The Triglyceride Composition, Structure, and Presence of Estolides in the Oils of *Lesquerella* and Related Species

Douglas G. Hayes*, Robert Kleiman and Bliss S. Phillips

New Crops Research, NCAUR, ARS, USDA, Peoria, Illinois 61604

ABSTRACT: Members of the genus *Lesquerella*, native to North America, have oils containing large amounts of hydroxy fatty acids and are under investigation as potential new crops. The triglyceride structure of oils from twenty-five *Lesquerella* species in the seed collection at our research center has been examined after being hydrolysis-catalyzed by reverse micellar-encapsulated lipase and alcoholysis-catalyzed by immobilized lipase. These reactions, when coupled with supercritical-fluid chromatographic analysis, provide a powerful, labor-saving method for oil triglyceride analysis. A comprehensive analysis of overall fatty acid composition of these oils has been conducted as well. *Lesquerella* oils (along with oils from two other Brassicaceae: *Physaria floribunda* and *Heliophilia amplexicaulis*) have been grouped into five categories: densipolic acid-rich (Class I); auricolic acid-rich (Class II); lesquerolic acid-rich (Class III); an oil containing a mixture of hydroxy acids (Class IV); and lesquerolic and erucic acid-rich (Class V). The majority of Class I and II triglycerides contain one or two monoestolides at the 1- and 3-glycerol positions and a C_{18} polyunsaturated acyl group at the 2-position. Most Class III and IV oil triglycerides contain one or two hydroxy acids at the 1- and 3-positions and C_{18} unsaturated acid at the 2-position. A few of the Class III oils have trace amounts of estolides. The Class V oil triglycerides are mostly pentaacyl triglycerides and contain monoestolide and small amounts of diestolide. Our triglyceride structure assignments were supported by ^{1}H nuclear magnetic resonance data and mass balances.

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Seed oils from the genus *Lesquerella*, members of the Brassicaceae family, have long been known to be rich in hydroxy fatty acids. Hydroxy acids and their derivatives have several applications, e.g., as lubricants, automotive greases, and surface coatings. Currently, the main source of hydroxy acids supplied by nature is castor (*Ricinus communis*) oil, all of which the United States must import. Ricinoleic (12-hydroxy 9-octadecanoic) acid, present in castor oil at *ca.* 90%, is considered "strategic and critical" material by the U.S. Department of Defense. Seed oils of *Lesquerella* species native to the American Southwest and northern Mexico contain significant amounts of lesquerolic (14-hydroxy 11-eicosenoic) acid, the C_{20} homolog of ricinoleic acid, while those native to Alabama and Tennessee possess large quantities of densipolic (15-hydroxy 9,15-octadecadienoic) acid (1,2). *Lesquerella fendleri*, whose oil contains 55% lesquerolic acid and 3% auricolic (14-hydroxy 11, 17-eicosadienoic) acid, is currently under development as a potential new U.S. crop (3). Perhaps other members of the genus *Lesquerella* may be sources of germplasm for new crop development. Currently, over 85 species of the genus *Lesquerella* have been found. Additional germplasm of *Lesquerella* is currently being collected (Thompson, A.E., private communication).

Although the fatty acid composition of several lesquerella oils, obtained from the seed collection at our research center, have been reported (1,2,4–6), modern capillary-column gas-chromatographic techniques provide greater detail than older packed-column technology. An updated, more accurate, and more detailed analysis of fatty acid composition is one of the main goals of this report. Additionally, the oil composition from five species of *Lesquerella* are reported here for the first time: *L. inflata, L. ludoviciana, L. mirandiana, L. purpurea*, and *L. tenella*. A second objective is analysis of the triglyceride (TG) structure of these oils. This information was obtained one way by subjecting small amounts of oil to reactions catalyzed by 1,3 positional-specific lipases. The success of this methodology for analyzing *L. fendleri* oil has already been demonstrated (7). However, supercritical fluid chromatography (SFC) has to be employed here for analysis of TG and the lipase-catalyzed reactions because of the larger molecular weights (MWs) involved. SFC is becoming more commonly employed for analysis of glycerides and their reactions (8–10; and Hayes, D.G., and R. Kleiman, manuscript in preparation). Previous analysis of TG from *L. auriculata* oil (11) indicated the presence of estolides: molecules containing two or more acyl groups joined *via* ester linkages between an -OH moiety on the hydrocarbon chain of one acyl group and the -COOH moiety of another (Fig. 1). High-pressure liquid chromatography (HPLC) has been traditionally

*To whom correspondence should be addressed at Dept. of Chemical Engineering, University of Alabama in Huntsville, Huntsville, AL 35899.

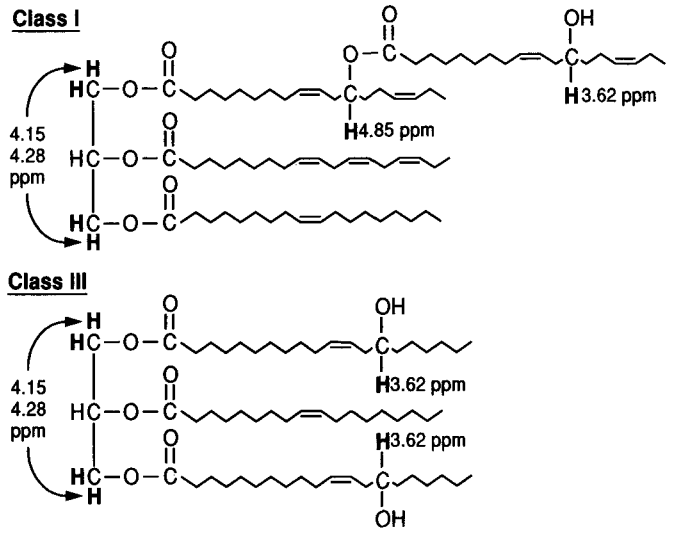

FIG. 1. Structure of common triglyceride (TG) from Class I (densipolic acid-rich) and III (lesquerolic acid-rich) oils with ^1H nuclear magnetic resonance assignments. The bond between the two hydroxy acyl groups in the Class I TG is referred to as an "estolide" bond.

employed to analyze estolide-containing TG (12–14). We believe that SFC is superior for estolide analysis because analyte MW can be determined through a near-universal retention time–molecular weight calibration (Hayes, D.G., and R. Kleiman, manuscript in preparation), and quantitation is more accurate due to the use of flame-ionization detection (FID).

