Production of Structured TAG Rich in 1,3-Dicapryloyl-2-γ-linolenoyl Glycerol from Borage Oil

Akiko Kawashima[a], Yuji Shimada[b,*], Toshihiro Nagao[b], Akihiro Ohara[c], Tsugio Matsuhisa[c], Akio Sugihara[b], and Yoshio Tominaga[b]

[a]Sonoda Women's University, Hyogo 661-8520, Japan, [b]Osaka Municipal Technical Research Institute, Osaka 536-8553, Japan, and [c]Meijo University, Faculty of Agriculture, Aichi 468-8502, Japan

ABSTRACT: γ-Linolenic acid (GLA) has the physiological functions of modulating immune and inflammatory responses. We produced structured TAG rich in 1,3-dicapryloyl-2-γ-linolenoyl glycerol (CGC) from GLA-rich oil (GLA45 oil; GLA content, 45.4 wt%), which was prepared by hydrolysis of borage oil with *Candida rugosa* lipase having weak activity on GLA. A mixture of GLA45 oil/caprylic acid (CA) (1:2, w/w) was continuously fed into a fixed-bed bioreactor (18 × 180 mm) packed with 15 g immobilized *Rhizopus oryzae* lipase at 30°C and a flow rate of 4 g/h. The acidolysis proceeded efficiently, and a significant decrease of lipase activity was not observed in full-time operation for 1 mon. GLA45 oil contained 10.2 mol% MAG and 27.2 mol% DAG. However, the reaction converted the partial acylglycerols to structured TAG and tricaprylin and produced 44.5 mol% CGC based on the content of total acylglycerols. Not only FFA in the reaction mixture but also part of the tricaprylin and partial acylglycerols were removed by molecular distillation. The distillation resulted in an increase of the CGC content in the purified product to 52.6 mol%. The results showed that CGC-rich structured TAG can efficiently be produced by a two-step process comprising selective hydrolysis of borage oil using *C. rugosa* lipase (first step) and acidolysis of the resulting GLA-rich oil with CA using immobilized *R. oryzae* lipase (second step).

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KEY WORDS: Acidolysis, borage oil, immobilized lipase, γ-linolenic acid, *Rhizopus oryzae*, selective hydrolysis, structured triacylglycerol.

γ-Linolenic acid (18:3n-6; GLA) is biosynthesized from linoleic acid (18:2n-6; LnA) by Δ6 desaturase, which is the rate-limiting enzyme in the essential FA cascade (1,2) and is an intermediate precursor of local hormones (prostaglandins, thromboxanes, and leukotrienes) (3). The FA has the physiological functions of modulating immune and inflammatory responses (4) and is effective for treating atopic eczema (5,6) and rheumatoid arthritis (7,8). Thus, GLA-containing oil, especially borage oil, is used as a health food and an ingredient in infant formulas (9).

Recently, structured TAG with medium-chain FA at the 1,3-positions and long-chain FA at the 2-position (MLM-type) were reported to be absorbed extensively into intestinal mucosa (10,11). Therefore, structured TAG containing functional FA are expected to act as nutrients for patients with poor digestion and malabsorption of lipids and as health foods for the elderly. Structured TAG can be produced by exchanging FA in natural oils and fats with medium-chain FA using an immobilized 1,3-specific lipase (12–15).

We reported that MLM-type structured TAG containing GLA were produced by acidolysis of borage oil with caprylic acid (8:0, CA) using immobilized *Rhizopus oryzae* (the former name of *R. delemar*) lipase (16). However, because borage oil consisted of TAG esterified with not only GLA but also LnA and oleic acid (18:1n-9; OA) at the 2-position, the content of 1,3- dicapryloyl-2-γ-linolenoyl glycerol (CGC) in the acidolysis product could not exceed 30 mol%. If TAG with LnA and OA at the 2-position can be eliminated from borage oil, the CGC content in the product should increase. It is known that lipases have TAG specificity (17); thus, selective hydrolysis of borage oil with a lipase acting weakly on GLA should result in enrichment of GLA in the undigested acylglycerols and removal of TAG lacking GLA. This paper shows that GLA-rich oil obtained by selective hydrolysis of borage oil with *Candida rugosa* lipase is an effective substrate for producing structured TAG rich in CGC, and that partial acylglycerols present in the substrate oil only slightly affect the TAG composition in the product.

