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Enzymatic Resolution of 1,2-Diols : Preparation of Optically Pure Dropropizine

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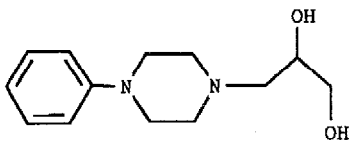
Key words: Lipase; stereoselective alcoholysis; 1,2-diols; Dropropizine; enzymes in organic solvent;

Abstract: Kinetic resolution of (R,S)-3-(4-phenyl-1-piperazinyl)--1,2-propanediol diacetate (2) by alcoholysis with *n*-propanol was carried out under lipase Amano PS catalysis in various organic solvents. Pure enantiomers of the corresponding diol (1) are useful as antitussive and central sedative therapeutic agents.

(R,S)-3-(4-phenyl-1-piperazinyl)-1,2-propandiol (Dropropizine) (1) is an antitussive agent used for a long time in the human therapy in form of racemic mixture. Recent studies have shown that the two separated isomers possess distinct pharmaco-therapeutic features which are particularly advantageous in comparison with the racemic mixture. Namely the levorotatory isomer possess the same antitussive activity as the racemic mixture, but is much more selective having lower activity degree on the central nervous system (CNS)[1]. The improvement in the activity/side effect (action on CNS) ratio obtained by means of the Dropropizine isomers involves an interest in the optical resolution of this drug.



(R,S) - 1

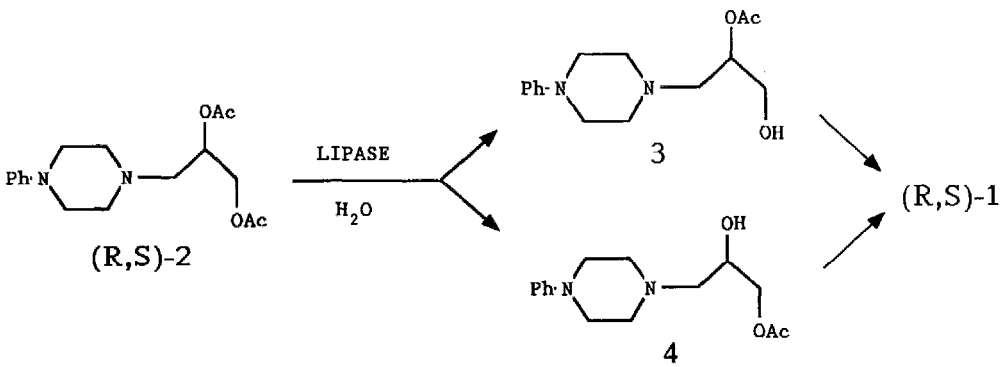
In the literature routes are reported for the synthesis of optically pure (1) starting from naturally occurring products, such as L-serine or D-mannitol[1,2]. However these methods require expensive chemical reagents and sometimes laborious procedures. In recent years have become apparent that enzymatic catalysis can offer significant advantages over classical chemical methods in the synthesis of optically active compounds[3].

In the present paper we report a new, efficient method for the

preparation of optically active (1) by lipase catalyzed stereoselective alcoholysis of the corresponding diacetate (2) with *n*-propanol.

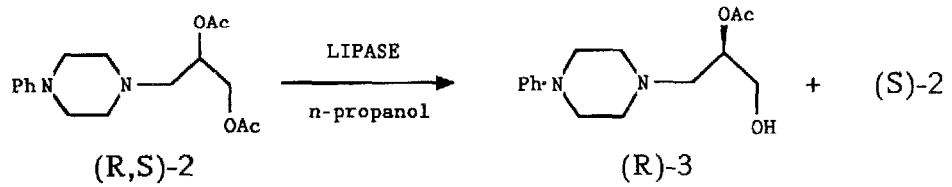
The resolution of the diester (2) was initially attempted via enzymatic hydrolysis using Lipase Amano PS from *Pseudomonas cepacia*[4] in water (Scheme 1). Hydrolytic reactions were carried out by adding 0.5 g (1.54 mmol) of (R,S)-2 and 30 mg of lipase to 20 ml of 0.5 M phosphate buffer pH 7, at 30°C. Periodically aliquots were withdrawn and analyzed by HPLC, using a chiral column Chiralcel OD (Daicel)[5]. The reaction was stopped after hydrolysis of 50% of (2). At this step the amounts of diol (1), secondary monoester (3), primary monoester (4) and diester (2) were quantitatively determined to be 5:10:35:50. The structures of primary and secondary monoesters were assigned by ^{1}H-NMR analysis[6], after isolation of the products by silica gel chromatography. The optical purity of unreacted (S)-(-)-2 was 73% according to HPLC analysis, and the absolute configuration was assigned by comparison of the optical rotation to the literature data[1], after alkaline hydrolysis to (S)-1. Both monoesters (3) and (4) underwent further enzymatic hydrolysis to yield racemic diol (1), proving the low regioselectivity and stereoselectivity of lipase PS in these reaction conditions.

