The Isolation of Hydroxy Acids from Lesquerella Oil Lipolysate by a Saponification/Extraction Technique

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ABSTRACT: The lipolysate from immobilized *Rhizomucor miehei* lipase (Lipozyme™)-catalyzed hydrolysis of lesquerella oil contains typically 35% free fatty acid (FFA), 2% monoglyceride, 25% diglyceride (DG), and 38% triglyceride (TG). Of the FFA, 75–80% are hydroxy acids (HFA). Various methods for isolating HFA from the lipolysate were examined, and a novel saponification/extraction method was developed. Lipolysate was mixed with 4 vol equivalents each of KOH/phosphate buffer and polar organic solvent. Hexane was then added to enhance phase separation. Three phases formed: a lower aqueous phase containing nothing of interest, a polar organic solvent middle phase that contained mostly fatty acid soaps, and a hexane-rich upper phase that contained mostly DG and TG, which can be recycled to a relipolysis step. The middle phase, when treated with concentrated hydrochloric acid, NaCl-saturated water, and hexane, released the FFA into the hexane. This fraction, referred to as the "Product," contained >99% of the FFA released in the lipolysis. "Product" consisted of 85–90% FFA, of which 75–80% was HFA. The other 10–15% of the "Product" consisted of partial glycerides and TG. The most critical parameters for the extraction are the pH of the aqueous solution and the polarity of the organic solvent (acetone was found to be the best choice). Additional purification steps for the "Product" are discussed.

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KEY WORDS: Extraction, fatty acid purification, hydroxy acids, lesquerella, lesquerolic acid, lipolysis, saponification.

Development of *Lesquerella fendleri* as a U.S. crop is being pursued by a partnership between the private sector and the U.S. Department of Agriculture. Lesquerella's seed oil is a rich source of hydroxy fatty acids (HFA): lesquerolic, 14-hydroxy-11-eicosenoic ($20{:}1^{11}\text{-}OH^{14}$) and auricolic, 14-hydroxy-11,17-eicosadienoic ($20{:}1^{11,17}\text{-}OH^{14}$) acids, present at 55 and 4%, respectively. Lesquerella oil's other acyl groups are largely C_{18} unsaturates. Development of lesquerella as a new crop has been discussed (1).

Full utilization of lesquerella fatty acids requires a process for isolating HFA from the seed oil fatty acid mixture. We have demonstrated that preparative-scale chromatography could be employed to acquire several hundred grams of lesquerella HFA (2). We have also split lesquerella oil and isolated an enriched HFA (>80%) fraction by high-vacuum molecular distillation of the free fatty acids (FFA), a process with greater industrial potential but where thermal sensitivity of HFA and polyunsaturated FFA is an important consideration (K.D. Carlson, unpublished data).

More recently, we reported that HFA are preferentially released during hydrolysis of the oil catalyzed by 1,3-positional specific lipases (3,4). Hydrolysis of lesquerella oil catalyzed by Lipozyme™, *Rhizomucor miehei* lipase adsorbed onto anion exchange resin, yields a lipolysate that contains 35% FFA, of which 75–80% are C_{20}-HFA (4). Obviously, to utilize this lipolytic process requires a method to separate FFA from mono-, di-, and triglycerides (MG, DG, and TG, respectively), and then the HFA from other FFA.

Coleman and Macrae (5) suggest that FFA and partial glycerides can be separated from TG by liquid-liquid extraction, alkali neutralization, molecular distillation, or crystallization. Quinlan and Moore (6) report that industry uses distillation to recover FFA from lipase-catalyzed acidolysis reaction mixtures. Derksen *et al.* (7) successfully isolated FFA from batch and continuous reactors during lipase-catalyzed hydrolysis of tricaprylin by using saponification techniques. They also isolated HFA from the lipolysates of *Dimorphotheca pluvialis* oil by crystallization in a cold trap (8). Separation of glyceride classes by chromatographic procedures is well known (9,10), but such technology would not be cost-effective on an industrial scale. For designing and selecting a separation scheme, we considered the following criteria: (i) efficient isolation of FFA, preferably HFA, from lipolysate; (ii) recovery of MG/DG/TG in unaltered form for re-lipolysis; (iii) inexpensive material and operating costs; (iv) acyl groups not degraded; (v) process safety. A separation method that combined alkali treatment (saponification) with liquid-liquid extraction, which we call "SAPEX," satisfactorily meets these criteria.