MATERIALS AND METHODS

Oils. Borage oil was a gift from Nippon Supplement Inc. (Osaka, Japan). GLA-rich oil was prepared according to a previous paper (18). In brief, borage oil (7 kg; GLA content, 22.5 wt%) was hydrolyzed at 30°C for 15 h with 20 units (U)/g of *C. rugosa* lipase in the presence of 50% water using a 30-L reactor (Mitsuwa Co. Ltd., Tokyo, Japan). To remove FFA from the reaction mixture, the oil layer was subjected to molecular distillation at 0.2 mm Hg and 170°C, but the acid value of the residue was still high, 38 mg KOH/g. Hence, the second cycle of distillation was conducted at 0.05 mm Hg and 190°C, and 2.5 kg residue (acid value, 6 mg KOH/g) was obtained; the GLA content was 44.7 wt% and its recovery was 69.9% of its initial content. The resulting GLA-rich oil was referred to as GLA45 oil. GLA45 oil consisted of 3.0 wt% FFA, 4.3 wt% MAG, 21.2 wt% DAG, and 71.5 wt% TAG. To

*To whom correspondence should be addressed at Osaka Municipal Technical Research Institute, 1-6-50 Morinomiya, Joto-ku, Osaka 536-8553, Japan. E-mail: shimaday@omtri.city.osaka.jp

TABLE 1
FA Compositions at 1,3- and 2-Positions of Borage Oil and GLA45 TAG

	FA composition (mol%)							
Oil	16:0	18:0	18:1	18:2	18:3n-6	20:1	22:1	24:1
Borage oil								
Total	11.3	3.9	16.0	36.0	22.5	3.7	2.3	1.4
1,3-Position	16.6	5.8	17.8	36.6	9.3	5.5	3.3	2.1
2-Position	0.6	ND	12.3	34.8	48.9	ND	0.3	ND
GLA45 oil[a]								
Total	6.4	3.3	10.1	22.7	45.4	5.2	3.0	1.7
GLA45-TAG[a]								
Total	6.1	3.7	10.9	22.6	45.1	5.3	3.1	1.7
1,3-Position	9.1	5.4	15.1	28.5	25.3	7.7	4.5	2.4
2-Position	ND	0.3	2.4	10.8	84.8	0.5	0.3	0.2

[a]Because GLA45 oil contained 37.4 mol% partial acylglycerols, TAG purified from GLA45 oil (GLA45-TAG) were analyzed. For preparation of GLA45-oil (oil containing 45% γ-linolenic acid), see text. ND, not detected.

remove partial acylglycerols in GLA45 oil, 20 g of the oil was subjected to a silica gel 60 column (120 g; 30 × 390 mm; Merck, Darmstadt, Germany), and TAG were eluted with a mixture of *n*-hexane/ethyl acetate (98:2, vol/vol) (recovery, 94.7%). The resulting oil was named GLA45-TAG (GLA content, 44.4 wt%). Table 1 shows FA compositions of borage oil, GLA45 oil, and GLA45-TGA, and the compositions at the 1,3- and 2-positions of borage oil and GLA45-TAG, determined by regiospecific analysis as described below. CA (purity, 98%) was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). The other chemicals were of analytical grade.

Lipases. *Candida rugosa* lipase (Lipase-OF) and *R. oryzae* lipase (Ta-lipase) were donated by Meito Sangyo Co. (Aichi, Japan) and Tanabe Seiyaku Co. Ltd. (Osaka, Japan), respectively. Lipase activity was measured by titrating FA liberated from olive oil (Wako Pure Chemical Ind. Co., Osaka, Japan) with 50 mM KOH as described previously (19): 1 U of lipase activity was defined as the amount of enzyme that liberated 1 μmol FA per minute. Hydrolysis activities of *C. rugosa* and *R. oryzae* lipases on borage oil were 59 and 103% of those on olive oil, respectively.

