Lipase-Catalyzed Synthesis of Polyhydric Alcohol-Poly(ricinoleic acid) Ester Star Polymers

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ABSTRACT: Polyesters of poly(ricinoleic acid) and polyol acyl acceptors (trimethylolpropane, pentaerythritol, and dimer diol), examples of lipophilic star polymers, were synthesized via bulk polymerization at 70°C in a 1 to 2-week period, using immobilized lipases from *Candida antarctica B*, CAL, and *Rhizomucor miehei*, RML (Novozyme and Lipozyme, respectively, from Novozymes North America, Franklinton, NC). In the screening of several synthesis procedures, the highest molecular weight and degree of conversion occurred when polyricinoleic acid, synthesized previously from ricinoleic acid using CAL as biocatalyst, was mixed with polyol and either CAL or RML. Such a procedure yielded pentaerythritol–poly(ricinoleic acid) tetraester with an average molecular weight of 4850 ± 440 Da, according to ^{1}H NMR analysis. Seventy-eight percent of the polyol acyl acceptor's hydroxyl groups were esterified, with the average degree of polymerization for its poly(ricinoleyl) chains being 5.4 ± 0.5. The product mixture contained 83% polyol ester and only 17 wt % nonesterified linear poly(ricinoleic acid). The rate-limiting step in the formation of poly(ricinoleic acid), propagation, was first-order with respect to monomer (ricinoleyl acyl groups); and, chain-transfer reactions were absent. The products formed possessed high viscosity and viscosity indices (155 for the pentaerythritol tetraester) and melting point temperatures below −7.5°C, suggesting their use as environmentally-friendly lubricant materials. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 1646–1656, 2006

Key words: star polymers; esterification; gel permeation chromatography; enzymes; MALDI; NMR

INTRODUCTION

There remains a great need to increase demand for agricultural feedstocks. Hydroxy fatty acids [e.g., ricinoleic (*R*-18:1^{9c}-OH^{12}), lesquerolic (*R*-20:1^{11c}-OH^{14}), and dimorphecolic (*S*-18:2^{10t,12t}-OH^{9}) acids from the oils of castor (*Ricinus comminus*), lesquerella (*L. fendleri*), and dimorphotheca (*D. pluvialis*), respectively] are bifunctional molecules that are potentially useful building blocks for chemical synthesis. Oligomers of hydroxy acids, also referred to as estolides, and their derivatives, may be useful materials for cosmetics, coatings, and food-related applications.[1,2] Moreover, due to their low melting point and high viscosity and viscosity index (the latter a measure of the resistance to viscosity increase with decreasing temperature), poly(hydroxy acids) may be valuable lubricant materials.[3–6] The esterification of the free —COOH terminus of poly(hydroxy acids) by fatty alcohol enhances their physical properties, namely, the viscosity was lowered and the viscosity index was increased with low melting point temperature retained.[6,7] Poly(hydroxy acid) esters are also biocompatible and biodegradable; for example, the monoester of poly(ricinoleic acid) and polyglycerol, "polyglycerol polyrcinoleate", is a common ingredient of cake mixes, toppings, and low-fat salad dressings, serves as a viscosity-reducing agent for chocolate, and is commonly used to lubricate cooking tins.[8]

Polyesters of polyols and poly(hydroxy acids) (Fig. 1), examples of branched star polymers, may also be effective lubricants. For instance, nonhydroxy fatty acid esters of polyol yield high viscosity indices and low pour point temperatures.[9] Diol diesters of poly(hydroxy acids) are reported to share the low melting point temperatures of the hydroxy acid starting materials but possess a higher viscosity and viscosity index.[7] Lipophilic star polymers may also have applications as drug delivery vehicles.

It has been suggested that poly(ricinoleic acid) be prepared enzymatically (using lipases) to avoid problems of discoloration, odor, and high energy costs that occur in high-temperature chemical processes.[10] Also, dehydration of ricinoleic acid can occur in chemical

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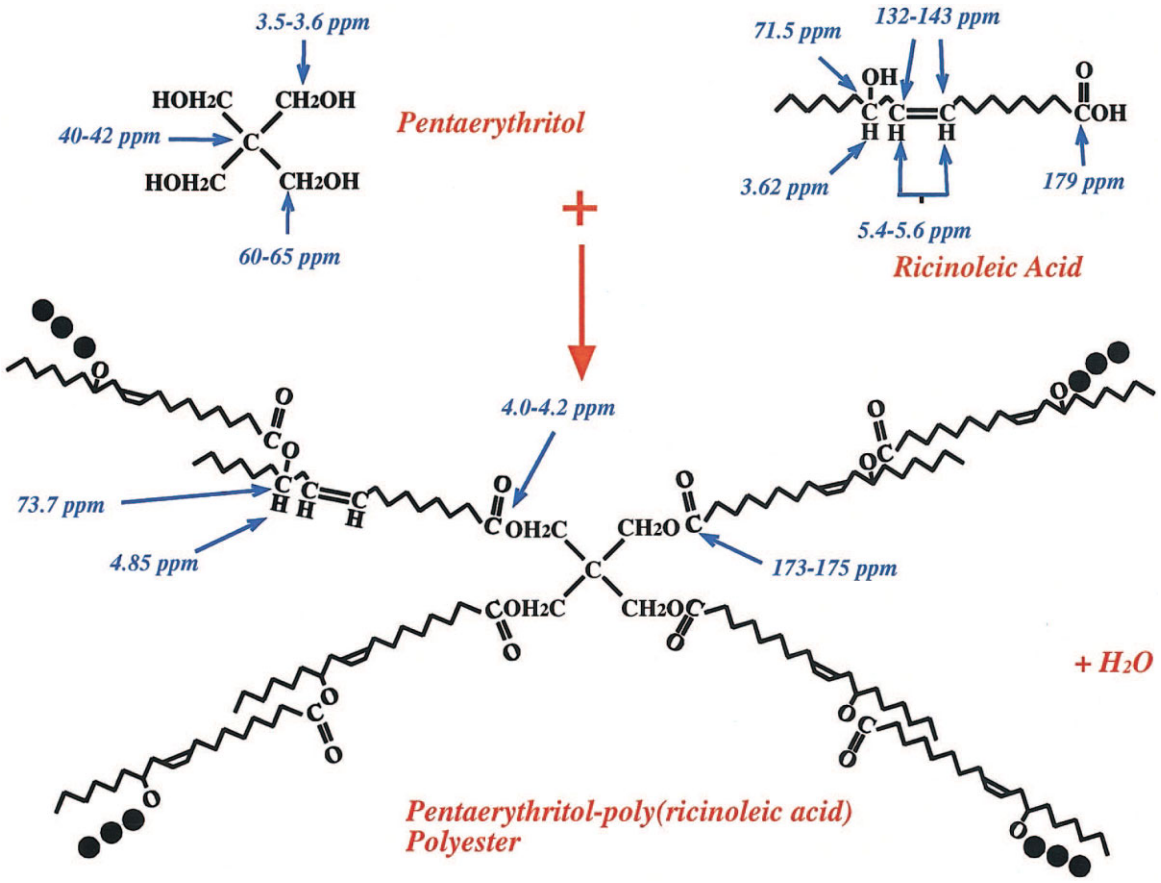