EXPERIMENTAL PROCEDURES

Materials. Refined lesquerella oil was obtained from our pilot plant studies. Castor oil was purchased from Sigma (St.

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Louis, MO). *Dimorphotheca pluvialis* oil, recovered from seed by supercritical fluid and solvent extraction, was a generous gift from Dr. J.T.P. Derksen (ATO-DLO, Wageningen, The Netherlands). Lipozyme and Palatase M, *R. miehei* lipase delivered in buffer solution (857 U/mL, based on olive oil-in-water assay), were generous gifts from Novo-Nordisk (Danbury, CT). Lipolysates of HFA-containing seed oils (lesquerella, castor, or *D. pluvialis*) were obtained from Lipozyme-catalyzed hydrolysis of water-saturated seed oil–isooctane mixtures or seed oil-containing water-in-oil microemulsion solutions formed by the surfactant sodium *bis* (2-ethylhexyl) sulfosuccinate (AOT) as described elsewhere (3,4). All organic solvents and reagents were of high purity (>98%) and used without further purification. Phosphate buffer solution, used in the pH range 10.5–14, was made from sodium phosphate dibasic and tribasic dodecahydrate purchased from Sigma. The pH of KOH/phosphate buffer was adjusted with concentrated HCl (aq.).

Lipolysate fractionation by SAPEX. The scheme for optimal performance of SAPEX is outlined in Figure 1. One volume part of lesquerella oil lipolysate is combined with three volume parts each of acetone and 1 M KOH in 0.5 M phosphate buffer at a pH of 11.5 and agitated. Each series of experiments employed a different batch of lesquerella oil lipolysate. Hexane or other lipophilic solvent was added to promote phase separation. Three phases formed: a lower phase that contained mostly water with no lipids (discarded), an acetone-rich middle phase that contained saponified FFA, and a hexane-rich upper phase that contained mostly DG and TG. This upper phase is referred to as the "Recycle." The middle phase, after treatment with acid and saturated salt solution, released FFA, which were taken up in hexane. The resulting FFA-rich hexane phase is referred to as the "Product." The proportion by weight of Product and Recycle was determined gravimetrically.

Chromatographic analyses. Composition of SAPEX phases was determined by supercritical fluid chromatography (SFC) with high-purity carbon dioxide (Air Products, Tamaqua, PA) as carrier fluid and a Dionex Model 600 chromatograph (Salt Lake City, UT). A nonpolar capillary column (10 m × 50 m i.d.) of SB-Methyl-100 stationary phase (Dionex) was employed. The following program that simultaneously increased column temperature and pressure was employed (11). The column temperature was held at 100°C for 9 min, then increased at 2.2°C/min up to 200°C, and held at 200°C for 1 min. The carrier fluid pressure was held at 12.7 MPa, then increased at 0.507 MPa/min up to 36.0 MPa. Gas chromatography (GC) on a neutrally polar (methyl 65% phenyl silicone) 25 m × 0.25 mm capillary column from Quadrex (New Haven, CT) was employed to separate and quantitate C_{20}-hydroxy fatty acids and C_{18} MG because they co-eluted during SFC. GC also was employed for separation of MG and DG positional isomers (3). The following conditions were used for separating samples derivatized with *bis*(trimethylsilyl)trifluoroacetamide (Sigma). The GC oven temperature was increased from 100 to 365°C at 10°C/min. Both the injector and flame-ionization detector temperatures were held at 380°C. The carrier gas, helium, was split at a ratio of 55:1.

Acetylation of lipolysate. Lesquerella oil lipolysate was pretreated with acetic anhydride before extraction to acetylate free hydroxyl groups in partial glycerides and hydroxy acyl groups (9). Five grams of lipolysate (after being dehydrated in a desiccator for several days) was combined with 200 mL acetic anhydride/pyridine (5:1, vol/vol) at room temperature and left overnight. Solvent was then evaporated. SFC analysis indicated that the resulting material was acetylated. This material was then subjected to SAPEX.

Further purification of SAPEX Product. Additional purification of Product from SAPEX was examined by hydrolysis with Palatase M in a stirred batch reactor that contained equal volumes of lipophilic material (Product plus hexane) and aqueous solution (phosphate buffer plus Palatase M). The reaction was analyzed by SFC and GC. In addition, Product was treated with hexane/acetonitrile liquid-liquid extraction. Volume amounts of each solvent were at least eight times the volume of (solvent-free) Product.

