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Enzymatic Polyesterification in Organic Media. Enzyme-Catalyzed Synthesis of Linear Polyesters. I. Condensation Polymerization of Linear Hydroxyesters. II. Ring-Opening Polymerization of ϵ-Caprolactone*

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SYNOPSIS

With the object to synthesize polyesters by enzymatic catalysis in organic media, two directions have been investigated: (1) the condensation polymerization of linear ω-hydroxyesters and (2) the ring-opening polymerization of lactones. The commercially-available crude porcine pancreatic lipase (PPL), suspended in organic solvents, was the preferred enzyme for the reactions. In order to determine the optimal conditions for the condensation polymerization, the bifunctional methyl 6-hydroxyhexanoate was used as a model compound to study the influence of the following parameters: type of the enzyme-catalyst, kind of solvent, concentration, temperature, duration, size of the reaction mixture, and stirring. Film-forming polyesters with a degree of polymerization (DP) up to about 100 were obtained from linear aliphatic hydroxyesters in n-hexane at reflux temperature (69°C). Yet concurrently with the intermolecular condensation polymerization, macrolactones were also formed by intramolecular reaction. Two aromatic hydroxyesters did not react under these conditions. For the ring-opening polymerization of lactones the reaction of ϵ-caprolactone with methanol as the preferred nucleophile, was studied. Polyesters with a DP of up to 35 were obtained in n-hexane at temperatures between 25 and 40°C. The degrees of polymerization of the polyesters were determined by comparative analyses of the end groups in the ^{1}H-NMR spectra and by determination of molecular weights either by vapor phase osmometry, gel permeation chromatography, or intrinsic viscosity. © 1993 John Wiley & Sons, Inc.

Keywords: enzymatic polymerization • organic solvents • polyesters • macrolactones

INTRODUCTION

Recently it has been shown that hydrolytic enzymes are highly stable in organic solvents and thus can be used for certain types of transformations which are difficult or impossible to carry out in water.[1] The most common reactions are lipase-catalyzed stereoselective esterifications and transesterifications, which were extensively used for the preparative resolution of chiral acids and alcohols. As an outgrowth of our interest in the use of enzymes in organic solvents,[2] we were led to explore the possibility of preparing polyesters by enzyme-catalyzed transesterification in organic media. This method could be used to prepare bio-like-polymers, and especially optically-active bio-like-polymers by enantioselective polymerization of chiral substrates.

Bio-like-polymers have gained growing interest lately due to their biocompatibility and biodegradability, properties that make them especially suitable for medical[3] and agricultural uses.[4] Optically-active polymers are of interest as reagents and catalysts in asymmetric syntheses[5] and as absorbents for the

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The paper is dedicated to the memory of Prof. D. H. Kohn who died before it was completed.

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chromatographic resolution of enantiomers, among other potential applications.[6] However, with the exception of naturally occurring polyesters such as poly(β-hydroxybutyrate),[7] and its industrially produced copolymer with β-hydroxyvalerate (BIOPOL),[8] there appears to have been little effort to prepare optically-active polyesters, or to prepare polyesters with enzymes. Only in three studies were there attempts to carry out the polymerization with enzymes in organic solvents.[9,10,11]

We have recently investigated the catalytic potential of the commercially-available crude porcine pancreatic lipase (PPL), suspended in organic solvents, towards esters of ω-hydroxyacids and found that PPL has a broad substrate specificity to this class of compounds. Since hydroxyesters are bifunctional molecules, they undergo condensation by two alternative routes: *intramolecular,* to give the corresponding lactone, or *intermolecular,* to give the dimer in the first step, which reacts further to give the trimer, and eventually polymer.

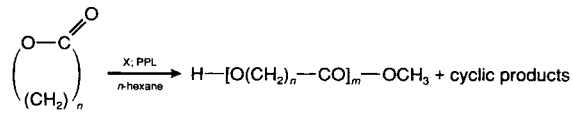
In the first part of this study, the potential of that methodology to the enzymatic synthesis of —[A–B]— type linear polyesters was explored, with a view to produce high-molecular-weight polymers. For this purpose we carried out a systematic study of the enzymatic polymerization, using as models two A–B type unbranched aliphatic hydroxyesters: methyl 5-hydroxypentanoate (**1**) and methyl 6-hydroxyhexanoate (**2**) (Scheme 1). The behavior of two A–B type aromatic hydroxyesters, methyl 4-hydroxybenzoate and methyl 4-(hydroxymethyl)-benzoate was also investigated.

In a second approach to obtain A–B type polyesters by enzymatic catalysis, the ring-opening polymerization of lactones in the presence of a nucleophile (X) was investigated (Scheme 2). This reaction was studied with ϵ-caprolactone only and hence $n = 5$.

1: $n = 4$.

2: $n = 5$.

Scheme 1.



1: X = CH_3OH.
2: X = $HO(CH_2)_3COOCH_3$.

Scheme 2.