Figure 1 Chemical structure of a pentaerythritol-(PE-) tetra[poly(ricinoleic acid)] polyester [PE-$(R_nH)_4$], its substrates, and ^1H- and ^{13}C NMR assignments used for identification and analysis. NMR peak assignments provided for PE agree with those corresponding to nonesterified —OH groups on mono-, di-, and triesters of PE. Assignments indicated in the figure are very similar to those of dimer diol, trimethylolpropane, and their esters. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

processes.[8] Reviewed elsewhere,[11–13] biocatalyst-directed synthesis of polymers has received increased interest due to its low energy usage, environmental friendliness, and the ability of enzymes to limit the product distribution through their inherent substrate, regio- and stereo-selectivity. Most of the reported research involving lipase-catalyzed polymerization has involved the esterification of diacids and α,ω-diols and the ring-opening polymerization of lactones, the latter producing polymers of ω-hydroxy acid and carbonate monomeric units. Product molecular weight (MW) in the 10,000–100,000 range has been reported. Many experimental parameters control the degree of polymerization and product distribution, such as water content, liquid phase polarity, temperature, and the diffusional mass transfer of the liquid phase through the pores of the solid, biocatalyst-containing, phase.[11,13,14]

Polyol-poly(hydroxy acid) polyesters similar in structure to that depicted in Figure 1 have been prepared via bulk polymerization at 70°C using immobilized biocatalysts in our laboratory. Poly(ricinoleic acid) served as the acyl donor and pentaerythritol (PE), trimethylolpropane (TMP), and dimer diol (DD) as acyl acceptors. In this paper, discussion focuses upon the preparation method, the characterization of products, and the product's physical properties.

EXPERIMENTAL

Ricinoleic acid, *R*-18:1^{9cis}-OH[12] (technical grade, originally containing 90% ricinoleic acid, 8% C_{18} mono- and dienes, and 2% saturates, allowed to slowly polymerize via condensation for several months, yielding a mixture of free fatty acid and polyester: 57% monomer, 33% dimer, 7% trimer, and 3% tetramer and higher oligomers, number-averaged molecular weight, M_n, of 458, and a polydispersity index, or PDI, of 1.31), TMP, and PE were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. Novozyme and Lipozyme-IM, *Candida antarctica* lipase B immobilized onto nylon and *Rhizomucor miehe*i lipase immobilized onto anion exchange resin, respectively, were kindly donated by No-

vozymes North America Inc. (Franklinton, NC). Dimer diol (MW 538 and PDI of 1.17 ± 0.18) and *L. fendleri* oil were kindly donated by Cognis Corp. (Cincinnati, OH) and International Flora Technologies (Gilbert, AZ), respectively. Solvents employed for HPLC analysis and work-up of reaction products were of high purity (HPLC grade) and used without further purification. Deionized water was employed throughout.

Polymer synthesis was conducted in an unstoppered beaker or glass jar placed on a hot plate/stirrer. Reactions took place in the absence of solvent in stirred batch mode on a 10–100 g scale at 60–80°C (i.e., "bulk polymerization"). For a typical reaction, reactant (ricinoleic acid and polyol, ~50 g total) was heated to 70°C; then, immobilized lipase (1.0 g) was added and stirred at 300 rpm (31.4 s^{-1}). The hot plate set point temperature was lowered initially to compensate for the increase of temperature caused by the exothermic heat of reaction, to maintain a nearly-constant temperature. The use of a reactor open to the atmosphere permitted evaporation of the product, water, increasing the thermodynamic equilibrium conversion into ester. Upon completion of the reaction, addition of solvent (acetone or ethyl acetate) was required to remove the immobilized enzyme from the product by microfiltration (0.25 μm), due to high viscosity. Solvent was removed in a rotary evaporator, and subsequently in a vacuum oven. One sample, dimer diol-poly(ricinoleic acid) polyester "DD-$(R_nH)_2$-1," was purified by column chromatography on a silica gel column using a positive gradient of ethyl acetate for an ethyl acetate-hexane solvent system.

Chemical characterization of product was conducted using thin layer chromatography (TLC), reversed phase high performance and gel permeation liquid chromatography (RP-HPLC and GPC, respectively), matrix-assisted laser deposition ionization-time of flight-mass spectroscopy, or MALDI-TOF-MS (MALDI for short), and ^{1}H- and ^{13}C NMR. TLC was conducted on thin silica gel-coated plates using hexane/acetone solutions for resolution and iodine vapor for detection. RP-HPLC and GPC were performed on a dual-pump gradient system (Varian, Inc, Walnut Grove, CA) equipped with a model MK-III evaporative light scattering detector, or ELSD (Alltech Associates, Deerfield, IL). Experiments demonstrated that the ELSD signal was approximately a linear function of concentration for all solutes encountered herein. RP-HPLC was performed with a 4.6 mm × 25 cm Microsorb C_{18} reversed phase column from Varian. An isocratic solvent system consisting of acetone/acetonitrile/acetic acid (45 : 45 : 10 v/v/v) was employed at a flow rate of 1.0 mL min^{-1}. GPC was performed on a Styragel HR-4E 300 × 7.8 mm ID column from Waters (Milford, MA). Dichloromethane at 1.0 mL min^{-1} was employed as mobile phase. Molecular weight was calculated from retention time using a semilog retention time-molecular weight calibration made from partially resolved oligoricinoleic acid peaks contained within the chromatogram (e.g., Fig. 2), or calibrations for TMP or PE esters made from several different runs, with molecular weight measured by ^{1}H NMR. Calibration line correlation coefficients were 0.99 or greater in absolute value. The chromatogram's underlying area was divided into a

