Low-Calorie Triglyceride Synthesis by Lipase-Catalyzed Esterification of Monoglycerides

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ABSTRACT: Monoglycerides of erucic acid ($C_{22:1}$, Δ13), prepared by conventional methods, were reacted with caprylic acid (octanoic acid, $C_{8:0}$) by using lipases as catalysts with the intention of synthesizing a triglyceride that contains two molecules of caprylic acid and one molecule of erucic acid (caprucin). The reaction was carried out by mixing lipase powder, a small quantity of water, and the reactants in a temperature-controlled stirred batch reactor. Organic solvents or emulsifying agents were not required. When the nonspecific lipase from *Pseudomonas cepacia* was used, a yield of approximately 37% caprucin was obtained, together with a complex mixture of di- and triglycerides that resulted from the random transesterification of the erucic acid. The fatty acid-specific lipase from *Geotrichum candidum* promoted minimal transesterification of erucic acid and resulted in a yield of 75% caprucin and approximately 10% interesterification products. Lipase from *Candida rugosa* exhibited a similar, although less pronounced, specificity to that from *G. candidum* and promoted more transesterification of erucic acid. Optimum conditions for *G. candidum* lipase were at 50°C and an initial water content of 5.5%. After the reaction, erucic acid was converted to behenic acid by hydrogenation, thereby converting caprucin into caprenin, a commercially available low-calorie triglyceride.
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KEY WORDS: Behenic acid, caprenin, caprucin, erucic acid, lipase, monoglyceride, synthesis, triglyceride.

Lipases have proven to be useful catalysts for the synthesis of triglycerides and partial glycerides because they possess high specificity and high activity at relatively low temperatures (1–5). Materials synthesized by lipase catalysis are therefore free of the by-products caused by high temperatures and inorganic catalysts, which are often used in chemical processes (6). Some lipases also have a unique selectivity not shown by chemical catalysts. Lipases that are 1,3-regioselective have been successfully used to synthesize structured lipids, such as cocoa butter substitute (1) and human milk-fat triglycerides (5).

A commercially-available structured lipid called caprenin has been shown to contain approximately half the calories of naturally occurring triglycerides due to incomplete absorption during digestion (7,8). This triglyceride, composed of one molecule of behenic acid ($C_{22:0}$) and two molecules of caprylic acid ($C_{8:0}$) or capric acid ($C_{10:0}$), may be synthesized by high-temperature reaction of monobehenin with the free acids (9) or reaction of monobehenin with a reactive form of the fatty acids, such as the anhydride, at lower temperatures (10). The object of the present work is to develop an enzyme-catalyzed procedure for the synthesis of caprenin that avoids high temperatures and expensive raw materials such as fatty acid anhydrides. Initially, caprucin was synthesized, a triglyceride that contains one molecule of erucic acid and two molecules of caprylic acid, which was subsequently converted to caprenin by hydrogenation of the erucic acid esters. With caprylic acid and monoerucin as starting materials, fatty acid-specific lipases were screened for ability to utilize caprylic acid but inability to hydrolyze ester bonds of glycerol with C_{22} fatty acids. In this way, transesterification of the C_{22} fatty acid should be minimized, thus eliminating by-product formation.

EXPERIMENTAL PROCEDURES

Materials. Lipase powders from *Geotrichum candidum* (GC-20) and *Pseudomonas cepacia* (PS-30) were obtained from Amano Pharmaceutical (Troy, VA). Lipase powder from *Candida rugosa* was a gift of Enzeco (Keyport, NY). The *bis*(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Regis Chemical Co. (Morton Grove, IL). Erucic acid (>95%) was purchased from Lancaster Synthesis (Wyndham, NH), and octanoic acid (caprylic acid, 99%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). The following gas chromatography standards were obtained from Sigma Chemical Co. (St. Louis, MO): monooctanoin, dioctanoin, trioctanoin, and dierucin; and the following from Aldrich Chemical Co.: octanoic acid and erucic acid.

