Lipase-Catalyzed Synthesis of Lesquerolic Acid Wax and Diol Esters and Their Properties

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ABSTRACT: Lesquerolic acid wax and α,ω-diol esters were synthesized *via* immobilized *Rhizomucor miehei* lipase-(Lipozyme) catalyzed esterification of lesquerolic acid and alcoholysis of lesquerella oil. For each wax ester synthesis, when alcohol substrate was present at a slight (*ca.* 20%) stoichiometric excess and water content was kept low, over 94% of the hydroxy acyl groups were esterified. The extent of reaction and the ratio of monoester to diester produced for α,ω-diol reactions was controlled by the solubility of diol in the medium. This latter quantity increased as alcoholysis proceeded due to the formation of partial glycerides and monoesters, which increased the polarity of the medium. Alcoholysis reactions were significantly slower when the medium diol content was above saturation. As the diol chainlength increased, diol solubilization decreased, the ratio of monoester to diester decreased, and the extent of hydrolysis increased. Alcoholysis reactions involving either fatty alcohols or diols suffered from acyl migration, which lowered the purity of lesquerolic acid esters. Several lesquerolic acid esters, synthesized on a preparative scale and purified *via* column chromatography, were evaluated for their properties: density, viscosity, and melting point. Potential applications for lesquerolic acid esters are discussed.

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KEY WORDS: α,ω-diol esters, esters, hydroxy acid esters, lesquerella oil, lesquerolic acid esters (properties of), lipase, *Rhizomucor miehei lipase.*

Lesquerella fendleri, native to the American Southwest, is being developed as a new U.S. crop. The progress of Lesquerella research has been recently summarized (1). Its oil is rich (59%) in hydroxy fatty acids, particularly lesquerolic ($20{:}1^{11}$-OH^{14}) and auricolic ($20{:}2^{11}$ 17-OH^{14}) acids, present at 55 and 4%, respectively (2). Hydroxy acids have several important industrial uses (3,4). Currently, the major commercial source of hydroxy fatty acids is ricinoleic ($18{:}19$-OH^{12}) acid from castor (*Ricinus communis*) oil, which the United States imports. Recently, we have designed a procedure that features 1,3-specific lipolysis to isolate lesquerella oil's hydroxy fatty acids (5). This method is successful because in *L. fendleri* triglycerides (TG), all of the C_{20} hydroxy acids are located at the 1- and 3- positions (5); moreover, 81% of TG have a structure consisting of C_{20}-hydroxy acyl groups at the 1- and 3-positions and a C_{18} unsaturate at the 2-position.

In this paper, we report the synthesis of lesquerolic acid esters from reactions catalyzed by 1,3-specific lipases. The use of 1,3-specific lipases is important because unlike random lipases, the former cannot catalyze ester formation at lesquerolic acid's C_{14} hydroxyl group. Hydroxy acid esters have a variety of uses, e.g., plasticizers, mold release agents, firming agents for cosmetics (hair products, lipsticks, lotions, etc.), waxes, surfactants, and chemical intermediates. Sulfonated wax esters of ricinoleic acid are promising surface-active compounds (6).

Recently, lipases with organic solvent-based media have been employed for the synthesis of hydroxy acid esters. Ghoshray and Bhattacharya (6) and Mukesh *et al.* (7) have produced ricinoleic acid wax esters from *Rhizomucor miehei* lipase-catalyzed esterification of ricinoleic acid or alcoholysis of castor oil at high yields. The latter investigators (7) discovered that 1-butanol inhibited the lipase. Wagner *et al.* (8) synthesized wax esters of aleuritic (C_{16}-trihydroxy) acid by a similar reaction scheme and achieved moderate (*ca.* 60%) yields. A polar solvent, *tert*-butyl methyl ketone, had to be employed for solubilization of the hydroxy acid (8). [Polar solvents are known to reduce enzymatic reaction rates (9)]. Lang *et al.* (10) formed wax esters of (*S*)-17-hydroxy stearic acid, (*R*)-3-hydroxy decanoic acid, and 2-hydroxy octanoic acid with various lipases. They found that the reaction rates were faster when *R. miehei* lipase was the biocatalyst, free fatty acids were acyl donors instead of fatty acid methyl esters, and the acyl group's -OH moiety was farther away from its -COO moiety (10). Furthermore, the stereochemistry of the first two hydroxy acids were conserved in their esters (10). Steffen *et al.* (11) produced monoglycerides of (*S*)-17-hydroxy and 12-hydroxy stearic acids at high yields (>80%) with either *R. miehei* or *Candida antarctica* lipase as biocatalyst. Several of the hydroxy acid wax esters produced in these investigations were found to be useful surfactants (6,8,10,11). Here, we produced lesquerolic acid wax esters *via* alcoholysis of lesquerella oil or direct esterification of the free acid by a protocol similar to that employed previously (6–8,10,11).

Synthesis of fatty acid polyhydroxyl alcohol-(or polyol-) fatty acid esters is difficult because polyols have poor solubility in the solvent class of choice—lipophilic organic solvents (e.g., hexane) (9). Moreover, polyol ester-producing re-