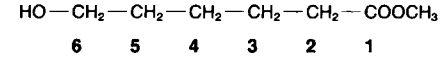
EXPERIMENTAL

Materials

Hydroxyesters

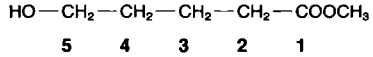
Methyl 6-hydroxyhexanoate (Me-6-HHX) and methyl 5-hydroxypentanoate (Me-5-HP) were prepared according to the synthesis of 4-hydroxybutyrates by Brown and Keblys.[12]

Methyl 6-Hydroxyhexanoate (Me-6-HHX). ϵ-Caprolactone (22 g, 0.2 mol) (Fluka product, > 99%) was dissolved in methanol (100 mL) in an Erlenmeyer flask equipped with a $CaCl_2$-drying tube. Sulfuric acid (98%) (0.25 mL) was added and the mixture was magnetically stirred for 16 h. After addition of calcium carbonate (3 g) and stirring for 1 h, the mixture was filtered and the methanol was evaporated. The residue was dissolved in chloroform (100 mL) and washed with aqueous $NaHCO_3$ (0.5*N*, 25 mL), with water (30 mL) and then dried over anhydrous magnesium sulfate. Following filtration and evaporation of the chloroform, the hydroxyester Me-6-HHX (16.9 g, 58% yield) was distilled at 83°C/0.1 mm Hg (lit.[13] bp at 90°C/1.5 mm Hg). Spectroscopic data:



^{1}H-NMR: (OCH_3) 3.63 ppm, s; (**2**) 2.26 ppm, t, J = 7.2 Hz; (**3**) 1.62 ppm, m; (**4**) 1.33 ppm, m; (**5**) 1.62 ppm, m; (**6**) 3.59 ppm, t, J = 10 Hz.

Methyl 5-Hydroxypentanoate (Me-5-HP). This hydroxyester was synthesized from δ-valerolactone according to the procedure for methyl 6-hydroxyhexanoate from ϵ-caprolactone. Thus, a mixture of δ-valerolactone (20.0 g, 0.2 mol), methanol (100 mL), and sulfuric acid (98%) (0.25 mL) was stirred for 20 hs. Worked-up as described, the hydroxyester (15.9 g) (bp 66–69°C at 0.1 mm Hg) was obtained in a yield of 65% (lit.[14] bp 61–65°C/0.5 mm Hg). Spectroscopic data:



^{1}H-NMR: (OCH_3) 3.62 ppm, s; (**2**) 2.30 ppm, t, J = 6.2 Hz; (**3**) + (**4**) 1.56 ppm, m; (**5**) 3.58 ppm, t, J = 9.8 Hz.

Methyl 4-hydroxybenzoate (> 98%) was a Fluka product.

Methyl 4-(hydroxymethyl)benzoate (> 98%) was obtained from Aldrich.

Enzymes. Porcine pancreatic lipase (PPL) (E.C. 3.1.1.3) [Sigma Chemical Co., No. L 3126, crude powder, containing 27.5% protein (biuret)] was used for the systematic study. Activity: 35–70 unit/mg protein, using triacetin at pH 7.4 after 60 min incubation.

Preliminary polymerization experiments with the following four enzymes were unsuccessful: Candida cylindracea lipase (CCL), liver acetone powder horse (LAPH), liver acetone powder porcine (LAPP), all from Sigma Chemical Co., and papain corolase from SERVA Feinbiochemica Heidelberg.

Solvents. All solvents were distilled and dried before use. Solvents for the enzymatic reactions were stored over type 4 molecular sieves.

Polymerization

The first series of experiments were carried out in closed vials in a thermostatic bath with shaking at ambient conditions. A number of preliminary studies at higher temperatures were performed in reaction flasks with reflux condenser.

To follow polymerizations at higher temperatures and to avoid losses of solvent, most of the polymerization experiments were carried out in Pyrex tubes, ca. 25 cm long and 2 cm wide with a bulb at the lower end and a constriction near the upper end. After introduction of the reactants, the solvent and of a small magnetic bar, the tubes were degassed and sealed. The reaction tubes were placed in a thermostatic oil-bath at 70 ± 1°C and their content was magnetically stirred. In a typical experiment, the monomer (0.1 mmol) was suspended in distilled and dried *n*-hexane (2 mL) in the reaction tube. Porcine pancreatic lipase (0.15 g), was added and the tube sealed. After periods of time, ranging from 3 to 30 days, the tubes were opened and the hexane phase separated from the enzyme by filtration. The enzyme was washed with more hexane (ca. 2 mL), and the two hexane solutions were united. The solid, remaining after washing with hexane, was stirred with two portions of chloroform (ca. 2 mL) for 2 h each. The *n*-hexane extracts and the chloroform extracts (ca. 80% yield in total) were each purified by chromatography.

The chromatographic separation of the reaction products was performed qualitatively on TLC plates with silica gel F-254, 60, Merck product, 0.25 mm thick. Quantitatively it was carried out either on preparative glass plates, 20 × 20 cm, covered with silica gel F 254, 60, 1 mm thick or on glass columns, packed either with Kieselgel 40 (0.063–0.20 mm) for separation of the products or with Florosil for filtration.

The various fractions from the hexane and chloroform extracts were examined by proton-NMR spectra.

Determination of the Activity of the Enzymes

The activity of PPL was estimated prior to the polymerization reaction and sometimes also at the end of it to perceive any reduction of the activity. The chosen standard reaction was the lactonization of methyl γ-hydroxybutyrate. The progress of this reaction was based on the relative changes of the IR bands of the ester carbonyl at 1730 cm^{-1} and the lactone carbonyl absorption at 1770 cm^{-1}.[2a]

Physical Test Methods

Spectroscopic Measurements

Proton NMR spectra were carried out in $CDCl_3$ solution on either a Bruker NR/200 AF FT-NMR or on a Bruker AM 400.

