Communications to the Editor

Enzymatic Syntheses of Alkyds. II: Lipase-Catalyzed Polytransesterification of Dichloroethyl Fumarate with Aliphatic and Aromatic Diols

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"All-trans" alkyds (unsaturated polyesters) were prepared by lipase-catalyzed polytransesterifications of diesters of fumaric acid and 1,4-butane diol. Industrially prepared alkyds, however, contain aromaticity in their backbone, which is introduced by phthalic acid derivatives and/or isomers. Thus, in this study, the specificity of lipases toward aromatic esters, diesters, and diols was investigated in order to find a means of introducing aromaticity in the lipase-catalyzed synthesis of "all-trans" alkyds. It was found that lipases from *Pseudomonas* and *Mucor* efficiently catalyzed the polytransesterification of dichloroethyl fumarate with aromatic diols such as benzene dimethanol. Crystallinity was found in the "all-trans" alkyds synthesized in acetonitrile. In order to determine whether this property was due to the nature of diol or the frequency of the double bond in the backbone of the polyester, other alkyds were prepared in which the length of the diol was changed as well as the number of carbons (odd or even) in the diol and the chemical nature of the diol (primary or secondary). In all cases crystallinity was found. It thus seems that the stereochemistry of the double bond is responsible for this property. Molecular weights, which were determined by gel permeation chromatography (GPC) analyses, were comparable with those of the industrially prepared alkyds.

INTRODUCTION

The first step in the industrial preparation of "general-purpose polyesters" involves the condensation of an unsaturated dicarboxylic acid (or anhydride or ester derivative) and/or an aromatic dicarboxylic acid (or one of its derivatives) with a diol to produce an alkyd. The alkyd is then cured to produce the thermoset general-purpose polyester. During the conventional preparation of alkyds, unsaturated constituents undergo extensive cis/trans isomerization. In contrast, in alkyd syntheses that are catalyzed by lipases, an "all-trans" product is obtained.[3] The presence of trans double bonds in the polymer backbone both improves the quality of the final product[2] and facilitates a faster curing reaction in the subsequent processing of the alkyd.

Biocatalyzed reactions in organic solvents,[5-7,10,12] including enzyme-catalyzed transesterification of diesters with diols,[1,8,11] have previously been reported. We have applied this type of biosynthetic method in an ongoing study of lipase-catalyzed syntheses of polyesters. In our first set of experiments fumaric acid esters were reacted with 1,4-butane diol.[3] Fumaric acid was chosen as the model compound, since it is one of the starting materials often used in the synthesis of general-purpose polyesters. In the lipase-catalyzed transesterification of fumaric acid diesters with 1,4-butane diol in acetonitrile, a crystalline solid alkyd insoluble in most organic solvents was obtained.[3] When tetrahydrofuran (THF) was used as the solvent, a low molecular weight soluble alkyd was obtained. We thus deemed it necessary in future studies to investigate the effects of the nature of the diol and the frequency of the double bond in the backbone of the polyester on crystallinity or solubility of the final polyester in organic solvents.

The only functionality lacking in our unsaturated polyester which is often present in general-purpose polyesters is aromaticity, which is generally introduced by using phthalic acid derivatives or their isomers. Thus, we sought a way of introducing the aromatic function into our "all-trans" polyester backbone.

The aim of the present study was thus twofold: to examine the specificity of lipases from different sources toward aromatic esters and diesters and to a variety of aliphatic and aromatic diols in the transesterification reaction and to investigate the reaction of different types of diol (primary, secondary, long-chain, or aromatic) with our model ester, dichloroethyl fumarate, in two different organic solvents, acetonitrile and THF.

MATERIALS AND METHODS

Chemicals

Dichloroethyl fumarate, chloroethyl benzoate, chloroethyl phenyl acetate, chloroethyl hydrocinnamate, and

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dichloroethyl phthalate were prepared by azeotropic esterification of the corresponding acids with 2-chloroethanol in toluene. Aliphatic and aromatic diols were purchased from Aldrich. Dianol 33 (4,4′-isopropylidenediphenoxy-1-propane-2-ol) was a gift from Makhteshim Ltd.), Beer-Sheva, Israel.