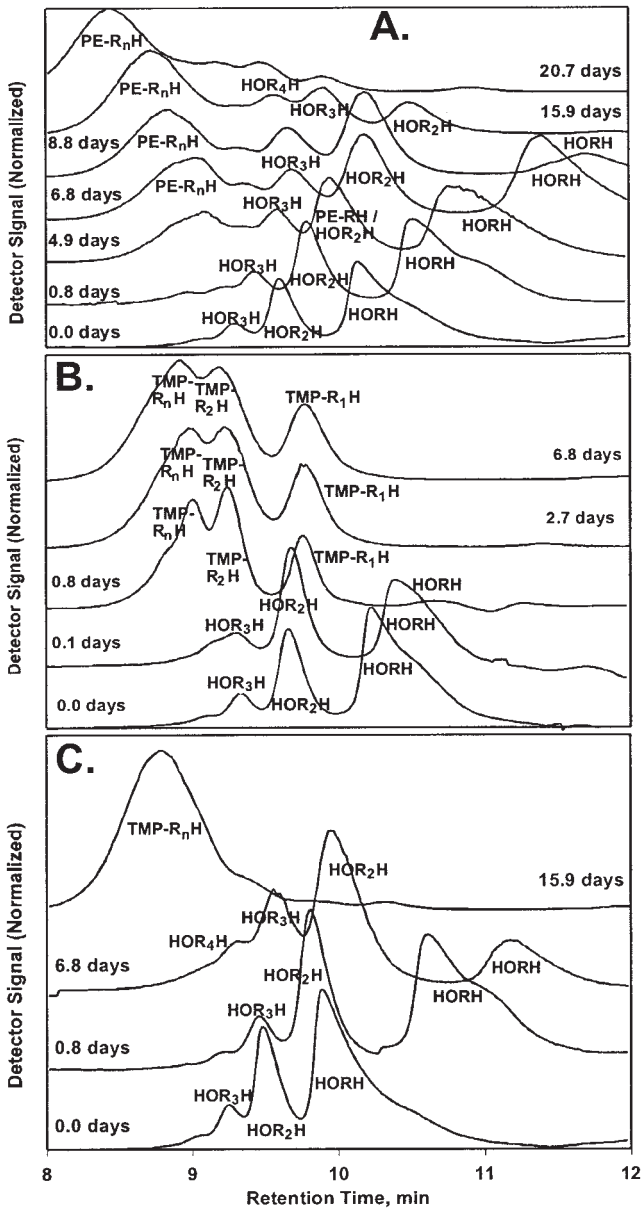

Figure 2 Overlaid GPC chromatograms for the formation of (A) PE-$(R_nH)_4$ via the method of eq. (4) and (B) TMP-$(R_nH)_3$ via the method of eq. (5) and (C) eq. (6) (second step catalyzed by *C. antarctica* B lipase). Reaction conditions: 50 g ricinoleic acid, 13 g polyol, and 1 g immobilized lipase at (69.1 ± 9.3)°C, with the transition from the first to the second step occurring at 6.8 days. Note that fresh lipase replaced used lipase at 6.8 days for all reactions.

TABLE I
Physical Properties of Polyhydric Alcohol-poly (ricinoleic acid) Esters and Selected Substrates

Sample[a]	Protocol	% Esterification of R_n chains to polyol	T_{melt} (°C)	SG (20°C)
HOR_nH-1[b,c]	Eq. 6(a)		<-17.7	0.935
HOR_nH-2[d]	Eq. 6(a)		<-17.6	0.935[e]
TMP-$(R_nH)_3$-1[b]	Eq. 4	>95[f,g]	<-16.6	0.961[e]
TMP-$(R_nH)_3$-2[b]	Eq. 5	100[f,g]	<-18.9	0.966[e]
TMP-$(R_nH)_3$-3[b,h]	Eq. 6(a)	97.9 ± 7.6[f,g]	<-14.0	N.D.[j]
TMP-$(R^nH)_3$-4[b,h]	Eq. 6(b)	90.3 ± 4.0[f]	<-16.6	N.D.[j]
PE-$(R_nH)_4$-1[b]	Eq. 4	61.7 ± 4.3[f]; 81[g]	<-14.6	0.941[e]
PE-$(R_nH)_3$-2[i]	Eq. 6(a)	82.7[f]	−7.5 to −13.3	0.973
DD			<-17.9	0.881
DD-$(R_nH)_2$-1	Eq. 4	100[j]	<-17.6	0.913
DD-$(R_nH)_2$-2[i]	Eq. 6(a)	79.7 ± 5.0[f]; 100[g]	<-17.9	0.899

[a] R, TMP, PE, and DD refer to ricinoleyl acyl groups, trimethylolpropane, pentaerythritol, and dimer diol, respectively; sample names correspond to those used in Tables II and III.
[b] GPC chromatograms and time course data depicted in Figs. 2–5
[c] 18% $n = 1$, 47% $n = 2$, 20% $n = 3$, 15% $n \geq 4$.
[d] 3% $n = 1$, 26% $n = 2$, 31% $n = 3$, 40% $n \geq 4$.
[e] 21°C.
[f] GPC Analysis.
[g] ^{1}H-NMR analysis.
[h] HOR_nH-1 is resultant product from 1st step of eq. 6 synthesis protocol.
[i] HOR_nH-2 is resultant product from 1st step of eq. 6 synthesis protocol; [j]purified using column chromatography.
[j] N.D. refers to "not determined.".

series of trapezoids; then, the number- and weight-averaged molecular weight (M_n and M_w, respectively) were calculated using the following formulae:

$$M_n = \sum y_i MW_i \quad (1)$$

$$M_w = \sum y_i MW_i^2 \quad (2)$$

where the subscript i refers to the ith trapezoid and MW_i to the average molecular weight of trapezoid i. The index of polydispersity, PDI, is then calculated from the ratio of the two:

$$PDI = M_w/M_n \quad (3)$$

The partial resolution of peaks for nonesterified ricinoleic acid and its oligomers from the symmetrically shaped polyester peak (Fig. 2) allowed for estimation of the percent esterification of poly(ricinoleic acid) chains to polyol, as listed in Table I. Error limits, based upon replicate experiments, were calculated from the student t-distribution employing 95% confidence levels.

MALDI was performed on an Omniflex instrument from Bruker (Billericka, WA). Samples were prepared by first dissolving 10 mg of each polymer sample into 1.0 mL of tetrahydrofuran (THF). The sample solution was then mixed with matrix (15 mg of *trans*-3-indoleacrylic acid dissolved in 1.0 mL of THF) and sodium chloride solution at the volume ratio of 3 : 10 : 3. 0.5 μL of each sample mixture was spotted on a target, which was dried *in vacuo* for ~2 min. The resultant sample/matrix was analyzed against a standard, the latter made by mixing adrenocorticotropic hormone, ACTH (18–39), and ubiquitin with α-cyano-4-hydroxycinnamic acid. The MALDI-TOF spectrometer was set on reflector and positive modes.

NMR spectra were performed on a 400 MHz Eclipse spectrometer from JEOL (Japan) or a Varian 500 MHz spectrometer using a 20-s delay time. ^{1}H- and ^{13}C NMR analyses employed 400 MHz and a 45° pulse width, and 100 MHz and a 30° pulse width, respectively. All samples were dissolved in $CDCl_3$. Heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC), and correlation spectroscopy (COSY) were also performed on a few samples to confirm peak assignments. Error limits, based upon replicate experiments, were calculated from the student t-distribution employing 95% confidence levels.