Infrared spectra in chloroform were performed on a Perkin-Elmer 237 grating IR spectrometer.

Mass spectra were performed on an Atlas CH4 spectrophotometer.

Molecular Weight Measurements

End-Group Analysis with Proton-NMR. The progress of the polyesterification of hydroxyesters was followed by the steady decrease of the absorptions of the two end groups: the methyl ester group of the monomer and the hydrogens bonded to the carbon carrying the —OH group, versus the steady increase of the absorptions corresponding to the hydrogens of the newly-formed ester —COO—CH_2—. By comparing the integrations of the decreasing proton absorptions against the increasing ones, one can calculate the average number of mer units (DP) in the chains. Yet with increasing molecular weight, this method loses its accuracy, because the absorptions of the end groups become too small to enable accurate integration and calculations.

Gel Permeation Chromatography (GPC). A Waters 410 apparatus was used: (1) with a low molecular weight set, calibrated with eight polystyrene stan-

dards in a molecular weight range from 106 to 12600 daltons, and (2) with a high-molecular-weight set, calibrated with polystyrene for a MW range from 900 to 1,800,000 daltons. With tetrahydrofuran as mobile phase, a refractive index detector was used. By calculations based on the retention times, the number-average molecular weight (M_n), the weight-average molecular weight (M_w), and the distribution values of the samples were obtained.

Vapor Phase Osmometry. A Hitachi-Perkin-Elmer 115 molecular weight apparatus was employed. It was calibrated with benzil in chloroform at 38.3°C (main oven) and 38°C (sub oven) and in toluene at 55 and 51°C, respectively.

Viscosity. The intrinsic viscosity of the polyesters was determined from viscosity measurements of their solutions in toluene with an Ubbelohde viscometer at 30 ± 0.1°C.

RESULTS AND DISCUSSION

Condensation Polymerization of Linear ω-Hydroxyesters

As far as formation of high-molecular-weight polyesters is concerned, the A–B type monomers have the advantage over the A–A and B–B monomers from viewpoint of equimolarity of the functional groups, i.e., the hydroxy and the acid or ester groups. Whereas equimolarity is inherent for the A–B type monomers, high purity and very precise weighing of the A–A and B–B types are necessary to obtain a polyester with a high degree of polymerization.

In this article the enzymatic polymerization of four A–B type unbranched hydroxyesters is reported. Two substrates were aliphatics, the linear methyl 5-hydroxypentanoate and methyl 6-hydroxyhexanoate, with the latter serving as a model for a systematic study of the conditions of enzymatic polyesterification. The two other substrates contained a phenyl ring, the "linear" methyl 4-hydroxybenzoate and methyl (4-hydroxymethyl)benzoate.

Generally, it can be stated, that whereas the two aliphatic monomers, especially methyl 6-hydroxyhexanoate polymerized under the given conditions to a film-forming high-molecular-weight polymer, the two aromatic hydroxyesters did not react. This lack of reactivity of aromatic monomers in enzymatic polyesterification was also observed by Wallace and Morrow.[11]

Polymerization of Methyl 6-Hydroxyhexanoate

In the enzymatic polyesterification of this monomer (Me-6-HHX) the influence of the following parameters was investigated: type of the enzyme (see "Experimental: Enzymes"), kind of the solvent, concentration, temperature, duration, size of the reaction mixture, and stirring. The molecular weights of the obtained polyesters were determined by ^{1}H-NMR from the ratio of the integrations of the starting end-groups (singlet for $-OCH_3$ at 3.64 ppm and triplet for $-\underline{CH_2OH}$ at 3.58 ppm) and of the product polyester (triplet for $\underline{CH_2OCO}-$ at 4.04 ppm) or by one of the other methods, VPO, GPC and intrinsic viscosity.

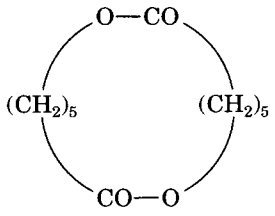
In addition to the linear polyesters, three macrolactones—dilactone (**I**), trilactone (**II**), and tetralactone (**III**)—were formed in total yields of 5–9% (Table I).

The Influence of the Solvent

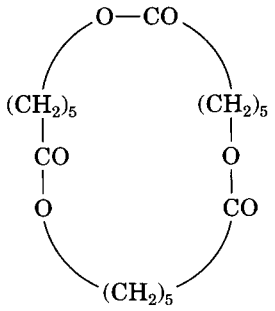
In the preliminary study on the influence of solvent on enzymatic polyesterification of Me-6-HHX (Table II), *n*-hexane and diethyl ether were found to be somewhat preferable to diisopropyl ether, whereas in toluene and THF only very low oligomers were formed. In a more thorough investigation of this parameter on the first stage of the reaction at two temperatures, the results were quite similar (Table III). The highest degree of polymerization was obtained in bulk, i.e., without solvent, followed by isooctane, diisopropyl ether, and *n*-hexane. In carbon tetrachloride the DP was much lower and it decreased further in benzene and in acetonitrile.