Immobilization of *R. oryzae* lipase was performed as described previously (20). In brief, after 50 g Dowex WBA (Dow Chemical Co., Midland, MI) was suspended in 40 mL of *R. oryzae* lipase solution (125 mg/mL; 6100 U/mL); immobilized lipase was prepared by drying under reduced pressure. The lipase preparation was activated by incubation in a substrate mixture containing a small amount of water (21). The pretreatment was performed as follows. In batch reaction, a mixture of oil/CA (1:2, w/w), 2% water, and 5% immobilized lipase by weight of the reaction mixture was incubated at 30°C for 48 h with shaking at 130 oscillations/min. The subsequent reactions were conducted by transferring the immobilized lipase into the same amount of substrate mixture without addition of water, followed by shaking under the same conditions as those for the pretreatment. In continuous flow reaction, a 200-mL mixture of oil/CA (1:2, w/w) saturated with water (water content, 1.15%) was fed into a fixed-bed bioreactor (18 × 180 mm) packed with 15 g immobilized lipase, and a 150-mL mixture of oil/CA without addition of water (water content, <500 ppm) was then fed into the reactor.

Reactions. Batch acidolyses of borage oil, GLA45 oil, and GLA45-TGA were conducted with two weight parts (12.6–14.3 mol parts) of CA using 5% immobilized *R. oryzae* lipase by weight of reaction mixture. The reactions were carried out at 30°C in a 7- or 50-mL screw-capped vessel with shaking at 130 oscillations/min. Repeated acidolysis was performed as follows. Acylglycerols were first extracted from the reaction mixture with *n*-hexane according to a previously reported procedure (20,22): They were extracted with 100 mL *n*-hexane after adding 70 mL of 0.5 N KOH (20% ethanol solution) to 4 to 8 g of reaction mixture. The resulting acylglycerols were then subjected to acidolysis with two weight parts of CA under conditions similar to those in the first reaction.

Continuous flow reaction was performed in a fixed-bed bioreactor packed with 15 g immobilized *R. oryzae* lipase that had been activated as described previously. The substrate mixture of GLA45 oil/CA (1:2, w/w) was continuously fed into the reactor by a peristaltic pump at 30°C and a flow rate of 4.0 g (4.5 mL)/h.

Analysis. FA in acylglycerols were methylated in methanol with sodium methylate as a methylating reagent. These methyl esters were analyzed with a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) connected to a DB-23 capillary column (0.25 mm × 30 m; J&W Scientific, Folsom, CA) as described previously (20). The column temperature was raised from 150 to 210°C at 2°C/min, and the temperatures of the injector and detector were set at 250°C.

The contents of MAG, DAG, TAG, and FFA were measured with a TLC/FID analyzer (Iatroscan MK-5; Iatron Laboratories Inc., Tokyo, Japan) after developing with a mixture of *n*-hexane/ethyl acetate/acetic acid (90:10:1, by vol).

TAG compositions were analyzed by GC and HPLC. The GC analysis was performed with a DB-1ht capillary column (0.25 mm × 5 m; J&W Scientific). The column temperature was raised from 120 to 270°C at 25°C/min, and from 270 to 360°C at 4°C/min. The temperatures of injector and detector

were set at 390°C. The carrier gas was helium at a flow rate of 0.88 mL/min. The HPLC analysis was performed with an octadecyl silica column (4.6 × 250 mm, Cosmosil 5C18-AR; Nacalai Tesque Inc., Kyoto, Japan). The mobile phase of acetone/acetonitrile (1:1, vol/vol) was used at a flow rate of 0.4 mL/min and 40°C, and the peaks of TAG were detected with a refractometer. The positional isomers of dicapryloyl-γ-linolenoyl glycerol were analyzed on a Chrompack silver ion chromatography column (4.6 × 250 mm; Chrompack, Middelberg, The Netherlands) as described by Irimescu *et al.* (23).

Regiospecific analysis of TAG was conducted by Grignard degradation with allyl magnesium bromide (24), followed by isolation and analysis of the 1,3-DAG fraction. The 1,3-DAG were isolated by TLC with a silica gel 60 plate (Merck), which was developed with a mixture of chloroform/acetone/acetic acid (94:4:1, by vol). FA located at the 2-position were calculated from the FA compositions of TAG and 1,3-DAG.

Water contents of the substrate and reaction mixture were determined by Karl Fischer titration (Moisture Meters CA-07; Mitsubishi Chemical Corp., Tokyo, Japan).