Estolides have been encountered in only a few other seed oils (12–22) and in epicuticular waxes from evergreen trees (23–28). Here, we report for the first time that seed oils from the genus *Lesquerella* rich in densipolic acid contain estolides. In addition to *Lesquerella*, we report on oils from *Physaria floribunda* (native to New Mexico), which has a fatty acid composition similar to several lesquerella oils, and from *Heliophilia amplexicaulis*, a plant native to Spain. In a previous report (12), *H. amplexicaulis* oil was shown to have large quantities of lesquerolic and C_{18}–C_{24} acyl groups and to contain estolides.

EXPERIMENTAL PROCEDURES

Materials. 1-Decanol (98% pure), the surfactant Aerosol-OT (AOT), or sodium *bis* (2-ethylhexyl) sulfosuccinate (99% pure), *bis* (trimethylsilyl) trifloroacetamide (BSTFA)/trichloromethyl silane (97:3), and *Rhizopus arrhizus* lipase (Type XI) were purchased from Sigma (St. Louis, MO). Isooctane (2,2,4-trimethylpentane) was of analytical grade. Lipozyme IM20, *Rhizomucor miehei* lipase immobilized onto anion exchange resin, was a generous gift from Novo-Nordisk (Danbury, CT). Lesquerella oils were recovered from seed contained in the National Center for Agricultural Utilization Research (NCAUR) (Peoria, IL) seed collection by the following procedure: About 0.1 g of seed was ground with a micro-head tissue grinder from Virtis (Gardiner, NY) at 100 rpm for *ca.* 1 min. Oil was extracted with petroleum ether. The extract was then filtered *in vacuo* and dried first under nitrogen gas, then in a vacuum oven for 2 h. Ricinoleic acid estolide standard was extracted from a ricinoleic acid sample, which had polymerized (20 mL material), with hexane (250 mL) and acetonitrile (100 mL). The estolides remained in the hexane phase, while the vast majority of free acid partitioned to the acetonitrile phase. Lesquerolic acid-containing monoestolides and their decyl esters were produced from lipase-catalyzed reactions (Hayes, D.G., and R. Kleiman, manuscript in preparation). Other glyceride standards were obtained from lipase-catalyzed reactions involving *L. fendleri*, industrial rapeseed, and castor oils.

Overall free fatty acid (FFA) composition of oils. For oils not containing estolides, the following procedure was employed: *ca.* ten seeds were crushed in a vial and covered with a 1-mL solution of 1% sodium methoxide in methanol and allowed to stand for 15 min. Hexane (1 mL) and 1% (wt/vol) NaCl in water (1 mL) were then added and mixed. The hexane layer, containing the fatty acid methyl esters (FAME) was isolated and used for gas chromatography (GC) analysis. The sodium methoxide method, however, did not sufficiently break the estolide's ester bond (SFC analysis). For these samples, the following procedure was used: Extracted oil, wetted with hexane, was contacted with 5 mL 0.5 M KOH in methanol and refluxed on a steam bath for 1 h. Then 5 mL BF_3 in methanol (14% wt/vol) was added. The resulting mixture was refluxed for 15 min. After the addition of 5 mL NaCl-saturated water, the FAME were extracted with 3 × 5 mL hexane. The hexane rinsates were treated with 3 × 5 mL water to remove acidic/basic impurities, then dried over Na_2SO_4. SFC analysis of these FAME samples indicated that all estolide and TG bonds were broken.

FAME were analyzed by GC on a model 5890 instrument from Hewlett-Packard (Avondale, PA) containing an FID and either a CP-Sil 88 (Chrompack, Ratitan, NJ) or a BPX70 (SGE, Austin, TX) fused-silica capillary column (both 25 m × 0.25 mm, and 0.21-μm film thickness). The column oven was programmed from 120 to 225°C at 1°C/min for the first column and from 125 to 245°C at 3°C/min for the second column. Peaks were identified by comparison with known standards and by the use of equivalent chainlengths.

Oil content. Oil content was determined gravimetrically by Butt extraction with petroleum ether.

1,3-Specific lipolysis. Lesquerella oils were hydrolyzed in water/AOT/isooctane reverse micelles with *R. arrhizus* lipase at room temperature. This procedure employed small quantities of material: 5 μL seed oil and 56 μL solution overall. The medium consisted of 8.9% (vol) seed oil, 1.6% aqueous lipase solution (50 mM sodium phosphate buffer at pH of 6.9), 87.7% isooctane, and 1.8% pentadecane (added as an internal standard), and it contained AOT at 89.3 mM. This produces a water–AOT molar ratio, or w_o value, of 8.7. The overall lipase concentration was 381 Units (U)/mL as measured by the linoleic acid–butanol esterification assay in reverse micelles (7) and 1980 U/mL with respect to the standard olive oil hydrolysis assay in oil/water emulsions. The mixing of lipase-

containing water–AOT–isooctane solution with seed oil initiated the reaction. Small aliquots were taken from each reaction after *ca.* 12 h for chromatographic analysis (based on Ref. 7 data, this was a suitable duration). In a few experiments, a second sample was taken after 24 h to verify that the FFA distribution did not change significantly. This held true for all tests, indicating that acyl migration did not occur to any significant extent and that the free acid distribution obtained at 12 h was not dependent upon the lipase's substrate specificity, as it would be during the initial stages of the reaction.