Viscosity of polyol–poly(ricinoleic acid) products and substrates was measured using Cannon-Fenske viscometers (models 450, 350, and 200) placed in a high-precision constant-temperature water bath. Viscometer constants were determined using *L. fendler*i oil as a standard with its published density and viscosity data.[15,16] At least two measurements were performed for each viscosity value reported herein. Error limits for were based on the 95% confidence level provided by the student t distribution. Specific gravity (SG) was determined using a pynchometer.

RESULTS AND DISCUSSION

GPC analysis

GPC was employed to monitor the time course of polyol–poly(ricinoleic acid), or polyol–R_nH, ester formation to calculate the number–averaged molecular weight (M_n), and the polydispersity index (PDI), where *R* refers to ricinoleyl acyl groups [—C(═O)—C_7H_{14}—CH═CH—CH_2—CH(C_6H_{13})O—] (Fig. 2). The chromatographic peaks for ricinoleic acid, HORH, and its di- and trimers (HOR_2H and HOR_3H, respectively) were partially resolved, but underwent tailing (Fig. 2). Although the peak positions of HORH, HOR_2H, and HOR_3H at time zero agreed with retention times predicted by polystyrene molecular-weight standards, the positions of these peaks shifted to longer retention time when polyol esters [Figs. 2(A) and 2(B)] or large-MW HOR_nH products]Fig. 2(C)] were formed. Moreover, for replicate measurements of a given sample, polyol ester peak positions remained constant (and possessed nearly Gaussian peak shape); but, HORH, HOR_2H, and HOR_3H peak positions varied according to the amount of reaction mixture placed on the column (data not shown). This suggests that the molecules with COOH functionality adsorbed to the GPC stationary phase. HORH, HOR_2H, and HOR_3H peak maxima obeyed a linear log MW versus retention time relationship, with correlation coefficients being 0.99 or greater. The correlation was used to calculate MW for the regions of chromatograms associated with HORH, HOR_2H, and HOR_3H (retention time $>$ ~9.2 min) and for polymerization of HORH in the absence of polyol (c.f. Fig. 2(C), 0.0–6.8 days). Log (MW)-retention time calibrations for TMP- and for PE-poly(ricinoleate) esters, derived from several different samples with MW estimated via ^{1}H NMR, were used to estimate MW for GPC peaks occurring before ~9.2 min. GPC peaks for polyol ester species that possessed one or two ricinoleyl groups [e.g., TMP-R_1H and -R_2H in Fig. 2(B)] were partially resolved from the broad, Gaussian polyol–poly(ricinoleic acid) peak [e.g., TMP-R_nH in Fig. 2(B)].

Values of M_n calculated via GPC were reasonably close to values of average MW calculated from ^{1}H NMR data [Fig. 3(A) and Table II]. GPC-derived M_n values for HOR_nH [e.g., Fig. 2(C)] also agreed with those provided by RP-HPLC, which resolved peaks for HOR_nH, $n = 1,\ldots,4$ (data not shown). (Note that a small fraction of peak area attributed to HORH, HOR_2H, and HOR_3H reflects oligomers with terminal oleic or linoleic acyl groups, present in the technical grade ricinoleic acid employed, as detected by RP-HPLC and MALDI.) Thus, the partial resolution of HOR_nH peaks from polyol ester peaks via GPC allowed for the accurate calculation of MW. Therefore, no attempts were made to suppress the apparent adsorption of the free COOH groups of poly(ricinoleic acid) onto the GPC column. Calibrations based on standards of polystyrene or poly(ethylene glycol), or PEG, produced M_n values that strongly disagreed with MW values predicted by ^{1}H NMR; thus, neither set of standards was employed. In agreement, a recent report suggests calibration with polystyrene standards can overestimate M_n by about 25%.[17]

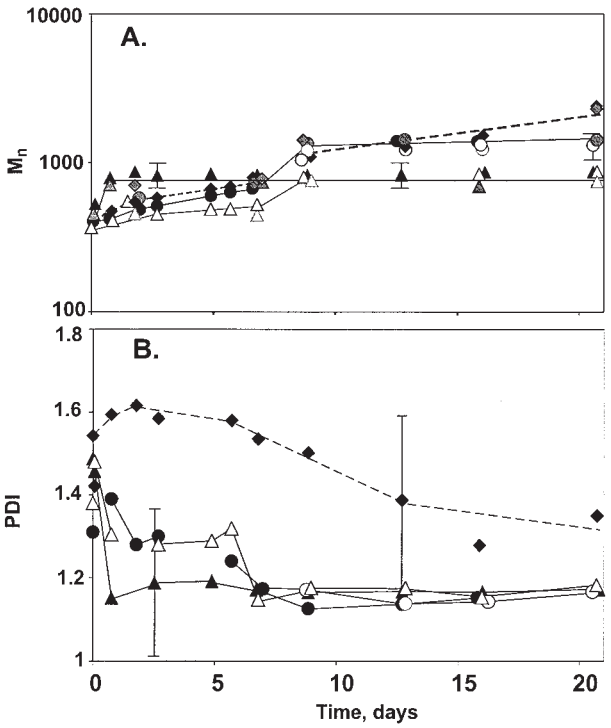

Figure 3 Change in (A) number-averaged molecular weight, M_n, and (B) polydispersity index (PDI) as a function of time for synthesis of TMP- or PE-R_nH polyesters. Reaction conditions are listed in Figure 2. Reaction schemes: (open triangle) TMP, eq. (4); (filled triangle) TMP, eq. (5); (Closed circle) TMP, eq. 6(a); (open circle) TMP, eq. 6(b); (diamond) PE, eq. (4). Data points with gray coloring and/or outline represent average MW estimated by ^{1}H NMR; data points with black color/outline represent GPC-derived M_n.

Comparison of synthesis protocols and kinetics

The following reaction schemes were compared for maximizing MW of TMP- and PE-poly(ricinoleic acid):

$$3\ \mathrm{HORH} + \mathrm{H_3CC(CH_2OH)_3} \xrightarrow{\mathrm{RML}} \mathrm{H_3CC(CH_2ORH)_3} + 3\mathrm{H_2O}$$

$$m\ \mathrm{HORH} + \mathrm{H_3CC(CH_2ORH)_3} \xrightarrow{\mathrm{CAL}} \mathrm{H_3CC(CH_2OR_iH)(CH_2OR_jH)(CH_2OR_kH)} + m\ \mathrm{H_2O}\ (i + j + k = m + 3) \quad (4)$$

TABLE II
Estimates of Chemical Structural Properties of Polyhydric Alcohol-poly (ricinoleic acid) Esters and Selected Substrates Based on ^{1}H-NMR, MALDI, and GPC Analysis