Molecular distillation. The reaction mixture obtained by the acidolysis of GLA45 oil was dehydrated at 70°C and 3 mm Hg for 30 min before applying molecular distillation. The water content was reduced to <100 ppm by the dehydration step. Removal of FFA was performed with a molecular distillation apparatus, Wiprene type 2-03 (Shinko Pantec Co. Ltd., Hyogo, Japan), by three-step distillation: at 130°C and 0.2 mm Hg, at 190°C and 0.05 mm Hg, and at 210°C and 0.05 mm Hg.

RESULTS

Acidolysis of GLA45 oil with CA. The reaction conditions were the same as those determined in the acidolysis of borage oil (16): A mixture of GLA45 oil/CA (1:2, w/w) and 5 wt% immobilized 1,3-specific *R. oryzae* lipase was shaken at 30°C. Figure 1 shows a typical time course of the main FA contents in acylglycerols. The contents of palmitic acid (PA; 16:0), OA, and LnA decreased rapidly and reached constant values after 20–30 h. Meanwhile, the GLA content decreased gradually, and the decrease continued even after 30 h. These facts indicated that the lipase acted strongly on PA, OA, and LnA and weakly on GLA. In addition, the CA content increased with decreasing contents of constituent FA, showing that the acidolysis proceeded efficiently.

Although the contents of the other constituent FA, saturated FA (18:0; initial content, 3.3 mol%) and monoenoic FA (20:1, 5.2 mol%; 22:1, 3.0 mol%; 24:1, 1.7 mol%) were not plotted in Figure 1, their contents were <1.0 mol% after 45 h of reaction. The result showed that the saturated and monoenoic FA were good substrates of *R. oryzae* lipase and were esterified at the 1,3-positions of TAG. The results agreed with the regiospecific localization of constituent FA of GLA45-TAG (Table 1).

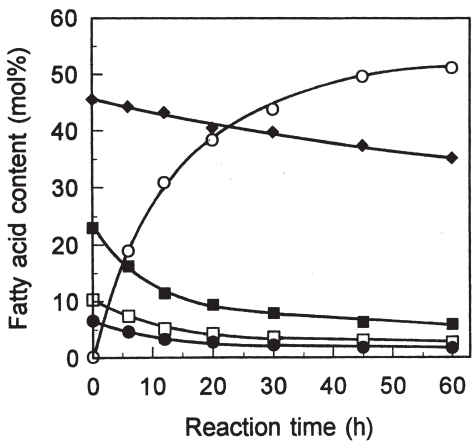


FIG. 1. Acidolysis of GLA45 oil with caprylic acid (CA) using immobilized *Rhizopus oryzae* lipase. A mixture of 12 g GLA45 oil, 24 g CA, and 1.8 g immobilized lipase was shaken at 30°C. An aliquot of reaction mixture (*ca.* 1 g) was periodically withdrawn to analyze the FA composition in acylglycerols. ○, CA; ●, palmitic acid; □, oleic acid (OA); ■, linoleic acid (LnA); ◆, γ-linolenic acid (GLA). For preparation of GLA45 oil (oil containing 45% GLA), see text.

Determination of TAG composition. TAG species are represented by three capital letters: e.g., CGO means TAG with CA and OA at the 1,3-positions and with GLA at the 2-position, and the 1,3-positions are not distinguished. The capital letters show the following FA; C, CA; O, OA; L, LnA; G, GLA. CXX shows CLL, CLO, COO, or their mixture. XXX indicates TAG not containing CA.

A gas chromatograph equipped with a DB-1ht capillary column can analyze TAG species with different M.W. An acylglycerol product obtained by single acidolysis of GLA45 oil with CA was subjected to GC analysis. As shown in Figure 2A, almost all TAG were separated, but CLC and COC were not completely separated. Meanwhile, TAG species can also be separated by HPLC equipped with an ODS column. The acylglycerol product was analyzed by HPLC (Fig. 2B). The peaks of CLC and COC were separated, although COC was eluted together with CGG. The COC content is thus calculated by subtracting the CLC content (HPLC analysis) from the total contents of CLC and COC (GC analysis). The COC content agreed with that calculated from the difference between the total contents of CGG and COC (HPLC analysis) and the CGG content (GC analysis). Based on these results, we analyzed TAG composition mainly by GC analysis and determined the contents of CLC and COC by GC and HPLC analyses.