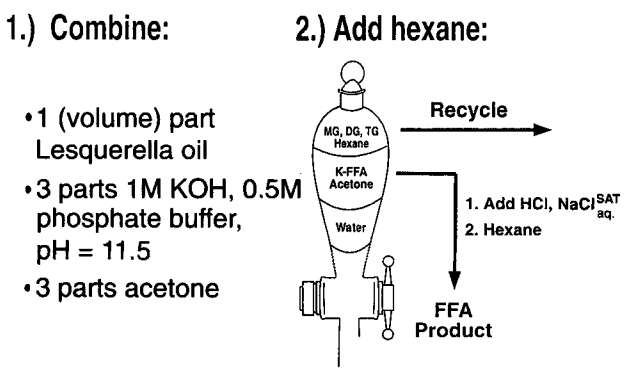

FIG. 1. Scheme used for saponification/extraction of fatty acid from lesquerella (lesq.) oil lipolysate with KOH/phosphate buffer and acetone. MG, DG, TG, mono-, di-, and triglycerides; FFA, free fatty acids; SAT, saturated.

RESULTS AND DISCUSSION

Screening methods to isolate hydroxy acids from lipolysate. Several methods were examined against the abovementioned criteria for HFA isolation. Thin-layer chromatography and column chromatography with silica gel and hexane/ethyl acetate in varying proportions as eluate were successful at isolating HFA. However, care had to be taken in separating HFA from DG that contained hydroxy acyl group(s) because both have similar polarities. Chromatographic methods mentioned previously are expensive and difficult to scale up, making them poor choices based on the above criteria. Both liquid-liquid extraction with solvent systems, such as hexane/acetonitrile, well-known for separating lipids that differ in polarity (12–14), and precipitation with chilled organic solvents yielded only partial isolation of HFA. All initial attempts to

isolate FFA from lipolysate *via* soap formation with NaOH or KOH in free form or in solution led to precipitation of the entire lipolysate. This was not surprising because of the many ionizable groups present in MG, DG, and hydroxy acyl groups in addition to the targeted COOH moiety of FFA. Therefore, we examined the effect of pH buffering and addition of organic solvent to reduce or eliminate these problems. The following novel method was developed.

SAPEX. The SAPEX procedure, shown in Figure 1, was quite successful in isolating FFA from other products of lipolysis. The most successful technique involved vigorous mixing of lesquerella oil lipolysate (not containing surfactant) with KOH/phosphate buffer and a polar organic solvent, preferably acetone. Hexane or petroleum ether was added in small amounts (hexane/acetone ratio of <1), either before or after the mixing step to promote separation into three phases. The amount of hexane added had little effect on the SAPEX results. The bottom aqueous phase contained nothing of value and was discarded. The middle organic phase consisted mostly of fatty acid–potassium soaps, which were acidified with concentrated HCl (aq.) and saturated salt solution. The FFA were then extracted with hexane (or other lipophilic solvent) at nearly 100% efficiency from the acidified layer to give the Product. Other concentrated acids examined for the acidification step, such as sulfuric and nitric acids, were unsatisfactory because they promoted a color change of the FFA. The top (Recycle) phase contained mostly DG and TG in hexane (lipophilic solvent), with a small amount of polar organic solvent also present, and can be directly relipolysized to recover additional HFA. Solvent can be recovered easily from this phase for reuse. The Recycle typically contained <1% FFA at optimum conditions, which indicated that the recovery of FFA in the Product is quite high (See Tables 1–5). SAPEX Product contained 85–90% FFA, the composition of which was nearly the same as that of the FFA portion of the original lipolysate. The remaining 10–15% of SAPEX Product was DG and MG, present in nearly equal proportions, and a small amount of TG. These glyceridic materials in the Product all were enriched in C_{20}-hydroxy acyl groups, as demonstrated by the higher proportion of hydroxy acid-containing MG (H-MG) among all MG (Table 1), and of DG that contained two hydroxy acyl groups (H,H-DG) among the DG (Tables 3 and 4). Thus, the tendency is for the more polar glycerides, e.g., MG, and especially H-MG and H,H-DG, to partition into the Product. In addition, the materials recovered from the extraction process were not altered by the alkali nor did further hydrolysis occur. However, MG and DG underwent isomerization, i.e., acyl migration. This will be discussed below. SAPEX has been scaled up successfully to treat 100-g quantities of lipolysate.