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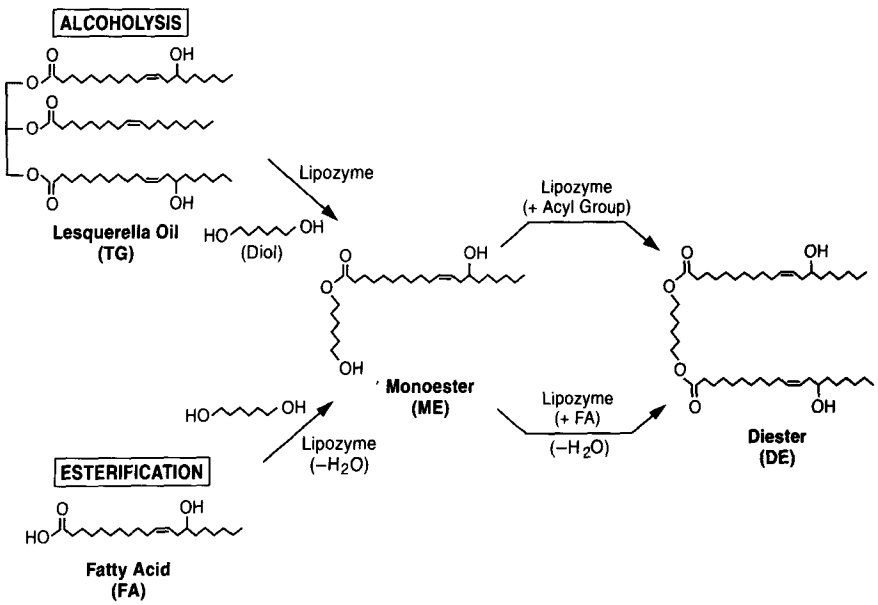
actions in lipophilic organic solvents are slow (12,13) and may require extremely high agitation rates to improve mass transfer between the polyol and lipase (14). Polyol esterification reactions have been performed successfully in more polar solvents, such as *tert*-butanol, 2-pyrrolidone, or tetrahydrofuran (15,16). In addition, polar organic solvents shift thermodynamic equilibrium, usually toward the formation of monoesters over di- and triesters (17). However, for most cases, enzyme reactions in polar organic solvents are slow (9), and care must be taken to prevent polar solvents from stripping away the enzymes' bound water layer needed for catalysis (18). As an alternative, diol esters have been successfully synthesized by reacting ethyl esters of short-chain (C_1-C_8) carboxylic acids with polyols. The ethyl ester, present in great stoichiometric excess, acted as solvent (19,20). However, this procedure has not been done with long-chain fatty acid ester substrate. Other methods involve employment of modified reaction medium for hosting polyol reactions. For example, reverse micelles—surfactant-controlled dispersions of water and polyol in lipophilic organic solvent—promote high concentrations of diol and provide relatively fast rates of reaction and extents of regioselectivity (21–23). But, recovery of product is difficult due to the presence of surfactant, and yields are moderate (*ca.* 50–60%). A second method is the employment of derivatized polyol, which is more soluble in lipophilic solvents. This increases reaction velocity. One derivitization method includes complexation with phenylboronic acid. This method has been applied in solubilizing glycerol to synthesize monoglycerides (11) and sugars to synthesize fatty acid ester biosurfactants (24) at high yields. A second derivitization procedure includes acid-catalyzed esterification of polyol with isopropylidene (25,26). Both phenylboronic acid and isopropylidene are easily removed from the products once the reaction is completed. A third alteration of reaction medium employs diol immobilized onto a solid matrix (e.g., silica gel). The immobilized diol and lipase are then stirred in a lipophilic solvent–fatty acid solution. Berger *et al.* (27) found that this method accelerated esterification by a factor of ten. In this report, we synthesize lesquerolic acid–diol esters *via* alcoholysis or direct esterification (Scheme 1). Immobilized lipase is magnetically stirred in diol-saturated ether containing lesquerella oil (or lesquerolic acid). When the reaction reached a plateau, the medium was resaturated with diol to allow the reaction to proceed.

MATERIALS AND METHODS

Lipozyme IM20 and IM60, *R. miehei* lipase immobilized onto anion exchange resin, were generous gifts from Novo Nordisk (Danbury, CT). α,ω-Diols, purchased from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI), were of high purity (>95%) and stored over $CaSO_4$ to reduce water content. 1-Decanol (99% pure) was purchased from Sigma. Oleyl alcohol (technical grade, 85% pure) was purchased from Aldrich. All solvents used for conducting enzymatic reactions and for column-chromatographic purification were of high purity. Refined lesquerella oil was donated by K.D. Carlson of our the NCAUR. Lesquerolic acid (*ca.* 65–85% pure) was obtained *via* lipolysis of lesquerella oil (5). 3A molecular sieves were purchased from Sigma. Silica gel, 230–400 mesh, was from Aldrich.

Enzyme reactions were conducted in 1–100 gram-size batch sizes under magnetic stirring or shaking to disperse Lipozyme. Stir rates were 150–200 rev min^{-1} unless otherwise stated. Reactions involving monohydric alcohols were conducted either in solventless mode at elevated temperatures (55–65°C) or in isooctane at ambient temperature (22 ± 1°C). For the former case, the batch reactor was left open to air to allow evaporation of water. For the latter case, the reaction



SCHEME 1

medium was pretreated with molecular sieves to remove water. Reactions involving diols were conducted in ethyl ether. Before the reactions began, diol was added in saturating (or in one case, oversaturating) amounts to the medium, then the medium was treated with molecular sieves. For several reactions, ester formation was briefly interrupted at one or two instances by temporarily removing Lipozyme. The remaining solution was treated with molecular sieves and resaturated with diol. Pentadecane or hexadecane was contained in several reaction solutions as internal standard. In several cases, Lipozyme used in the reactions was stored over $CaSO_4$ to reduce its water content.

Reactions were analyzed primarily by supercritical-fluid chromatography (SFC) with high purity carbon dioxide (Air Products, Tamaqua, PA) as carrier fluid in a model 600 chromatograph from Dionex (Salt Lake City, UT). Nonpolar capillary columns (10 m × 50 μm i.d.) of SB-Methyl-100 and SB-Phenyl-5 stationary phases (from Dionex) were employed. Pressure programming of the carrier fluid was used to separate peaks (28). The use of these nonpolar columns allows separation based on molecular weight, but no separation based on degree of unsaturation (28). Separation of fatty acid (FA), monoglyceride (MG), diglyceride (DG), and esters based on degree of unsaturation and separation of partial glyceride positional isomers was determined by gas chromatography on a methyl 65% phenyl silicone 25 m × 0.25 mm capillary column from Quadrex (New Haven, CT) (29). Lipozyme, recovered from the medium *via* filtration, was reusable. Removal of ether *via* evaporation, followed by centrifugation, allowed nearly complete separation of the insoluble diol from the ester products/remaining substrates. Isolation of products at high purity was conducted by silica gel column chromatography with hexane/ethyl acetate at varying proportions as eluate. Fractions collected were analyzed by thin-layer chromatography and SFC. Trace amounts of solvent were removed from the resulting product in a vacuum oven.