Thin-layer chromatography (TLC) was done on 0.25-mm silica gel 60 plates purchased from Merck (Montreal, Canada). Nuclear magnetic resonance spectra (^1H and ^{13}C NMR) were obtained on a JEOLCO JNM-GX400 FT NMR spectrometer (Jeol, Peabody, MA) with deuteriochloroform as solvent and tetramethylsilane (TMS) as an internal standard. For ^{13}C NMR, only diagnostic signals are reported, and the signals are given as ppm downfield from TMS. Infrared (IR) spectra were obtained on a Perkin-Elmer 1310 spec-

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trophotometer (Norwalk, CT) with 1% solutions in carbon tetrachloride. All solvents were of high-performance liquid chromatography (HPLC) grade, and all reagents were purchased either from Aldrich Chemical Co. or from Lancaster Synthesis. HPLC was accomplished with a Spectra-Physics (Piscataway, NJ) Model 8800 ternary pump, an SP8480XR ultraviolet (W) detector, and a Supelco (Bellefonte, PA) 5μ silica gel column (4.6 mm i.d. × 25 cm) with solvents and flow rates as indicated.

Synthesis of 1-monoerucin, rac *1-erucoyl-3-capryloylglycerol,* rac *1-erucoyl-2-capryloylglycerol and* rac *1-erucoyl-2,3-dicapryloylglycerol.* Glycerol (13.8 g, 0.15 mmol) and acetone (22 mL, 0.30 mmol) were heated in benzene (100 mL), containing *p*-toluenesulfonic acid (0.15 g), under reflux to azeotropically remove water. After 4 h, water was no longer being removed from the reaction, erucic acid (16.9 g, 0.050 mmol) was added, and heating was continued for another 4 h. The reaction mixture was diluted with 50 mL ether, washed with aqueous $NaHCO_3$ and with water (2 × 100 mL), and dried over $MgSO_4$. The solvent was removed by flash evaporation, and then the acetonide group was cleaved by heating the crude erucoylated product in 2-methoxyethanol (80 mL) containing H_3BO_3 (30 g, 0.48 mmol) at 90–100°C for 2 h. The reaction product was diluted with water and extracted with ether. After the ethereal extract had been washed thoroughly with water, it was dried ($MgSO_4$) and concentrated. The crude monoerucin was crystallized under hexane (40 mL), filtered, and recrystallized from methanol (20 mL), yielding 1-monoerucin (5.4 g, 27%): m.p. = 48–49°C, TLC (4% ethyl acetate–chloroform) R_f 0.11; IR 3600b, 1740 cm^{-1}; ^1H NMR δ 5.34 (*bt*, 2H, HC=CH), 4.23 and 4.16 [ABX, 2H, J_{AB} = 11.6 Hz, J_{AX} = 5.0 Hz, J_{BX} = 5.6 Hz, $CH_2OC(=O)R$], 3.93 [*m*, 1H, $OCC(OH)H_XCO$], 3.69 and 3.62 [CDX, 2H, J_{CD} = 11.5 Hz, J_{CX} = 4.0 Hz, J_{DX} = 5.8 Hz, $CH_2OC(=O)R$], 2.35 (*t*, 2H, $CH_2C=O$), 2.01 (*m*, 4H, $CH_2C=CCH_2$), 1.63 (*m*, 2H, $CH_2CH_2C=O$), 1.27 (CH_2 env.), 0.88 (*bt*, 3H, CH_3) ppm; ^{13}C NMR δ 174.32 (C=O), 129.91 (C=C), 70.32, 65.18, 63.37 (C–O) ppm.

Monoerucin (1.03 g, 2.50 mmol) was dissolved in $CHCl_3$ (10 mL) that contained pyridine (0.65 mL, 8.0 mmol). A solution of capryloyl chloride (1.3 mL, 7.5 mmol) in $CHCl_3$ (5 mL) was added dropwise with ice bath cooling. The resulting mixture was stirred at ambient temperature for 3 d, and then worked up in the usual manner. The crude product was purified by flash chromatography (11) with 7% ethyl acetate–hexane and yielded *rac* 1-monoerucoyl-2,3-dicapryloylglycerol (1.47 g, 88.6%): TLC (solvent above) R_f 0.74; IR 1740 cm^{-1}; ^1H NMR δ 5.34 [*m*, 3H, HC=CH and $CH_XOC(=O)R$], 4.30 and 4.14 [A_2B_2X, 4H, J_{AB} = 12.0 Hz, J_{AX} = 4.3 Hz, J_{BX} = 5.9 Hz, $CH_2OC(=O)R$], 2.31 (*t*, 6H, $CH_2C=O$), 1.99 (*m*, 4H, $CH_2C=CCH_2$), 1.61 (*m*, 6H, $CH_2CH_2C=O$), 1.27 (CH_2 env.), 0.88 (*bt*, 9H, CH_3) ppm; ^{13}C NMR δ 173.22 and 172.82 (C=O), 129.87 (C=C), 68.92 and 62.10 (C-O) ppm.