Enzymes

Candida cylindracea lipase and porcine pancrease lipase were purchased from Sigma. Lipases AP6 from *Aspergillus niger* and *Rhizopus* and Lipase P from *Pseudomonas fluorescens* were gifts from Amano Pharmaceutical Co., Ltd., Nagoya, Japan. Mold lipases from *Mucor* immobilized on anion exchangers were gifts from Novo Industries, Bagsvaerd, Denmark.

Enzymatic Activity

The hydrolytic activity of a lipase is defined as the amount of free fatty acid liberated from a triglyceride per unit time. In our assay for lipase activity the liberation of butyric acid from tributyrin was followed with a pH meter at pH 7.5. The changes in pH were corrected with a standard solution of sodium hydroxide. This analysis was performed routinely to confirm that the lipase had not lost its activity after being used as a catalyst. Even after a number of repeated uses we did not find any meaningful decrease in hydrolytic activity of the enzymes investigated.

It is important to mention that there is no direct correlation between the hydrolytic activity and the transesterification activity, the latter being much lower. In a typical polytransesterification reaction between a diester and a diol, the transesterification activity of a lipase was defined by us as the appearance of the monotransesterification product per unit of time or the disappearance of the diester per unit of time. Usually, higher oligomers formed after 24 h, and a qualitative comparison was sufficient for choosing the most efficient lipase. Since all the enzymes were crude preparations, specific activities were usually very low. Thus, relatively large amounts of enzyme, 1 g, were added in a typical reaction.

Reaction

The reaction system contained a 1:1 molar ratio of diester to diol in a given volume of organic solvent. Usually 0.01 mol diester and 0.01 mol diol were dissolved in 100 mL solvent. Reactions could be performed even in concentrated solutions (up to 2*M*) when THF was used as the solvent. The limiting factor for more concentrated solutions was the solubility of the diester in the organic solvent. The solid enzyme preparation was added to the reaction mixture without further manipulation. The mixture was shaken at 260 rpm on a gyrorotatory shaker at 37°C. At intervals, samples were withdrawn and analyzed by thin-layer chromatography (TLC) or by high-performance liquid chromatography (HPLC). At the end of the reaction, the enzyme was filtered off, the solvent and the leaving alcohol were evaporated off, and the remaining mixture of oligomers was analyzed by ^{1}H nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC). The enzyme was washed first in the solvent in which the reaction was performed and then with ether, dried, and stored for reuse.

The TLC was performed on silica-gel-coated plastic sheets (Merck). Elution was accomplished with a mixture of petroleum ether, 60–80°, and ethyl acetate (5:3). Spots were visualized with a UV lamp or by exposure to iodine vapor. The HPLC was performed on a Spectra Physics instrument with a C-8 reverse-phase column (Supelco) utilizing an isocratic eluting mixture of acetonitrile:water (80:20). Peaks were detected at 220 nm and recorded on a 4290 Integrator (Spectra Physics). For the HPLC analyses, HPLC-grade acetonitrile (Merck) and doubly distilled water were used. The GPC was also performed on a Spectra Physics instrument equipped with a PSM-400 column or with two connected μ-styragel columns (500 and 1000 Å). Samples were prepared by dissolving 40 mg of the oligomer mixture in 10 mL THF. The HPLC-grade THF (Bio-Rad) was used as the eluting solvent at a rate of 1.5 mL/min. Peaks were detected at 254 nm. The ^{1}H NMR spectra were recorded on a Bruker WP-200-5Y FT-nmr (200 MHz) spectrometer ($CDCl_3$, tetramethylsilane as standard). X-ray powder diffractometry was performed with a Philips instrument, PW 1050/70 (Radiation CuKα, scintillation counter detector. Data were collected by step scanning with 0.02°/step every 2 s).