Repeated acidolyses of borage oil, GLA45 oil, and GLA45-TAG. GLA45 oil contained 10.2 mol% MAG and 27.2 mol% DAG based on the content of total acylglycerols. We studied the effect of these partial acylglycerols on the composition of structured TAG obtained by acidolysis of GLA45 oil with CA. Not all of the FA at the 1,3-positions of TAG were exchanged with CA by a single acidolysis (Fig. 1). Hence, the acidolysis degree was increased by repeated acidolysis.

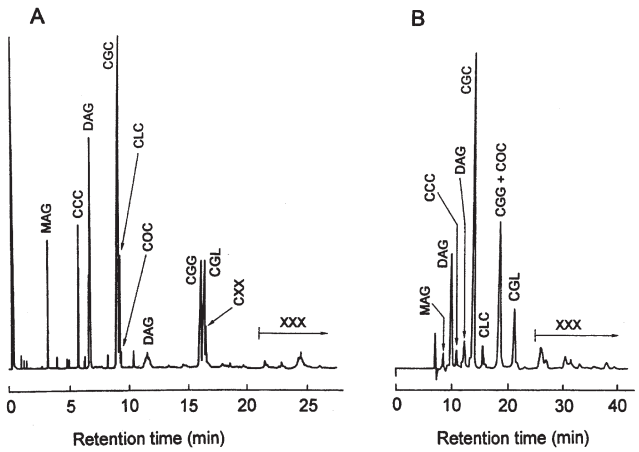


FIG. 2. GC and HPLC of acylglycerols obtained by single acidolysis of GLA45 oil with CA using immobilized *R. oryzae* lipase. (A) Chromatogram of GC analysis on DB-1ht capillary column (0.25 mm × 5 m; J&W Scientific, Folsom, CA). (B) Chromatogram of HPLC analysis on Cosmosil 5C18-AR (4.6 × 250 mm; Nakalai Tesque Inc., Kyoto, Japan). Acylglycerols were eluted at 40°C and 0.4 mL/min with a mixture of acetone/acetonitrile (1:1, vol/vol). TAG are expressed by three capital letters: C, CA; O, OA; L, LnA; G, GLA. CXX shows CLL, CLO, COO, or their mixture. XXX indicates TAG not containing CA. See Figure 1 for other abbreviations.

Acidolyses of borage oil, GLA45 oil, and GLA45-TAG with CA were repeated as follows. A mixture of 4 g oil/CA (1:2, w/w) and 0.2 g immobilized *R. oryzae* lipase was shaken at 30°C for 48 h. The acylglycerols were extracted with *n*-hexane from the reaction mixture and were allowed to react again with two weight parts of CA under similar conditions. The acidolysis was repeated three times in total using the same immobilized lipase. Table 2 shows FA compositions of products obtained by repeated acidolysis. The CA contents in acylglycerol fractions were increased by repeating acidolysis of the three oils and reached approximately 60 mol% after three repetitions. If all FA at the 1,3-positions were exchanged with CA, the content would be 66.7 mol%. These results thus indicated that part of the GLA esterified at the 1,3-positions remained even after three repetitions because *R. oryzae* lipase acted on GLA weakly.

Table 3 shows the TAG composition in products obtained by repeated acidolysis. The CGC contents were increased by repeating the acidolysis. When acidolyses of borage oil, GLA45 oil, and GLA45-TAG were repeated three times, the CGC contents reached 34.7, 50.8, and 61.3 mol%, respectively. In the repeated acidolysis of the three oils, CGG remained even after three repetitions. It was confirmed from the result that *R. oryzae* lipase shows weak activity on GLA at the 1,3-positions.

The contents of CLC and COC after three acidolyses of borage oil were 30.3 and 11.9 mol%, respectively (Table 3). The contents of these structured TAG were significantly lower in acidolyses of GLA45 oil and GLA45-TAG, showing that TAG with LnA and OA at the 2-position of borage oil are efficiently eliminated by selective hydrolysis of borage oil with *C. rugosa* lipase.