Sample[a]	Average acyl groups per chain[b]	Esterification of polyol —OH groups[c]	Estimated average MW[d]	M_n (GPC)[e]	PDI (GPC)[f]	Chemical species (MALDI)[g,h]
HOR_nH-1[i]	2.1 ± 0.3		724 ± 80	667	1.19 ± 0.18	HOR_nH: n = 1–6
HOR_nH-2	3.6 ± 0.3		1040 ± 80	916	1.10 ± 0.17	HOR_nH: n = 2–9
TMP-$(R_nH)_3$-1[i]	1.7 ± 0.2	0.42	746 ± 110	872	1.18 ± 0.18	TMP-R_m; m = 2–7
TMP-$(R_nH)_3$-2[i]	1.7 ± 0.2	0.40	720 ± 110	859	1.17 ± 0.18	TMP-R_m; m = 2–7
TMP-$(R_nH)_3$-3[i,j]	2.4 ± 0.5	0.64	1420 ± 120	1402	1.15 ± 0.18	TMP-R_m; m = 2–7; HOR_3H
TMP-$(R_nH)_3$-4[i,j]	2.7 ± 0.5	0.55	1330 ± 120	1310	1.17 ± 0.17	TMP-R_m; m = 2–10
PE-$(R_nH)_4$-1[i]	2.7 ± 0.3	0.82	2300 ± 180[h]	2410	1.35 ± 0.18	PE-R_m; m = 4–11; HOR_nH: n = 2–6
PE-$(R_nH)_4$-2[k]	5.4 ± 0.5	0.78	4850 ± 440	4530	1.46 ± 0.18	PE-R_m; m = 4–16
DD-$(R_nH)_2$-1[l]	1.6 ± 0.5	1.00	1460 ± 130	1342	1.08 ± 0.17	DD-R_m; m = 1–7
DD-$(R_nH)_2$-2[k]	3.0 ± 0.5	1.00	2220 ± 200	2078	1.13 ± 0.17	DD-R_m; m = 3–6

[a] Sample names correspond to those used in Tables 1 and 3; nomenclature given in Table I.
[b] Estimated using $C_{12}HOH$ (3.62 ppm), $C_{12}HOR$ (4.85 ppm), and $C_9H{=}C_{10}H$ (5.4–5.6 ppm) of acyl donor.
[c] Estimated using CH_2OH (3.4–3.5 ppm) and CH_2OR (4.0 ppm) groups of the polyol acyl acceptor.
[d] Based on the average acyl groups per chain (Column 2), the number of —OH groups per polyol molecule, the fraction of polyol —OH groups esterified (Column 3), and the percent esterification of polyricinoleyl chains to polyol (Table I), and assumes the degree of polymerization of free polyricinoleyl chains and those esterified to polyol are the same; error limits based on 95% confidence interval using student t distribution.
[e] Number–averaged molecular weight, error limits for $\log(M_n)$: ± 3%, according to the t-distribution employing 95% confidence levels.
[f] Polydispersity index, with error limits calculated according to the t-distribution employing 95% confidence levels.
[g] Species with MW <~600 were not detectable due to interference by the MALDI matrix spectral peaks.
[h] Small amounts of oligomers with nonhydroxy acyl terminal groups were detected.
[i] GPC chromatograms and time course data depicted in Figs. 2–5.
[j] HOR_nH-1 is product from 1st step of eq. 6 synthesis protocol.
[k] HOR_nH-2 is product from 1st step of eq. 6 synthesis protocol.
[l] Purified using column chromatography.

$$n\ \mathrm{HORH} + \mathrm{H_3CC(CH_2OH)_3} \xrightarrow{\mathrm{CAL}} \mathrm{H_3CC(CH_2OR_iH)} \times(\mathrm{CH_2OR_jH})(\mathrm{CH_2OR_jH}) + n\ \mathrm{H_2O}\ (I + j + k = n) \quad (5)$$

$$n\ \mathrm{HORH} \xrightarrow{\mathrm{CAL}} \mathrm{HOR_nH} + (n - 1)\ \mathrm{H_2O}$$

$$\mathrm{HOR_nH} + \mathrm{H_3CC(CH_2OH)_3} \xrightarrow{\text{(a)CAL or (b)RML}} \mathrm{H_3CC(CH_2OR_nH)_3} + 3\mathrm{H_2O} \quad (6)$$

where RML and CAL refer to immobilized *R. miehei* and *C. antarctica* B lipases, respectively. The initial hypothesis was that the method outlined in eq. (4) (a "divergent" synthetic approach employing terminology from dendrimer synthesis, meaning that the polymer is synthesized from the central core outwards[18]) would maximize conversion, hence MW, because the first step would direct the consumption of ricinoleic acyl substrate for polyol ester formation, leading to the improved solubility of polyol in the ricinoleic acid-rich media. Moreover, the inability of RML and most other 1,3-selective lipases to utilize secondary —OH groups as acyl acceptors prevents HOR_nH formation, but permits esterification of the polyols' primary —OH groups.[19] Frequently, the low solubility of polyol controls the rate of reaction for lipase-catalyzed polyol-fatty acid esterification in nonaqueous media.[20] In the second step, the "random"-positional selective biocatalyst, CAL, catalyzes attachment of ricinoleyl groups to the monoricinoleyl chains of the first step polyol–ricinoleic acid ester product. CAL is commonly employed to catalyze ring-opening polymerization of lactones at temperatures between 60 and 80°C[13,21]; lipases derived from members of the *Candida* family (e.g., *C. rugosa*) are reported to catalyze HOR_nH synthesis.[19,22,23]

Results demonstrated the maximization of MW occurred from a "convergent" approach, referring to the covalent attachment of poly(ricinoleic acid) to a central core molecule,[18] in contrast to the proposed hypothesis [eq. (6); c.f. Fig. 3]. The "convergent" approach consisted of HOR_nH formation catalyzed by CAL, then attachment of HOR_nH to polyol catalyzed by either CAL or RML. The successful employment of the convergent approach suggests HOR_nH chains readily penetrated the active sites of both lipases, lead-

ing to the formation of acyl-enzyme intermediate. In agreement, others recently demonstrated that oligomeric acyl donors and acceptors penetrate the active site of CAL.[24–26] Both enzymes catalyzed the second step of the "convergent approach" [eq. (6)] at a similar rate [Fig. 3(A)]. The time course demonstrates that an average MW value of 724 was achieved in about 7 days for the first step of the "convergent" approach [Fig. 2(A); *HOR$_n$H*-1 entry in Table II], an increase in MW of 48% from that of the acyl donor substrate. In the absence of lipase, an 8% increase of MW was measured for a 1-week period (data not shown). The rate of lipase-catalyzed polymerization of ricinoleic acid was much slower than that for ring-opening polymerization of lactone, the difference due to the presence of primary —OH groups on the latter's acyl groups, the lower viscosity for poly(ε-caprolactone) at a given MW, and the activation of the ω-hydroxy acyl group when existing as a lactone. Employment of a longer duration (10 d) resulted in an average MW of 1040 for HOR$_n$H (Table II, *HOR$_n$H*-2, ^{1}H NMR analysis). Matsumura et al. achieved an apparent M_n value of 1260 for this reaction[22]; however, their method of analysis may have been quite inaccurate, because of the use of GPC calibrated by polystyrene standards (discussed earlier). In addition, the consistent increase in MW with time reflects the thermostability of CAL and RML preparations at 70°C,[27,28] the absence of kinetic limitation by the product, water, because of its free evaporation at the elevated reaction temperature, and the limited ability of lipases to hydrolyze HOR$_n$H.[19]