SCHEME 1



In a second approach we studied the enzymatic alcoholysis of the diester (2) in organic solvent, using *n*-propanol as nucleophile (Scheme 2). The reactions were carried out by suspending 0.1 g of lipase PS in a solution of 0.5 g (1.54 mmol) of (R,S)-2 and 0.74 ml (12.32 mmol) of *n*-propanol in 10 ml of hexane. The suspension was shaken on an orbital shaker at 200 rpm at 30°C.

SCHEME 2



Surprisingly the behavior of lipase PS in these reaction conditions was dramatically different than in water. As shown in scheme 2, the reaction spontaneously stopped after the stereoselective cleavage of the primary ester group, affording 50% of (S)-(-)-**2**; {$[\alpha]_D^{25}$=-6.4° (C=1, EtOH), ee= 95%} and 47% of (R)-(+)-**3**; {$[\alpha]_D^{25}$=+7.7° (C=1, EtOH), ee= 95%[8]}. It is noteworthy that in the alcoholysis reaction only small traces of monoester (**4**) (less than 3%) were detected, indicating a higher regioselectivity than that displayed by the same enzyme in the hydrolytic conditions. Changes in chemoselectivity and stereoselectivity upon a transition from water to organic media were previously observed with lipase PS for other type of reactions[9]. In order to investigate the effects of the nature of the organic media on the selectivity of the enzyme, the alcoholysis reactions were carried out in various solvents with different hydrophobicity (log P) and dielectric constant (ϵ), using *n*-propanol as nucleophile. The resulting enantioselectivity (expressed as enantiomeric ratio, E)[10] is showed in table 1. For all the tested solvents the regioselectivity displayed in *n*-hexane was confirmed.

Table 1. Effect of the Solvent on Enantioselectivity of Lipase PS.

SOLVENT	log P	ϵ	NUCLEOPHILE	E
H_2O	-	78.54	H_2O	17
hexane	3.5	1.89	*n*-propanol	146
CCl_4	3.0	2.24	*n*-propanol	502
toluene	2.5	2.37	*n*-propanol	120
iso-propylether	1.9	3.88	*n*-propanol	152
2-methyl-2-butanol	1.45	5.82	*n*-propanol	589
2-methyl-2-butanol	1.45	5.82	H_2O	63
n-propanol	0.28	20.1	*n*-propanol	181
acetonitrile	-0.33	36.2	*n*-propanol	82
1,4-dioxane	-1.1	2.2	*n*-propanol	164

It can be seen that there was no correlation between the properties of the solvents and the selectivity of lipase PS. However the enantioselectivity displayed in organic media was always higher than that measured in water. This difference is presumably due to the higher rigidity of the enzyme in anhydrous organic solvent respect to the water, where the increased protein's conformational flexibility results in a relaxation of stereoselectivity[11]. This hypothesis was confirmed by carrying out the hydrolysis of (R,S)-**2** in 2-methyl-2-butanol presence of 1% of water acting as nucleophile. In this conditions only the primary ester was hydrolyzed and the enantiomeric ratio increased from 17 (calculated for the hydrolysis in 100% aqueous solution) to 63.

In conclusion the method here reported illustrates the crucial role of the reaction media in the lipase PS-catalyzed resolution of Dropropizine. The extension of this procedure for the preparation of optically active 1,2-diols is currently under investigation.

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4. Comparable results were obtained using porcine pancreatic lipase (Sigma) and lipase from *Candida cylindracea* (Sigma).
5. HPLC analysis. Eluent: Hexane\2-propanol 9:1; Flow: 0.8 ml\min. Retention times: (S)-2 12.33 min; (R)-2 15.03 min; (R,S)-3 18.12; (R,S)-4 22.27.
6. ^{1}H-NMR analysis (200 MHz, $CDCl_3$). Monoester (3) δ: 3.9 ($C\underline{H}_2OH$, m), 5.2 ($C\underline{H}OAc$, m); monoester (4) δ: 4.0 ($C\underline{H}OH$, m), 4.1 ($C\underline{H}_2OAc$, m). Porcine pancreatic lipase catalyzed transesterification of (R,S)-1 in ethyl acetate, carried out using the procedure described by Cesti et al.[7] afforded (R,S)-4, confirming the assignation of the structures.
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8. The optical purity of (R)-(+)-3 was determined by HPLC after transformation into (R)-(+)-2 by reaction with acetic anhydride.
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