SAPEX operational parameters. The most important parameter in the SAPEX process is the pH of the aqueous solution. Without buffering, the pH of a concentrated KOH solution is over 13, and at such high pH a large amount of solidification/precipitation occurred. The optimal buffer pH must be lower than the pKa of OH groups present in MG, DG, and hydroxy acyl groups, so they do not ionize (e.g., the pKa of the OH group in tyrosine is 10.9), but higher than the pKa of COOH groups (pH >3), so that fatty acid soaps form. Within this range, the pH selected should be as high as possible to increase the extent of FFA saponification. Phosphate and glycine buffers, with pKa values of 11.8 and 9.7, respectively, were examined. Because the more expensive glycine buffers gave poorer recoveries of FFA from the middle phase after its acidification, they were abandoned in favor of the phosphate buffers. As shown in Table 1, pH values >12 resulted in a significant amount of precipitation, which limited recovery of all components. At pH values <11, recovery of FFA in the Product was significantly reduced (Table 1), and larger amounts of FFA appeared in the Recycle (Table 2). However, within the pH range of 10.6 to 13.5, HFA content of the FFA

TABLE 1
The Effect of pH of KOH/Phosphate Buffer on the Composition of Product from SAPEX of Lesquerella Oil Lipolysate[a]

	%						
pH	FFA	MG	DG	TG	Wt[b]	HFA/FFA	H-MG /MG
13.5	97.0	0.0	1.1	1.9	27.0[c]	70.0	—
12.4	89.8	3.6	4.0	2.6	21.6[c]	75.5	39.3
12.0	87.8	3.8	5.3	3.2	24.8[c]	72.7	34.6
11.4	86.6	3.4	6.3	3.7	30.2	73.5	36.9
11.1	88.4	3.1	5.1	3.4	21.4	75.4	37.4
10.6	82.8	4.0	6.9	6.4	18.6	81.0	37.1
9.9	—	—	—	—	[d]	—	—
9.2	—	—	—	—	[d]	—	—
Orig[e]	19.7	1.2	21.1	58.0	—	72.8	18.1

[a]Conditions: 5 mL lipolysis mixture, 15 mL acetone, 15 mL 1 M KOH/0.5 M phosphate buffer. FFA = free fatty acid, MG = monoglyceride, DG = diglyceride, TG = triglyceride, HFA = hydroxy fatty acid, H-MG = hydroxy acid monoglyceride; SAPEX, saponification/extraction method.
[b]Product weight percentage of total weight recovered.
[c]Due to formation of precipitate, less than 100% of original medium recovered.
[d]Small recoveries; analysis of product not performed.
[e]Composition of original lipolysis medium.

TABLE 2
The Effect of pH of KOH/Phosphate Buffer on the Composition of the Recycle from SAPEX of Lesquerella Oil Lipolysate[a]

	%			
pH	FFA	MG	DG	TG
13.5[b]	<0.5	<0.5	23.1	76.6
12.4[b]	<0.5	<0.5	22.0	78.1
12.0[b]	<0.5	<0.5	22.6	77.0
11.4	<0.5	<0.5	24.2	75.9
11.1	0.3	0.1	23.7	75.7
10.6	0.5	0.1	23.7	75.7
9.9	5.8	0.6	23.7	70.0
9.2	11.8	1.0	21.1	66.1
Orig[c]	19.7	1.2	21.1	58.0

[a]Conditions and abbreviations given in Table 1; data from same experiments used to derive Table 1.
[b]Due to formation of precipitate, less than 100% of original medium recovered.
[c]Composition of original lipolysis medium.

was significantly enriched (74.7 ± 3.7%; Table 1) compared to the bulk oil HFA content (*ca.* 55%). An optimum pH value of *ca.* 11.5 was chosen for all subsequent experiments, based on the high recovery of FFA in the Product and the absence of precipitation (Tables 1 and 2) at this pH.

The SAPEX process was also affected by the type of alkali used. Among the bases examined [NaOH, KOH, and $Ca(OH)_2$], KOH was the alkali of choice because of its greater solubility in phosphate buffer and higher solubility in water at ambient temperature (15).

Buffer ionic strength was also examined. The inclusion of saturating amounts of KOH in 1 M KOH/0.5 M phosphate buffer caused all fatty acid soaps to partition along with glyceridic products to the upper (hexane-rich) phase during SAPEX, and isolation of a HFA-rich FFA fraction was not achieved. On the other hand, use of a buffer with lower ionic strength than 1 M KOH/0.5 M phosphate buffer lowered the recovery and purity of HFA in the Product. Therefore, 1M KOH and 0.5M phosphate buffer gave the best extraction performance without precipitation and was employed in subsequent experiments.