The density, ρ, of several materials was determined gravimetrically. Melting points were determined in a standard capillary melting point apparatus. Kinematic viscosity, ν, was measured in a temperature-controlled oil bath with Cannon-Fenske viscometers from Cannon Instruments (State College, PA). Viscometer constants were determined with lesquerella oil as standard. Density and viscosity values for lesquerella oil were taken from the data of Noureddini *et al.* (30,31). The viscosity index (VI) of lesquerolic acid and two wax esters was determined by a standard procedure (32), which required values of ν at 40 and 100°C. These values were estimated from viscosity measurements at alternate temperatures from the following equations

$$\ln \nu = A + B \exp(T^{-1}) \quad [1]$$

where T is the temperature in Kelvin, and A and B are constants. Correlation coefficients were >0.99. This relationship did not fit data for lesquerolic acid well. For this case, values of ν at 40 and 100°C were estimated *via* extrapolation.

RESULTS AND DISCUSSION

Synthesis of lesquerolic acid wax esters. Synthesis of lesquerolic acid oleyl ester was achieved *via* lipase-catalyzed alcoholysis of lesquerella oil and esterification of lesquerolic acid. The time course of reaction for alcoholysis and esterification are depicted in Figures 1 and 2, respectively. [Fatty alcohols were present at a slight (10–50%) stoichiometric excess for all reactions discussed here. Both reactions yielded high conversions of hydroxy acyl groups, over 94%. Ideally, alcoholysis would produce 66.7% (acyl group molar equivalents) of FA oleyl ester and 33.3% MG, the bulk of which would be unsaturated monooctadecanoates. However, for our case, the ester (nonhydroxy acid ester, X-E, plus hydroxy acid ester, H-E) content after 42 h was 81.2%, and the MG content never became more than 8%. In addition, the percentage of H-E among all FA esters, which should theoretically be higher than 80% (28), was only 64% after 42 h. These results reflect acyl migration, or the isomerization of 1,2- (2,3-) DG and 2-MG to 1,3-DG and 1-MG, respectively. Acyl groups present originally at the 2-position can then, as a result of acyl migration, be attacked by the 1,3-specific biocatalyst. Acyl migration also has been reported for Lipozyme-catalyzed hydrolysis (5), glycerol–oleic acid esterification (33), and acidolysis (34). However, little or no acyl migration occurred for Lipozyme-catalyzed transesterification between triolein and ethyl palmitate, presumably because of the absence of partial glycerides and fatty acid, the latter of which promotes acyl migration (34). Other promoters of acyl migration are discussed elsewhere (5,33,34). The presence of significant amounts of undesired X-E in the reaction end-product makes inclusion of a purification step necessary. Alcoholysis was also investigated in other solvents (ethyl ether and butyl ether) and with other fatty alcohols (1-octanol and 1-decanol). The time course of reaction for each alcohol and solvent type

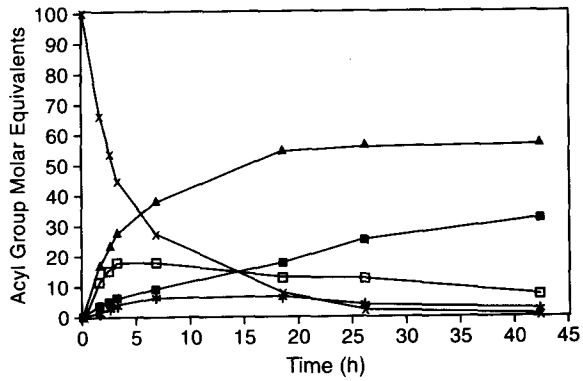


FIG. 1. Oleyl alcoholysis of lesquerella oil catalyzed by Lipozyme-IM20 (Novo Nordisk, Danbury, CT) at 42.8°C. Equal weights of lesquerella oil and oleyl alcohol (1.20 mol oleyl alcohol per mol acyl group) were added to isooctane. The resulting solution (0.20 vol/vol oleyl alcohol, 0.18 v/v lesquerella oil, and 0.62 vol/vol isooctane) was treated with molecular sieves. Lipozyme stored over $CaSO_4$ was added at a concentration of 0.21 g/mL (0.062 g/g substrates). (■) X-E, (▲) H-E, (*) MG, (□) DG, (X) TG.

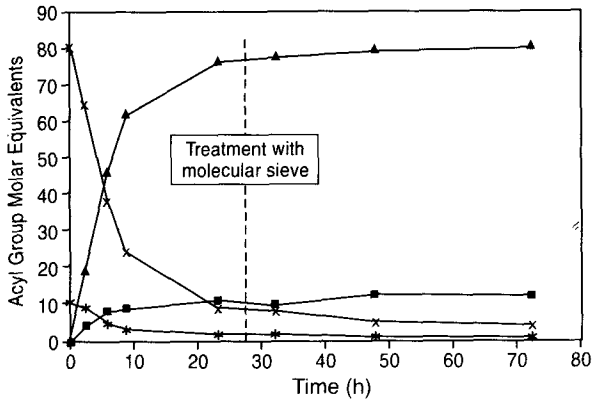


FIG. 2. Lipozyme-IM20-catalyzed esterification of lesquerolic acid and oleyl alcohol at 60.0°C in the absence of solvent. Reaction mixture: 17.6 g (.055 mol acyl groups) lesquerolic acid (82% pure), 20.2 g (.075 mol) oleyl alcohol (technical grade), and 2.81 g Lipozyme stored over $CaSO_4$. (*) X-FA, (X) H-FA, (■) X-E, (▲) H-E. See Figure 1 for company source.