Monoerucin (2.06 g, 5.0 mmol) was treated as above with pyridine (0.26 mL, 3.2 mmol) and capryloyl chloride (0.51 mL, 3.0 mmol) to produce a mixture of diglycerides. These were separated and purified by flash chromatography with 14% ethyl acetate–hexane while monitoring the eluant by TLC (4% acetone–$CHCl_3$). Obtained in this manner was *rac* 1-erucoyl-3-capryloylglycerol as an oil (1.7 g, 63%): HPLC (2% *iso*-propanol-hexane at 0.5 mL/min) R_t = 9.4 min; TLC (4% acetone–chloroform) R_f 0.43; IR 3600, 1740 cm^{-1}; ^1H NMR δ 5.34 (*bt*, 2H, HC=CH), 4.14 (*m*, 5H, CH_2O and CHO), 2.34 (*t*, 4H, $CH_2C=O$), 2.01 (*m*, 4H, $CH_2C=CCH_2$), 1.62 (*m*, 4H, $CH_2CH_2C=O$), 1.27 (CH_2 env.), 0.88 (*bt*, 6H, CH_3) ppm; ^{13}C NMR δ 173.88 (C=O), 129.88 (C=C), 68.39 and 65.03 (C–O) ppm; and *rac* 1-erucoyl-2-capryloylglycerol as an oil (0.4 g, 15%): R_t = 15.5 min; TLC (same solvent) R_f 0.26; IR 3600, 1740 cm^{-1}; ^1H NMR δ 5.35 (*bt*, 2H, HC=CH), 4.2 (*m*, 5H, CH_2O, CHO), 2.35 (*t*, 4H, $CH_2C=O$), 2.0 (*m*, 4H, $CH_2C=CCH_2$), 1.63 (*m*, 4H, $CH_2CH_2C=O$), 1.28 (CH_2 env.), 0.88 (*bt*, 6H, CH_3) ppm; ^{13}C NMR (sample contained the 1,3-diglyceride) δ 173.91 (C=O's not distinguishable), 129.91 (C=C), 72.16, 62.04, 61.60 (C–O) ppm.

Hydrogenation of caprucin to caprenin. Caprucin (1.0 g) was dissolved in absolute ethanol (10 mL) and swirled briefly with 0.1 g of PtO_2. The mixture was filtered, a fresh portion of catalyst was added, and the mixture was then hydrogenated at *ca.* 20 lb pressure for 0.5 h. The mixture was diluted with water and extracted with ether. The ethereal layer was dried ($MgSO_4$) and concentrated to produce caprenin in quantitative yield: m.p. = 30–31°C.

Lipase-catalyzed esterification of monoerucin with caprylic acid. One g of monoerucin, prepared as described above, was mixed with 0.7 g caprylic acid in a flat-bottom glass tube, 3 × 5 cm, resulting in a mole ratio of caprylic acid/monoerucin of 2:1. The tube was placed in a glass mantle through which water was circulated from a constant-temperature water bath. A small quantity of distilled water was added to the reactants, typically 100 μL, which were then mixed for 3 min. Lipase powder, 50 mg, was then added to start the reaction. The reaction mixture was agitated by magnetic stirring at 600 rpm throughout the reaction.

Analysis of the reaction mixture. During the course of the reaction, samples of approximately 5 mg were removed from the reaction mixture and mixed with 50 μL dry pyridine and 100 μL BSTFA. Partial glycerides and the free fatty acids were converted to their TMS derivatives by heating the sample for 15 min at 100°C. After dilution with 5 mL hexane, the samples were analyzed by gas chromatography as follows: a 15-m long, nonpolar high-temperature capillary column, i.d. 0.32 mm, film thickness 0.1 micron, was used (DB1-HT; J&W Scientific, Folsom, CA). An aliquot of 0.5 μL of derivatized sample was injected directly on-column in a Hewlett-Packard 5610 gas chromatograph (Palo Alto, CA); helium carrier gas flow rate was 5.5 mL/min, and flame-ionization detection with an initial oven temperature of 70°C was followed by a temperature program of 20°C/min to a final temperature of 350°C, which was held for 3 min. Peaks in the chromatograms were identified by comparing retention times to those of standards, purchased commercially (see the Experimental Procedures

section) or synthesized as described above (monoerucin, 1-erucoyl-2-octyl-glycerol, 1-erucoyl-3-octyl-glycerol, 1-erucoyl-2,3-dioctyl-glycerol, and 1-behenoyl-2,3-dioctyl-glycerol). Other peaks were identified by inference. Relative response factors were calculated for the major components based on seven injections of a standard mixture and were used to convert the integrator response to wt%: erucic acid = 0.69, monoerucin = 0.71, 1-erucoyl-3-octyl-glycerol = 0.91, and 1-erucoyl-2,3-dioctyl-glycerol = 1.00.