GLA45 oil contained 10.2 mol% MAG and 27.2 mol% DAG (Table 3). Repeated acidolysis of the oil decreased the contents of partial acylglycerols and increased the CCC content (the possible conversion pathway is described in the Discussion section). Three acidolyses of GLA45 oil increased the CGC content to 70.5 mol% based on the content of total TAG except CCC. The content coincided well with CGC content in the product obtained by three acidolyses of GLA45-TAG, i.e., 68.6 mol%. In addition, the contents of the other struc-

TABLE 2
FA Compositions of Reaction Products Obtained by Acidolyses of Borage Oil, GLA45 Oil, and GLA45-TAG with Caprylic Acid (CA)[a]

Substrate / Treatment	FA composition (mol%)								
	8:0	16:0	18:0	18:1	18:2	18:3n-6	20:1	22:1	24:1
Borage oil									
Original	—	11.3	3.9	16.0	36.0	22.5	3.7	2.3	1.4
First	48.1	2.1	0.7	7.2	17.5	21.9	0.8	0.5	0.3
Second	58.3	0.6	0.2	5.5	13.9	20.0	0.3	0.1	0.1
Third	60.6	ND[b]	ND	5.3	13.7	20.0	ND	ND	ND
GLA45 oil									
Original	—	6.4	3.3	10.1	22.7	45.4	5.2	3.0	1.7
First	46.3	1.8	0.9	2.7	6.2	38.9	1.3	0.8	0.5
Second	58.3	0.9	0.5	1.5	3.4	33.4	0.7	0.4	0.2
Third	62.9	0.5	0.3	1.1	2.8	30.0	0.5	0.2	0.1
GLA45-TAG									
Original	—	6.1	3.7	10.9	22.6	45.1	5.3	3.1	1.7
First	41.9	2.0	1.0	3.1	6.8	41.9	1.5	0.9	0.5
Second	52.5	1.2	0.5	1.7	3.5	38.8	0.6	0.3	0.2
Third	58.1	0.7	0.3	1.2	2.7	35.6	0.4	0.1	ND

[a]Acidolyses of the three oils were performed with two weight parts of CA using 5 wt% immobilized *Rhizopus oryzae* lipase. After the reactions, acylglycerols were recovered with *n*-hexane and were then allowed to react again under similar conditions. The acidolysis was repeated three times in total.
[b]Not detected.

TABLE 3
Acylglycerol Compositions of Reaction Products Obtained by Acidolyses of Borage Oil, GLA45 Oil, and GLA45-TAG with CA[a]

Substrate Treatment	MAG	DAG	TAG composition (mol%)[b] CCC	CGC	CLC	COC	CGG	CGL	CXX
Borage oil									
First	ND	4.2	0.6	25.4	26.4	9.2	7.8	11.6	7.2
Second	ND	4.6	3.0	32.4	31.7	10.4	6.9	7.3	2.2
Third	ND	4.7	4.8	34.7	30.3	11.9	5.7	5.4	1.0
GLA45 oil									
Original	10.2	27.2	—	—	—	—	—	—	—
First	7.7	18.7	3.4	37.1	4.4	1.5	10.1	9.7	2.1
Second	4.8	10.7	10.2	47.8	5.6	1.7	9.0	5.3	0.8
Third	2.4	7.9	17.6	50.8	6.3	1.5	8.5	3.5	0.5
GLA45-TAG									
First	ND	4.1	0.5	44.6	4.2	1.4	20.9	12.4	2.9
Second	ND	5.3	2.5	58.7	6.2	1.4	16.8	6.5	1.3
Third	ND	5.2	5.5	61.3	7.4	1.8	13.4	4.2	0.6

[a]The products were the same as those employed in Table 1.
[b]TAG are expressed by three capital letters: C, CA; O, oleic acid; L, linoleic acid; G, γ-linolenic acid (GLA). CXX means CLL, CLO, COO or their mixture. See Figure 1 for other abbreviation.

tured TAG obtained by repeated acidolysis of GLA45 oil were almost the same as those obtained by the acidolysis of GLA45-TAG. These results showed that CGC-rich structured TAG can be produced efficiently by a two-step process: selective hydrolysis of borage oil using *C. rugosa* lipase (first step), and acidolysis of the resulting GLA-rich oil with CA using immobilized *R. oryzae* lipase (second step). Furthermore, partial acylglycerols in GLA-rich oil scarcely affected the increase in the CGC content, although the amount of a by-product, CCC, increased.

