*Tetrahedron* Vol. 49, No 22, pp. 4939-4944, 1993
Printed in Great Britain

0040-4020/93 $6 00+ 00


# Enzymatic Separation of *Cis/Trans* 1,4–Cyclohexanedimethanol Mixtures by Mono– and Polytransesterification

**Shimona Geresh,[1]* Elizabeth Elbaz,[2] and Robert Glaser[2]**

[1]The Institutes for Applied Research, and [2]Department of Chemistry
Ben–Gurion University of the Negev, Beer–Sheva 84110, ISRAEL

(*Received in UK* 23 *March* 1993)

***Abstract:*** Lipase-catalyzed poly- and monotransesterification reactions were used in kinetic separations of a commercial mixture of *cis,trans*–1,4–cyclohexanedimethanol The reactions were performed with lipases from various sources and with mono- and diesters as the acylating reagents In a series of monotransesterifications, the highest diastereoselectivity (1 6 5) was obtained with β–chloroethyl hydrocinnamate as the acyl donor and lipase from *Pseudomonas fluorescens* Polycondensations with fumarate esters using lipase from porcine pancreas afforded moderate diastereoselectivity (about 1 4 5) for the *cis/trans* monocondensate, and markedly increased diastereoselectivity (about 1 24) for the *cis/trans* dicondensate product

## INTRODUCTION

Biotransformations and kinetic resolutions may be performed efficiently with enzymes, especially hydrolases. Lipases are the most commonly used hydrolases since they are readily available, relatively stable, and may be obtained from a variety of sources Kinetic and enantioselective resolutions have been performed using lipase-catalyzed hydrolyses, esterifications, transesterifications, and polyesterifications [1-7]

Recently, we have shown that "all–trans" unsaturated polyesters (alkyds) can be prepared by lipase-catalyzed polycondensation of fumarate esters with a variety of aliphatic and aromatic diols [8,9] The mild conditions required in enzymatic reactions permit the synthesis of "all–trans" alkyds in which the *trans* configuration of the double-bond appears to influence the overall physicomechanical properties of cured polyesters [10] The "all–trans" fumarate polyesters differ from the olefinic polyesters prepared as commodity chemicals for general purpose use (the latter contain a mixture of *cis* and *trans* double-bonds) Another example in the polymer industry in which the *cis/trans* isomeric nature of monomer has not received special consideration is that of 1,4–cyclohexanedimethanol Polyester films and fibers with interesting properties (such as high resistance to hydrolysis) have been prepared from the polycondensation of mixtures of *cis*– and *trans*–1,4–cyclohexanedimethanol (**1** and **2**) with aromatic diacids and/or hydroxycarboxylic acid,[11-13] but not with pure diastereomers

To determine how the configuration of the *cis/trans* diol influences the final properties of the polymers, polyesters have to be synthesized from each diastereomer, and then characterized Since neither pure *cis*– nor pure


1


2

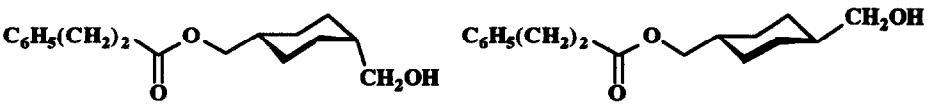
*trans*-1,4-cyclohexanedimethanol is commercially available,[14] the ability of of various lipases to differentiate between the two diastereomers was investigated in transesterifications of the diol mixture with mono- and diesters In this paper we describe the kinetic separation of a commercially available mixture of *cis*- and *trans*-1,4-cyclohexanedimethanol (ratio *ca.* 1·2 5). The reaction studied was the lipase-catalyzed transesterification of the diol mixture with various acyl donors: an activated ester (2-chloroethyl hydrocinnamate), an activated diester [di(2-chloroethyl) fumarate], and vinyl acetate