Even though esterification of TMP and ricinoleic acid catalyzed by CAL [eq. (5)] resulted in the formation of significant amounts of HOR$_n$H by-product during the earlier stages of the reaction (RP-HPLC analysis; data not shown), the majority of product formed after 2 weeks of reaction time consisted of TMP-poly(ricinoleic acid) ester (Table I). The resultant M_n and PDI values were nearly identical with that achieved using the 2-enzyme divergent method [eq. (4)]; but, the reaction kinetics were more rapid for the former (Fig. 3). Note the replacement of enzyme with fresh CAL at 6.8 h did not further increase MW [Fig. 3(A)]; ester did not form between polyol and ricinoleic acid or its oligomers in the absence of lipase (data not shown), further supporting the absence or near-absence of enzyme inactivation.

TMP, PE, and DD were employed successfully as acyl acceptors, leading to high degrees of conversion (Table I). The replacement of triol, TMP, with the tetra-ol, PE, resulted in an increased M_n and PDI throughout the course of reaction, when comparing reactions performed using the method of eq. (4) (Fig. 3(A) and Table II). To increase the degree of polymerization, the procedure outlined in eq. 6(a) was employed using a larger MW HOR$_n$H acyl donor (*HOR$_n$H*-2 in Table I) than that employed for the experiment of Figure 3 (*HOR$_n$H*-1 in Table I), resulting in a PE-poly(ricinoleic acid) ester product of similar chemical structure to that illustrated in Figure 1 with an average MW of 4850 (Table II, *PE-(R$_n$H)$_4$*-2).

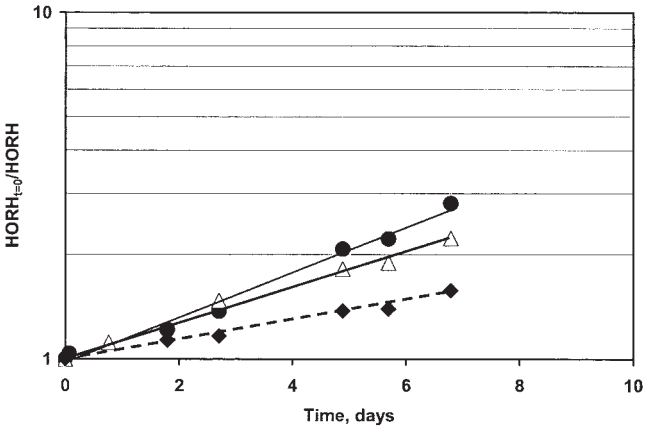

Figure 4 First order reaction plot [ratio of monomer (ricinoleic acid, HORH) concentration to monomer at time zero to its value at time *t* versus reaction time] for synthesis of TMP- or PE-R$_n$H polyesters. Symbols listed in Figure 3; reaction conditions listed in Figure 2. Ordinate values derived from GPC.

Figure 4 depicts a first-order kinetic plot of the ratio of monomer (HORH) concentration at time zero to that at time *t* on a log scale versus time. It appears that the formation of HOR$_n$H [eq. (6), first step] and the esterification of polyol OH groups by a single ricinoleyl group [eq. (4), first step] for both TMP and PE polyol substrates are represented by a straight-line relationship between 0.0 and 6.8 days (up to 64, 55, and 36% conversion of hydroxy acid monomer for eq. (6), eq. 4-TMP, and eq. 4-PE experiments, respectively), suggesting these steps are first-order with respect to monomer concentration, as occurred for CAL- and porcine pancreatic lipase-catalyzed ring-opening polymerization of ε-caprolactone.[29,30] The cited references state the linearity of the first-order plots indicate the absence of chain termination substeps and lipase activity loss. The slow, linear increase of M_n with conversion of ricinoleic acid monomer up to 60−80% conversion (Fig. 5), similar to lipase-catalyzed ring-opening polymerization of lactone,[29,30] suggests the absence of chain transfer substeps for the first step of all synthesis schemes given in eqs. (4–6); moreover, the sequential growth of polyol esters [eq. (4)] and poly(ricinoleic acid) [eqs. (5) and (6)] by the addition of hydroxy-acyl monomer. This result is further suggested by the time course of TMP ester formation by the "convergent" approach [eq. (6)]; moreover, the degree of polymerization of the poly(ricinoleic acid) chains increased slowly, and did not change during the transition between steps 1 and 2 [Fig. 3(A)] and NMR data not shown). These results agree with re-

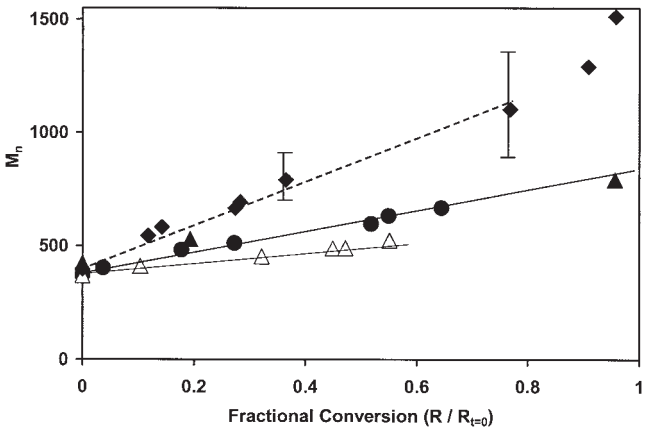

Figure 5 Number-averaged molecular weight, M_n, versus fractional conversion of monomer, HORH, for synthesis of TMP- or PE-R_nH polyesters. Symbols listed in Figure 3; reaction conditions listed in Figure 2. Values for M_n determined via GPC.