RESULTS

Reaction at 35°C. The minimum temperature at which the reaction mixture was liquid was approximately 35°C. Initially, experiments were conducted at this temperature as described above with lipase from *G. candidum* in tightly-stoppered reaction vessels. Because the rate of synthesis of 1-erucoyl-2,3-dicapryloyl-glycerol (caprucin) was extremely slow, the reaction was then carried out under the same conditions but in an open vessel. The time course of the reaction for each system is compared in Figure 1. In the reaction in a tightly-stoppered vessel (Fig. 1A), approximately 80% of the monoerucin has been converted, compared to 97% in the unstoppered reaction (Fig. 1B) after 96 h. The diglyceride intermediate, erucoyl–octyl–glycerol, reaches a maximum of 65% in the stoppered vessel and 80% in the unstoppered vessel. The conversion of this diglyceride to caprucin occurs more slowly in a stoppered vessel, reaching a maximum of 22% by the end of the reaction period, while in the unstoppered vessel, the maximum of 54% is reached in 50 h. Because of the higher reaction rates and higher conversions, all subsequent reactions were carried out in unstoppered vessels.

Variation of initial water content. To optimize the reaction conditions further, the initial water concentration in the reaction mixture was varied from 3 to 10.5% of the total weight of the reaction mixture. Table 1 shows the concentration of caprucin in the reaction mixture after 48 h and the initial rates of consumption of monoerucin and production of erucoyl–octyl–glycerol and caprucin. Unstoppered reaction vessels and *G. candidum* lipase at 35°C were used. A maximum in both the rate of caprucin synthesis and the concentration of caprucin after 48 h was observed at an initial water content of 5.5%. At initial water contents of 4.5% or lower, almost no synthesis of caprucin occurred. Although the rate of caprucin synthesis at 5.5% initial water content was three times higher than at 5.0 or 8.0% water content, a high concentration of caprucin was detected in these samples after 48 h reaction (43 and 49% caprucin, respectively). At all initial water contents,

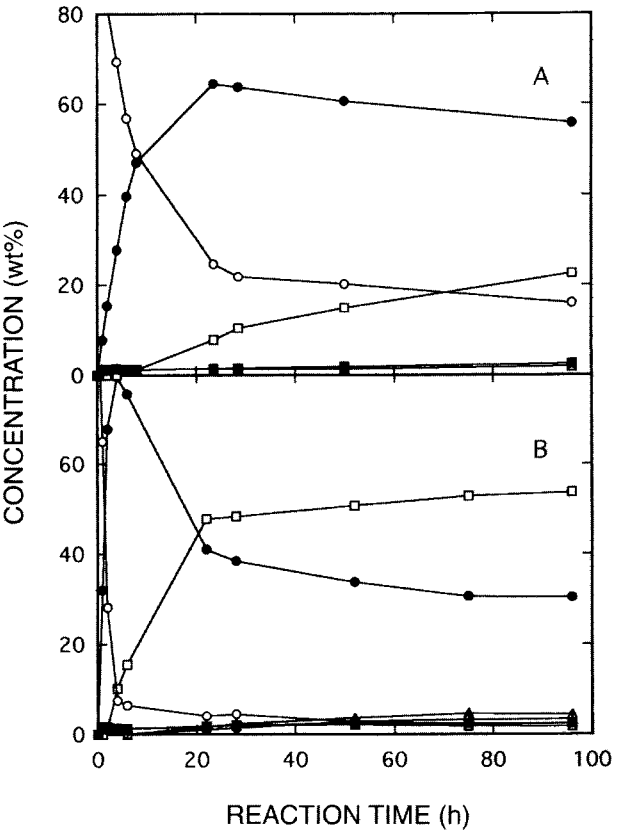

FIG. 1. Composition of the reaction mixture during esterification of monoerucin with caprylic acid at 35°C and *Geotrichum candidum* lipase as catalyst: A = reaction carried out in stoppered vessel, B = reaction in unstoppered vessel. Legend: ○, monoerucin; ●, erucoyl–capryloyl–glycerol; □, erucoyl–dicapryloyl–glycerol (caprucin); ■, erucic acid; △, dierucin; ▲, dierucoyl–capryloyl–glycerol; ▽, monocaprylin; ▼, dicaprylin; ⊕, tricaprylin.