## RESULTS AND DISCUSSION

*Cis/trans configurational assignment.* $^1$H NMR showed the 1,4-cyclohexanedimethanol starting material to contain two diastereomers in the ratio of 1·2 4 The -CHC$\underline{H}_2$OH methylene protons afforded major and minor intensity signals at δ 3.45 (d, 6 3 Hz) and δ 3 53 (d, 6 8 Hz), respectively. The *cis/trans*-mixture was non-enzymatically converted to the corresponding dibenzoate esters containing the same ratio of isomers [major species. δ 4 10 (d, 6.2 Hz, -CHC$\underline{H}_2$OC=O) and minor species δ 4 20 (d, 7 2 Hz, -CHC$\underline{H}_2$OC=O)] *Trans*-1,4-cyclohexanedimethanol dibenzoate [mp 122-124° C, lit [15] 125° C, δ 4 10 (d, 6 2 Hz, -CHC$\underline{H}_2$OC=O)] was prepared by fractional crystallization of the *cis/trans*-mixture Hydrolysis of the *trans*-dibenzoate afforded the pure *trans*-dimethanol [δ 3 45 (d, 6.3 Hz, -CHC$\underline{H}_2$OH)], and enabled the assignment of *trans*-stereochemistry for the major species of the commercial cyclohexanedimethanol mixture. *Cis/trans* mixtures of the various esters used in this study were non-enzymatically prepared from the known *cis:trans* = 1 2 4 ratio 1,4-cyclohexanedimethanol starting material In the limited series of esters used in our study, the major component in the non-enzymatically prepared isomeric mixture of esters gave the higher field (closer to TMS) -CHC$\underline{H}_2$O methylene doublet signal, and thus was assigned as the *trans*-isomer

*Lipase-catalyzed transesterification of the diol mixture with 2-chloroethyl hydrocinnamate* Lipases from porcine pancreas and *Pseudomonas fluorescens* were used to test a possible kinetic separation of the commercial mixture of 1,4-cyclohexanedimethanol isomers through a transesterification reaction. The results presented in Table 1 show the expected decrease in diastereoselectivity as a function of time The best results were obtained in one day with *P. fluorescens* lipase (*cis/trans* =1 6 5) No dicondensate product was observed, probably as a result of the 1 1 mole ratio of diol to chloroethyl hydrocinnamate used in the reaction. In addition, changes in enzyme quantity or substrate concentration were found to influence the rate of product formation, but did not significantly change the degree of diastereoselectivity

$^1$H NMR spectroscopy was used to estimate the *cis/trans* ratio in both the ester monocondensates (**3**,**4**), and in the remaining unreacted diols, as well as the extent of the reaction (ratio of monocondensates to unreacted ester starting material), see Figure 1 $^1$H NMR spectroscopy ($CDCl_3$) **3** (*cis*) δ 3 93 (d, 7 2 Hz, -CHC$\underline{H}_2$OC=O) and δ 3 47 (d, 6 8 Hz, -CHC$\underline{H}_2$OH), **4** (*trans*) δ 3 83 (d, 6 4 Hz, -CHC$\underline{H}_2$OC=O) and δ 3 38 (d, 6 3 Hz, -CHC$\underline{H}_2$OH)




**3** **4**

Table 1. Diastereoselectivity of the lipase-catalyzed transesterification of a mixture of *cis/trans*-1,4-cyclohexanedimethanol with 2-chloroethyl hydrocinnamate.[a]

| lipase | reaction time (days) | *cis/trans* ratio monocondensate ester (**3**:**4**)[b] | monocondensate (**3**+**4**):unreacted 2-chloroethyl hydrocinnamate[c] |
|---|---|---|---|
| Porcine pancreas | 1 | 1 3 2 | 1.5 1 |
| Porcine pancreas | 3 | 1:2 7 | 34·1 |
| *Pseudomonas fluorescens* | 1 | 1:6.5 | 17·1 |
| *Pseudomonas fluorescens* | 3 | 1.4.3 | 33:1 |

[a]1,4-Cyclohexanedimethanol 2-chloroethyl hydrocinnamate mole ratio = 1 1, *cis:trans* 1,4-cyclohexanedimethanol starting material mole ratio = 1 2 5 [b]Measured from δ 3 93 [d, 7 2 Hz, **3** (*cis*)] and δ 3 83 [d, 6 4 Hz, **4** (*trans*)] -CHC$H_2$OC=O $^1$H NMR resonances [c]Measured from δ 3 93 and 3 83 -CHC$H_2$OC=O $^1$H NMR resonances for **3** and **4**, respectively, relative to the δ 4 23 -OC$H_2$C$H_2$Cl signal for 2-chloroethyl hydrocinnamate

