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Biotransformations with *Rhizopus arrhizus* and *Geotrichum candidum* for the Preparation of (*S*)-Atenolol and (*S*)-Propranolol

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Abstract—(±)-Atenolol/(±)-propranolol and their acetates were incubated with the fungus *Rhizopus arrhizus* and *Geotrichum candidum* separately for different time intervals to afford (*S*)-atenolol/(*S*)-propranolol in good optical yield. The time and pH for this biotransformation was optimised. The present biodegradations using *Rhizopus arrhizus* and *Geotrichum candidum* provides a simple and useful method to obtain (*S*)-atenolol and (*S*)-propranolol which are active enantiomers of the β-adrenergic blockers. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

The biological activity exhibited by the drug molecule is directly related to its absolute stereochemistry.[1] Atenolol and propranolol, the β-adrenergic blockers used to treat cardiovascular disorders are characterised by the aryloxypropanolamine structure with one chiral centre and the activity resides in the (*S*)-enantiomer.[2,3] On the contrary the opposite (*R*)-enantiomer may be responsible for the undesirable effects.[4]

The synthesis of (*R*)- and (*S*)-atenolol has been achieved by lipase catalysed hydrolysis[5] of the intermediate *O*-acetyl esters. The synthesis of (*R*)- and (*S*)-propranolol has been achieved by using lipase catalysed hydrolysis[6] of the intermediate *O*-acetyl esters and also by using lipase catalysed acylation[7] of the intermediate secondary alcohols. *R. arrhizus* has been utilised in the reduction of prochiral ketones,[8] hydrolysis of racemic acetates[9] and in the biosorption studies of radionuclides.[10] *G. candidum* has been utilised in the reduction of various prochiral ketones.[11] Excellent catalytic asymmetric synthesis of the optically active β-blockers by using chiral catalysts[12a–j] and industrial scale synthesis by utilising above-mentioned methods have been reported. The processes consisting of whole cells being economical and operationally simple and recent use in biosorption studies prompted us to develop this methodology. To our knowledge, this is the first report on the biodegradation studies for the preparation of (*S*)-atenolol and (*S*)-propranolol using above whole cells.

Results and Discussions

Optimisation of microbial transformations of (±)-atenolol and (±)-propranolol with respect to optical purity and chemical yield have been carried out. (±)-Atenolol (**1a**) or (±)-propranolol (**1b**) were incubated with *R. arrhizus* or *G. candidum* separately at different pH conditions and varying time intervals, 2–8 days. It was interesting to observe that the yield of recovered substrate was found to decrease during the course of biotransformation. Therefore optical rotation of the recovered substrate was taken and found to be optically active. The best results were obtained at pH = 7.0 (Fig. 2a–d) and after 6 days (Fig. 1a–b). The absolute configuration ((*S*)-atenolol (**2a**) and (*S*)-propranolol (**2b**)) was assigned after correlating with literature data. So it is clear that (*R*)-alcohol gets preferentially metabolised.

In another approach similar experiments were carried out with (±)-atenolol acetate (**3a**) and (±)-propranolol acetate (**3b**). It was interesting to note that substrate (*R*)-atenolol acetate and (*R*)-propranolol acetate were metabolised maybe after hydrolysis as per earlier observations. However (*S*)-atenolol acetate and (*S*)-propranolol acetate can be recovered. The recovered acetates were chemically hydrolysed (with K_2CO_3–MeOH) to give (*S*)-atenolol and (*S*)-propranolol, respectively. In this case also, the maximum enantiopurities were obtained at pH = 7.0 and after 6 days (Fig. 1a–b). The absolute configuration