RESULTS AND DISCUSSION

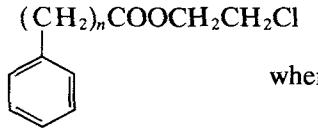
Specificity of Lipases toward Aromatic Esters

The biocatalaytic activity of lipases from different sources was studied in transesterification reactions of chloroethyl aromatic esters and *n*-butanol (Table I). Homologs of benzoic acid esters I (phenylacetic acid and hydrocinnamic acid) were accepted as substrates by some of the lipases, especially Novo's *Mucor* enzyme and the lipase from *Pseudomonas*. The reaction was also catalyzed to some extent by the yeast enzyme (*C. cylindraceae*). The same behavior was observed with chloroethyl benzoate as substrate. Since dichloroethyl phthalate (**II**), the substrate that was of greatest interest to us, underwent only a monotransesterification reaction, we sought other means of introducing aromaticity into the alkyd backbone, as described below.

Table I. Effect of the type of lipase on the transesterification between chloroethyl aromatic esters and *n*-butanol in THF.

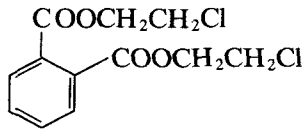
Lipase	Supplier	Chloroethyl esters of[a]			
		Benzoic acid	Phenyl acetic acid	Hydrocinnamic acid	Phthalic acid[b]
P. fluorescens	Amano	+	+	+++	+
C. cylindraceae	Sigma	+	+	+	–
Porcine pancreas	Sigma	–	–	–	+
Rhizopus	Amano	–	–	–	–
A. niger	Amano	–	–	+	–
Mold	Amano	–	–	++	–
M. miehei	Novo	+	++	+++	+

[a] The extent of appearance of product after 24 h was graded from + (small amount) to +++ (end of transesterification reaction); –, no reaction.
[b] Only monotransesterification.



where $n = 0$ (benzoate)
$n = 1$ (phenylacetate)
$n = 2$ (hydrocinnamate)

I

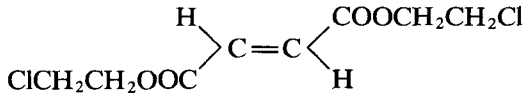


II, dichloroethyl phthalate

Specificity of Lipases toward Unsaturated and Aromatic Diols

Since we wanted to compare the lipase-catalyzed synthesis product with the industrial polyester, we attempted to introduce the aromatic function into our polyester by using an aromatic diol. Since the lipases from *Pseudomonas* and *Mucor* had accepted the aromatic esters, it was decided to perform this part of the study with those two lipases.

Unsaturated diols, analogs of 1,4-butane diol, were also investigated, mainly for purposes of comparison. Table II presents the results of reactions between the model ester, dichloroethyl fumarate (**III**), and a variety of diols. It can be seen that both unsaturated and aromatic diols underwent transesterification with dichloroethyl fumarate. With the aromatic diols high oligomers were detected after longer periods of time (24–48 h).



III, dichloroethyl fumarate

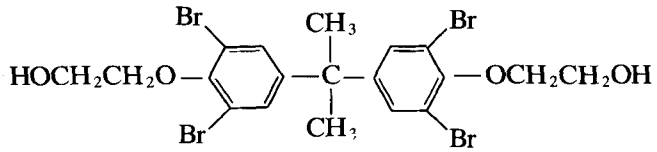
The extent of oligomerization, expressed in terms of the molecular weight of the product mixture, was determined in the benzene dimethanol diols in reactions performed in THF as solvent (Table III). For purposes of comparison the molecular weights of alkyds obtained with 1,4-butane diol are also listed.

Table II. Lipase-catalyzed transesterification between dichloroethyl fumarate and a variety of diols in THF.

Diol	Reaction[a] after	
	24 h	48 h
Mucor		
1,4-Butene diol	–	+
1,4-Butyne diol	–	+
1,3-Benzene dimethanol	+	++
1,4-Benzene dimethanol	+	+++
2,6-Pyridine dimethanol	+	+
Pseudomonas		
1,4-Butene diol	+	++
1,4-Butyne diol	–	+
1,3-Benzene dimethanol	++	+++
1,4-Benzene dimethanol	+	+++
2,6-Pyridine dimethanol	++	++

[a] The occurrence of a reaction was followed by the appearance of monomer or dimer (+) or higher oligomers (++) or the complete disappearance of the starting diester (+++).