The relationships between the degree of polymerization (DP), obtained in the various solvents (Table III), and both the solubility parameter (δ) and log P are shown in Figures 1 and 2, respectively. The dependence of DP on δ (Fig. 2) shows a distinct influence of δ on the reaction, resulting in higher DP values at $\delta < 8$ and lower values at $\delta > 8$. These results are in accordance with the work of Brink and Tramper[16] who also found a higher biocatalytic activity at $\delta < 8$. Plotting DP vs. log P (Fig. 2) gave an S-shaped curve, which according to Laane et al.[17] is typical for various enzymatic systems in organic solvents. Yet it should be noted that Laane et al.[17] did not get a good correlation for their PPL system. Generally, according to Klibanov[1a] and to Laane et al.,[17] the dependence of the catalytic activity of the enzymes on the polarity of solvents is attributed to the solvent's ability to strip the enzyme from its essential water layer, or to penetrate into the water

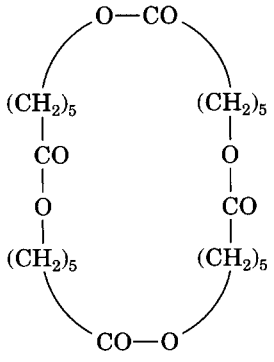
Table I. Enzymatic Formation and Structure Determination of Macrolactones **I**, **II**, and **III**



I



II



III

	I	II	III
^{1}H-NMR, δ (ppm):	1.30–1.70 (m, 6X2H); 2.35 (t, 2X2H, J = 6.3 Hz); 4.14 (t, 2X2H, J = 5.3 Hz).	1.30–1.70 (m, 9X2H); 2.30 (t, 3X2H, J = 6.0 Hz); 4.05 (t, 3X2H, J = 6.0 Hz).	1.30–1.70 (m, 12X2H); 2.30 (t, 4X2H, J = 5.8 Hz); 4.05 (t, 4X2H, J = 6.0 Hz).
Mass spectrum molecular ion:	228.1 (1%)	342.2 (2.5%)	456.3 (5%)
Molecular weight (VPO):	238.2	—[a]	—[a]
Melting point:	106–109°C[b]	—[a]	—[a]
Weight (mol) ratio between macrolactones in lactone fraction:	58% (78%)	23% (15.5%)	19% (6.4%)

[a] Insufficient quantities for determination.
[b] Literature[15] 111–113°C.

Table II. Enzyme-Catalyzed Polymerization of Me-6-HHX[g]

Experiment No.	Solvent	Time (h)	Temperature (°C)	Substrate[a] (mol)	Yield[b] (%)	Polymer MW[c]	Polymer [η] (dL/g)	Polymer mp (°C)
2/1[d]	THF	430	25	0.01	98	380	—	—
2/2[e]	toluene	410	70	0.02	83	600	—	—
2/3[e]	diisopropyl ether	600	67.5	0.02	83	2900	0.12	52–57
2/4[e]	diethyl ether	550	34.6	0.02	62	4300	0.133	58–63
2/5[d]	*n*-hexane	1030	25	0.02	98	1200	0.063	46–48
2/6[e]	*n*-hexane	890	69	0.02	79	4600	0.138	54–58
2/7[e,f]	*n*-hexane	890	69	0.02	71	7000	0.245	57–61
2/8[e,f]	—	265	25	0.01	89	550	—	—

[a] Composition of starting reaction mixtures: 0.01 mol (1.46 g) substrate (Me-6-HHX), 1.5 g enzyme-PPL, and 15 mL solvent.
[b] Total yield of isolated products.
[c] Determined by NMR.
[d] In shaker, 200 rpm.
[e] With heating and magnetic stirring.
[f] At the start only 1.0 g enzyme; afterwards 0.3 g portions were added every 3–4 days.
[g] In the control experiments, without enzyme, no reaction occurred.

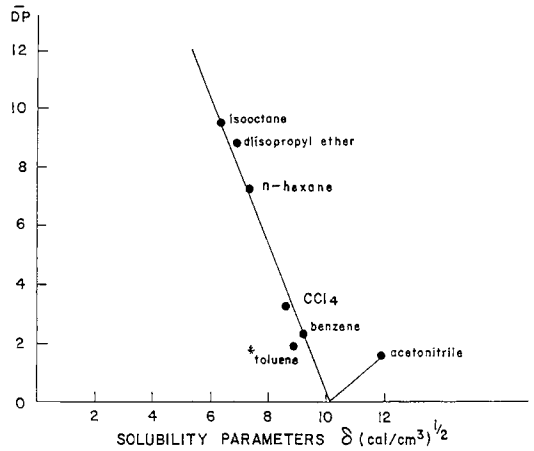


Figure 1. Degree of polymerization of Me-6-HHX (Table III) versus solubility parameter δ (⁻Taken from Table II).