Polar organic solvents with high dielectric constants (15) and low values of Log P (the logarithm of the water–octanol partition coefficient) (16,17) yielded three-phase systems and the best results. Less polar solvents ethyl ether [log P = 0.85 (16)] and *tert*-butanol [log P = 0.36 (17)] did not yield three-phase systems. The least polar solvent examined that produced three phases, tetrahydrofuran (THF), yielded Product that recovered little of the FFA (Table 3). Other polar solvents gave high recoveries of FFA in the Product, and the Recycle consisted almost entirely of DG and TG (Table 3). Acetonitrile and acetone, which have similar values of Log P, yielded Product with the highest percentage of FFA. Solvents more polar than acetone, such as methanol and isopropanol, yielded Product less pure in FFA (Table 3). Interestingly, a significant amount of fatty acid isopropyl esters were formed during the SAPEX process with isopropanol as polar solvent (Table 3). When acetonitrile was employed, two organic phases rich in fatty acid soaps were formed: a lower, "normal," acetonitrile soap-laden phase between the bottom aqueous and hexane Recycle phases, and an "upper" soap-laden phase above the hexane Recycle phase. The Product from the "lower" phase was much purer in FFA than that from the "upper" phase. The formation of a second soap-laden phase may be related to the large degree of immiscibility that exists between hexane and acetonitrile compared to that between hexane and the other solvents. A small, but significant, amount of polar organic solvent is present in the Recycle for all polar organic solvents examined. Based on these results, acetone was the organic solvent of choice and employed in all subsequent experiments.

Data in Table 4 show the effect of the acetone proportion on the SAPEX process. When the volume ratio of acetone to KOH/buffer was 0.33, the percentage of FFA in the Product

TABLE 3
Effect of Organic Solvent Type on Composition of Product and Recycle from SAPEX of Lesquerella Oil Lipolysate[a]

Solvent[b]		Methanol	ACN		Acetone	2-Propanol	THF
Log P[d]		−0.76	−0.33		−0.23	0.05[e]	0.49
∈[f]	Orig[c]	32.6	37.5[g]		20.7	18.3	7.6[g]
Product composition (%)							
FFA	26.4	48.8	80.6[h]	48.5[i]	82.9	59.1	22.5
Esters	0.0	trace	0.0	0.0	0.0	4.2	0.0
MG	5.2	10.8	11.1	6.4	9.6	7.6	4.2
DG	29.0	24.8	7.0	24.6	5.2	20.2	32.6
TG	38.7	15.4	1.2	20.3	2.3	9.1	43.2
HFA/FFA	79.4	74.0	81.2	65.9	76.4	75.0	77.1
H,H-DG/DG[j]	24.8	31.2	0.0	21.9	33.7	28.6	19.6
Wt% Product	—	53.9	23.6	16.3	34.0	36.9	1.2
Recycle composition (%)							
FFA		0.0	1.6		0.0	2.2	40.7
MG		0.0	0.5		0.0	1.4	5.2
DG		36.3	38.5		41.6	40.6	30.4
TG		66.0	61.8		61.4	57.6	24.2

[a]Conditions: 5 mL lipolysis medium, 20 mL each organic solvent and 1 M KOH/0.5 M phosphate buffer (pH = 11.5). Abbreviations as in Table 1; H,H-DG = DG containing two hydroxy acyl groups.
[b]Abbreviations: ACN = acetonitrile; THF = tetrahydrofuran. See Table 1 for other abbreviations.
[c]Original lipolysis medium.
[d]From Reference 16.
[e]From Reference 17.
[f]Dielectric constant, 25°C; from Reference 15.
[g]20°C.
[h]Lower product phase from extraction with ACN as solvent (discussed in text).
[i]Upper product phase from extraction with ACN as solvent (discussed in text).
[j]Product weight percentage of total weight recovered (the latter of which was within 5% of the weight of lipolysis medium extracted).