Lipase-catalyzed alcoholysis. Lesquerella oil TG was reacted with 1-decanol in the presence of 1,3-specific lipase to produce fatty acid decyl ester (FAE), estolide decyl ester (E^iE), where *i* refers to the number of estolide bonds per estolide molecule, monoglyceride (MG), and diglyceride (DG). Oil (200 μL) and 30 μL pentadecane (as internal standard) were dissolved in 2 mL isooctane/decanol (1:1, vol/vol). The solution was then treated with 0.3-nm molecular sieves to reduce water content. After the sieves were removed, approximately 0.1 g Lipozyme was added. The reaction was conducted at 25°C (*L. auriculata, L. lescurii, L. ludoviciana*, and *P. floribunda* oils) or 43°C (all others) in a vortex-evaporator unit from Haake-Bucheler (Saddlebrook, NJ), which provided sufficient agitation to disperse the biocatalyst. Aliquots were withdrawn during the course of reaction for chromatographic analysis. As was true for lipolysis, little acyl migration was detected, and results reported and used in the calculations were at or near final (equilibrium) conditions. The occurrence of hydrolysis side-reaction during decanolysis was minimal.

Chromatographic analysis of lipase-catalyzed reactions. GC was employed primarily for determining the FFA and MG distribution obtained during lipolysis. Aliquots were trimethylsilane-derivatized through the addition of BSTFA reagent, followed by incubation at 80°C for at least 10 min. GC was performed on a neutrally polar (methyl 65% phenyl silicone) 25 m × 0.25 mm capillary column from Quadrex (New Haven, CT). The equipment and operating conditions match those described previously (10). SFC was performed on a model 600 chromatograph, equipped with an FID and a (nonpolar) SB-Phenyl-5 (10 m × 50 μm i.d.) capillary column. All SFC equipment was from Dionex (Salt Lake City, UT). The carrier fluid was SFC/SFE-grade CO_2 from Air Products (Tamaqua, PA). The oven temperature was maintained at 100°C, while the FID temperature was held at 350°C. The carrier fluid pressure was programmed as follows: 6 min isobaric hold (8 min for SB-Methyl-100 column) at 125 atm, followed by a 5 atm/min pressure ramp to 400 atm or until all analytes eluted. Time-split injection was employed.

^1H Nuclear magnetic resonance (NMR) analysis. ^1H NMR analysis was employed to measure the ratio of hydroxy acids and estolides to TG in the oil. A 300-MHz model WM 300 instrument from Bruker (Billericka, MA) was employed on samples of oil in $CDCl_3$ with a dual 5-mm probe. The spectral width was 3597 Hz, and 16–64 scans were taken. Peak assignments, shown in Figure 1, are consistent with previous investigations (29,30).

Thin-layer chromatography (TLC). TLC was employed to separate (E^iE) containing free hydroxy groups (e.g., a monoestolide ester composed of two hydroxy acids) from those without free hydroxyl groups from *L. auriculata* (Class II) and *L. lescurii* (Class I) oils after 1,3-specific decanolysis. A similar procedure was shown to be successful for this type of separation (31). The separations employed Kieselgel 60 preparative plates (2 mm thick) from Merck (Darmstadt, Germany). The solvent system was methylene chloride/methanol/acetic acid at 97.5:2.5:1 (vol/vol/vol). Bands were detected with iodine on the outside edge of the plate. Materials not in contact with iodine were recovered from preparative plates by extraction with methylene chloride/methanol at 80:20 (vol/vol). R_f values for analytes present in the alcoholysis reactions include: MG (0.12), decanol (0.40), E^iE with free hydroxyl group (0.42), hydroxy acid decyl ester (0.42), DG and TG containing hydroxy acid or estolide with free hydroxyl group (0.43–0.65), nonhydroxy acid decyl ester (0.80), E^iE without free hydroxyl (0.84), and TG without hydroxy acid (0.96).

RESULTS AND DISCUSSION

Oil classification. For the sake of organization, the *Lesquerella* species have been classified based on their oil composition and TG structure: Class I, densipolic acid-rich; Class II, auricolic acid-rich; Class III, lesquerolic acid-rich; Class IV, a mixture of hydroxy acids. Oil from *H. amplexicaulis* is designated Class V. The list of *Lesquerella, Physaria*, and *Heliophilia* species examined here with their NCAUR seed location numbers, or NU number, are contained in Table 1, along with their fatty acid composition and oil content. Of the 27 species examined here, 19 have oils that belong to Class III. Five belong to Class I, and one each to Classes II, IV, and V. Class I, II, and V oils differed from Class III and IV oils in their high estolide content.

Determination of TG structure. Rhizopus arrhizus lipase-catalyzed hydrolysis in reverse micelles, analyzed by GC, was successfully employed here to help determine the fatty acid positional distribution in lesquerella oil TG. However, this technique is not successful in detecting the presence of estolides because our GC technique poorly resolves estolides and estolide-containing glycerides. Thus, SFC, a method shown to successfully resolve oligomers (8,9), was used. Lipase-catalyzed decanolysis of oil TG was performed in addition to hydrolysis because fatty acid and E^iE SFC peaks are sharper and more Gaussian in shape than free acid and estolide peaks. This is apparent from the chromatograms shown in Figure 2. In addition, comparison of chromatograms from hydrolysis and decanolysis reactions helps differentiate TG, DG, MG, FFA, and E^i from the decyl esters of the latter two.

Neither *R. arrhizus* lipase or immobilized *Mucor miehei* lipase (Lipozyme) was able to attack estolide ester bonds of synthetic mono- and polyestolide-containing samples (data not shown). Other 1,3-specific lipases possess the same inability (Ref. 11; and Hayes, D.G., and R. Kleiman, manu-