ports for lipase-catalyzed synthesis of long-chain non-ω hydroxy acid polymers[19] and contrast with the reported middle-chain cleavage of large-MW poly(ε-caprolactone) during its lipase-catalyzed synthesis.[30] Figure 4 demonstrates that HOR_nH formation catalyzed by CAL is more rapid than the RML-catalyzed esterification of polyol —OH groups, and, for the latter, that esterification of the triol TMP was more rapid than that for the tetra-ol PE. The average MW increase with fractional conversion was greater for ricinoleic acid esterification of PE than for TMP because of the presence of one additional poly(ricinoleic acid) chain per polyol molecule for the former (Fig. 5). The increase of MW as a function of conversion is nearly identical for the reaction of eq. (5) and for polymerization of ricinoleic acid [step 1 of eq. (6)] as expected since the data points plotted in Figure 5 for the former reaction represent the initial period of the reaction (0–1 day.), during which the amount of free ricinoleyl and poly(ricinoleyl) —OH groups greatly outnumber the free polyol —OH groups, hence indicating the dominance of HOR_nH formation over polyol esterification. The latter trend is further supported by NMR data, which demonstrates an increase of the degree of polymerization for the poly(ricinoleic acid) chains and a low (20%) esterification of polyol —OH groups during the initial period of the eq. (5) reaction (data not shown). The nonlinear increase of MW for PE ester formation for conversions above 80% indicated in Figure 5 (eq. (4), second step) reflects the growth of poly(ricinoleic acid) chains by addition of R_nH groups ($n \geq 2$) rather than by monomeric hydroxy-acyl units due to the depletion of ricinoleic acid. A similar observation and conclusion were made for poly(ε-caprolactone) formation.[30]

Structural characterization of final products

To confirm the structure of the polymeric products, MALDI, ^{1}H-, and ^{13}C NMR were performed. MALDI spectra contained major peaks corresponding to MW values of polyol-(R_nH) esters and the absence or near absence of HOR_nH peaks, with the exception of a few samples such as PE-$(R_nH)_4$-1, which contained significant amounts of HOR_nH impurity, according to GPC and ^{1}H NMR analyses given in Table I (data not shown). Trace amounts of polyol esters with nonhydroxy acyl-terminated poly(ricinoleyl) chains were also detected. The polyol ester species indicated by MALDI results are consistent with ^{1}H NMR-generated predictions. The average MW values calculated from MALDI data were lower than the values determined by GPC and ^{1}H NMR for the high-MW materials [e.g., PE-$(R_nH)_4$-2 and DD-$(R_nH)_2$-2] for reasons not yet determined. MALDI could not be employed to accurately predict MW for lower-MW samples because of the inability to detect chemical species with MW lower than 500 due to interference by the spectral peaks of the matrix. (An exception is for purified sample DD-$(R_nH)_2$-1, where the MALDI-predicted M_n and PDI, 1318 and 1.06, respectively, compare favorably with NMR and GPC-derived values contained in Table II.) MALDI (nor any other method employed herein) was not able to detect the distribution of the *n* ricinoleyl groups between the multiple poly(ricinoleyl) chains attached to the polyol's OH groups.

^{1}H NMR was employed to obtain structural information for the polyol ester products formed, and their average MW, as performed previously.[31] ^{1}H- and ^{13}C NMR spectra for HOR_nH were similar to that reported previously for this specific oligomer[22] and other poly(hydroxy acids)[7,31,32] (Fig. 1). ^{1}H NMR spectra for DD, PE, and TMP polyesters of -R_nH were identical to that of HOR_nH except for additional peaks at 4.0–4.2 and 3.4–3.5 ppm for protons attached to the primary hydroxyl-containing carbon atoms of the acyl acceptors (data not shown). The former is attributed to protons near ester bonds, while the latter represent protons near free —OH groups (Fig. 1), in agreement with previously published data for PE-erucic acid mono-, di-, and triesters.[33] In many cases, more than one singlet peaks occurred in the 4.0–4.2 ppm region, suggesting the difference in poly(ricinoleyl) chain lengths esterified to a given polymer molecule's polyol group or perhaps heterogeneity in the number of poly(ricinoleyl) chains esterified to the polyol.[33] The heterogeneity was also reflected by multiple ^{13}C peaks for the —OH-containing and interior carbon atoms of PE and TMP, located at 60–62 and 40–42 ppm, respectively, as reported for PE-erucic acid mono-, di-, tri-, and -tetra-esters.[33] ^{13}C NMR also indicated the presence of carbonyl carbons for the polyol esters participating in ester bond formation (173–174 ppm),

TABLE III
Viscosity of Polyol–Poly (ricinoleic acid) Esters and Selected Substrates as a Function of Temperature

Sample[a]	μ_{23} ($\times 10^3$ Pa s)[b]	μ_{40} ($\times 10^3$ Pa s)[b]	μ_{60} ($\times 10^3$ Pa s)[b]	μ_{80} ($\times 10^3$ Pa s)[b]	A[c] ($\times 10^3$)	B[c]	Correl Coeff[c]
HOR_nH-1	477 ± 50	174 ± 8	58.5 ± 1.5	27.5 ± 0.3	5.29	−11.7	0.999
HOR_nH-2	559 ± 6	223 ± 3	91.6 ± 0.1	43.5 ± 3.8	4.68	−9.51	1.000
TMP-$(R_nH)_3$-1[d]	944 ± 35	311 ± 16	97.8 ± 0.2	42.0 ± 0.0	5.81	−12.8	0.998
TMP-$(R_nH)_3$-2[d]	1396 ± 1	426 ± 18	139 ± 24	55 ± 2.0	5.88	−12.7	0.999
PE-$(R_nH)_4$-1[d]	1170 ± 2	398 ± 13	149 ± 13	70.1 ± 5.4	5.20	−10.6	0.998
PE-$(R_nH)_4$-2[e]	2540 ± 60	861 ± 10	325 ± 13	164 ± 1.0	5.04	−9.37	0.997
DD	2500 ± 32	693 ± 5	174 ± 8	58.9 ± 0.4	6.91	−15.5	1.000
DD-$(R_nH)_2$-1[f]	675 ± 4	253 ± 18	97.7 ± 3.2	46.4 ± 5.1	4.92	−10.1	0.999
DD-$(R_nH)_2$-2[e]	965 ± 7	370 ± 6	153 ± 19	69.7 ± 3.6	4.80	−9.37	1.000

[a] Sample names correspond to those used in Tables 1 and 2; nomenclature given in Table I.
[b] viscosity at temperature (°C) indicated by subscript; standard errors based on 95% confidence limits, calculated using the student *t* distribution.
[c] Parameters and correlation coefficient for equation $\ln(10^3\ \mu\ \text{Pa s}) = A/T(K) + B$, eq. 7.
[d] GPC chromatograms and time course data depicted in Figs. 2–5.
[e] HOR_nH-2 is resultant product from 1st step of eq. 6 synthesis protocol.
[f] Purified by column chromatography.

while the presence of free COOH groups (179 ppm) was not detected for final polyol ester products, confirming that free poly(ricinoleic acid) is not a major component.[32,34]

