Found: 296.1876 $[M + H]^+$; 318.1689 $[M + Na]^+$. $[\alpha]_{D2}^{20} = +285$ (C = 1, CH_3OH), $[\alpha]_{D1}^{20} = +116$ (C = 1, CH_3OH).

Compound 6. 1H NMR: 7.14, 6.8 (each 2H, d, $J = 8.5$ Hz, Ar-H); 3.82 (2H, t, $J = 6.6$ Hz, H-1); 2.82 (2H, t, $J = 6.6$ Hz, H-2); 3.60 (1H, dd, $J = 1.44$ Hz, $J = 14.0$ Hz, H-3a); 3.35 (1H, dd, $J = 7.5$ Hz, $J = 14.0$ Hz, H-3b); 4.46 (1H, Ws, H-4); 4.10 (1H, dd, H-5a); 4.03 (1H, dd, H-5b); 3.70 (1H, m, H-6); 1.26, 1.22 (each 3H, d, $J = 6.76$ Hz, $J = 6.56$ Hz, CH_3*2); 2.24 (6H, s, -$COCH_3$).

ESI-MS m/z: calcd. for $C_{18}H_{27}NO_5$ 337.1889. Found: 338.1958 $[M + H]^+$; 360.1774 $[M + Na]^+$. $[\alpha]_{D2}^{20} = -2700$ (C = 1, CH_3OH), [α]D201# = −711 (C = 1, CH_3OH), $ee = 95\%$.

The data of compound **12** are the same as those of compound **10**, except $ee = 85\%$.

Preparation and Characterization of S-Betaxolol

Preparation of S-betaxolol. Compound **6** and compound **12** were dissolved in methanol and then stirred with excessive NaOH together, until the completion of the reaction (TLC control). After excess methanol was removed, the residue was dissolved in ethyl acetate. The organic layer was washed first three times with saturated $NaHCO_3$, then three times with saturated NaCl, and last with water. The organic layer obtained was then dried over Na_2SO_4 and concentrated under vacuum pressure.

Characterization of S-betaxolol. The IR and ^1H NMR data of S-betaxolol were identical with those described by Di Bono et al. ESI-MS m/z: calcd. for $C_{18}H_{29}NO_3$ 307.2147; found: 308.2229 $[M+H]^+$. $[a]_D^{20} = -13.0$ (c 1.0, $CHCl_3$), and -19.6 (C = 1, CH_3OH), $ee > 99\%$, which are identical to those described in the literature.[1,5,7]

HPLC Conditions for Resolved Betaxolol and Related Intermediates

The HPLC conditions for intermediates **5** and **6** are as follows: n-hexane–isopropanol = 85:15, OJ, 1.0 mL min^{-1}, 273 nm. $T_{5R} = 21.7$ min, $t_{5S} = 23.5$ min, $t_{6R} = 28.1$ min, $t_{6S} = 32.0$.[11]

The HPLC conditions for resolved betaxolol are as follows: n-hexane/isopropanol/diethylamine 85:15:0.05, OJ, 1.0 mL min^{-1}, 273 nm. $t_R = 7.3$ min, $t_S = 9.3$ min. The results are identical to those described in the literature.[5,7]

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REFERENCES

1. Di Bono, G.; Scilimati, A. chemoenzymatic route to both enantiomers of betaxolol. *Synthesis* **1995**, *6*, 699.
2. (a) Osborne, N. N.; Wood, J. P.; Chidlow, G.; Casson, R.; De Santis, L.; Schmidt, K. G. Effectiveness of levobetaxolol and timolol at blunting retinal ischaemia is related to their calcium and sodium blocking activities: Relevance to glaucoma. *Brain Res. Bull.* **2004**, *62*, 525; (b) Aoyama, Y.; Motoki, M.; Hashimoto, M.; Aoyama, Y.; Motoki, M.; Hashimoto, M. Nippon Ganka. Effect of various antiglaucoma eyedrops on human corneal epithelial cells. *Gakkai Zasshi* **2004**, *108*(2), 75.
3. Binet, J.; Manoury, P. S. S-Isomer of betaxolo: Its preparation and its application in therapy. Patent GB2130585, 1985.
4. Muthukrishnan, M.; Garud, D. R.; Joshi, R. R.; Joshi, R. A. Concise synthesis of β-blockers (S)-metoprolol and (S)-betaxolol using hydrolytic kinetic resolution. *Tetrahedron* **2007**, *6*, 1872.
5. Joshi, R. A.; Garud, D. R.; Muthukrishnan, M.; Joshi, R. R.; Gurjar, M. K. A convenient synthesis of the enantiomerically pure β-blocker (S)-betaxolol using hydrolytic kinetic resolution. *Tetrahedron: Asymmetry* **2005**, *16*, 3802.
6. Joshi, R. A.; Murugan, M.; Garud, D. R.; Borikar, S. P.; Gurjar, M. K. S-(−)-1-{4-[2-(allyloxy)-ethyl]phenoxy}-3-isopropylamino propan-2-ol, and process for preparation thereof and process for preparation of S-(−)betaxolol. U.S. Patent 6,989,465, 2006.

7. Joshi, R. A.; Murugan, M.; Garud, D.; Borikar, S. P.; Gurjar, M. K. Process for preparation of S-(−)-betaxolol and salts thereof. U.S. Patent 2006004109, 2006; U.S. Patent 7019172, 2006.
8. Ippolito, R. M.; Vigmond, S. Process for the preparation of betaxolol and related 1-[4-[2-(cycloalkylalkoxy)ethyl]phenoxy]-3-(alkylamino)-2-propanol pharmaceuticals. GB Patent 2187190, 1987; Process for the preparation of phenoxyisopropanolamine derivatives useful as α-adrenergic blocking agents. ES Patent 544803A1, 1986.
9. Jung, S. H.; Linh, P. T.; Lim, H. K.; Kim, H. J.; Kim, K. H.; Kang, J. S. Enantioselective preparation of metoprolol and its major metabolites. *Arch. Pharmacal Res.* **2000**, *23*, 226.
10. Li, Y. H.; Huang, L. H.; Liu, H. M.; Huang, K. X. Screening and identification of microorganism for enantioselective hydrolysis of betaxolol intermediate. *Chin. Ind. Microb.* **2009**, *39*, 17.
11. Li, Y. H.; Yu, M. Y.; Liu, H. M. Determination of the *ee* values and content of β-amino alcohol chiral drug intermediates by HPLC. *Chin. J. Z. Zhou Univ. (Med. Sci.)* **2009**, *44*, 427.