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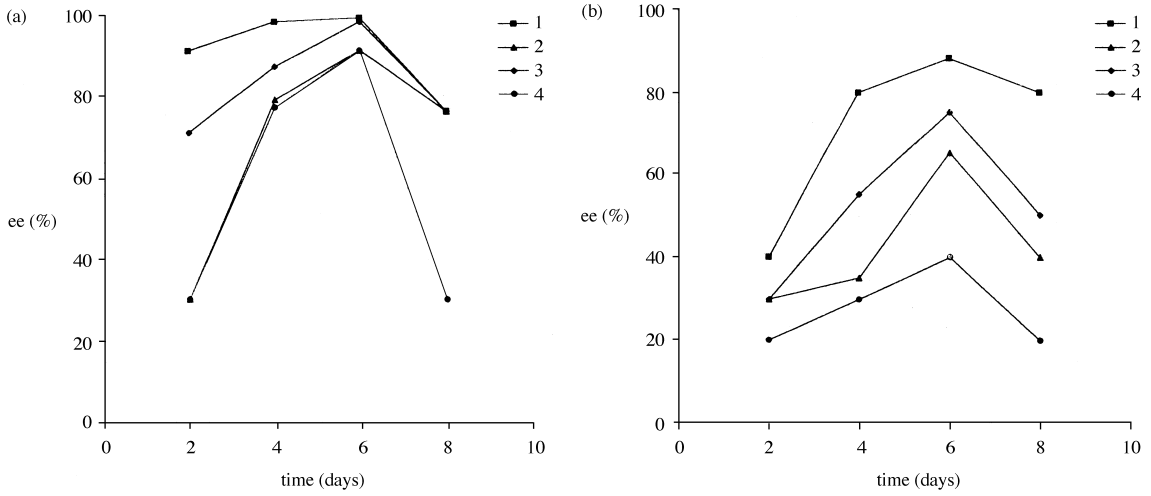


Figure 1. (a) Biotransformation of atenolol and atenolol acetate; 1 Atenolol; *R. arrhizus*; 2 Atenolol; *G. candidum*; 3 Atenolol acetate; *R. arrhizus*; 4 Atenolol acetate; *G. candidum*. (b) Biotransformation of propranolol and propranolol acetate; 1 propranolol; *R. arrhizus*; 2 Propranolol; *G. candidum*; 3 Propranolol acetate; *R. arrhizus*; 4 Propranolol acetate; *G. candidum*.

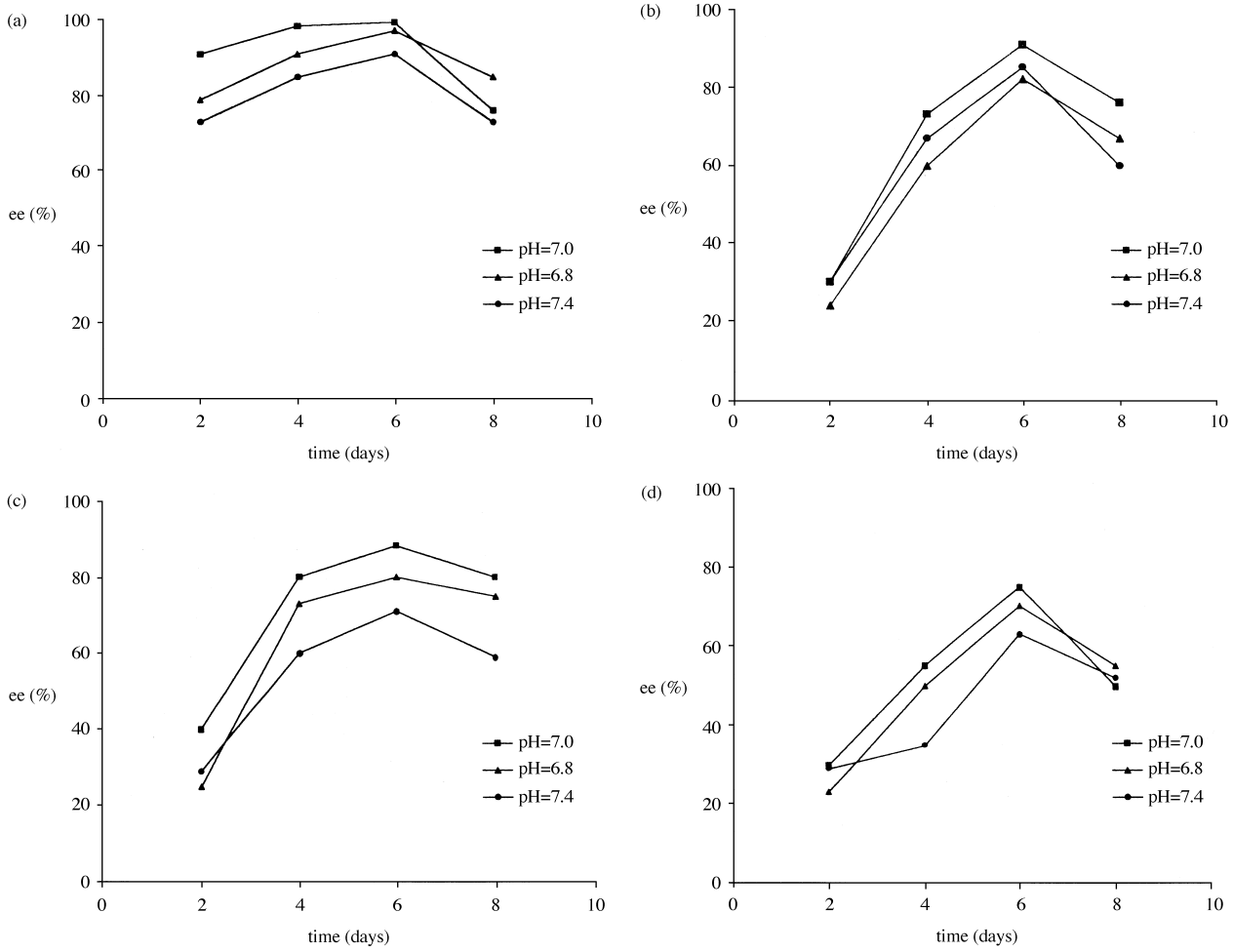
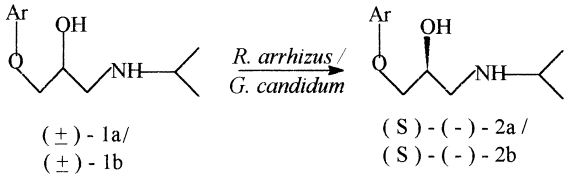