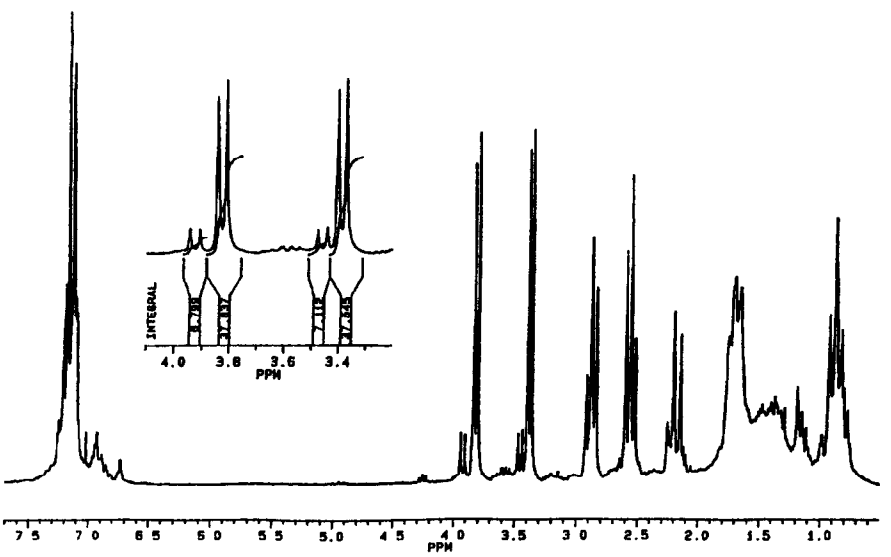



Figure 1 $^1$H NMR spectrum ($CDCl_3$) of a mixture of *cis/trans* monocondensates **3**,**4** obtained from the *Pseudomonas fluorescens* lipase catalyzed transesterification of a *cis,trans*-1,4-cyclohexanedimethanol 1·2 5 mixture with 2-chloroethyl hydrocinnamate

*Lipase-catalyzed transesterification of the diol mixture with vinyl acetate.* The enzymatic reaction was very fast and less diastereoselective when excess[3] vinyl acetate was used as the acyl donor in the lipase-catalyzed reaction of the diol mixture [vinyl acetate:diol molar ratio = 4·1]. Changes in diastereoselectivity or percent conversion as a function of enzyme quantity are shown in Figure 2 for the lipase from *P. fluorescens*. When 1.0 g lipase was utilized, for example, 43% of the unreacted starting diol mixture remained (*i.e.* 57% conversion) after 0 5 hours reaction time. Reaction product analysis showed only traces of the monoester. The primary product was found to be the diacetates [low (*ca.* 4%) diastereoselectivity]. After longer reaction times [1.5 hours] the diastereomeric ratio of the diester products was measured to be the same as that of the starting commercial mixture of diols (*cis:trans* = 1:2.5) Thus, diastereoselectivity was obtained only for low conversions using vinyl acetate as the acyl donor in the presence of *P. fluorescens* lipase. The same reaction performed with 1.0 g of *Mucor mehei* lipase (an immobilized lipase from Novo) afforded only 3% unreacted diols (*i.e.* 97% conversion) after one-half hour reaction time and no monoester products were detected. Also in this case, the diastereomeric diester product ratio was found to be the same as that of the starting commercial mixture of diols (*cis:trans* = 1 2 5) since the reaction was very fast

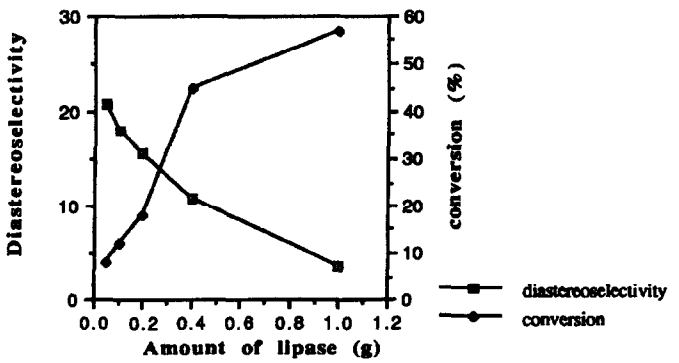
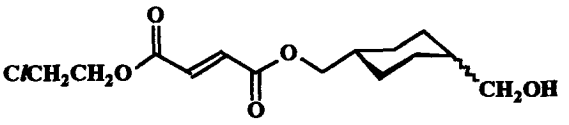
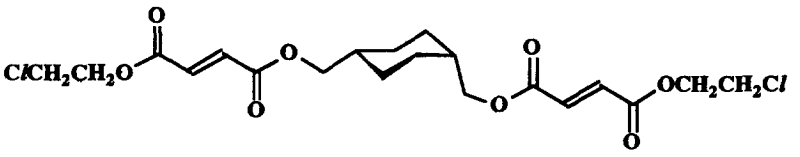