TABLE 1
Effect of Initial Water Content on Initial Rates of Conversion of Monoerucin and Production of Erucoyl–Octyl–Glycerol and Caprucin (wt%/h) Catalyzed by *Geotrichum candidum* Lipase (the concentration of caprucin after 48 h reaction is also shown)

Initial water content (wt%)	Initial rate (wt%/h)			Caprucin content (wt% after 48 h)
	Monoerucin	Erucoyl–octyl–glycerol	Caprucin	
3.0	0.3	0.3	0.0	0.0
3.5	0.8	0.6	0.0	0.0
4.0	1.5	1.6	0.0	0.0
4.5	2.0	2.0	0.0	1.0
5.0	18.9	15.9	0.9	48.9
5.5	35.0	32.0	3.5	53.6
8.0	22.3	22.4	1.1	42.6
10.5	16.4	16.7	0.7	38.7

production of erucoyl–octyl–glycerol could be detected. The rate of production increased with increasing water content up to 5.5% and thereafter decreased again. A dramatic increase occurred between 4.5 and 5.0% initial water, with relatively small changes at higher water levels. The rate of decrease in monoerucin concentration paralleled the changes observed with erucoyl–octyl–glycerol.

Effect of reaction temperature. Synthesis of caprucin with *G. candidum* lipase in unstoppered reaction vessels was carried out under the conditions described above (5.5% initial water content) but at 35, 50, and 60°C. At 60°C, the concentration of caprucin in the reaction mixture only reached 14% after 48 h reaction time (data not shown). This result is similar to that obtained at 35°C in a stoppered reaction vessel (Fig. 1A). As shown in Figure 2, a reaction temperature of 50°C resulted in a faster and more complete reaction than obtained at 35°C (Fig. 1B). A concentration of 75% caprucin was reached, the monoerucin concentration was less than 0.5%, and the concentration of erucoyl–octyl–glycerol was reduced to approximately 10% after reaching a peak of 80%. Figure 2 also shows that, under these reaction conditions, the concentrations of dierucoyl-octyl-glycerol and trioctanoin also increase, with the combined concentration of these and other minor components reaching approximately 15% after 100 h.

Effect of octanoic acid concentration. Optimum conditions for reaction vessel (unstoppered), initial water content (5.5%), and reaction temperature (50°C) were used in the investigation of the effect of octanoic acid concentration on the *G. candidum* lipase-catalyzed synthesis of caprucin. The mole ratio of octanoic acid-to-monoerucin at the beginning of the reaction was varied from 1:1 to 6:1. As shown in Table 2, the initial rate of synthesis of caprucin is highest at a mole ratio of 2:1. At mole ratios higher than 2:1, the rate of synthesis is lower, but the concentration of caprucin after 120 h approaches that obtained at 2:1. A mole ratio less than 2:1 results in both a lower rate of synthesis and a low caprucin concentration after 120 h reaction.

Fatty acid selectivity. Saturated fatty acids with even carbon numbers ranging from C_6 to C_{14} were reacted singly with monoerucin at a fatty acid/monoerucin mole ratio of 2:1 under the optimized conditions for octanoic acid as described above. All reaction mixtures were liquid under those conditions. Figure 3 shows the relative initial rates of conversion