Phenol was not found to be a suitable substrate for the lipases used in this study, perhaps because of its acidity. Derivatives of phenol, such as bisphenol A (4,4′-isopropylidene diphenol) were, however, found to react with the fumaric acid ester. By HPLC analysis a monomer, a dimer, and a trimer were detected. Better results were obtained when a primary diol derivative of bisphenol A, i.e., 4,4′-isopropylidenebis[(2-(2,6-dibromophenoxy) ethanol] (BBPAE) (**IV**) was used.



IV, BBPAE

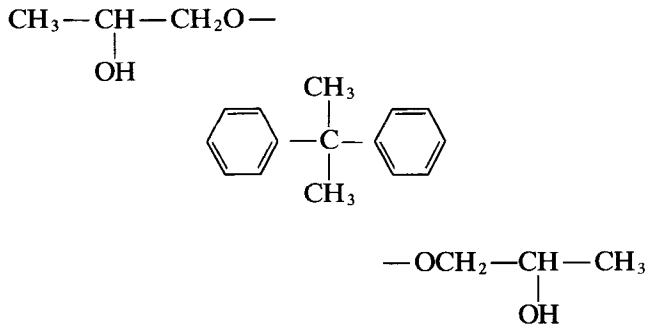
Table III. Molecular weight and dispersivity factors of polyester oligomers.

Diol (solvent in which the reaction was performed)	$\overline{M}_w$ [a]	Dispersivity factor[b]
1,4-Butane diol (in THF)	600–800	1.33
1,4-Butane diol (in acetonitrile)	1200–1400	1.05
1,3-Benzene dimethanol (in THF)	768	1.49
1,4-Benzene dimethanol (in THF)	1260	1.55
BBPAE[c] (in THF)	872	1.39
BBPAE (in acetonitrile)	2208	1.75

[a] Determined by GPC.
[b] Dispersivity factor is defined as the ratio $\overline{M}_w/\overline{M}_n$.
[c] BPAE, 4,4'-isopropylidenebis[2-(2,6-dibromophenoxy)ethanol].

The enzyme-catalyzed reactions were performed in both THF and acetonitrile. Molecular weight determinations by GPC showed a molecular weight of 872 for the soluble oligomeric mixture produced in THF and an average molecular weight of 2208 (m.p. 128–131°C) for the solid alkyd synthesized in CH_3CN.

In the polycondensation reaction between Dianol 33 (V) and dichloroethyl fumarate, the nonspecific mold lipase was found to be the most efficient catalyst.



V, Dianol 33

Specificity of Lipases toward Aliphatic Diols

The all-trans unsaturated polyester, poly(butylfumarate), obtained by lipase-catalyzed transesterification of 1,4-butane diol with dichloroethyl fumarate was analyzed by powder X-ray diffractometry (Fig. 1). The diffraction lines at definite (Bragg) angles were characteristic of crystalline matter. When a sample of the industrial unsaturated polyester was subjected to X-ray analysis (Fig. 2), a pattern typical of an amorphous polymer was obtained. It was possible that the crystallinity revealed in poly(butylfumarate) (Fig. 1) was related to the preservation of the trans configuration of the double bond along the polyester chain. However, since this property could possibly be attributed to the nature of the diol (primary or secondary, chain length, odd or even number of carbon atoms), the specificity of the lipases from *Pseudomonas* and mold toward different diols was investigated by following their polytransesterifications with dichloroethyl fumarate (Table IV). It was found that polyethylene glycol (PEG-300) and a homologous series of diols with a skeleton of 2 to 10 carbons reacted with the model diester (Table IV).

Table IV shows that in all the enzymatic transesterifications about half of the starting diester reacted in the first 8 h, irrespective of the nature of the diol. After 24 h higher oligomers were usually formed. With PEG-300 only the monotransesterification product was obtained. In the series of longer chain diols (C_6, C_8, C_{10}, PEG-300) the reaction seemed faster with the 1,8-octane diol, although the differences were relatively small. The product obtained in the polytransesterification of dichloroethyl fumarate with 1,8-octane diol was isolated, and the average molecular weight was found by GPC to be 1781, with a dispersivity factor of 1.44. X-ray powder diffractometry revealed that this product was also crystalline. Thus, it seems that crystallinity in the polyester is not an expression of the length of the diol or the density (frequency) of double bonds in the macromolecular chain.