Figure 2 Diastereoselectivity and conversion in the lipase catalyzed acylation of a *cis,trans*-1,4-cyclohexanedimethanol 1·2.5 mixture with vinyl acetate as a function of the amount of enzyme The *trans* acetate ester values were normalized relative to the *cis* product. The conversion was calculated from the ratio of mono- and diester formed (formed with vinyl acetate) to remaining unreacted diols.

*Lipase-catalyzed transesterification of the diol mixture with di(2-chloroethyl) fumarate* The enzymatic reaction of the commercial mixture of diols with a diester was also investigated. In this case, di(2-chloroethyl) fumarate was chosen for reaction catalyzed by lipases from *P. fluorescens*, and porcine pancreas, as well as the immobilized lipase from *M. mehei* Oligomers were detected in all the reactions, but the reaction was usually stopped at the mono- and dicondensate level. The monocondensate fraction showed a *cis/trans* mixture (**5**:**6**) of *ca.* 1:4.5 using porcine prancreas lipase The second transesterification catalyzed by porcine pancreas lipase yielded the *trans*-dicondensate with considerable diastereoselectivity [**7**,**8** *cis/trans* ratio = *ca.* 1:19] Similar results were obtained when diethyl fumarate was utilized as the acyl donor [monocondensate *cis:trans* = 1 4.5, dicondensate *cis:trans* = 1:24] $^1$H NMR spectroscopy ($CDCl_3$)· **5** (*cis*) δ 4 07 (d, 7 2 Hz, -CHC$\underline{H}_2$OC=O) and δ 3 48 (d, 6 7 Hz, -CHC$\underline{H}_2$OH); **6** (*trans*) δ 3 97 (d, 6.4 Hz, -CHC$\underline{H}_2$OC=O) and δ 3 39 (d, 6.2 Hz, -CHC$\underline{H}_2$OH), **7** (*cis*) δ 4.13 (d, 7 3 Hz, -CHC$\underline{H}_2$OC=O); **8** (*trans*) δ 4.03 (d, 6 2 Hz, -CHC$\underline{H}_2$OC=O), see Figure 3




**5 (cis); 6 (trans)**




**7**


**8**

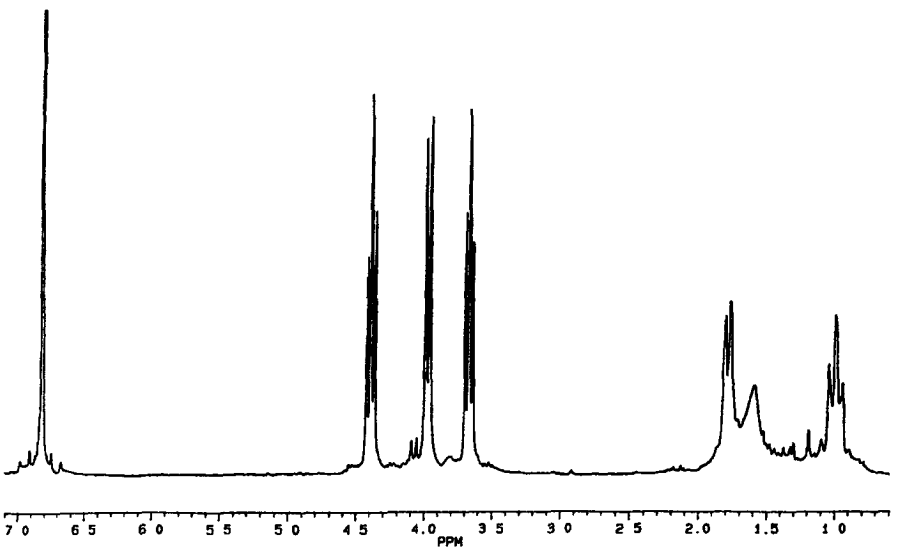



Figure 3 $^1H$ NMR spectrum ($CDCl_3$) of a mixture of *cis/trans* dicondensates **7,8** obtained from the procine pancreas lipase catalyzed transesterification of a *cis,trans*-1,4-cyclohexanedimethanol 1:2.5 mixture with di(2-chloroethyl) fumarate