TABLE 1
Overall Fatty Acid Composition of Oils from the Genus *Lesquerella*

NU#	Species	Class	% Oil	16:0	18:0	18:1^{9}	18:11^{11}	18:2	18:3	20:1	18:2=O[a]	16:1-OH	18:1-OH	18:2-OH	20:1-OH	20:2-OH
40872	*L. angustifolia*	III	26	2.5	1.4	16.0	3.2	12.0	2.3	0.6	0.2	0.1	3.8	0.1	54.2	0.1
49337			29	1.0	1.0	11.5	2.0	7.2	2.2	0.7	0.0	0.0	6.8	0.0	65.6	1.9
60665				1.5	1.3	12.0	1.2	8.0	2.2	0.7	0.0	0.0	5.4	0.0	67.0	0.0
40814	*L. argyraea* subsp. *argyraea*	III	26	1.0	1.8	15.2	2.9	6.3	5.7	0.8	0.7	0.0	0.5	0.0	63.2	1.6
40928			27	1.2	1.7	11.3	2.7	10.2	6.4	0.7	0.3	0.1	1.1	0.1	60.1	1.3
60692			28	1.3	2.1	13.2	2.4	9.2	5.6	0.8	0.0	0.0	0.5	0.0	61.2	1.2
49322	*L. auriculata*	II	33	3.6	4.6	24.7	2.2	1.8	7.6	2.9	0.3	0.0	4.2	2.7	9.2	31.8
60661	*L. densipila*[b]	I		4.3	3.7	17.3	2.9	1.8	9.7	0.3	0.0	1.4	9.6	46.5	0.5	0.0
40910	*L. engelmanii*	III	11	1.4	1.8	15.4	4.0	8.1	7.2	0.7	0.7	0.3	0.7	0.0	56.7	1.8
40925			21	0.9	1.4	11.9	2.8	6.2	8.6	0.7	0.3	0.1	1.0	0.2	60.3	2.8
40926			20	1.0	1.3	12.6	3.1	7.8	7.5	0.7	0.2	0.0	0.9	0.1	59.1	2.6
40817	*L. fendleri*	III	20	0.9	1.7	11.8	2.4	5.2	11.1	0.6	0.8	0.0	0.4	0.0	59.7	4.8
40821			28	1.0	1.8	14.3	3.6	6.5	9.7	0.8	0.6	0.1	0.4	0.0	54.7	3.0
40826			23	0.8	1.4	10.9	3.1	6.4	10.7	0.7	0.5	0.1	0.4	0.0	58.1	3.8
41689				1.1	1.7	11.6	3.2	6.0	11.2	0.6	1.1	0.1	0.3	0.1	55.1	1.9
64261				1.6	1.9	14.5	1.8	8.7	12.9	0.9	0.0	0.0	0.0	0.0	53.7	3.1
40848	*L. globosa*	III	39	1.0	1.0	8.8	2.2	9.2	3.7	0.7	0.5	0.0	4.5	0.0	65.7	0.4
40816	*L. gordonii*	III	29	1.1	1.6	19.0	2.9	5.2	5.8	1.2	0.6	0.3	0.6	0.1	59.0	1.8
40869			26	1.1	1.1	6.6	2.7	4.0	7.1	0.9	0.4	0.2	0.7	0.1	60.7	3.8
45461			28	1.5	1.7	19.8	3.6	5.6	5.4	1.0	0.5	0.1	0.5	0.1	55.1	1.9
40873	*L. gracilis* subsp. *gracilis*	III	33	1.0	1.0	9.7	1.4	4.1	2.4	0.9	0.2	0.0	5.4	0.2	73.0	0.3
49338				1.4	1.1	9.7	1.1	4.0	3.3	1.0	0.0	0.0	8.0	0.0	70.1	0.0
60670			26	0.8	0.4	7.4	1.0	2.8	2.5	0.7	0.0	0.0	5.6	0.1	78.3	0.0
40875	*L. grandiflora*	III	27	1.4	3.7	24.4	4.6	7.2	1.2	1.3	0.4	0.2	0.9	0.0	54.2	0.0
60659			25	1.0	2.6	23.6	3.6	5.8	1.0	1.4	0.0	0.0	0.6	0.0	59.6	0.4
49618	*L. inflata*	III		1.0	1.5	7.8	0.7	11.8	4.6	0.9	0.0	0.0	2.9	0.0	67.4	0.6
33090	*L. lasiocarpa*	III		1.8	3.0	24.9	2.2	5.5	2.3	1.4	0.0	0.0	1.5	0.0	56.0	0.0
40812	*L. lasiocarpa* subsp. *lasiocarpa*	III	30	1.1	2.6	23.7	3.1	4.6	1.5	1.9	0.0	0.0	0.7	0.0	60.4	0.0
60648			28	1.3	3.1	24.6	2.9	5.0	1.5	1.9	0.0	0.0	0.8	0.0	58.3	0.0
40843	*L. lescurii*	I	28	4.6	3.7	17.7	2.7	2.1	9.7	0.3	0.2	1.6	12.0	40.7	0.8	0.0
41683			29	4.2	3.8	19.5	2.8	2.1	9.8	0.3	0.2	1.7	10.7	42.0	0.7	0.0
33082	*L. lindheimeri*	III		1.7	2.1	14.0	0.9	4.2	1.2	1.9	0.2	0.0	0.6	0.0	72.1	0.0
40904			21	0.7	1.4	11.4	1.3	3.5	0.1	1.7	0.4	0.0	0.0	0.7	0.0	78.7
61380	*L. ludoviciana*	IV	25	1.6	0.5	15.3	2.4	4.9	18.2	0.8	0.3	0.0	12.9	10.2	27.4	4.0
41681	*L. lyrata*	I	26	4.1	4.6	20.5	2.7	2.0	9.0	0.3	0.3	1.5	10.7	41.2	0.5	0.0
44760	*L. mirandiana*	III	22	1.3	2.1	15.1	2.8	7.8	6.8	0.6	0.3	0.3	0.4	0.2	57.9	1.4
40946	*L. ovalifolia* subsp. *ovalifolia*	III		0.9	0.9	11.9	3.1	6.4	12.0	0.8	0.4	0.3	0.7	0.3	57.6	4.2
41679	*L. perforata*	I	23	5.1	3.7	18.3	3.4	1.9	9.6	0.2	0.2	2.2	11.0	41.8	0.4	0.0
60662			24	4.0	3.4	17.5	2.8	1.6	9.7	0.3	0.2	1.7	10.7	45.4	0.7	0.0
64507	*L. pinetorum*	III		2.6	1.9	19.4	2.7	11.8	3.9	1.1	0.0	0.0	0.7	0.0	50.6	0.4
64508	*L. purpurea*	III		1.2	1.9	11.0	1.3	8.7	4.6	1.1	0.0	0.4	0.4	0.0	66.3	1.2
44505	*L. recurvata*	III	19	0.7	1.5	11.4	1.6	5.5	4.5	0.9	0.6	0.2	0.6	0.1	70.8	1.1

(Continued)

TABLE 1 (*Continued*)