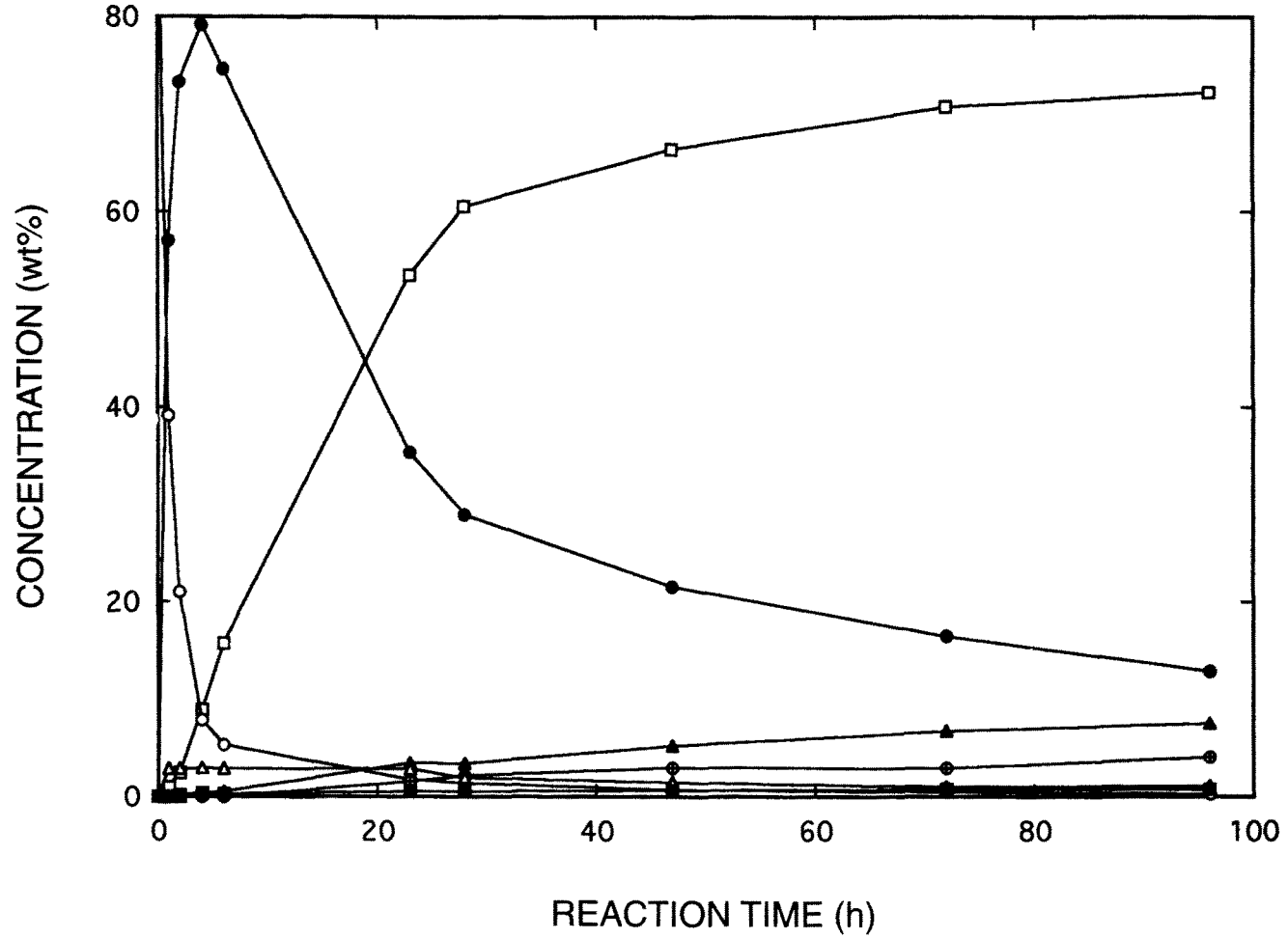

FIG. 2. Time course of the reaction between caprylic acid and monoerucin at 50°C with *Geotrichum candidum* lipase as catalyst. Composition of the reaction mixture. Legend as in Figure 1.

TABLE 2
Effect of Initial Octanoic Acid Concentration on Initial Rates of Conversion of Monoerucin and Production of Erucoyl–Octyl–Glycerol and Caprucin (wt%/h) Catalyzed by *Geotrichum candidum* Lipase (the concentration of caprucin after 120 h reaction is also shown)

Mole ratio C_8/monoerucin	Initial rate (wt%/h)			Caprucin content (wt% after 120 h)
	Monoerucin	Erucoyl–octyl–glycerol	Caprucin	
1.0:1	12.0	12.2	0.0	1.8
1.5:1	32.0	32.1	0.3	12.6
2.0:1	60.9	57.0	3.3	75.3
3.0:1	42.7	39.2	2.1	75.6
4.0:1	33.7	29.9	1.4	69.4
6.0:1	18.8	15.6	0.7	46.0

of monoerucin for each acid, with the rate for octanoic acid set to 1.0. Clearly, *G. candidum* lipase is highly selective for octanoic acid and utilized the other fatty acids between 10 and 30 times more slowly.