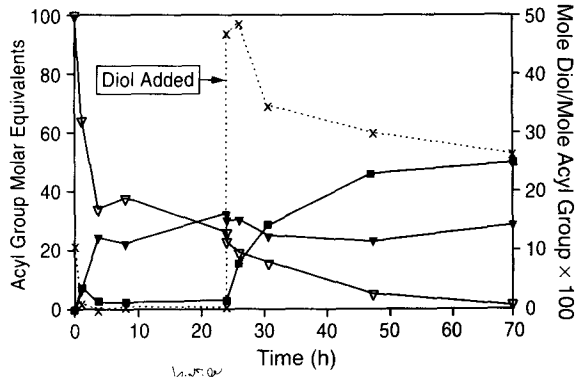


FIG. 3. 1,8-Octanediolysis of lesquerella oil catalyzed by Lipozyme-IM60 at 22°C in ethyl ether. Reaction medium initially: 5 mL 10% (vol) lesquerella oil in ethyl ether that contained saturating amounts of diol and 0.1 g untreated Lipozyme. The solution was treated with molecular sieves before addition of Lipozyme. At 24.2 h, the solution was momentarily removed from Lipozyme, resaturated with diol, and treated with molecular sieves. (▽) TG, (■) ME, (▼) DE, (X) diol (secondary *y*-axis). See Figure 1 for company source.

was quite similar to that displayed for oleyl alcoholysis in Figure 1. Alcoholysis would not occur in pyridine, even with large quantities (0.75 wt%) of water. This suggests that pyridine denatures lipase rather than stripping away its essential water layer.

Esterification was attempted with an FA sample that contained 80 (mole) % hydroxy fatty acids (H-FA), the vast majority of which was lesquerolic acid, 10% nonhydroxy fatty acids (X-FA), and the remainder MG and DG. Because water is a product of the reaction and its presence limits the extent of esterification, steps to remove it are essential. Employment of open-air reactors, operating at elevated temperature (60°C), promotes low water content (35). However, water removal *via* molecular sieves was successful in accelerating the reaction rate, as indicated by the significant decrease of H-FA concentration after the treatment (Fig. 2). Mass balances and chromatographic analysis indicated that essentially all partial glycerides present initially were consumed by the reaction. The final product on an alcohol-free basis contains 80% H-E, which would be of a purity acceptable for industrial applications. However, due to the excess of alcohol present initially, the final product contained *ca.* 0.7–0.8 moles of oleyl alcohol per mole of acyl group; thus, purification was necessary.

Synthesis of α,ω-diol-lesquerolic acid esters. The time course of lipase-catalyzed 1,8-octanediolysis of 10% lesquerella oil (Scheme 1) in ethyl ether (22°C) is depicted in Figure 3. Ether was chosen as solvent because of its ability to solubilize diol; moreover, more nonpolar solvents, such as hexane and isooctane, solubilize diols poorly. In addition, due to its volatility and low boiling point, ether is easily removed from the medium upon completion of the reaction. As mentioned above, more polar solvents, such as pyridine, which better solubilize diol but denatured Lipozyme, were not used. The reaction initially contained diol at saturating quantities, which was only 0.097 moles per mole of acyl group (Fig. 3). The reaction reached a plateau (33.5% esterification) between 10 and 20 h, presumably due to depletion of diol (Fig. 3). At this plateau, the ratio of diester (DE) to monoester (ME) was greater than 10:1. This is due to the relative abundance of ME as acyl acceptor compared to diol in the initial stages of the reaction (Scheme 1). At 24.1 h, the medium was resaturated with diol and treated with molecular sieves. The amount of diol contained in the medium then rose more than seven-fold compared to its initial amount (Fig. 3 and Table 1). This probably occurred because of the depletion of TG and formation of esters and partial glycerides, which together increase the polarity of the medium, hence enhancing the diol solubility. Moreover, the ratio of diol to ME increased, which promoted the formation of ME over DE (Fig. 3). The reaction was stopped at *ca.* 70 h, with 96.0% of C_{20}-hydroxy acyl groups esterified.

Reduction of medium water content is quite important for this reaction, as it was for lesquerolic acid wax ester formation (see above). Alcoholysis of lesquerella oil by 1,6-hexanediol and 1,10-decanediol was conducted under identical conditions to those used for 1,8-octanediolysis (Fig. 3). The results for all three reactions are contained in Table 1. These results show that a significant amount of free fatty acids (FFA) is present at 24.1 h, the time by which the first plateau was reached. This indicates the occurrence of the hydrolysis side-reaction. However, after treatment with molecular sieves (24.1 h), the FA content for all three reactions was significantly lessened (Table 1). When water was added to the medium, the diol concentration at saturation increased. For example, when 3.2% (vol) water was added to ether containing 0–10% lesquerolic acid, the saturating diol concentration rose by a factor greater than eight (data not shown). However, when 0.25% water was added, the increase in diol solubilization was slight (data not shown). In addition, when the reaction of Figure 3 was performed in the presence of 0.25%

TABLE 1
Results from Lipozyme IM60-Catalyzed Alcoholysis of Lesquerella Oil with α,ω-Diols[a]

	C_6 Diol[b] 24.1 h	C_6 Diol[c] 69.8 h	C_8 Diol[b] 24.1 h	C_8 Diol[c] 69.8 h	C_{10} Diol[b] 24.1 h	C_{10} Diol[c] 69.8 h	C_8 Diol[c,d] 31.3 h
FFA, %[e]	2.9	0.0	3.7	0.1	4.8	2.1	0.0
MG, %[e]	9.7	17.0	6.1	14.8	5.5	10.4	15.7
DG, %[e]	34.1	14.5	32.3	7.5	32.3	13.8	13.0
TG, %[e]	13.6	11.5	23.4	0.7	28.2	2.3	7.4
ME, %[e]	5.8	37.1	2.9	49.1	1.5	20.2	45.4
DE, %[e]	33.9	19.9	31.6	27.9	27.6	51.2	18.5
Diol initially, mol/mol acyl group * 100	9.74		5.01		4.11		22.2
Diol, mol/mol acyl group * 100[f]	0.0	72.1	0.2	26.1	0.0	1.8	
Diol, mol/mol acyl group * 100[g]	79.6		47.2		17.0		33.5
H-ME/ME	71.8	78.2	65.7	73.0	83.8	63.6	78.2
DE(18,L)/DE	33.2	32.0	35.3	37.4	26.6	41.1	26.9
DE(L,L)/DE	64.4	66.9	60.3	56.4	63.5	50.5	70.8
% Esterification hydroxy acyl group	53.6	77.1	44.9	96.0	38.0	83.4	86.7