For the short-chain diols (C_2–C_4) the fastest reaction was obtained with 1,4-butane diol. The reaction also proceeded well with ethylene glycol, although the hydroxyl groups in this diol are vicinal with no methylene groups separating them. The parity of the number of carbons in the diol did not seem to have a special effect on the unsaturated polyesters. Crystallinity was found in all the products.

Since all the diols used in this study were primary diols and the industry uses mainly propylene glycol (1,2-propane diol), we thought it important to prepare an enzymatically catalyzed polyester with this diol. In this reaction two enzymes were used, the Amono *Pseudomonas* lipase and a nonspecific mold lipase from Novo. The reaction rates were almost identical with the two enzymes, but even after long periods of time (~2 weeks) starting materials were still present in the oligomeric mixture, preventing further examination of molecular weights or crystallinity. However, from Table IV it can be seen that the rate of reaction was slower with the secondary diol than with the isomeric 1,3-propane diol.

In all the examined polytransesterification reactions the trans configuration of the double bond was preserved. A patent was then issued based on the results

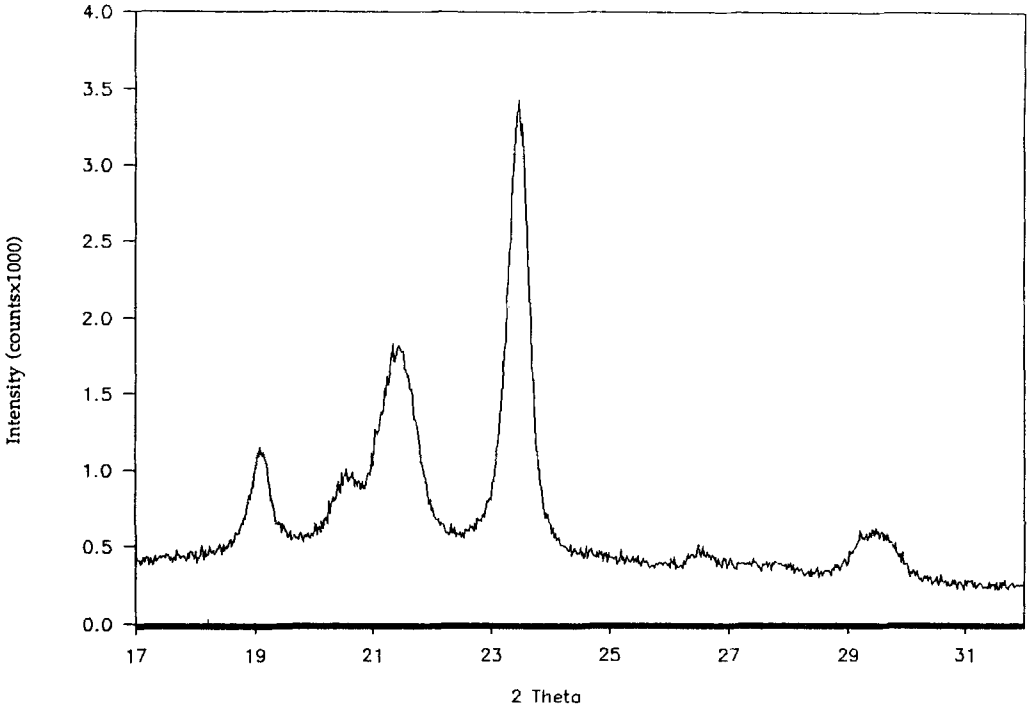


Figure 1. X-ray powder diffraction of all-trans poly(butylfumarate).