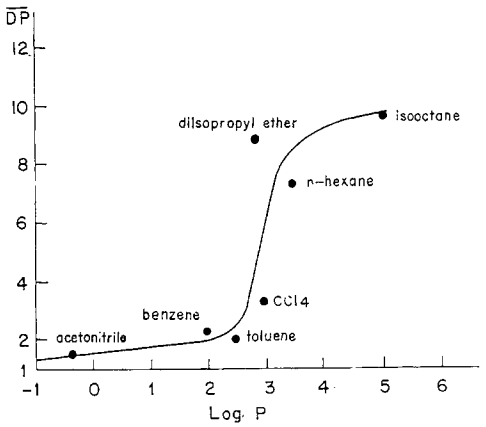


Figure 2. Degree of polymerization of Me-6-HHX (Table III) vs. log P (⁻taken from Table II).

layer in order to distort the interaction between the water and the enzyme molecule. In our polyesterification systems, which involve repetition of the same reaction of steadily changing substrates in respect to their DP and their mobility, a further factor should be considered. Polyesters of higher molecular weight were only obtained in poor solvents, whereas in good solvents only low oligomers were formed. This latter finding could be explained by the good solubility and consequent removal of the product oligomers from the enzyme surface resulting in a lowering of the concentration of the oligomer-substrate near the enzyme. However, in a poor solvent, the growing substrate concentrates near the enzyme and thus enables further reaction between the functional hydroxy and ester groups of larger molecules, and consequently higher-molecular-weight polyesters were produced. Carrying out the reaction at maximal concentration—in bulk without solvent—afforded polyesters with still higher DP.

Table III. Enzyme-Catalyzed Polymerization of Me-6-HHX[a]

Experiment No.	Solvent	Temperature (°C)	Polymer MW[b]	Polymer Yield (%)	Macrolactone Yield (%)
3/1	— (in bulk)	70	1900 (16.4)	95	1.2
3/2	isooctane	70	1130 (9.6)	62	7
3/3	diisopropyl ether	70	1035 (8.8)	59	10.5
3/4	*n*-hexane	70	850 (7.2)	61	8.5
3/5	CCl_4	70	395 (3.2)	69	8.8
3/6	benzene	70	295 (2.3)	73	11.3
3/7	acetonitrile	70	200 (1.5)	90	0
3/8	— (in bulk)	30	305 (2.4)	95	1.0
3/9	isooctane	30	260 (2.0)	62	1.1
3/10	diisopropyl ether	30	260 (2.0)	48	3
3/11	*n*-hexane	30	260 (2.0)	76	1.7
3/12	diethyl ether	30	260 (2.0)	69	1.3
3/13	CCl_4	30	260 (2.0)	46	1.9
3/14	benzene	30	240 (1.8)	66	3
3/15	acetonitrile	30	200 (1.5)	69	0

[a] Composition of starting reaction mixtures: 0.002 mol (0.29 g) hydroxyester, 0.3 g enzyme (PPL), 3 mL solvent; carried out in closed reaction tubes with magnetic stirring; duration 69 h.
[b] Determined by NMR (number of mer units).

Table IV. Dependence of the Degree of Enzymatic Polymerization upon the Concentration of Me-6-HHX[a] in Toluene

Experiment No.	Toluene (mL)	Polymer MW[b] (DP)	Macrolactones (% mol)
4/1	0	760 (6.4)	2
4/2	7.5	400 (3.2)	23.4
4/3	15	270 (2.1)	46.7

[a] Composition of starting reaction mixture: 0.001 mol (0.146 g) hydroxyester Me-6-HHX and 0.15 g enzyme PPL; duration: 357 h.
[b] Determined by NMR.

The Concentration of the Substrate

The concentration of the hydroxyester in the solvent (toluene), influences the DP of the obtained polymer and also the formation of macrolactones (Table IV). At low concentration (exp. 4/3, Table IV) about half of the product consisted of very low-molecular-weight oligomers—mainly dimers—and the other half were cyclic macrolactones. In bulk (exp. 4/1) mainly polymers were obtained.

The Reaction Temperature

By raising the reaction temperature, a sharp increase in the DP of the product polyesters could be observed. Comparing the results of experiments 2/5 and 2/6 (Table II), carried out at prolonged durations at 25 and 69°C, the molecular weights of the obtained polymers were about 1200 and 4600 respectively. Similar results were obtained for various solvents at shorter durations (Table III), when the oligomers formed at 70°C had a higher molecular weight than those from reactions at 30°C. Also, the content of the macrolactones was higher at 70°C which is in agreement with findings reported by Zhi-Wei and Sih.[18] Further, the influence of the solvents on the DP of the products was more pronounced at the higher temperature.

The Size of the Reaction Mixture and the Influence of Stirring

Scaling up the enzyme catalyzed polymerization of Me-6-HHX, resulted in a subsequent decrease of the molecular weights of the polyesters (Table V). Thus, with 1 mmol hydroxyester, a film-forming polymer with a DP = 82 (exp. 5/1) was obtained after a comparatively short time, whereas with 200 mmol substrate (exp. 5/4) the product-polyester had a DP of about 20 after more than a doubled duration. Attention should be drawn to the product of exp. 5/2 with 10 mmol substrate, yet the high DP was obtained after a four-fold duration of the reaction time in comparison to exp. 5/1. A similar behavior of decreasing molecular weight with scaling up was also found in the polyesterification of Me-5-HP. The change of scale did not seem to influence much the formation of macrolactones (Table V).