[a]Reaction conducted under conditions listed in Figure 3 (medium saturated with diol initially) unless otherwise indicated. Lipozyme IM60 from Noro Nordisk (Danbury, CT).
[b]Reaction stopped (plateau in reaction reached). Medium treated with molecular sieves and resaturated with diol.
[c]Final concentrations (at or near equilibrium).
[d]Conditions listed in Figure 4. Medium oversaturated with diol.
[e]Acyl group molar equivalents. FFA, free fatty acid; MG, monoglyceride; DG, diglyceride; TG, triglyceride; ME, monoester; DE, diester.
[f]Before resaturation.
[g]After resaturation.

added water, over 9% of the acyl groups were hydrolyzed (data not shown).

The effect of diol chainlength was significant in two areas. First, diols of longer chainlength are solubilized to a lesser extent. This increased the ratio of DE to ME produced (Table 1). Second, the water present in the medium that contains long-chain diols must be more difficult to remove than that in medium containing short-chain diol. Moreover, the FFA content in the decanediol medium (resulting from hydrolysis) is higher than that in the hexanediol or octanediol-containing medium (Table 2). The presence of water and carboxylic acid generally increased the extent of acyl migration for reactions occurring in organic solvents or in solventless mode (see above). Indeed, a larger extent of acyl migration is observed for the decanediol reaction, as evidenced by smaller MG content and smaller percentages of 20-hydroxy moieties in the ME and DE (Table 2).

Figure 4 displays octanediolysis of lesquerella oil under the same conditions used previously (Fig. 3), except the diol concentration here was above saturation, i.e., solid diol was also present in the medium. When comparing this reaction (Fig. 4) with one where no insoluble diol is present (Fig. 3), the initial rate of the former (39.8 μmol TG/h) is much slower than the latter (159 μmoles TG/h). However, diol concentration in solution increases as the reaction procedes, then reaches a plateau at *ca.* 0.33 moles per mole acyl group (Fig. 4). Because of the large concentration of diol present at all times, the ratio of ME to DE is always high (Table 1). In addition, this reaction was less susceptible to acyl migration than that of Figure 3, as indicated by the higher percentages of lesquerolic acyl groups in ME and DE. Perhaps the suspended diol, which we discovered, was quite hygroscopic (Karl Fischer titration data), and may have adsorbed the small quantities of water present in the medium, which would reduce acyl migration (see above). In conclusion, it would be desirable to operate reactions employing diols under conditions where diol concentrations were at, but not above, saturation. Berger *et al.* (27) conducted Lipozyme-catalyzed alcoholysis successfully by suspending silica gel that contained diol. This technique was successful because the organic solvent remained saturated, but did not become oversaturated, with diol.

One could control the relative amount of ME to DE by adjusting the ratio of diol to acyl group in the medium (27). For example, to produce DE, the reaction was stopped at an intermediate point in the reaction, and diol was removed by evaporation of ethyl ether, followed by centrifugation. The supernatant, containing only minute amounts of diol, was then reacted in a lipophilic organic solvent or in solventless mode to rapidly produce DE (data not shown).

Figure 5 depicts the formation of C_{20}-hydroxy acid-1,8-octanediol esters *via* Lipozyme-catalyzed esterification in ethyl ether (Scheme 1). The lesquerolic acid substrate contained ca. 64.8% (acyl group molar equivalents) C_{20}-H-FA, 24.3% X-FA, and the remainder MG, DG, and TG. The figure demonstrates that the initial (medium-saturated) diol con-

TABLE 2
Physical Properties: Density (ρ) and Melting Point (T_{melt}) of Hydroxy Acid Esters

Material	Source/method[a]	Purity[b]	ρ, g/mL (25°C)	T_{melt} (°C)
Ricinoleic acid	[c]		0.945	5.5–7.7
Ricinoleic acid decyl ester	[d]		0.880	2.0
Monoricinolein	[c]		0.985	20[e]
Ricinoleic acid–decanediol monoester	Esterification[f]	81.1[g]		9.5–11.5
Lesquerolic acid	Hydrolysis[h]	84.5 (90.5)	0.93	17.0–17.8
Lesquerolic acid decyl ester	Alcoholysis[i]	86.1 (92.1)	0.88	12.5
Lesquerolic acid oleyl ester	Esterification[i]	83.6 (98.7)	0.86	10.0–11.0
Monolesquerolin	Esterification[j]	91.9 (98.6)[k]		44.0–45.2
Dilesquerolin	Esterification[j]	(66.1)[l]		22.0–24.5
Diglyceride (one C_{18} and one lesquerolic acyl group)	By-product[m]	93.8		(–2) – (+1)
Lesquerolic acid–decanediol Monoester	Alcoholysis[f]	71.9[n]		26.0–26.5