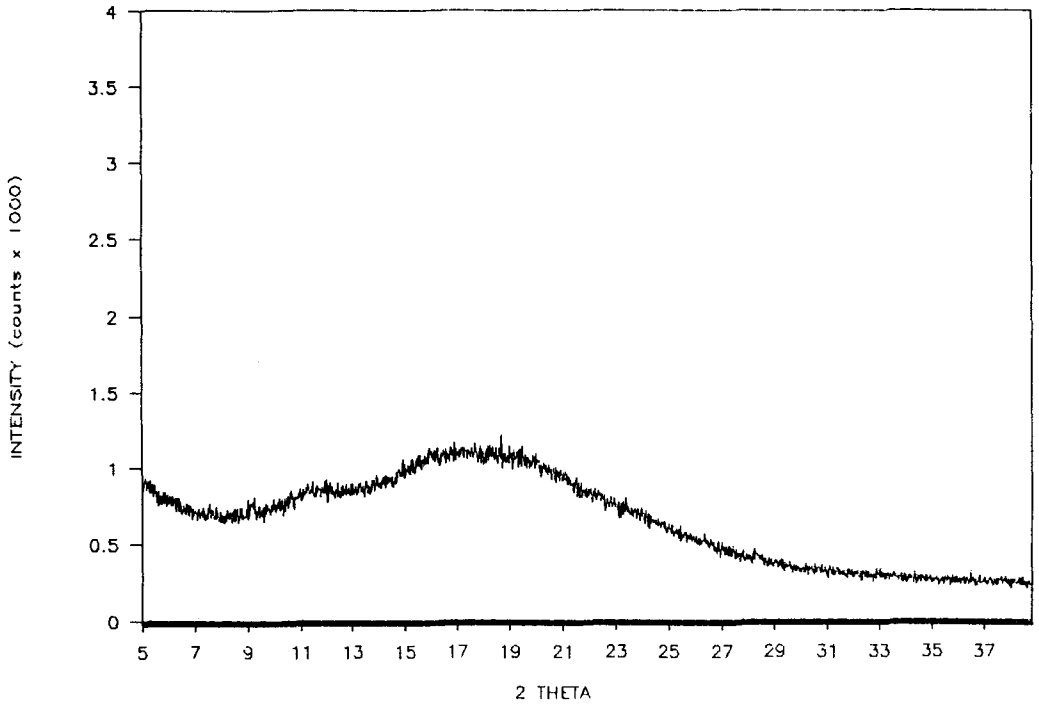


Figure 2. X-ray powder diffraction of an industrial unsaturated polyester.

presented in this article as well as on the physico-mechanical properties of these alkyds.[4]

CONCLUSIONS

The specificity of a variety of lipases to unsaturated and aromatic diols was investigated in the enzymatic polytransesterification with dichloroethyl fumarate. It was found that lipase-catalyzed polycondensations do occur with aromatic diols. By this method we overcame the difficulty of using aromatic diesters (phthalic or isophthalic) which are the conventional starting materials for unsaturated polyesters. The average molecular weight was found to compare favorably with the values obtained from chemical polymerization.[9] The main advantage in using lipases as catalysts in the polyconden-

Table IV. Relative rates of lipase-catalyzed transesterifications between dichloroethyl fumarate and a variety of diols.[a]

Diol	Remaining diester	
	8 h	24 h
Ethylene glycol	42.9	22.0
1,3-Propane diol	45.3	16.0
1,2-Propane diol (propylene glycol)[b]	56.0	30.0
1,4-Butane diol	38.0	9.5
1,6-Butane diol	55.0	27.7
1,8-Octane diol	45.3	18.2
1,10-Decane diol	51.3	20.6
PEG-300	54.6	32.0

[a] 0.01 mol diester and 0.01 ml diol dissolved in 100 mL THF were shaken on a gyrorotatory shaker at 37°C in the presence of lipase from *Pseudomonas* (Amano). The quantities of the remaining starting material were determined by HPLC analysis (a calibration curve of dichloroethyl fumarate was used).

[b] Since propylene glycol has a secondary hydroxyl group, the reaction was also performed with the nonspecific mold lipase from Novo. The results obtained were very similar to those with *Pseudomonas* lipase.

sation reactions is the mild conditions used which preserve the absolute configuration, thus imparting new and interesting qualities to the synthesized alkyds.

We express our appreciation for the helpful scientific discussions and advice from Professor R. Wachs and Dr. I. Barzilay. Professor Wachs, who was the President of Bio-Koor Ltd., brought the subject to our attention. We also wish to thank Amano for the unlimited supply of *Pseudomonas* lipase and Novo for the mold lipases and Dr. Y. Bar-Yaacov of Machteshim Ltd. for his help in molecular weight determinations as well as his willingness to impart to us his experience in the field of polyesters. Our thanks to Professor L. Zevin and Ms. S. Lach for the X-ray analysis.

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