NU#	Species	Class	% Oil	16:0	18:0	$18:1^{9}$	$18:1^{11}$	18:2	18:3	20:1	18:2=O[a]	16:1-OH	18:1-OH	18:2-OH	20:1-OH	20:2-OH
41680	*L. stonensis*	I	24	4.4	3.8	16.8	2.8	1.5	10.1	0.2	0.3	2.0	8.8	46.7	0.3	0.0
60663			27	4.7	4.1	17.7	2.8	1.8	9.8	0.3	0.2	1.5	8.7	45.0	0.9	0.0
45462[c]	*L. tenella*	III	31	1.6	2.1	20.6	3.9	5.1	5.8	1.1	0.2	0.2	0.4	0.1	53.6	1.8
46427[c]			30	1.5	1.9	19.1	3.7	5.2	5.1	1.1	0.0	0.0	0.7	0.0	58.6	2.8
64485[c]				1.1	1.6	17.7	3.5	4.1	5.6	0.8	0.0	0.0	0.4	0.0	62.5	2.5
64486[c]				1.6	2.0	20.7	4.4	5.5	5.8	1.1	0.0	0.0	0.5	0.0	52.3	2.0
64487				1.5	1.8	19.4	3.7	5.4	5.9	1.0	0.0	0.0	0.5	0.0	58.6	1.9
48625	*Physaria floribunda*	III	31	1.3	1.5	13.2	2.7	11.1	12.3	1.0	0.2	0.0	0.6	0.1	51.3	2.0
52582				1.6	1.2	17.1	1.7	11.1	9.5	1.0	0.0	0.0	0.0	0.0	53.5	1.6
62350	*Heliophilia amplexicaulis*[d]	V		0.9	0.6	9.4	0.3	3.4	6.6	7.2	0.0	0.0	0.3	0.0	35.1	0.2

[a]15,16-Epoxy,*cis*-9,*cis*-12,-octadecadienoic acid.

[b]National Center for Agricultural Utilization Research number (NU #) 41682 (*L. densipila*) used for analysis of triglyceride structure. Class explanations: I, densipolic acid-rich oil; II, auricolic acid-rich oil; III, lesquerolic acid-rich oil; IV, an oil containing a mixture of hydroxy acids; V, lesquerolic and erucic acid-rich oil.

[c]Originally identified as *L. palmeri* but is *L. tenella* (Dierig, D., private communication).

[d]Other prominent fatty acids: 20:0 (2.4%), 22:0 (7.2%), 22:1 (23.0%), 22:2 (0.9%), 24:0 (0.2%), 24:1 (0.4%), 22:-hydroxy (0.8%).

script in preparation). Thus, the structural retention of estolides during the hydrolysis and alcoholysis reactions is assumed.

Our assignments of TG structure, based on SFC analysis of lipase-catalyzed reactions, were compared against ^{1}H NMR data. The results, comparing estolides per TG and free hydroxyl (-OH) groups per TG, show strong agreement (Table 2). In addition, the percentage of acyl group types (e.g., C_{18} fatty acid, 20-OH FA), calculated from our assumed TG structures, strongly agrees (mostly within 5%) with the measured percentages taken from Table 1 (data not shown). Furthermore, fatty acid decyl ester composition, determined by SFC during alcoholysis, agrees with FFA composition determined by GC during lipolysis. The analysis of the reaction medium after esterification by TLC supports our assignments of estolide structure. For example, TLC analysis confirmed that almost all estolide esters formed from Class I and II oils contained a free hydroxyl group.

Class I (densipolic acid-rich) oils. The overall fatty acid composition of oils from the five members of Class I (*L. densipila, L. lescurii, L. lyrata, L. perforata,* and *L. stonensis*) are listed in Table 1. All of these oils have quite similar fatty acid compositions. Of the oils from the genus *Lesquerella*, Class I oils have the highest content of saturated and hydroxy acids of C_{16}s and C_{18}s (particularly densipolic acid), and the lowest C_{20}-hydroxy acid content. In addition, Class I oils are unique among the lesquerella oils for possessing significant amounts (0.4–0.6%) of a C_{16} diene, as observed previously (4). The content of ricinoleic (8–12%) and densipolic (41–47%) acids reported here are higher than those previously found (4,7), with the exception of Bender and Lee (5) for *L. densipila* oil, where our results closely match theirs. We attribute the discrepancies to our improved methodology, where during FAME formation all estolide bonds were broken (demonstrated *via* SFC). The seeds from Class I species contain 23–29% oil, with *L. lescurii* having the highest content (Table 1). These percentages are comparable with other members of *Lesquerella* (Table 1).

SFC/lipase reaction analysis indicated that the majority of TG from Class I oils contained four (77–84%) or five (13–19%) acyl groups, suggesting the presence of estolides (Fig. 2a; Table 3). For example, oil from *L. perforata* contains 1.12–1.30 estolides per TG (Table 2). To our knowledge, this is the first report of naturally-occurring estolides present in these oils. Analysis of the (1,3-specific) lipase-catalyzed reactions shows that the only MG species formed contain C_{18} unsaturated acyl groups (Fig. 2a), indicating that the estolides are present solely at the 1- and 3-glycerol positions. Of the estolide esters and estolides formed during decanolysis and hydrolysis, respectively, all contain only two acyl groups (Fig. 2a). Moreover, only monoestolides (E^{1}) occur. The most common estolides present (84–87%) possess two 18-OH groups (Table 3). Thus, the majority of estolides contain a free hydroxyl group. Table 3 also lists the composition of the acyl groups released during lipolysis, i.e., acyl groups at the 1- (3-) position not part of estolides. A large percentage of