[a]Except where indicated, all products synthesized from reactions catalyzed by Lipozyme (Novo Nordisk, Danbury, CT). See Table 1 for abbreviations.
[b]Numbers in parentheses are the purities of C_{20}-hydroxy (lesquerolic plus auricolic) esters.
[c]From product data, CasChem Co (Bayonne, NJ).
[d]From Reference 6.
[e]Pour point.
[f]Reactions performed in ethyl ether at 22°C.
[g]Impurities present: ricinoleic acid monoestolide decanediol monoester at 8.9% and free monoestolide at 7.7%. Monoestolide was present as an impurity in the ricinoleic acid substrate.
[h]Major impurities were C_{20}-hydroxy FA (15.5%) and ricinoleic acid–decanediol monoester (3.9%).
[i]Solventless, 55–65°C.
[j]Both monolesquerolin and dilesquerolin produced from the same reaction (solventless, 60°C); a high rate of magnetic stirring (200 rev min–1) was needed to improve mass transfer between immiscible glycerol and lesquerolic acid phases and Lipozyme.
[k]Of the 20-hydroxy MG, 81.6% was 1-(3-)monolesquerolin, 11.6% 2-monolesquerolin, and 6.8% 1-(3-)monoauricolin.
[l]Major impurities were C_{20}-hydroxy fatty acids (FA) (24.8%), other DG (5.3%), and trilesquerolin (2.0%).
[m]Produced from alcoholysis reaction that yielded lesquerolic acid decyl ester as major product.
[n]Major impurities were C_{20}-hydroxy FA (15.5%) and ricinoleic acid–decanediol monoester (3.9%).

centration is much higher than for the alcoholysis reaction. This is most probably due to the lesquerolic acid-containing medium being more polar. At 4.73 h, diol substrate was depleted, the production of ME reached a maximum and began to decrease, while the level of DE increased. At this point, the medium was resaturated with diol and treated with molecular sieves, in similar fashion to that performed during alcoholysis. The new concentration of diol is slightly less than that ob-

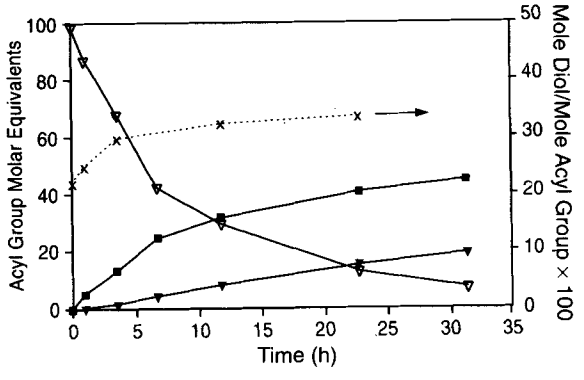


FIG. 4. 1,8-Octanediolysis of lesquerella oil catalyzed by Lipozyme-IM60 at 22°C in ethyl ether at a diol concentration greater than saturation. Symbols, conditions as in Figure 3, except a larger amount of diol (0.29 g, i.e., 4.0 moles of diol per mole acyl group) is present initially. See Figure 1 for company source.

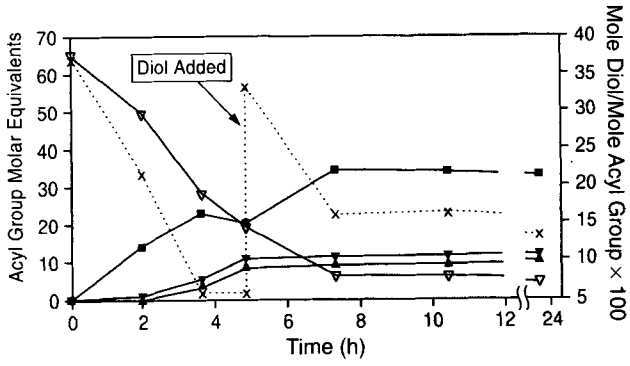


FIG. 5. Lipozyme-IM60-catalyzed esterification of 1,8-octanediol and lesquerolic acid at 22°C in ethyl ether. Formulation of medium as in Figure 3, with lesquerolic acid replacing lesquerella oil. At 4.73 h, the solution was momentarily removed from Lipozyme, resaturated with diol, and treated with molecular sieves. (▽) H-FA, (■) H-ME, (▼) DE(H,H), (▲) DE(X,H), (X) diol (secondary *y*-axis). See Figure 1 for company source.

tained originally, indicating that the medium becomes less polar upon esterification. The replenishment of diol led to further production of ME, while the DE amount remained constant. The experiments contained in Figures 3 (alcoholysis) and 5 (esterification) are comparable, i.e., both employ identical initial concentrations of acyl groups and enzyme activity. The initial rate of esterification is slower than that for alcoholysis (cf. Fig. 5 vs. Fig. 3). The overall conversion of acyl groups here is high, over 92%. Perhaps a second treatment with molecular sieves would provide further esterification. Most of the partial glycerides present initially have been converted to ester (Table 3).

Table 3 demonstrates the percentage of C_{20}-hydroxy monoesters (H-ME) among ME and DE contain one and two hydroxy acyl groups [DE(H,H) and DE(H,X)], respectively, among DE(H,H) are 69.5, 41.6, and 50.2%, respectively. Moreover, the percentage of hydroxy acyl groups in [DE(H,H) 72.7%] is nearly identical to the percentage of hydroxy acyl groups in ME and the percentage of H-FA among free acids. The compositions of ME and DE are comparable to those obtained *via* 1,8-octanediolysis of lesquerella oil (Table 1).

Properties and potential applications for lesquerolic acid esters. Lesquerolic acid esters were produced *via* Lipozyme-catalyzed reactions on a preperative scale and purified by silica gel column chromatography. The melting point, T_{melt}, and density, ρ, for several lesquerolic acid esters are included in Table 2. Properties of ricinoleic acid esters are also included for reference. The substitution of lesquerolic for ricinoleic acyl groups (i.e., the addition of a $-CH_2$ group to the tail of the ricinoleic acyl group) raises the melting point of the free acid and esters but does not significantly affect the values of density (Table 2). Esterification with fatty alcohols significantly lowers the melting point and density of hydroxy acids, and esterification with diol (forming monoester) slightly raises the melting point (Table 2).