The distribution of ricinoleyl acyl groups between those with hydroxyl groups esterified and not esterified to another acyl group was calculated using the NMR bands at 4.85 and 3.62 ppm, respectively, (Fig. 1). The number of acyl groups per sample was calculated by two different methods: the 5.4–5.6 ppm multiplets associated with the double bonds of ricionleyl acyl groups, and the sum of ricinoleyl acyl groups suggested by the 4.85 ppm plus 3.62 ppm bands (Fig. 1). The two estimates agreed within 15% of their mean value. The distribution of the ricinoleyl groups divided by the average number of acyl groups per sample provided an average degree of polymerization for the poly(ricinoleyl) chains (Table II, column 2). In addition, differentiation of esterified and free hydroxyl groups of the acyl acceptors (as discussed earlier) allowed for estimation of the percent esterification of polyol OH groups (Table I). The combination of the above-mentioned quantities, along with the fraction of poly(ricinoleyl) chains esterified to polyol (bands at 4.0–4.2, 4.85, and 3.62 ppm), led to estimation of average MW. The calculation procedure assumes the degree of polymerization for HR_nOH and R_nH chains esterified to polyol are identical. As discussed earlier, the highest MW products obtained, PE-$(R_nH)_4$-2 and DD-$(R_nH)_2$-2, both produced using the "convergent" approach, had high degrees of esterification for the polyol —OH groups, and similar or larger degrees of polymerization for the poly(ricinoleic acid) chains compared to their step 1 product, HOR_nH-2 (Table III). Estimates of MW derived from ^1H NMR and GPC agreed with each other reasonably well.

Physical properties

Table I depicts the melting point temperature range and the specific gravity (SG) of products. The esterification of -R_nH chains to TMP or PE led to an increase of SG relative to HOR_nH (0.935); however, DD-R_nH esters possessed SG values lower than HOR_nH, but above DD (0.881). The low-melting characteristics of HOR_nH and DD were not lost due to esterification, with all but one species remaining as a liquid when stored in the freezer (−14 to −18°C). The exception was *PE-*$(R_nH)_4$*-2*, the highest MW product, which melted between −13.3 and −7.5°C.

Viscosity measurements as a function of temperature are contained in Table III. Generally, viscosity increased with MW, as would be expected. Note that the attachment of -R_nH chains greatly reduced the viscosity of DD. The major aspiration for this work was to form polyol-R_nH ester products with a viscosity index (VI)[35] above 150 as desired for lubricant materials.[36] The highest molecular weight esters, *PE-*$(R_nH)_4$*-2* and *DD-*$(R_nH)_2$*-2*, possessed VI values of 155 and 132, respectively, while HOR_nH and DD feedstocks have VI values of 113 and 24, respectively. Moreover, the esterification of viscous lipidic feedstocks such as HOR_nH and DD led to a significant and desirable increase in VI, as occurred previously.[7]

The change in viscosity, μ, with absolute temperature *T*, strongly obeyed the following well-known Andrade Equation[37] (correlation coefficients > 0.997 for all species):

$$\ln(\mu) = A/T + B \qquad (7)$$

Values of the slope, *A*, and intercept, *B*, are given in Table III. Note that values of *A* and *B* for polyol-R_nH esters prepared by a convergent approach [i.e., eq. (6)]

are very close to those of the corresponding HOR_nH feedstock.

When the viscosity of the samples listed in Table III [with the exceptions of TMP-$(R_nH)_3$-1 and -2 and DD] at 23°C were plotted against MW, a linear relationship was obtained with a slope of 2.26 Pa s Da^{-1} and a correlation coefficient of 0.998. Thus, one can employ this result to determine the molecular structure of polyol–poly(ricinoleic acid) that will match a targeted value of viscosity. The samples also shared similar trends with respect to the change in viscosity with temperature; moreover, the slope values of the Andrade Equation [coefficient A from eq. (7)] were similar: 4990 ± 600. (Error limits represent 95% confidence intervals predicted by the *t*-distribution.) In contrast, TMP-$(R_nH)_3$-1 and -2 possessed higher viscosity values than that predicted by 2.26 Pa s Da^{-1} and an Andrade slope coefficient value of about 5850. The higher viscosity of the two TMP esters relative to the other polyol ester products listed in Table III is probably due to a significant amount of their polyol —OH groups remaining unesterified (Table II). DD is of a different chemical structure than the polyol polyesters and would not be expected to share similar viscosity-related properties.

Polyol-R_nH polyesters are high viscosity and VI, low-melting materials that are most probably highly biodegradable, based on the high biodegradability reported for poly(hydroxy acids) and their esters and for TMP-fatty acid esters.[36,38,39] Such materials may have utility as lubricants for high performance vehicles and for environmentally sensitive applications such as for food processing equipment, lawn mowers, transformers, marine equipment, etc. The market for biodegradable lubricants is expected to grow as environmental regulations in Europe and North America expand.[36] To improve the applicability of polyol-poly(hydroxy acids) as lubricants, one would use a hydroxy acid that lacked double bonds, to improve oxidative stability. One can substitute 12-hydroxy stearic acid for ricinoleic acid and achieve a similar product to that reported here, based on the similar kinetics of lipase-catalyzed polymerization of the two hydroxy acids.[22] (Ricinoleic acid was employed in this work because its double bond assisted in the calculation of MW by ^1H NMR.) An additional lipase-catalyzed step, the "end-capping" of free —OH groups on the poly(ricinoleic acid) chains by saturated nonhydroxy fatty acid, would further improve the viscosity properties.[6]

CONCLUSIONS

Polyol–poly(ricinoleic acid) polyesters were synthesized in bulk using trimethylolpropane, pentaerythritol, and dimer diol as acyl acceptors when catalyzed by immobilized *Candida antarctica* B lipase. A convergent synthetic approach, where poly(ricinoleic acid) is first synthesized in bulk; then, polyol is added [eq. (6)], yielded the highest conversion of ricinoleic acid acyl donor and polyol acyl acceptor, and the highest molecular weight product. For instance, PE-$(R_nH)_4$ was synthesized at 82.7% purity (GPC analysis) and possessed an average MW of 4850 (NMR analysis). The polyol–poly(ricinoleic acid) polyesters possessed melting points below −7°C, high viscosity, and high VI values, suggesting their potential use as biodegradable lubricant materials.

NOMENCLATURE

CAL	*Candida antarctica* B lipase immobilized onto nylon (Novozyme, Novo-Nordisk)
ELSD	Evaporative light scattering detector
GPC	Gel permeation chromatography
MALDI	MALDI-TOF-MS, or Matrix-assisted laser desorption ionization- time-of-flight mass spectroscopy
MW	Molecular weight
M_n	Number-averaged molecular weight (eq. (1))
M_w	Weight-averaged molecular weight (eq. (2))
NMR	Nuclear magnetic resonanace
PDI	Index of polydispersity (eq. (3))
R	Ricinoleyl acyl groups, —C(═O)—C_7H_{14}—CH═CH—CH_2—$CH(C_6H_{13})$O
RML	*Rhizomucor miehei* lipase immobilized onto anion exchange resin (Lipozyme IM, Novo-Nordisk)
RP-HPLC	Reversed phase high performance liquid chromatography
SG	Specific gravity
T	Temperature
THF	Tetrahydrofuran
VI	Viscosity index
m_i	Viscosity at temperature *i*, Pa s

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