The difference in diastereoselectivity obtained with lipases from various sources points to the subtle spatial differences in their active sites which enable them to discriminate between the *cis* and *trans* isomers of 1,4–cyclohexanedimethanol The almost pure *trans*–dicondensate obtained in this study will be hydrolyzed chemically, and the resulting *trans*–1,4–cyclohexanedimethanol will be used in polymer synthesis

## EXPERIMENTAL

*General.* Melting points are uncorrected and were determined in open-ended capillaries. $^1$H NMR spectra (4.7 T, $CDCl_3$, 298 K) were recorded at 200 MHz on a Bruker WP-200-SY FT-NMR spectrometer Tetramethylsilane was added as an internal standard. Chemicals and solvents were purchased from commercial sources. Lipase from porcine pancreas was bought from Sigma. Lipase from *Mucor miehei* was obtained as a gift from Novo Industri, Denmark. Lipase from *Pseudomonas fluorescens* was purchased from Amano, Japan Esters used as substrates were prepared according to known esterification methods.

*Enzymatic reaction.* A commercially available lipase (0.5 g) was added to a mixture of *cis/trans*–1,4–cyclohexanedimethanol and the corresponding ester (mole ratio dimethanol:ester = 1:1 or 2:1) in 40 mL of methyltert-butyl ether. The mixture was shaken on a gyrorotatory shaker at 250 rpm and 38° C. Aliquots were removed for TLC analysis and in different runs the reaction was stopped after various periods of time. The enzyme was then filtered off, the solvent was removed at reduced pressure, and the reaction mixture was separated by silica-gel column chromatography using a gradient of ethyl acetate in petroleum ether (60-80° C).

## REFERENCES

1. Cambou, B.; Klibanov, A M. *J. Am. Chem. Soc.* **1984**, *106*, 2687-2692.
2. Whitesides, G. M.; Wong, C. -H. *Angew. Chem. Intl. Ed. Engl.* **1985**, *24*, 617-638.
3 Wang, Y. -F., Chen, S. -T.; Liu, K. K. -C.; Wong, C. -H. *Tetrahedron Lett.* **1989**, *30*, 1917-1920.
4. Bianchi, D.; Cesti, P; Battistel, E. *J. Org. Chem.* **1988**, *53*, 5531-5534.
5. Bevinakatti, H. S.; Banerji, A. A. *J. Org. Chem.* **1991**, *56*, 5372-5375
6. Wallace, J S.; Morrow, C. J *J. Polym. Sci. Part 4: Polym. Chem.* **1989**, *27*, 2553-2567.
7. Gutman, A. L., Oren, D.; Boltanski, D ; Bravdo, T *Tetrahedron Lett.* **1987**, *28*, 5367-5368.
8. Geresh, S., Gilboa, Y. *Biotech. Bioeng.* **1990**, *36*, 270.
9. Geresh, S ; Gilboa, Y. *Biotech. Bioeng.* **1991**, *37*, 373.
10 Geresh, S., Gilboa, Y.; Abrahami, S., Bershadsky, A. *J. Adv. Polym.* **1993**, in press.
11. Manley, T R., Williams, D. A. *Polymer* **1971**, *12*, 2-26.
12. Toyo Rayon Co. Ltd , **1961**, Japan Pat. No. 15,395. *Chem. Abstr.* **1962**, *56*, 11802a.
13. Eastman Kodak Co. Inc , **1962**, U.S. Pat. No 3,033,822. *Chem. Abstr.* **1962**, *57*, 4903g
14 Andruzzi, F.; Ceccarelli, G., Paci, M. *Polymer* **1980**, *21*, 1180-1184.
15 Haggis, G A.; Owen, L. N *J. Chem. Soc.* **1953**, 404-407.

## ACKNOWLEDGEMENT

Gratitude is expressed to the Kreitman Family Endowment Fund, Ben-Gurion University of the Negev, for the purchase of a Bruker WP-200-SY FT-NMR spectrometer. Presented by E E. in partial fulfillment of the M Sc degree requirements of Ben-Gurion University of the Negev