TABLE 3
Results from Lipozyme IM60-Catalyzed Esterification of 1,8-Octanediol and Lesquerolic Acid[a]

Material	0.00 h	1.88 h	4.73 h[b]	23.48 h
FA, %[c]	89.2	70.2	26.6	6.4
ME, %[c]	0.0	24.2	29.2	47.2
DE, %[c]	0.0	1.8	41.3	45.9
MG + DG + TG, %[c]	10.8	3.7	2.9	0.5
Diol, mol/mol acyl group * 100	36.9	21.9	33.0[d]	13.6
H-FA/FA	72.7	70.2	70.5	66.9
H-ME/ME		57.9	68.3	69.5
DE/(X,H)/DE		38.1	41.6	41.6
DE(H,H)/DE		36.0	51.1	50.2
% Esterification hydroxy acyl group[e]	0.0	20.6	68.2	89.9

[a]Conditions listed in Figure 5. Company source and abbreviations as in Tables 1 and 2.
[b]Medium resaturated with diol and treated with molecular sieves.
[c]Percentages of acyl group molar equivalents.
[d]After resaturation.
[e]Includes hydroxy acyl groups initially present in MG + DG + TG.

TABLE 4
Values of Kinematic Viscosity (ν) and Viscosity Index (VI) for Lesquerolic Acid and Its Wax Esters

Material[a]	ν (25.3°C), cSt	ν (41.3°C), cSt	ν (5.80°C), cSt	ν (97.5°C), cSt	VI
Lesquerolic acid	278.7	93.1[b]	46.9	12.4	77
Lesquerolic acid decyl ester	54.8	30.8	18.8	5.9	116
Lesquerolic acid oleyl ester	65.0	32.6	18.9	8.0	162

[a]Purities and methods of derivation listed in Table 2.
[b]42.9°C.

Values of kinematic viscosity, ν, for lesquerolic acid and two of its wax esters are included in Table 4. The dynamic viscosity of lesquerolic acid, μ, equal to ρ multiplied by ν, is 258.0 cp at 25°C, which is a factor of 10 larger than viscosities for nonhydroxy fatty acids, such as oleic acid (31). Esterification of lesquerolic acid significantly lowers viscosity. VI, is a measure of the resistance of a material's viscosity to temperature. It is desirable that a material have a viscosity that changes little with temperature; i.e., a good lubricant will have a VI above 150 (35). Thus, lesquerolic acid oleyl ester may be a good candidate as a lubricant or lubricant additive (Table 4). On the positive side, lesquerolic acid oleyl ester as a lubricant additive would be biodegradable (36); however, its high melting point (10–11°C) would be a disadvantage. Hydrogenation of the ester would significantly lower the melting point.

Monoricinolein is employed as a plasticizer, wetting agent, and a mold-release agent. But monoricinolein must be hydrogenated, producing mono-12-hydroxystearate, to be used as an emulsifier or firming agent in cosmetic products, such as lipstick and hair preparations (product information from CasChem, Bayonne, NJ). This is because monoricinolein is a liquid at room temperature, while mono-12-hydroxystearate is a solid up to 69°C. Perhaps monolesquerolin, a waxy material that melts at 44–45°C, can fulfill many of these needs. In addition, due to its longer chainlength, monolesquerolin will be more hydrophobic than monoricinolein, which may be an advantage. Lesquerolic acid MG and DG and diol ME are multi-hydroxy compounds that may have use as surface-active or wetting agents. Their properties require further evaluation. Ricinoleic acid wax esters, such as palmityl ricinoleate or octyl 12-hydroxystearate, are employed in various skin care products. Perhaps homologous lesquerolic acid wax esters may provide suitable substitutes. Sulfonation of lesquerolic acid wax esters may produce useful surfactants, as suggested previously (6). Investigation of new lesquerolic acid esters, derivatives thereof, and their properties and applications will continue in our laboratory.