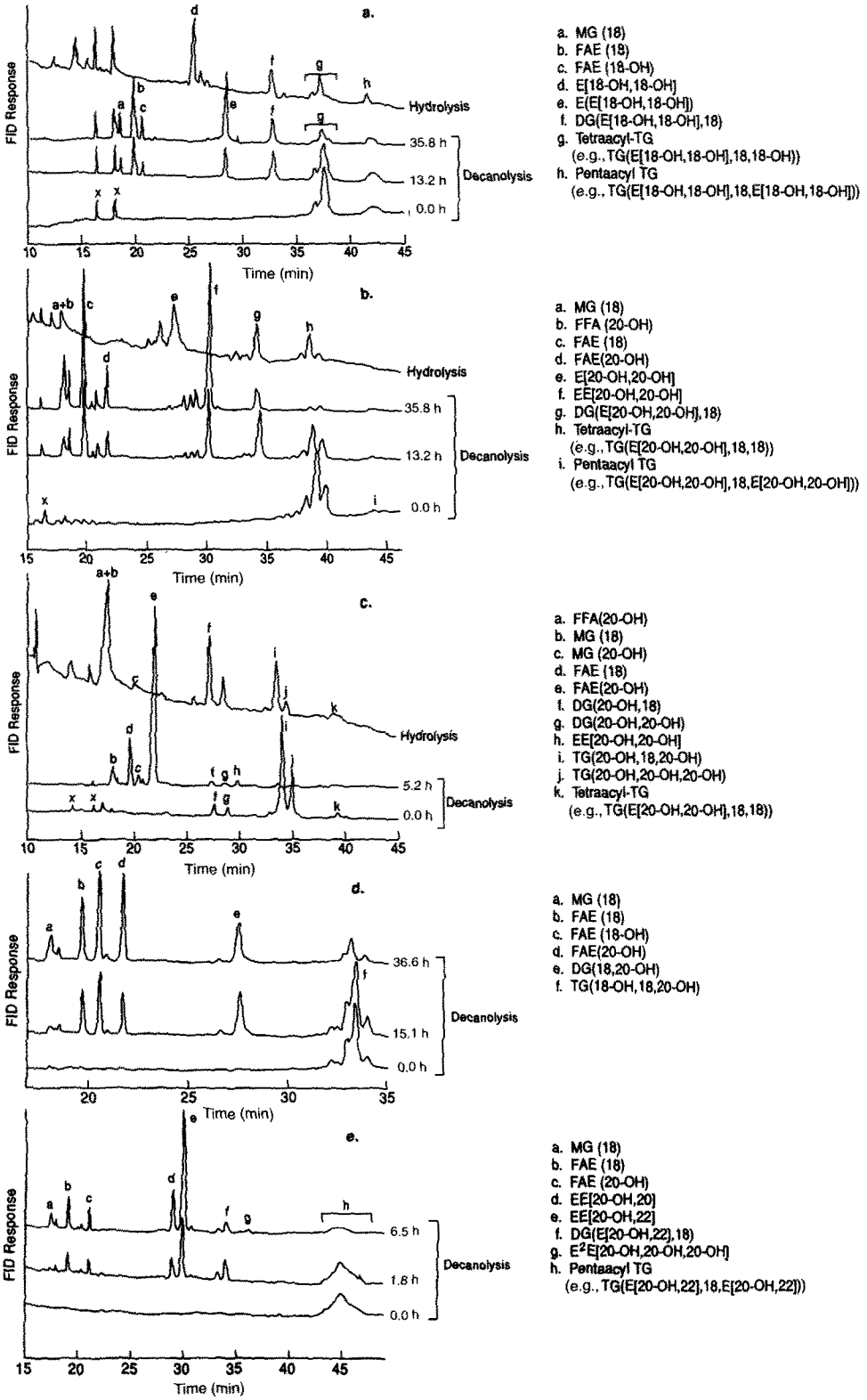

FIG. 2. Supercritical fluid chromatography traces for lipase-catalyzed decanolysis (with reaction times listed) and hydrolysis of *Lesquerella* oils at 25°C. (a) *L. lescurii* (Class I) oil; (b) *L. auriculata* (Class II, auricolic acid-rich) oil; (c) *L. recurvata* (Class III) oil (performed at 43°C); (d) *L. ludoviciana* (Class IV, a mixture of hydroxy acids) oil; and (e) *Heliophilia amplexicaulis* oil. Peaks labelled x are contaminants resulting from the seed oil extraction step. These materials played no role in the lipase-catalyzed reactions and could be used as internal standards. Abbreviations: MG, monoglycerides; FAE, fatty acid decyl ester; DG, diglyceride; TG, triglyceride; FFA, free fatty acid; FID, flame-ionization detector; E[x,y], monoestolide containing acyl groups x and y; EE[x,y], monoestolide decyl ester; $E^2E[x,y,z]$, diestolide decyl ester containing acyl groups x, y, and z. See Figure 1 for Class I and III explanations.

TABLE 2
A Comparison of Results Measured by ^{1}H Nuclear Magnetic Resonance (NMR) and Calculated from Our Determined Triglyceride (TG) Structure for *Lesquerella* Oils

Species	Class	NU#	Free hydroxyls/ TG (NMR)	Free hydroxyls/ TG (calc)[a]	Estolide/ TG (NMR)	Estolide/ TG (calc)[a]
Soybean oil			0.00	0.00	0.00	0.00
L. perforata	I	41679[b]	1.12	1.30	0.80	1.15
L. auriculata	II	49322	1.08	1.07	0.96	1.01
L. fendleri	III	[c]	1.76	1.72	0.00	0.00
L. ludoviciana	IV	61380	1.55	1.57	0.00	0.00
Heliophilia amplexicaulis	V	62350	0.00	0.00	2.00	1.96

[a]Determined from composition of fatty acid and monoglyceride released during lipolysis and estolide esters formed during lipase-catalyzed decanolysis and number of acyl groups per TG from supercritical fluid chromatography analysis. See Table 1 for Class and NU# explanations.
[b]Oil composition used in calculations was from NU# 60662.
[c]Refined *Lesquerella* oil.

