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LIPASE-MEDIATED ESTERIFICATION OF RACEMIC ALCOHOLS: A COMPARISON OF 2-SUBSTITUTED CYCLOHEXANOLS AND CYCLOPENTANOLS

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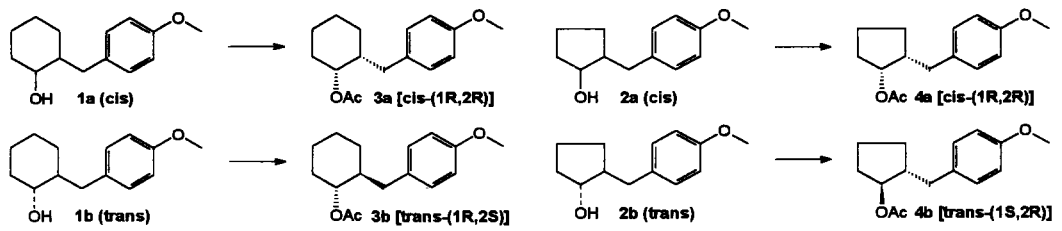
SUMMARY
Esterification of five- and six-membered-ring-containing alcohols catalysed by three different lipases (those from porcine pancreas, *Candida cylindracea* and *Geotrichum candidum*) was studied. Some conversions gave high stereochemical purity, but all gave low yields.

INTRODUCTION

Continuing research on enzyme-mediated synthesis of chiral precursors of biologically active compounds, our attention was focused on comparable experiments using 2-substituted cyclohexanols and cyclopentanols under identical reaction conditions. The current experiments followed the recently obtained results with the respective *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)-1-cyclohexanol (Zarevúcka *et al.*, 1993, 1994). The substrates used in this part of research, i.e. 2-(4-methoxybenzyl)-1-cyclohexanol and 2-(4-methoxybenzyl)--1-cyclopentanol, are the source of their respective enantiomers, which are convenient intermediates in the synthesis of biologically active insect juvenile hormone analogs. However, synthesis of pure enantiomers of 2-substituted cyclohexanols and cyclopentanols might also have more advantages. The basic question on the applicability of the results of enzyme--mediated resolution reactions obtained with 2-substituted cyclohexanols to 2-substituted cyclopentanols would have to be answered in this stage of research. **Scheme 1** shows the general reaction pathway of the enzymic reactions engaged. Only the main products are shown in the **Scheme 1**, while the opposite enantiomers, which occur in the reaction as alcohols, are not depicted there. The selectivity of the enzymic reactions is always higher with respect to the main product(s), while the remaining product(s) (especially those in the enzyme-mediated resolution reactions) are less favored considering their stereochemical purity.



Scheme 1: Lipase mediated resolution of racemic alcohols **1a**, **1b**, **2a** and **2b**

MATERIALS AND METHODS

Enzymes used

Porcine pancreatic lipase, PPL (135U.mg^{-1}) and lipase from *Candida cylindracea*, CCL (1010U.mg^{-1}) were purchased from Sigma. Lipase from *Geotrichum candidum* (GCL) was purchased from Amano.

Transesterification - general procedure I

A lipase (10 mg) was added to a magnetically stirred solution of **1a**, **1b**, **2a** or **2b** (0.23mmol) in 2 ml of vinyl acetate, and the reaction mixture was stirred at laboratory temperature. Periodically, 20 µl aliquot amounts of the liquid phase were withdrawn and analyzed by HPLC. When no more reaction progress was observed, the reaction was stopped. The solid enzyme was filtered off and the solvent was evaporated. The residue was purified by column chromatography to afford the unreacted alcohols in its enantiomerically enriched form, and the corresponding acetates **3a**, **3b**, **4a** and **4b**. The optical purity [i. e. the enantiomeric excess (ee) values] of acetates were determined by HPLC analysis of their 3,3,3-trifluoro-2-methoxy--2-phenylpropanoic acid (MTP) ester derivatives after their alkaline hydrolysis.

Transesterification - general procedure II

Vinyl acetate (2ml), 10mg of lipase and 10mg of powdered molecular sieves (4A) were added sequentially to 0.23mmol of racemic alcohols **1a**, **1b**, **2a** or **2b**. The resulting suspension was stirred at laboratory temperature and monitored by HPLC. The solid enzyme and the molecular sieves were filtered off, and the solvent was evaporated. The residue was purified by column chromatography. The ee values of the acetates **3a**, **3b**, **4a** and **4b** were determined by HPLC analysis of their MTP ester derivatives after their alkaline hydrolysis.

Alkaline hydrolysis of the esters 3a, 3b, 4a and 4b

The following procedure is representative: The esters **3a**, **3b**, **4a** or **4b** were dissolved in a 1M NaOH in absolute ethanol (1ml). The solution was stirred for 4h at laboratory temperature. The solvent was evaporated, and the residue was purified by a short column chromatography on silica gel.