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REFERENCES

1. Roetheli, J.C., K.D. Carlson, R. Kleiman, A.E. Thompson, D.A. Dierig, L.K. Glaser, M.G. Blase and J. Goodell, *An Assessment of Lesquerella as a Source of Hydroxy Fatty Acids for Industrial Products,* Office of Agricultural Materials, Cooperative Research Service, USDA, Washington, D.C., 1991.
2. Carlson, K.D., A. Chaudhry and M.O. Bagby, Analysis of Oil and Meal from *Lesquerella fendleri* Seed, *J. Am. Oil Chem. Soc. 67*:438–442 (1990).
3. Naughton, F.C., Production, Chemistry, and Commercial Applications of Various Chemicals from Castor Oil, *Ibid. 51*:65–71 (1974).
4. Schwitzer, M.K., Castor Oil, in *Proceedings of the World Conference on Oleochemicals into the 21st Century*, edited by T.H. Applewhite, American Oil Chemists' Society Press, Champaign, 1991, pp. 111–118.
5. Hayes, D.G., and R. Kleiman, 1,3-Specific Lipolysis of *Lesquerella fendleri* Oil by Immobilized and Reverse Micellar Encapsulated Lipases, *J. Am. Oil Chem. Soc. 70*:1121–1127 (1993).
6. Ghoshray, S., and D.K. Bhattacharya, Enzymatic Preparation of Ricinoleic Acid Esters of Long-Chain Monohydric Alcohols and Properties of the Esters, *Ibid. 69*:85–88 (1992).
7. Mukesh, D., R.S. Iyer, J.S. Wagh, A.A. Mikashi, A.A. Banerji, R.V. Newadkar and H.S. Bevinakatti, Lipase Catalyzed Transesterification of Castor Oil, *Biotechnol. Lett. 15*:251–256 (1993).
8. Wagner, F., F. Kleppe, W. Lokotsch, A. Ziemann and S. Lang, Synthesis of Uncommon Wax Esters with Immobilized Lipases, *Ann. N.Y. Acad. Sci. 672*:484–491 (1992).
9. Laane, C., S. Boeren, K. Vos and C. Veeger, Rules for Optimization of Biocatalysis in Organic Solvents, *Biotechnol. Bioeng. 30*:81–87 (1987).
10. Lang, S., R. Multzsch, A. Passeri, A. Schmeichel, B. Steffen, F. Wagner, D. Hamann and H.K. Cammenga, Unusual Wax Esters and Glycolipids: Biocatalytical Formation and Physico-Chemical Characterization, *Acta Biotechnol. 11*:379–386 (1991).
11. Steffen, B., A. Ziemann, S. Lang and F. Wagner, Monoacylation of Trihydroxy Compounds, *Biotechnol. Lett. 14*:773–778 (1992).
12. Gatfield, I.L., The Enzymatic Synthesis of Esters in Non-Aqueous Systems, *Lebensm. Wiss. Technol. 19*:87–88 (1986).
13. Margolin, A.L., J.Y. Crenne and A. M. Klibanov, Stereoselective Oligomerizations Catalyzed by Lipases in Organic Solvents, *Tetrahed. Lett. 28*:1607–1610 (1987).
14. Osada, K., K. Takahashi and M. Hatano, Polyunsaturated Fatty Glyceride Syntheses by Microbial Lipases, *J. Am. Oil Chem. Soc. 67*:921–922 (1990).
15. Geresh, S., and Y. Gilboa, Enzymatic Synthesis of Alkyds, *Biotechnol. Bioeng. 36*:270–274 (1990).
16. Khaled, N., D. Montet, M. Pina and J. Graille, Fructose Oleate Synthesis in a Fixed Catalyst Bed Reactor, *Biotechnol. Lett. 13*:167–172 (1991).
17. Janssen, A.E.M., A. Van der Padt, H.M. Van Sonsbeek and K. Van't Riet, The Effect of Organic Solvents on the Equilibrium Position of Enzymatic Acylglycerol Synthesis, *Biotechnol. Bioeng. 41*:95–103 (1993).
18. Zaks, A., and A.M. Klibanov, The Effect of Water on Enzyme Action in Organic Media, *J. Biol. Chem. 263*:8017–8021 (1988).
19. Cesti, P., A. Zaks and A.M. Klibanov, Preparative Regioselective Acylation of Glycols by Enzymatic Transesterification in Organic Solvents, *Appl. Biochem. Biotechnol. 11*:401–407 (1985).
20. Hajjar, A.B., P.F. Nicks and C.J. Knowles, Preparation of Monomeric Acrylic Ester Intermediates Using Lipase Catalysed Transesterifications in Organic Solvents, *Biotechnol. Lett. 12*:825–830 (1990).
21. Chang, P.S., J.S. Rhee and J.J. Kim, Continuous Glycerolysis of Olive Oil by *Chromobacterium viscosum* Lipase Immobilized on Liposome in Reversed Micelles, *Biotechnol. Bioeng. 38*:1159–1165 (1991).
22. Hayes, D.G. and E. Gulari, Formation of Polyol-Fatty Acid Esters by Lipases in Reverse Micellar Media, *Ibid. 40*:110–118 (1992).
23. Rees, G.D., T.R.J. Jenta, M.G. Nascimento, M. Catauro, B.H. Robinson, G.R. Stephenson and R.D.G. Olphert, Use of Water-in-Oil Microemulsions and Gelatin-Containing Microemulsion-Based Gels for Lipase-Catalysed Ester Synthesis in Organic Solvents, *Indian J. Chem. 32B*:30–34 (1993).
24. Oguntimein, G.B., H. Erdmann and R.D. Schmid, Lipase Catalyzed Synthesis of Sugar Ester in Organic Solvents, *Biotechnol. Lett. 15*:175–180 (1993).
25. Pecnik, S., and Z. Knez, Enzyme Solvent-Free Synthesis, *J. Am. Oil Chem. Soc. 69*:261–265 (1992).
26. Fregapane, G., D.B. Sarney and E.N. Vulfson, Enzymic Solvent-Free Synthesis of Sugar Acetal Fatty Acid Ester, *Enzyme Microb. Technol. 13*:796–800 (1991).
27. Berger, M., K. Laumen and M.P. Schneider, Lipase-Catalyzed Esterification of Hydrophilic Diols in Organic Solvents, *Biotechnol. Lett. 14*:553–558 (1992).
28. Hayes, D.G. and R. Kleiman, Supercritical Fluid Chromatographic Analysis of New Crop Seed Oils and Their Reactions, *J. Am. Oil Chem. Soc.*, in press, 1996.
29. Hayes, D.G., and R. Kleiman, Recovery of Hydroxy Fatty Acids from Lesquerella Oil with Lipases, *J. Am. Oil Chem. Soc. 69*:982–985 (1992).
30. Noureddini, H., B.C. Teoh and L.D. Clements, Densities of Vegetable Oils and Fatty Acids, *Ibid. 69*:1184–1188 (1992).
31. Noureddini, H., B.C. Teoh and L.D. Clements, Viscosities of Vegetable Oils and Fatty Acids, *Ibid. 69*:1189–1191 (1992).
32. *Annual Book of ASTM Standards*, Part 24, ASTM Press, Philadelphia 1981, pp. 277–285, Method D 2270–79.
33. Lortie, R., M. Trani and F. Ergan, Kinetic Study of the Lipase-Catalyzed Synthesis of Triolein, *Biotechnol. Bioeng. 41*:1021–1026 (1993).
34. Bloomer, S., P. Adlercreutz and B. Mattiasson, Triglyceride Interesterification by Lipases: 2. Reaction Parameters for the Reduction of Trisaturated Impurities and Diglycerides in Batch Reactions, *Biocatalysis 5*:145–162 (1991).
35. Ergan, F., M. Trani and G. Andre, Production of Glycerides from Glycerol and Fatty Acid by Immobilized Lipases in Non-Aqueous Media, *Biotechnol. Bioeng. 35*:195–200 (1990).
36. Shubkin, R.L., (ed.), *Synthetic Lubricants and High-Performance Functional Fluids*, Marcel Dekker, New York, 1992.

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