This behavior could be explained by the fact, that the enzyme is insoluble in the solvent and tends to agglomerate, reducing the surface of the catalysis-effecting area in heterogeneous systems. This assumption was confirmed by comparing reactions at different scales with and without stirring (Table VI). At a 1 mmol scale of the substrate, there is actually no difference in the molecular weight of the products between the stirred (exp. 6/1) and the nonstirred reaction (exp. 6/2). Doubling the added enzyme (exp. 6/3) gave an increase of the molecular weight by only about 10%. Scaling up to 100 mmol substrate (exp. 6/4), gave in the case of the stirred reaction

Table V. Dependence of the Degree of Polymerization (DP) of Me-6-HHX on Scaling Up of the Starting Mixture

Experiment No.	Substrate (mol)	Enzyme (g)	Solvent (mL)	Time (h)	Polymer MW[a] (DP)	Polymer DP/24 h	Polymer [η] (dL/g)	Macrolactone Yield (% mol)
5/1[b]	0.001	0.15	1.5	355	9300 (82)	5.54	0.404	9
5/2[b]	0.01	1.5	15	1400	12000 (105)	1.80	0.54	9
5/3[b]	0.02	3	30	888	4600 (40)	1.08	0.138	9.1
5/4[b]	0.2	30	300	790	2400 (21)	0.64	0.084	8.9
5/5[c]	0.01	1.5	15	580	9100 (80)	3.3	0.37	7
5/6[c]	0.02	3	30	436	4300 (38)	2.09	0.133	7

[a] Determined by NMR.
[b] Carried out in *n*-hexane under reflux (69°C) with magnetic stirring.
[c] Carried out in diethyl ether under reflux (35°C) with magnetic stirring.

Table VI. Dependence of the Degree of Polymerization of Me-6-HHX on Stirring[a]

Experiment No.	Substrate (mol)	Enzyme (g)	Solvent (mL)	Stirring	Time (h)	Polymer MW[b] (DP)	Macrolactone Yield (% mol)
6/1	0.001	0.15	1.5	magnetic	96	5600 (49)	8.3
6/2	0.001	0.15	1.5	none	96	5900 (52)	8.8
6/3	0.001	0.3	1.5	magnetic	96	6500 (57)	9.1
6/4	0.1	15	150	mechanical	165	2900 (25)	8.5
6/5	0.1	15	150	none	165	400 (3)	8

[a] All experiments were carried out in *n*-hexane at reflux temperature (69°C).
[b] Determined by NMR.

polyester with a molecular weight about six times higher than the oligomers obtained in an unstirred reaction (exp. 6/5). No influence on the formation of macrolactones was observed.

The Duration of the Reaction

The time dependence of the polyesterification in four different solvents, followed by taking samples from the reaction mixtures during the progress of the reaction, showed the general trend of increasing the degree of polymerization with time (Fig. 3). However due to the heterogeneous nature of the reaction mixture and to the decreasing solubility of the growing polyester, the samples, examined with progressing time do not accurately represent the real average molecular weight, but rather a lower value. This was due to the fact that very high-molecular-weight molecules precipitated on the enzyme and were not included in the samples withdrawn. Indeed, when the enzyme was washed with chloroform at the end of the reaction, polymers with higher molecular weight were obtained after evaporation of the solvent.

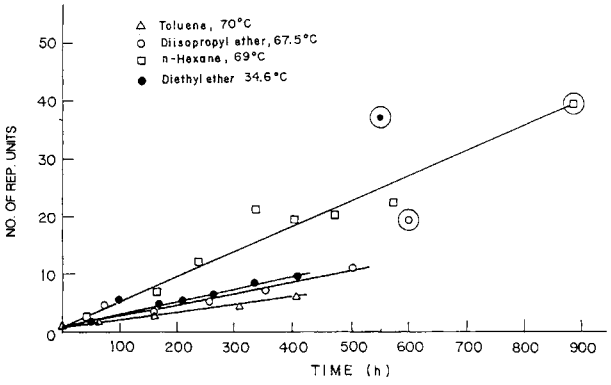


Figure 3. Time dependence of the enzyme-catalyzed polymerization of Me-6-HHX in various solvents. The circled points are the products from the chloroform extracts of the enzymes.

Polymerization of the Dimer of Me-6-HHX and of Poly(Me-6-HHX)

In polyesterification of A–B type monomers, the two functional groups A and B are also available in the corresponding dimer and polymer. Consequently, to get polyesters with higher molecular weights, both these A–B-type substrates were used for further polyesterification.

The dimer of Me-6-HHX was prepared by enzymatic oligomerization of the monomer up to an average DP of 1.5. The reaction mixture was separated by chromatography on a silica gel column, and examination of the fractions by NMR and VPO revealed that a mixture of oligomers up to five repeating units was obtained. The remaining monomer and dimer constituted 50 and 25% (weight percentage), respectively; and the trimer, tetramer, and pentamer constituted 15, 6, and 4%, respectively, of the reaction mixture.

The dimer fraction reacted in boiling *n*-hexane (69°C) in the presence of fresh PPL to produce after 26 h a polyester with an average molecular weight of 4300 (determined by NMR) and macrolactones (11% by weight). For comparison, the monomer yielded under the same conditions and after 33 h a polyester with an average MW of 1800 (by NMR) and ca. 5% macrolactones.