TABLE 4
Effect of Ratio of Acetone to KOH/Phosphate Buffer Volume on SAPEX of Lesquerella Oil Lipolysate[a]

		Composition as a function of acetone/buffer ratio				
Ratio	Orig[b]	0.33	0.67	1.0	2.0	3.0
Product composition (%)						
FFA	31.4	61.7	83.3	86.0	86.4	82.2
MG	2.4	3.9	3.7	4.9	1.6	2.7
DG	26.4	18.1	8.8	6.7	9.4	11.5
TG	39.8	16.2	4.3	2.4	2.6	3.6
HFA/FFA	75.0	76.3	78.2	79.4	77.4	79.0
H,H-DG/DG	8.6	12.1	18.4	20.8	37.2	22.6
Wt% Product[c]	—	42.6	23.7	31.5	29.5	28.5
Recycle composition (%)						
FFA	31.4	0.0	0.0	0.4	0.5	0.6
MG	2.4	0.0	0.0	0.7	0.9	0.6
DG	26.4	37.9	38.8	38.8	40.0	39.3
TG	39.8	62.1	61.2	60.2	58.7	59.5

[a]Conditions: 5 mL medium, 15 mL 1 M KOH/0.5 M phosphate buffer solution, pH = 11.5. Abbreviations as in Tables 1–3.
[b]Composition of original lipolysis medium.
[c]Product weight percentage of total weight recovered.

was lowest. Ratios higher than 0.67 did not significantly alter the higher content of FFA in the Product and the effectiveness of SAPEX. Thus, a ratio of acetone to KOH/buffer of 1.0 (vol/vol) was selected. The proportion of hexane added to induce phase separation did not affect the extraction results (hexane/acetone ratios between 0.33 and 2.0 were examined). Without hexane, phase separation was less rapid.

Data in Table 5 show the effect of the volume ratio of KOH/phosphate buffer to lipolysate at a fixed ratio of acetone to buffer (1:1, vol/vol). At a buffer/lipolysate ratio of 2, the proportion of FFA in the Product (78%) and the percentage recovery of FFA (72%) are unacceptably low. The purity and recovery of FFA worsen further as the ratio is decreased from 2. At ratios ≥4, the FFA proportion in the Product is acceptable (>85%). As mentioned above, SAPEX promotes "acyl migration," i.e., isomerization among the DG and MG, in both Product and Recycle, as detected by GC (Fig. 2). Acyl migration occurred to a larger extent among MG than among DG. The occurrence of acyl migration has important consequences. For example, when the Recycle is relipolysized, the percentage of HFA among FFA decreases. Moreover, during isomerization, C_{18} unsaturated acyl groups migrate from the 2-position of MG and DG to the outer position, thus enabling 1,3-specific lipase to release them. To illustrate, Recycle phase was relipolysized with Lipozyme as before (4), and the lipolysate was then subjected to SAPEX as described above.

TABLE 5
Effect of Volume Ratio of KOH/Phosphate Buffer to Lipolysis Medium on SAPEX of Lesquerella Oil Lipolysate[a]

		Composition as a function of buffer/lipolysate ratio			
Ratio	Orig[b]	1.0	2.0	4.0	6.0
Product composition (%)					
P-FFA	31.4	—	77.9	85.5	92.5
MG	2.4	—	6.7	6.1	4.4
DG	26.4	—	10.0	6.4	1.7
TG	39.8	—	5.5	2.1	1.4
HFA/FFA	75.0	—	85.2	80.5	78.5
H,H-DG/DG	8.6	—	16.8	27.5	21.5
Wt% product[c]	—	[d]	20.1	31.0	36.0
Recycle composition (%)					
FFA		18.8	7.5	0.1	0.4
MG		3.4	1.1	0.3	1.4
DG		31.7	35.7	38.3	37.5
TG		46.1	55.8	61.3	60.8

[a]Conditions: 5 mL medium, ratio of acetone to 1 M KOH/0.5 M phosphate buffer, pH = 11.5, fixed at 1:1 (vol/vol). Abbreviations as in Tables 1–3.
[b]Composition of original lipolysis medium.
[c]Product weight percentage of total weight recovered.
[d]Small recovery; analysis of product not performed.