Comparison of lipases. Lipase from *C. rugosa* and *P. cepacia* were used to catalyze the reaction between octanoic acid and monoerucin (mole ratio 2:1) under the optimum conditions devised for *G. candidum* lipase. *Candida rugosa* lipase showed little activity at 50°C, and it was necessary to carry out this reaction at 35°C. The time course of the reaction is shown in Figure 4A and is similar to the time course obtained at 35°C with *G. candidum* lipase (Fig. 1A). However, with *C. rugosa* lipase, a lower final concentration of caprucin was obtained (45%), although conversion of both monoerucin and erucoyl–octyl–glycerol was approximately the same. This difference can be explained by the appearance of 10% erucic acid and 13.5% C_8 glycerides in the reaction mixture. Lipase from *P. cepacia* was active at 50°C, and the reaction time course is shown in Figure 4B. In contrast to lipases from both *G. candidum* and *C. rugosa*, a complex mixture of products was formed. Clearly, the erucic acid was transesterified during the reaction, resulting in a high concentration of dierucoyl–octyl–glycerol (23%) and trioctyl–glycerol (14%). The random mixture of products formed is consistent with the known nonspecific nature of *P. cepacia* lipase.

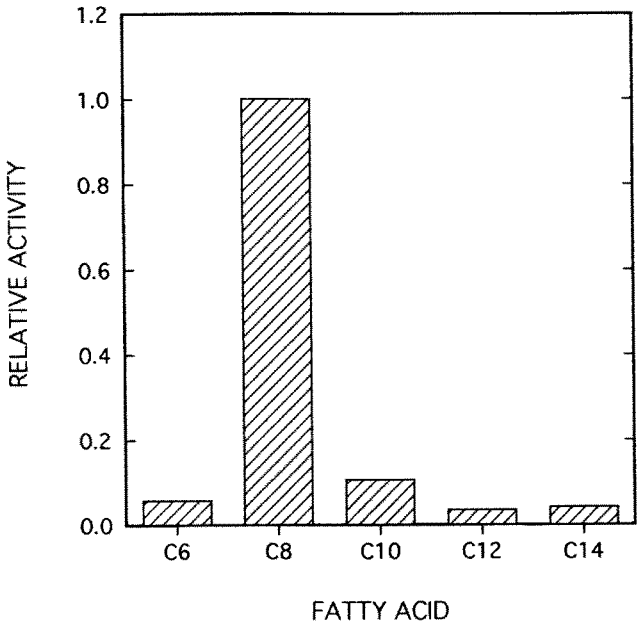

FIG. 3. Initial rates of conversion of monoerucin during *Geotrichum candidum* lipase-catalyzed reaction of monoerucin with saturated fatty acids of differing chainlength. Results are expressed relative to C_8 (value set to 1).

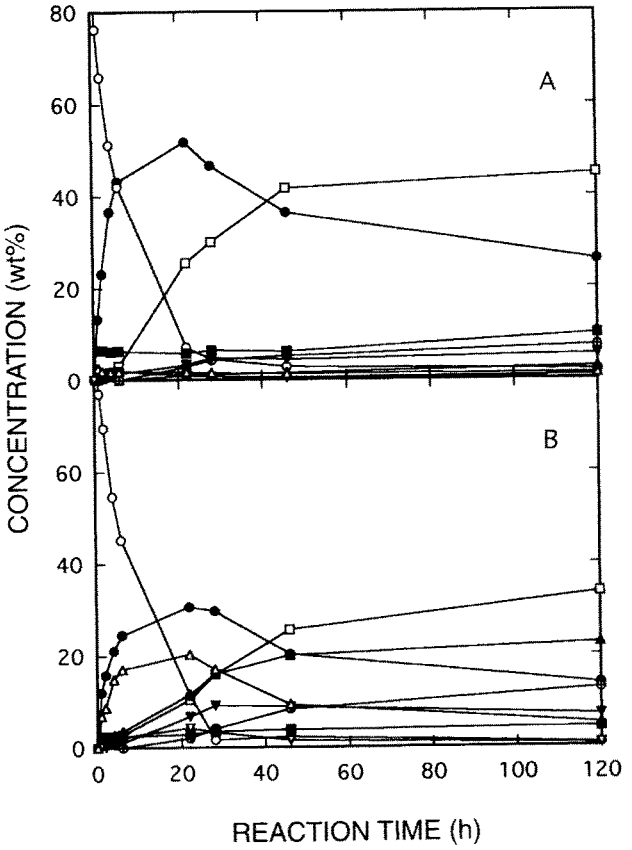

FIG. 4. Comparison of lipases as catalysts for the esterification of monoerucin with caprylic acid. A, *Candida rugosa* lipase at 35°C; B, *Pseudomonas cepacia* lipase at 50°C. Legend as in Figure 1.