It was shown that also polyesters from previous experiments could be used as substrates for additional polymerization with fresh enzyme (PPL) in *n*-hexane (Table VII). At 25°C no reaction occurred; presumably due to the higher melting range of the substrate-polyester, at about 50°C. At the reflux temperature (69°C) however, a significant rise of the molecular weight was observed in all runs. It is interesting to note, that although the starting

Table VII. Further Polymerization of Poly(Me-6-HHX)

	Substrate[a]				Products		
	Polymer				Polymer		
Experiment No.	MW (NMR)	[η] (dL/g)	Time (h)	Temperature (°C)	MW (NMR)	[η] (dL/g)	Lactone (% mol)
7/1[b]	1200	0.063	620	25	1200	0.063	—
7/2[c]	2400	0.084	1200	69	9000	0.39	5.8
7/3[c]	7000	0.245	888	69	8000	0.28	5.6
7/4[d]	2400	0.084	96	69	4800	0.165	6.1
7/5[d]	2400	0.084	166	69	6000	0.213	6.6
7/6[d]	2400	0.084	266	69	6500	0.234	7.1
7/7[d]	2400	0.084	311	69	5800	0.212	5.9

[a] The substrate contained no lactones.
[b] 1.14 g (0.01 mol repeating units), PPL (2 g), *n*-hexane (20 mL); on a shaker.
[c] 2.28 g (0.02 mol repeating units), PPL (4 g), *n*-hexane (40 mL); under reflux.
[d] 0.155 g (0.001 mol repeating units), PPL (0.15 g), *n*-hexane (2 mL); in closed reaction tubes.

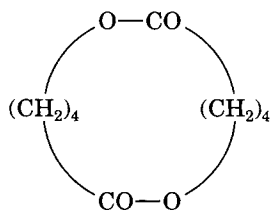
substrates did not contain any lactones, lactones did appear in the product. Their formation may be due to partial hydrolysis of the polymers by the enzyme into smaller oligomers followed by "re-equilibration" toward the development of a ring-chain equilibrium.

Polymerization of Methyl 5-Hydroxypentanoate (Me-5-HP)

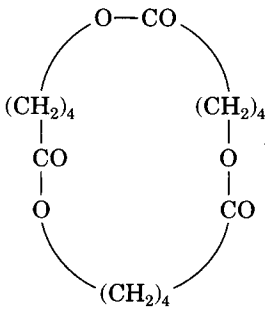
Enzymatic polymerization of Me-5-HP in organic solvents was catalyzed by PPL in a similar way to that of Me-6-HHX. The degree of polymerization was monitored as before by ^{1}H-NMR analysis of the end groups. Polyesters up to 29 mer units were obtained after 60 days at the reflux temperature of *n*-hexane, which again was found to be the preferred medium.

Further, cyclic by-products (about 2% of the product), a dilactone (**IV**) and a trilactone (**V**) were formed by intramolecular cyclization of the dimer and trimer respectively (Table VIII).

Table VIII. Enzymatic Formation and Structure Determination of Macrolactones **IV** and **V**



IV



V

^{1}H-NMR, δ (ppm) ($CDCl_3$):	1.40–1.85 (m, 4X2H); 2.43 (t, 2X2H, *J* = 4.8 Hz); 4.11 (t, 2X2H, *J* = 4.8 Hz).	1.30–1.75 (m, 6X2H); 2.35 (t, 3X2H, *J* = 6 Hz); 4.10 (t, 3X2H, *J* = 6 Hz).
Mass spectrum molecular ion:	200.1 (7%)	301.1 (2.6%)
Weight (mol) ratio between macrolactones in lactone fraction:	62% (70.5%)	38% (29.5%)

Table IX. Enzyme-Catalyzed Polymerization of ϵ-Caprolactone in the Presence of Methanol or Me-6-HHX

Experiment No.	Reactants[a] (Molar Ratio)	Polymer MW NMR	Polymer MW VPO	Polymer Yield (% mol)	Macrolactone Yield (% mol)
8/1	ϵ-caprolactone + methanol (50 : 1)	4400	2630	95	5
8/2	ϵ-caprolactone + methanol (1 : 1)	1480	1125	95	5
8/3	ϵ-caprolactone + Me-6-HHX (3 : 1)	2900	1800	96	4

[a] Reactants (0.02 mol), PPL (3 g), *n*-hexane (20 mL); carried out in a shaking apparatus at 25°C during 1100 h. No reaction was observed in the absence of the enzyme.

Enzymatic Polyesterification by Ring-Opening of ϵ-Caprolactone

To polymerize lactones by enzymatic catalysis, the lactone has to be ring-opened by a nucleophile concurrently with the progress of the polyesterification. Two nucleophiles, methanol and methyl 6-hydroxyhexanoate (Me-6-HHX), were used in the preliminary series and both compounds yielded polyesters (Table IX), showing the feasibility of this reaction. Furthermore, by increasing the ratio ϵ-caprolactone/nucleophile, polyesters with higher molecular weight were formed. For the consequent follow-up of the reaction by NMR, methanol was the preferred nucleophile, as the chemical shifts of the components of the resulting reaction mixtures—ϵ-caprolactone, macrolactone, and polymer—are quite different and thus enable to determine their compositions. With Me-6-HHX on the other hand, there are some common absorptions with the polyester-product, which prevent the use of NMR for the follow-up. The chosen absorptions were the triplet of ϵ-caprolactone at