MTP esters

Preparation of the diastereoisomeric MTP esters of alcohols studied in a microscale using MTPCl (3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride) as reagent has already been described in details (Dale *et al.*, 1969; Dale and Mosher, 1970; 1973; Sullivan *et al.*, 1973; Minamikawa and Brossi, 1978). The results of the determination of the optical purity of the alcohols **3a**, **3b**, **4a** and **4b** obtained are summarized in **Table 1**.

Analytical methods used

The ^{1}H NMR spectra were recorded on a Varian UNITY 500 spectrometer (in a FT mode) at 499.8 MHz frequency in deuteriochloroform using tetramethylsilane as internal reference. The ^{19}F NMR measurements were taken on the same spectrometer at 470.27 MHz frequency in deuteriochloroform using hexafluorobenzene as external reference (δ = -162.9 ppm). Column chromatographies were performed on a silica gel (Herrmann, Köln-Ehrenfeld, FRG). Precoated silica plates were used for TLC analyses. HPLC analyses of the reaction mixtures were performed on a Waters HPLC instrument consisting of a Waters 600 Controller, a Waters 717 plus Autosampler, a Waters 486 Tunable Absorbance Detector (215 nm), and a Novo Pak C_{18} (3.9 (i. d.) x 150 mm) reverse phase column, using water/methanol (2 : 3) as mobile phase, flow rate 1.5 $ml.min^{-1}$.

HPLC analyses of the MTP esters were performed on a Thermoseparation Products HPLC instrument equipped with a ConstaMetric 4100 Bio pump, a SpectroMonitor 5000 UV DAD and a series of 3 columns (4 (i. d.) x 250 mm each) filled with a Separon (5 mm) silica gel phase, using light petroleum with ether (10 %) as mobile phase, flow rate 2.5 $ml.min^{-1}$.

RESULTS AND DISCUSSION

The reaction course is shown in **Scheme 1**, in which only the main products of the reactions are shown. It is summarized in **Table 1** that the lipases worked with very variable results, based on the chemical yields and the enantiomeric excess values. With the substrates **1a** and **1b**, PPL and GCL gave much more favorable results than CCL did. However, acceptable chemical yields were only obtained using GCL to esterify **1b**. Enzymic esterification of **1a** gave low yields in each case. The absolute configuration of the products **3a** and **3b** shown in **Scheme 1** was assigned *via* their MTP esters (cf. Materials and Methods).

Measurement of the reaction kinetics proved a typical reaction course of this enzymic process showing no deviations. Samples of the reaction were analyzed under the conditions given in Materials and Methods. The course of the curves obtained corresponded with the yield of the products after final work-up of the reaction mixture.

With the substrates **2a** and **2b,** GCL again seems to be the most favorite enzyme to proceed the title reaction. However, the yields are generally lower than those with **1a** and **1b**. Assigning the absolute configuration (*via* MTP esters), we found that **4a** was formed with the same absolute configuration as **3a**, while **4b** is the opposite enantiomer to the analogous six-membered-ring-containing compound **3b (Scheme 1)**. This finding was very important to start a separate study on determination of the absolute configuration of 1,2-disubstituted cyclopentanols, the topic of a separate paper (Zarevúcka *et al.*, 1995a). The optical purity of the products **3c**, **3b**, **4a** and **4b** was determined (after their two-step conversion into the corresponding MTP esters) by HPLC on a silica gel phase. Identity of the UV spectra of both

(the major and the minor) components of the diastereoisomeric mixture was used to identify both components in a mixture. The results are also summarized in the **Table 1**.

The ^{1}H and ^{19}F NMR spectra recorded are subjects of a more detailed analysis presented in a separate paper (Zarevúcka *et al.*, 1995b).

Table 1: Esterification of the alcohols **1a - 2b**

general proced.	substrate	enzyme	yield [%]	ee [%][a]	substrate	enzyme	yield [%]	ee [%][a]
I		PPL	3	98		PPL	6	74
	1a	CCL	0.3	80	**2a**	CCL	3	72
		GCL	5	> 99		GCL	2	94
		PPL	7	96		PPL	4	> 99
	1b	CCL	7	15	**2b**	CCL	8	48
		GCL	24	98		GCL	3	44
II		PPL	0.2	99		PPL	3	90
	1a	CCL	3	75	**2a**	CCL	4	55
		GCL	6	79		GCL	6	94
		PPL	5	93		PPL	12	> 99
	1b	CCL	18	20	**2b**	CCL	18	80
		GCL	23	97		GCL	11	96

[a] determined on the basis of the optical purity of the alcohol obtained from the ester by alkaline hydrolysis

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