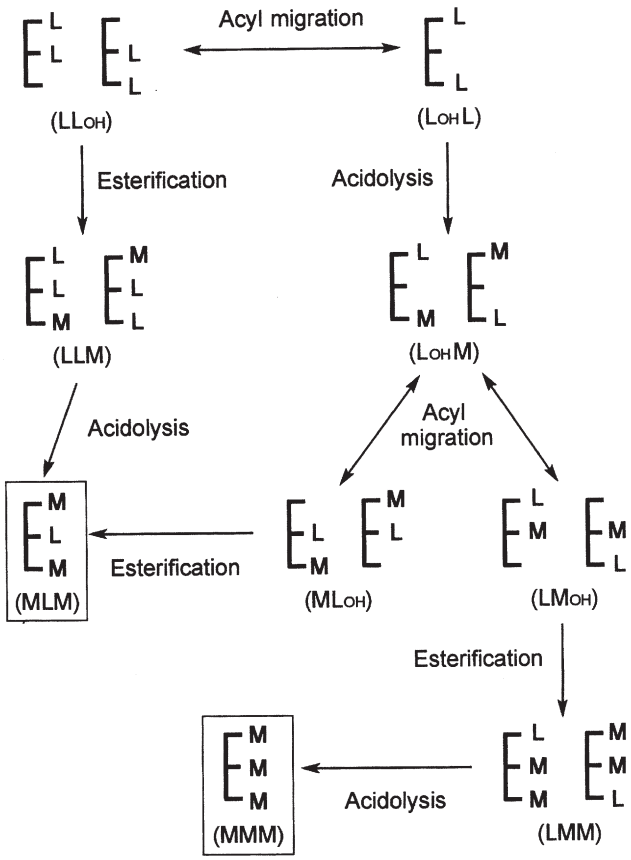
Continuous flow acidolysis of GLA45 oil. Table 3 shows that removing partial acylglycerols from GLA45 oil is not necessary to produce structured TAG rich in CGC. We thus attempted continuous flow acidolysis of GLA45 oil in a fixed-bed bioreactor with the aim of developing an industrial process. A substrate mixture of GLA45 oil/CA (1:2, w/w) was fed into a fixed-bed bioreactor (18 × 180 mm) packed with 15 g immobilized *R. oryzae* lipase at 30°C and a flow rate of 4.0 g (4.5 mL)/h. When the reaction was started, the contents of CA and GLA in the product acylglycerol fraction were 55.8 and 31.9 mol%, respectively. The contents after continuous full time operation for 1 mon were, respectively, 55.4 and 31.4 mol%, showing that the immobilized lipase was very stable under the conditions employed. The degree of acidolysis degree was higher than that in the single reaction of GLA45 oil (Table 2). The daily amount of substrate mixture in the flow reaction was 6.4 g/g lipase and equal to that in the 48-h batch reaction with 7.8 wt% immobilized enzyme. Because the batch reaction was conducted using 5 wt% immobilized lipase, the higher degree of acidolysis was assumed to be due to a larger amount of lipase.

Purification of structured TAG rich in CGC. Reaction mixture (1.0 kg) eluted from the above bioreactor was dehydrated and then subjected to molecular distillation at 130°C and 0.2 mm Hg. CA was recovered in the distillate (573 g) with a 94% recovery. The purity was 98.4%, showing that the preparation can be used as a substrate for the next reaction. FFA in the residue were removed by distillation at 190°C and 0.05 mm Hg. However, because the acid value of the residue was still high (18 mg KOH/g), the distillation was conducted again at 210°C and 0.05 mm Hg. The distillation recovered 222 g residue with acid value was 2.6 mg KOH/g (recovery of structured TAG, 97%). Table 4 shows the TAG composition before and after distillation. The CGC content was increased from 44.5 to 52.6 mol% by distillation, and the MAG, CCC, and DAG contents were decreased from 4.8, 7.6, and 11.1 mol% to 0.4, 2.9, and 6.4 mol%, respectively. GLA-rich oil contained partial acylglycerols, and its acidolysis with CA generated a by-product, CCC. But purification of structured TAG by molecular distillation removed part of the CCC as

TABLE 4
TAG Compositions of Reaction Products Before and After Molecular Distillation[a]

Molecular distillation	MAG	DAG	TAG composition (mol%) CCC	CGC	CLC	COC	CGG	CGL	CXX
Before	4.8	11.1	7.6	44.5	5.6	1.6	9.5	9.1	1.7
After	0.4	6.4	2.9	52.6	6.5	1.9	11.5	10.1	1.9

[a]Acidolysis of GLA45 oil was performed with two weight parts of CA in a fixed-bed bioreactor, and the structured TAG were purified by molecular distillation. See Tables 1 and 2 for abbreviations.