Hydrogenation. After reacting monoerucin with octanoic acid for 120 h (50°C), with *G. candidum* lipase as catalyst, a sample was removed and hydrogenated as described in the Experimental Procedures section. Hydrogenation converted the reaction mixture from a liquid at room temperature to a waxy, white solid. After hydrogenation, the retention time of glycerides that previously contained erucic acid, eluted approximately 6 s later on gas chromatograms. Glycerides that contained only octanoic acid had identical retention times before and after hydrogenation. The retention time of the shifted caprucin peak was identical to the retention time of chemically synthesized caprenin (described above). This indicated that the erucic acid had been converted to behenic acid. The concentrations of caprucin before hydrogenation and of caprenin after hydrogenation were almost identical (approximately 75%).

DISCUSSION

The lipase from *G. candidum* has a well-known fatty acid specificity (12) and hydrolyzes esters that contain fatty acids with a double bond at the Δ9 position more rapidly than other fatty acids. More detailed studies showed that this lipase also catalyzes hydrolysis of trioctanoin more readily than monoacid triglycerides that contain saturated fatty acids of other chainlengths (13). Another study demonstrated that fatty acids with chainlengths longer than 18 carbons were poor substrates for *G. candidum* lipase, regardless of the position of the double bond (14).

In the present study, this lipase was applied successfully as a catalyst for the synthesis of caprucin (monoerucoyl–dicapryloyl–glycerol), which was quantitatively converted to caprenin by hydrogenation of the double bond in the erucic acid ester. The lipase possessed high selectivity for caprylic acid and against erucic acid. As a result, caprylic acid was esterified to monoerucin with little synthesis of transesterification products. These products were either di- or triglycerides that contained either no erucic acid or two molecules of erucic acid due to the transfer of erucic acid to a glyceride that already contained erucic acid. Although reactivity of fatty acids other than C_8 was low, the reaction conditions used in this study were optimized for C_8. It may be possible to increase the reactivity of other fatty acids by specifically optimizing the conditions for these acids.

It is well known that the water content of the reaction medium is critical for the activity of the lipase and the equilibrium position of the reaction when enzymes are used to catalyze glyceride synthesis or transesterification (4,15). This also was found in the present work when a sharp maximum in activity was observed at 5.5% water content. Also, the reaction rate was significantly higher when the reaction vessel was open throughout the reaction. A similar observation was made by Ergan and co-workers (2) during the synthesis of triolein from fatty acids and glycerol. These workers suggested that water, which was produced by ester bond formation, was removed through evaporation, thus changing the reaction equilibrium toward ester synthesis.

Increasing the reaction temperature up to 50°C increased both reaction rate and yield of caprucin in the case of *G. candidum* lipase. Because higher temperatures resulted in inactivation of the enzyme, further improvements in caprucin yield that are based on temperature must be carried out with enzymes stabilized by immobilization. Due to the poor temperature stability of the lipase, esterification of monobehenin with caprylic acid could not be carried out because of the high melting point of monobehenin (85°C).

Two other lipases were evaluated in the present investigation. Lipase from *C. rugosa* is partially selective against erucic acid but not against shorter-chain fatty acids (14,16). When used as a catalyst for the synthesis of caprucin in the work described here, erucic acid was not transesterified extensively, but the content of transesterification products was approximately two times higher than obtained with *G. candidum* lipase at the same yield of caprucin. Also, a high conversion of substrates was not possible because the reaction was carried out at 35°C, due to instability of the lipase at 50°C (17). Optimization of the reaction conditions specifically for *C. rugosa* lipase may result in a higher yield of caprucin.

Pseudomonas cepacia lipase was used for comparative purposes as a catalyst for caprucin synthesis because it is known to be a nonspecific lipase. The results indicate that extensive transesterification of erucic acid had occurred, giving a complex mixture of mono-, di-, and triglycerides, which is not useful for the synthesis of caprenin. A similar complex mixture is obtained when interesterification of tribehenin and trioctanoin is carried out with a chemical catalyst such as sodium methoxide (18). The use of fatty acid-specific lipases for the synthesis of caprenin is clearly superior to nonspecific lipases or chemical catalysts and provides a practical route to the synthesis of structured lipids such as caprenin.

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