TABLE 3
Analysis of Class I and II Oil Triglycerides (TGs) from Supercritical Fluid Chromatography (SFC) and Lipase-Catalyzed Reactions

Species	*Lesquerella densipila*	*L. lescurii*	*L. lyrata*	*L. perforata*	*L. stonensis*	*L. auriculata*
NU#	60661	41683	41681	60662	60663	49322
Class	I	I	I	I	I	II
TG[a]						
Triacyl TG	2.9	3.2	4.8	2.3	4.3	1.4
Tetraacyl TG	84.1	79.9	76.8	80.8	77.6	96.1
Pentaacyl TG	13.0	17.0	18.4	16.9	18.1	2.5
Fatty acid composition, 1- and 3-position[b]						
16:0	11.0	12.0	10.9	10.3	12.2	12.2
16:1	1.1	1.3	1.8	1.7	1.4	1.6
18:0	8.6	11.2	9.0	8.8	9.0	13.9
$18:1^9$	28.8	32.2	37.5	30.2	27.0	32.4
$18:1^{11}$	6.0	7.4	7.4	6.7	8.5	4.2
18:2	0.0	1.3	2.1	2.6	0.9	1.3
18:3	6.8	8.8	8.5	9.5	7.3	6.7
20:0	0.8	1.6	1.0	0.9	0.8	0.9
20:1	0.7	1.1	0.8	0.8	0.8	5.0
16:1-OH	0.0	0.0	0.4	0.0	0.0	0.0
18:1-OH	4.3	2.8	3.0	3.9	3.3	0.7
18:2-OH	30.6	20.3	16.8	23.9	27.8	1.5
20:1-OH	0.9	0.0	0.7	0.5	1.1	6.8
20:2-OH	0.3	0.0	0.0	0.4	0.0	9.5
Estolide structure[c]						
E^1[16-OH,18-OH]	3.2	3.6	3.1	3.6	3.9	1.7
E^1[18-OH,18]/E^1[20-OH,16]	3.3	5.2	4.2	3.3	4.0	2.3
E^1[18-OH,18-OH]	85.9	84.0	85.4	86.7	83.9	5.1
E^1[18-OH,20]E^1[20-OH,18]	1.9	1.8	1.3	0.0	1.5	6.3
E^1[18-OH,20-OH]	0.0	0.0	0.0	0.0	0.0	8.1
E^1[20-OH,20-OH]	0.0	0.0	0.0	0.0	0.0	72.8

[a]From SFC analysis of TG. See Table 1 for NU# and Class explanations.
[b]Composition of fatty acids released during 1,3-specific lipolysis (gas chromatography analysis).
[c]Apparent estolide composition (products from lipase-catalyzed decanolysis of seed oils SFC analysis). E^1[x,y] refers to monoestolide containing acyl groups x and y.

these acyl groups are saturated fatty acids. Moreover, 56–68% of the palmitic acyl groups in the Class I oils are at the 1- (3-) position (and not part of estolides). In addition, of the C_{18} unsaturates, a much larger portion of oleic acyl groups (33–41%), belong to this grouping than C_{18} polyunsaturates, such as linolenic acid (16–22%). Thus, the most abundant TG component of Class I oils contains C_{18} acyl groups at the 1- (or 3-) and 2-positions and a monoestolide composed of two 18-OH groups at the remaining position [TG(18,18,E^1[18-OH,18-OH])]. This TG is diagrammed in Figure 1. The most

common pentaacyl TG contains monoestolides with two 18-OH groups at the 1- and 3-positions and a C_{18} unsaturate at the 2-position. Note also that the most common DG species formed has a MW that agrees with these TG assignments, namely DG(18,E^1[18-OH,18-OH]).

Class II (auricolic acid-rich) oil. Lesquerella auriculata oil is the only oil of the genus known at this time to be high (38%) in auricolic acid, the C_{20} analog of densipolic acid (Table 1). The oil's fatty acid composition is homologous to Class I oils. For example, *L. auriculata* oil contains a relatively high overall content of palmitic and stearic acids, and of the C_{18} unsaturates, oleic is the most common (Table 1). Indeed, the amounts of C_{18} unsaturates relative to each other in Class I and II oils are quite similar. The oil has several different acyl groups of interest present at small quantities, including 22:0, 22:1 (erucic acid), and 16:1-OH (determined by GC). In general, our fatty acid analysis agrees closely with the previously reported data (11), but is more detailed. *Lesquerella auriculata* oil's TG structure is also homologous to that just presented for Class I oils. Moreover, the oil contains 1.07 estolides per TG (Table 2) and contains mostly (96.1%) tetraacyl TG (Table 3). This latter percentage is slightly higher (*ca.* 85%) than reported previously (11). Perhaps the difference is due to the previous investigation (11) failing to take into account the presence of pentaacyl TG. The triacyl TG present probably does not contain any 20-OH acyl groups due to their low MW (SFC analysis). The vast majority (at least 81%) of 20-OH groups are present in estolides. Similar to that described for Class I oils, no polyestolides were detected, and the (mono-) estolides are located only at the 1- and 3-glycerol positions. The (2-)MG formed during the lipase reactions are mostly (over 80%) C_{18} unsaturates. Most of the estolides contain two hydroxy acyl groups; moreover, the most common estolide species (72.8%) is E^1[20-OH,20-OH] (Table 3). Based on our results and calculations, the most common TG is TG(18,18,E^1[20-OH,20-OH]), as identified in Figure 2b. [The peak to its immediate right, at retention time 39.5 min, is probably TG(20-OH,18,E^1[20-OH,20-OH]).] Also, like Class I oils, the majority of saturates, such as palmitic acid (82%), are located in the 1-(3-) positions (not containing estolides), and more oleic acid (32%) is located in this position than C_{18} polyunsaturates, such as linolenic acid (21%). The composition of DG from lipolysis/alcoholysis supports the determined TG structure; 75% of DG belong to a SFC peak with MW equivalent to DG(18,E^1[20-OH,20-OH]).

Class III (lesquerella acid-rich) oils. In general, the TG structure and fatty acid composition of the 19 Class III oils are quite similar to those described already for *L. fendleri* oil (7); namely, the oils have a large (50–90%) proportion of lesquerolic acid (Table 1), and the most common TG type has two 20-hydroxy acid groups at the 1- and 3-positions and a C_{18} unsaturated acyl group at the 2-position, as illustrated in Figure 1. [Minor components of *L. fendleri* oil are discussed elsewhere (32)]. Table 4 contains the relative amounts of fatty acids released during 1,3-specific lipolysis. The results show that 1,3-specific lipolysis greatly concentrates the C_{20} hydroxy acids and the C_{16} and C_{18} saturates, as was obtained previously for *L. fendleri* oil (10). Moreover, calculations show that 61-100% of the lesquerolic acyl groups are located at the 1- and 3-positions (Table 4). (Essentially all of the saturated acyl groups are located at the 1- and 3-positions.) In all but one case (*L. lindheimeri*), the most abundant TG was TG(20-OH,18,20-OH), while in most cases, the second most abundant was TG(18,18,20-OH) (Table 5). However, several oils contained significant amounts of TG(20-OH,20-OH,20-OH), which is homologous to the most common TG of castor oil, TG(18-OH,18-OH,18-OH). These oils (*L. lindheimeri, L. gracilis* subsp. *gracilis, L. recurvata, L. purpurea,* and *L. inflata*), as expected, have the largest proportions of lesquerolic acid (Table 1). However, with the single exception of *L. lindheimeri* oil, the percentage of TG(20-OH,20-OH,20-OH) is significantly less than the probability of this TG occurring based on random distribution of 20-OH acyl groups.