SCHEME 1

well as partial acylglycerols. This result can be explained by the fact that the total carbon numbers of MAG, CCC, and DAG were 21, 27, and 29, respectively, and were smaller than that of MLM-type structured TAG, 37 (see Fig. 2A).

To investigate the presence of positional isomers of CGC, the purified preparation was analyzed by HPLC with a silver ion column, which showed that CGC was composed of 94 mol% 1,3-dicapryloyl-2-γ-linolenoyl glycerol and 6 mol% 1(3),2-dicapryloyl-3(1)-γ-linolenoyl glycerol. These findings indicated that the use of GLA-rich oil is effective for the production of structured TAG rich in CGC.

DISCUSSION

Structured TAG containing nearly 50 mol% CGC can be produced efficiently by a two-step process, comprising selective hydrolysis of borage oil using *C. rugosa* lipase and its acidolysis with CA using immobilized *R. oryzae* lipase, as shown in this study. GLA45 oil prepared by selective hydrolysis contained 37.4 mol% (25.5 wt%) partial acylglycerols, which increased the amount of a by-product, CCC. However, the partial acylglycerols need not be removed, because some part of CCC was eliminated by molecular distillation.

Selective hydrolysis of borage oil. GLA45 oil was prepared by hydrolysis of borage oil with *C. rugosa* lipase. Although the degree of hydrolysis was 64.8%, the main component of the product was TAG. The phenomenon was the same as those in selective hydrolyses of tuna oil and arachidonic acid-containing single-cell oil. Some lipases, including *C. rugosa* lipase, catalyze esterification and transesterification simultaneously with hydrolysis even in the presence of large amounts of water. Based on those findings, we proposed a mechanism by which TAG was accumulated in the acylglycerol fraction after hydrolysis (25). The mechanism could also be applied to selective hydrolysis of borage oil.

Conversion of partial acylglycerols to MLM-type structured TAG. GLA-rich oil prepared by hydrolysis of borage oil contained 27.2 mol% DAG and 10.2 mol% MAG, but the acidolysis of GLA-rich oil decreased the content of partial acylglycerols, although the CCC content increased (Table 3). The conversion of partial acylglycerols, especially DAG, to MLM-type structured TAG can be explained by acyl migration, acidolysis, and simultaneous esterification. A possible conversion pathway is shown in Scheme 1. 1(3),2-DAG are esterified with CA and converted to TAG with long-chain FA at the 1(3),2-positions and CA at the 3(1)-position (referred to as LLM). Water was not added in the reaction system, and the water content after the reaction did not exceed 1500 ppm even though the water in the medium was not removed. Hence, *R. oryzae* lipase should catalyze not only acidolysis

but also esterification. The synthesized LLM subsequently undergo acidolysis with CA, and MLM-type structured TAG are generated.

1,3-DAG are converted to MLM-type structured TAG and tricaprylin through a pathway as shown in Scheme 1. 1,3-DAG first undergo acidolysis with CA and are converted to DAG with long-chain FA at the 1(3)-position and CA at the 3(1)-position (referred to as $L_{OH}M$, where the subscript OH indicates a hydroxy group not esterified with FA). The DAG are converted to ML_{OH} and LM_{OH} by acyl migration. The ML_{OH} are then esterified with CA, and MLM-type structured TAG are generated. If the other DAG, LM_{OH}, are esterified with CA, they are converted to LMM. Long-chain FA at the 1(3)-position of these TAG finally undergo acidolysis with CA, and MMM (tricaprylin) is generated.

In continuous flow acidolysis of GLA45 oil, the content of partial acylglycerols decreased from 37.4% to 15.9 mol%, and the content of tricaprylin generated was 7.6 mol% (Table 4). This indicated that 13.9 mol% partial acylglycerols were converted to MLM-type structured TAG. Hence, the use of GLA45 oil assured higher recovery of GLA than the use of GLA45-TAG as a substrate.

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