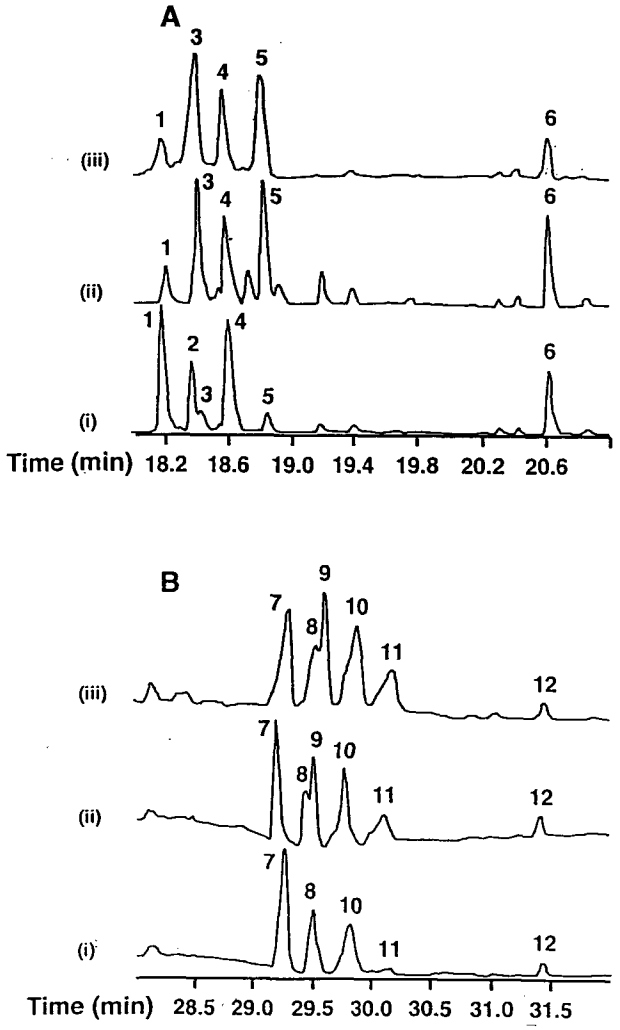

FIG. 2. The appearance of acyl migration *via* KOH extraction: among (A) monoglycerides (MG) and (B) diglycerides (DG). (i) Original lipolysate, (ii) Product, and (iii) Recycle. Extraction conditions: 220 mL medium, 700 mL acetone, 700 mL 1 M KOH/ 0.50 M phosphate, pH = 11.5. Peak assignments (Ref. 3): 1. 2-M18:1, 2. 2-M18:2, 3. 1-M18:1, 4. 1-M18:2/2-M18:3, 5. 1-M18:3, 6. 1-M-Lesq., 7. 1-(3)Lesq., 2-18:1 DG, 8. 1-(3)Lesq., 2-18:2 DG, 9. 1-(3)Lesq., 3-(1)18:1 DG, 10. 1-(3)Lesq., 3-(1)18:2 DG/1-(3)Lesq., 2-18:3 DG, 11. 1-(3)Lesq., 3-(1)18:3 DG, 12. 1,3-dilesquerolin. See Figure 1 for abbreviations.

Hydrolysis occurred at 36.7% , similar to that obtained from lipolysis of pure lesquerella oil (35%) (4). However, the SAPEX Product contained only 80.2% FFA (vs. 85–90% from SAPEX of whole oil lipolysate), and the proportion of hydroxy acids among the FFA in the SAPEX Product was only 56% (vs. 75–80% for SAPEX product from the whole-oil lipolysate).

Lesquerella oil lipolysate, containing the surfactant Aerosol-OT (3,4), was also subjected to SAPEX. The method failed to isolate HFA, most probably because of emulsification promoted by the surfactant.

SAPEX applied to other hydroxy acid oils. SAPEX was applied to castor (*Ricinus communis*) and *D. pluvialis* oils, which are rich in hydroxy acids. Castor oil contains 89% ricinoleic acid ($18:1^{9c}\text{-}OH^{12}$), and *D. pluvialis* contains 55% dimorphecolic acid [$S(+)\text{-}18:2^{10t,12t}\text{-}OH^{9}$]. Lipolysis of these two oils by the same procedure employed for lesquerella oil lipolysis (4) yielded 30–35% FFA (Table 6), which is similar to the final percentage lipolysis of lesquerella oil (Tables 1–5). SAPEX of castor and *D. pluvialis* oil lipolysates gave results similar to those for lesquerella oil lipolysate, e.g., similar weight recoveries and product distributions in both Product and Recycle phases. Also, the proportions of HFA in SAPEX Products from castor and *D. pluvialis* oils were slightly higher than the proportions of HFA in their respective lipolysates, as was also observed for SAPEX of lesquerella oil lipolysate. Furthermore, there was a tendency for castor and *D. pluvialis* Products to contain large amounts of hydroxy acid partial glycerides. Because of the high level of ricinoleic acid in castor oil (89%), the oil's lipolysate contains large amounts of hydroxy acid partial glycerides, which partition to the SAPEX polar solvent phase, and therefore, lower the percentage of FFA in castor's Product phase (79.5%) (Table 6). Of interest, the Recycle from SAPEX of *D. pluvialis* lipolysate contained a relatively large amount of FFA (5%). An explanation is not apparent. Although SAPEX successfully recovered FFA from castor and *D. pluvialis* oil lipolysates, further purification of Product in both cases would be required to obtain a commodity of acceptable purity. Further lipolysis of castor oil Product may yield a product of acceptable purity because most of its partial glycerides are enriched in hydroxy acids (see below). The purity of dimorphecolic acid in the Product from SAPEX of *D. pluvialis* oil lipolysate is low because of the low percentage of hydroxy acids among the lipolysate's FFA (63%).

