INCREASED RACEMATE RESOLUTION OF PROPRANOLOL ESTERS BY LIPASE IMMOBILIZED CATALYSIS

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Abstract - A screening of six different immobilized lipases (C. rugosa, C. antarctica, P. fluorescens, M. miehie, R. niveus) was done to determine the best for the stereospecific hydrolysis of RS-O-BP. An experimental design (2^3) where the enzymatic activity was a function of temperature, buffer concentration and solvent was selected. P fluorescens showed the best results for S(-) isomer, with an increase of the stereoselectivity ratio of 1.5, using tetrahydrofuran as solvent, and 15 mM buffer phosphate concentration at 37 °C. C. rugosa showed the best results for R(+) isomer, with an increase of the stereoselectivity ratio of 1.7, using acetone as solvent, and 25 mM buffer concentration at 15 °C. The enantiomeric excess of S(-) propranolol with P fluorescens was 87%, higher than those reported elsewhere.

Keyword - Candida rugosa, Pseudomona fluorescens, Enantioselectivity, Biotransformation, Lipase, Propranolol.

I. INTRODUCTION

Propranolol (1-isopropilamino-3-(1-naphtoxy)-2-propanol) is a known beta-adrenergic blocking agent commonly used for the treatment of arterial hypertension (AHT) and some cardiovascular disorders (Barret, 1985). However, its use has been overpassed by other beta-blocking agents, Barret (1985) found the side-effects, mainly in asthma patients. Muscle contraction effects have been the cause of bronchoconstriction observed in those patients by Goodman and Gillman (1985). As propranolol is a mixture of two stereoisomers, S(-) and separately on AHT and bronchoconstriction. Barret (1985) demonstrated that such side effect could be attributed to the R(+) propranolol isomer, and therefore proposed to develop enriched S(-) propranolol isomer galenic formulation of the highest possible “optical” purity to avoid the observed adverse effect of commercially available racemic mixtures of propranolol.

Yost and Holtzman (1979) proposed a highly attractive method of propranolol racemate resolution at preparative scale. Propranolol stereoisomers were separated by multiple-step recrystallization of its di-(p-tolyl) tartaric acid salts.

Silber and Riegelman, 1980; Matsuo and Ohno, 1985; Terao et al., 1988 and Bevinakatti and Banerji, 1991, have proposed the preparation of S(-)-propranolol through immobilized catalysis using mainly several lipases as stereospecific catalysts. The uses of immobilized enzymes versus other conventional chemical catalysis are being increasingly preferred by the pharmaceutical industry because of its higher selectivity and yield, with a less environmental impact.

Bevinakatti and Banerji (1991) have published examples about stereoselective esterification or hydrolysis of several drug racemates for its resolution using lipases. Chiral discrimination is a long-standing problem in the development, use and action of pharmaceutical agents. Since many drugs are chiral compounds and interact with a chiral receptor in the body, only one of the enantiomers will show the optimal therapeutic action. To avoid side effects of the unwanted enantiomer, the strongly regulated pharmaceutical industry increasingly demands drugs containing only the biologically active enantiomer. However, some synthetically produced chiral substances are still being sold in a racemic mixture. This is the case of Propranolol. Currently the commercially available preparation is a racemic mixture, in which only the S(-)-enantiomer has beta-adrenergic blocking activity (Barrett, 1985; Howe and Shanks, 1966; Potter, 1967; Rahn et al., 1974; Walle et al., 1984). Barrett (1985) indicated that its side effects on asthma patients have been attributed to the R(+) isomer, which produces bronchoconstriction. Therefore, the resolution of commercial propranolol racemate in order to obtain the bioactive S(-) isomer at its highest purity is a matter of concern for a better treatment of AHT and asthma patients.

Several research groups (Silber and Riegelman, 1980; Hermansson and von Bahr, 1980; Hermansson, 1982; Thompson, et al., 1982; Matsuo and Ohno, 1985; Terao, et al., 1988; Bevinakatti and Banerji, 1991) have reported the preparation of (S)-propranolol by lipase catalysed reactions. However, the length of the procedure and the low overall yields preclude the industrial application of these methods.

Lipase catalysed reactions are superior to conventional chemical methods owing to mild reaction