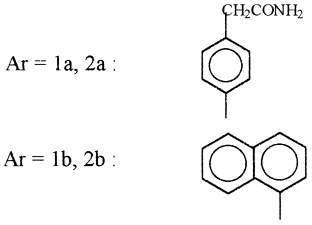
Figure 2. (a) Optimization of pH during the biotransformations (atenolol; *R. arrhizus*); (b) Optimization of pH during the biotransformations (atenolol; *G. candidum*); (c) Optimization of pH during the biotransformations (propranolol; *R. arrhizus*). (d) Optimization of pH during the biotransformations (propranolol; *G. candidum*).

((*S*)-atenolol (**2a**) and (*S*)-propranolol (**2b**)) was assigned after correlating with literature data. It could thus be inferred that both the fungus (*R. arrhizus* and *G. candidum*) preferentially metabolised the (*R*)-acetates.

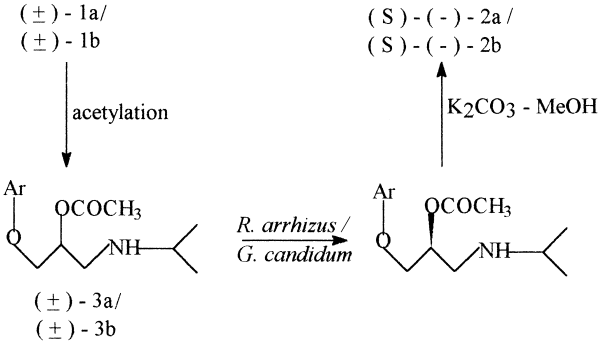
From the literature it appears that the lipase catalysed resolution of the intermediate chlorohydrin is the first report on the synthesis of chiral atenolol.[5] The method reports the optical yield >95% with the multi-step

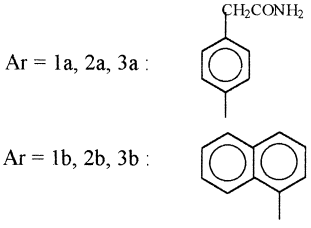
synthesis but with the use of expensive biocatalysts. On the contrary our present method describes the synthesis of enantiomerically pure β-blockers by microbial asymmetric destruction, with 88–99% ee. Our present approach is simple, single-step and totally new from the classical method of synthesis of chiral β-blockers.





Scheme 1.





Scheme 2.

The lipase catalysed (chemoenzymatic) preparation of (*S*)-propranolol via resolution of intermediates, cynohydrin and chlorohydrin were recently reported. Matsuo et al.[6] described the preparation of (*S*)-propranolol with 71% ee while Bevinakatti et al.[16] report good optical purity, 95% ee and overall 30% yield. The methods are multi-step synthesis and use expensive biocatalysts. Therefore our present method is simple, environmentally friendly, single-step producing (*S*)-propranolol with 40–88% ee, but suffers from the disadvantage like that of low atom efficiency. As far as our knowledge goes this is the first report on the synthesis of (*S*)-atenolol and (*S*)-propranolol employing the biodegradation approach.

From the experimental results (Scheme 1, Table 1), it clearly demonstrates that the incubation of the (±)-atenolol and (±)-propranolol itself with the fungus is a better method for the biodegradation of drugs mentioned above as compared with the method involving biodegradation of corresponding esters (Scheme 2, Table 1).