Table X. Enzyme-Catalyzed Polymerization of ϵ-Caprolactone with Methanol (Molar Ratio, 10 : 1)[a]

Sample No.[b]	Time (h)	Fraction Weight (%)	Reaction Mixture Constituents: Monomer (% mol)	Dilactone (% mol)	Polymer (% mol)	Polymer MW[c] (DP)
9/1-H	96	39	86	6.8	7.2	306 (2.4)
9/1-C	96	61	38	0	62	613 (5.1)
9/2-H	216	38	52.6	26.6	20.8	297 (3.2)
9/2-C	216	62	10.6	0	89.4	978 (8.3)
9/3-H	288	16	35.2	38.9	25.9	420 (3.4)
9/3-C	288	84	5.5	2.7	91.8	1058 (9.0)
9/4-H	360	16	21.7	43.9	34.4	534 (4.4)
9/4-C	360	84	3.6	2.1	94.3	1252 (10.7)
9/5-H	456	14	12.9	56.5	30.6	374 (3.0)
9/5-C	456	86	0	1.5	98.5	1434 (12.3)
9/6-H	624	10	0.2	68.4	31.4	408 (3.3)
9/6-C	624	90	0	2	98	1924 (16.6)
Control	624	100	95	2.5	2.5	260 (2.0)

[a] ϵ-Caprolactone, 0.001 mol (0.116 g); methanol, 0.0001 mol (0.0032 g); PPL (0.15 g); *n*-hexane (1.5 mL). The polymerizations were carried out in closed tubes in a shaking apparatus at 40°C.

[b] H: Fraction soluble in *n*-hexane. C: fraction obtained by extracting the filtered enzyme with chloroform when the reactions were stopped.

[c] Determined by ^{1}H-NMR.

4.21 ppm (J = 4.9 Hz), the triplet of the dilactone at 4.14 ppm (J = 5 Hz), the triplet of $-CH_2-OCO-$ for the polyester at 4.04 ppm (J = 6.2 Hz) and the singlet and triplet at 3.65 and 3.55 ppm for the end groups $-OCH_3$ and $-CH_2OCO-$, respectively. The reaction conditions and the results of the polyesterification are summarized in Tables IX and X and in Figures 4 and 5. The reaction products were separated and purified as described for the products obtained from hydroxyesters. Thus one finds a sharp increase of the percentage of polyester in the chloroform soluble fraction (Fig. 4), coupled with a steep decrease of the ϵ-caprolactone content and a very low content (ca. 2%) of dilactone. However, in the n-hexane-soluble fraction (Fig. 5), with the decrease of the monomer, the concurrent increase of the polymer is quite moderate reaching only 30%, while there is a steady rise of the dilactone content, reaching up to about 70%. Thus, the polymer, which is less soluble in n-hexane, remains near the enzyme where it continues to grow due to a steady diffusion of the monomer from the surrounding solution. The great difference between the dilactone content of the two fractions is to be ascribed to the solubility of the dilactone in n-hexane. It seems that the ring opening reaction of the ϵ-caprolactone is quicker than the polymerization and the dilactone formation. In any case, the polyester continues to grow steadily even at rather low concentration of the monomer probably due to the intermolecular condensation of the growing chains.

In conclusion, using methyl 6-hydroxyhexanoate, methyl 5-hydroxypentanoate, and ϵ-caprolactone with methanol as model compounds, it was shown that high-molecular-weight polyesters can be synthesized by enzymatic catalysis in organic media. In

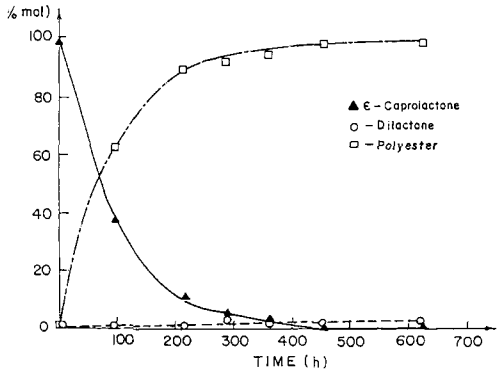


Figure 4. Enzyme-catalyzed polymerization of ϵ-caprolactone with methanol. Time dependence of reaction components in the chloroform-soluble fraction (% mol).

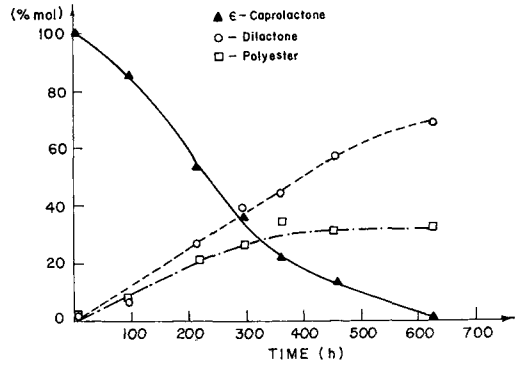


Figure 5. Enzyme-catalyzed polymerization of ϵ-caprolactone with methanol. Time dependence of reaction components in the n-hexane-soluble fraction (% mol).

continuation of this work,[19] it was also shown that it is possible to use the enantioselectivity of the enzyme in addition to its catalytic power, for preparing optically-active polyesters.

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