Other SAPEX experiments. In an attempt to reduce the proportion of hydroxy-MG and -DG in the Product, lesquerella oil lipolysate was pretreated with acetic anhydride to acetylate free OH groups. We anticipated that this would reduce the polarity of MG and DG and hence the degree to which they partition to the acetone-rich middle phase. Of course, the free hydroxyl group of lesquerolic acid would also be acetylated, to give acetoxy–lesquerolic acid (14-acetoxy-11-eicosenoic acid) as major lipolysate component. After

TABLE 6
SAPEX of Castor and Dimorphotheca Oil Lipolysates[a]

	Castor oil			Dimorphotheca oil		
(%)	Lipolysate	Recycle	Product	Lipolysate	Recycle	Product
FFA	31.1	1.0	79.5	34.0	5.2	84.9
MG	6.7	3.2	7.2	1.1	2.3	0.9
DG	30.1	45.2	10.1	24.7	33.4	6.5
TG	31.2	49.2	2.8	38.0	57.7	2.8
HFA/FFA	86.7	55.6	90.1	58.0	44.5	62.6
Wt%		60.2	39.8		66.6	33.4

[a]Conditions: 2 mL lipolysate, 3 mL each acetone and 1 M KOH/0.5 M phosphate buffer, pH = 11.5, petroleum ether replacing hexane for phase separation and for recovery of Product after addition of hydrochloric acid to acetone (middle) phase for Dimorphotheca lipolysate. Abbreviations as in Tables 1–3.

lipolysate described in Table 3 was acetylated, SAPEX was performed with 1 M KOH/0.5 M phosphate buffer (pH = 11.5) and acetone at a 4:1 (vol/vol) ratio with respect to acetylated lipolysate. Although the extraction yielded Product richer in FFA (90%), with a higher percentage of (acetylated) HFA among the FFA (84%), only 35% overall of the acetylated C_{20} hydroxy fatty acids were recovered in the Product. Thus, acetylation would not be a desirable pre-step for our SAPEX technique.

As discussed above, the typical Product derived from SAPEX of lesquerella lipolysate contains *ca.* 90% FFA, and of the FFA, *ca.* 75–80% are hydroxy acids (i.e., the overall percentage of C_{20}-hydroxy acids is 64–72%). Thus, depending on the needs of the user, further purification of the HFA fraction may be required. One additional step examined was the relipolysis of the Product to decompose the MG, DG, and TG present (18). To illustrate, Lesquerella SAPEX Product was treated with 1,3-specific ("free") Palatase M (*R. miehei* lipase) in a water–hexane two-phase medium, chosen because it leads to a higher percentage hydrolysis. With this medium, hydroxy acyl groups are released much more slowly than nonhydroxy acyl groups (4). We found that a significant amount of the partial glycerides were hydrolyzed, and the *overall* percentage of HFA increased from 65 to 68%, but the proportion of hydroxy acids among FFA actually decreased slightly. The migration of nonhydroxy acyl groups from the 2-position to the 1- (3-) position with MG and DG allows the 1,3-specific lipase to catalyze their release during relipolysis. Thus, with the elimination of partial glycerides from the mixture, methods for separating HFA from nonhydroxy-FFA, such as distillation, multistage liquid-liquid extraction, or crystallization/precipitation might be directly applied to the new Product. In our hands, a second purification step, acetonitrile–hexane liquid-liquid extraction, was not successful.

In conclusion, the combination of lipase-catalyzed hydrolysis of lesquerella oil and the SAPEX method presented here yields a technical grade of lesquerolic acid product (65–75%). The structural integrity of lesquerolic acid is retained. The technical-grade product has been used in the synthesis of novel lesquerolic acid derivatives, which have potential applications in lubrication and cosmetics (19).

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