Conclusion

Biodegradation using *R. arrhizus* and *G. candidum* provides a simple and useful method to obtain (*S*)-atenolol and (*S*)-propranolol.

Experimental

Materials and methods

Chemicals. All reactions were monitored by TLC (thin layer chromatography) on precoated silica gel sheet 60 F 254 (Merck). Compounds were visualised by iodine vapours or in UV light. Preparative TLC was carried out with silica gel (Merck). The substrate (±)-atenolol,[13] (±)-propranolol[14] and their corresponding *O*-acetyl esters were prepared by literature procedure.[15]

Micro-organisms. *Rhizopus arrhizus* (NCIM 997), *Geotrichum candidum* (NCIM 980) were obtained from National Collection of Industrial Micro-organisms, National Chemical Laboratory, Pune, India.

Instrumentation. IR spectra were recorded on Perkin Elmer 170-X Infrared Fourier Transform Spectro-

Table 1. Results of biotransformation after 6 days at pH = 7.0[a]

Compound	Fungus	$[\alpha]_D$ (c, solvent)	ee (%)	Recovered yield (%)	Configuration
atenolol	*R. arrhizus*	−13.6 (1%, ethanol)[5]	>99	75	(*S*)
atenolol acetate	*R. arrhizus*	−12.7 (0.25%, ethanol)[5]	96	69	(*S*)
propranolol	*R. arrhizus*	−9.0 (0.5%, ethanol)[16]	88	74	(*S*)
propranolol acetate	*R. arrhizus*	−6.9 (1%, ethanol)[16]	68	70	(*S*)
Atenolol	*G. candidum*	−12.0 (0.5%, ethanol)[5]	91	74	(*S*)
Atenolol acetate	*G. candidum*	−11.6 (0.5%, ethanol)[5]	88	67	(*S*)
propranolol	*G. candidum*	−8.0 (1%, ethanol)[16]	78	73	(*S*)
propranolol acetate	*G. candidum*	−4.0 (2%, ethanol)[16]	39	68	(*S*)

[a]Lit.[5] $[\alpha]_D = -13.10$ (0.9%, ethanol, >99%ee); Lit.[16] $[\alpha]_D = -10.2$ (1.02%, ethanol).

photometer. ^{1}H NMR spectra were recorded on (VRX) 300 MHz Spectrometer, using $CDCl_3$ or DMSO-d_6 as solvents (TMS as internal reference). Optical rotation measurements were carried out on Jasco-360 polarimeter.

Determination of absolute configuration and enantiomeric excess. The absolute configuration $[\alpha]_D$ and enantiomeric excess (ee%) of microbial products were determined by correlating the data with those reported in the literature.[5,16]

Incubation condition. The fungus was inoculated in an autoclaved (15 psi for 20 min) 500 mL cotton plugged erlenmeyer flask containing 250 mL of the medium (*G. candidum*: 50 g D-glucose, 10 g yeast extract, 10 g peptone in 1 L water ; *R. arrhizus*: 50 g D-glucose, 10 g peptone, 3 g soya meal in 1 L water). The flasks were shaken on a rotatory shaker operating at 220 rpm (at 28±1 °C).

After 48 h of growth, the cells were separated from culture medium by filtration and washed repeatedly with saline and distilled water. A 10 g portion of wet cells was placed in a cotton plugged erlenmeyer flask containing 75 mL 0.2 M phosphate buffer (pH = 7.0), previously autoclaved. The substrate (±)-atenolol (100 mg/1 mL *n*-butanol) was incubated separately with the fungus *R. arrhizus* and *G. candidum* for different time intervals (2, 4, 6 and 8 days, respectively).

Enzymatic (whole cell) resolution. At the end of the fermentation, the cells were filtered off. The filtrate was extracted with *n*-butanol (atenolol)/$CHCl_3$ (propranolol/acetates). The cells were also washed with the respective organic solvent and the combined organic solvent was washed with water, dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and the residue was subjected to preparative TLC. (Solvent system : [for atenolol] = $CHCl_3$:MeOH 8:2/[for propranolol] = $CHCl_3$:MeOH 9.8:0.2). Recovered yields being 75 mg with *R. arrhizus* and 74 mg when *G. candidum* was used.

In case of *O*-acetyl esters, the residue obtained was subjected to preparative TLC. The isolated pure *O*-acetyl ester was hydrolysed (with K_2CO_3–MeOH) to yield optically pure atenolol/propranolol. The compounds obtained were characterised by IR and ^{1}H NMR.

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