Oils from *L. angustifolia, L. globosa,* and *L. gracilis* subsp. *gracilis* are the only Class III oils that contain significant amounts of ricinoleic acid (Table 1). In a previous report (6), the ricinoleic acyl groups of *L. globosa* oil were found exclusively in a trihydroxy acid TG fraction. Likewise, when we separated *L. fendleri* oil TG by silica-gel column chromatography, we found all 18-OH acyl groups to be present in TG(20-OH,18-OH,20-OH) (Hayes, D.G., and R. Kleiman, submitted for publication). In agreement, for *L. gracilis* subsp. *gracilis* oil, 19.6% of TGs were TG(20-OH,18-OH,20-OH) (Table 5), but for *L. angustifolia* oil, the percentage of TG(20-OH,18-OH,20-OH) is reported as zero (Table 5). This occurred probably because the large TG(20-OH,18,20-OH) overlapped and hid the small TG(20-OH,18-OH,20-OH) peak. Results from 1,3-specific lipolysis of the trihydroxy acid TG fraction of *L. fendleri* oil indicate that ricinoleic acid is located mostly at the 2-position (Hayes, D.G., and R. Kleiman, submitted for publication). However, for many Class III oils, the proportion of ricinoleic acyl groups at the 1- and 3-positions is significant: >33.3% (Table 4).

Only *L. fendleri, L. ovalifolia, L. gordonii, L. tenella,* and *P. floribunda* oils contained significant amounts (1.5–5%) of auricolic acid (Table 1). It is interesting that auricolic acid is slightly less prone to be located at the 1- and 3-glycerol positions than is lesquerolic acid (Table 4).

Concerning C_{18} unsaturates, oleic acid was the most abundant, although all Class III oils had significant quantities of vaccenic ($18:1^{11}$), linoleic (18:2), and linolenic (18:3) acids. Among the C_{18} unsaturates, oils from *L. fendleri, L. ovalifolia* subsp. *ovalifolia, P. floribunda, L. mirandiana,* and *L. engelmanii* contained large percentages (20–35%) of linolenic acid, similar to Class I and II oils, while oils from *L. angustifolia, L. grandiflora, L. lasiocarpa* (including subsp. *lasiocarpa*), and *L. lindheimeri* contained only small quantities. It appears that oils with high linolenic acid also have high auricolic acid content. In agreement, fractionation of *L. fendleri* oil shows that auricolic acid and linolenic acid are more likely to belong in the same TGs (Hayes, D.G., and R. Kleiman,

TABLE 4
Analysis of Fatty Acids Released from 1,3-Specific Lipolysis and the Percent Distribution of Several Acyl Groups in the 1- and 3-Glycerol Positions for Class III and IV *Lesquerella* Oils

		Percentage among acyl groups released during 1,3-specific lipolysis										Proportion (%) of acyl group bonded to 1- and 3-TG positions				
Species	NU#	16:0	18:0	$18:1^{9}$	$18:1^{11}$	18:2	18:3	20:1	18:1-OH	20:1-OH	20:2-OH	18:1-9	18:3	18:1-OH	20:1-OH	20:2-OH
L. angustifolia	60665	2.7	2.0	4.9	2.1	2.2	0.5	1.2	1.1	82.2	0.2	0.27	0.15	0.14	0.82	
L. argyraea	60692	2.3	3.2	5.1	1.7	2.2	0.9	1.1	0.3	81.5	1.0	0.26	0.11		0.89	0.56
L. engelmanii	40926	2.2	1.9	7.0	2.3	2.7	1.5	1.1	0.5	76.6	3.2	0.37	0.13	0.37	0.86	0.82
L. fendleri	64261	1.4	2.1	3.9	1.9	1.4	2.1	1.0	0.2	80.5	5.1	0.18	0.11		1.00	1.10
L. gordonii	40816	1.9	1.9	6.6	2.1	1.5	1.2	1.1	0.3	80.3	2.1	0.23	0.14		0.91	0.78
L. gracilis	49338	2.1	1.4	3.9	1.4	1.0	0.6	1.3	1.7	85.6	0.0	0.27	0.12	0.14	0.81	
L. grandiflora	40875	2.2	4.2	10.3	2.9	1.9	0.5	1.7	0.4	74.8	0.3	0.29	0.33		0.84	
L. inflata	49618	1.4	1.9	2.7	7.8	2.6	0.8	1.5	0.0	80.9	0.2	0.23	0.12	0.0	0.80	
L. lasiocarpa	33090	3.3	5.1	13.2	3.2	2.6	0.9	2.3	1.4	66.1	0.5	0.35	0.26	0.62	0.79	
L. lasiocarpa	40812	2.8	4.5	9.6	2.6	2.1	1.2	2.6	1.0	72.1	0.6	0.27	0.53		0.80	
L. lindheimeri	33082	3.8	2.0	13.9	2.6	5.8	0.8	2.5	0.7	66.2	0.0	0.66	0.44		0.61	
L. mirandiana	44760	3.7	3.2	13.6	2.7	5.1	3.0	1.2	0.5	63.1	1.3	0.60	0.29		0.73	0.62
L. pinetorum	64507	5.8	3.7	17.1	4.5	7.9	2.0	1.7	0.9	54.0	0.0	0.59	0.34		0.71	
L. purpurea	64508	2.6	3.5	5.6	3.0	3.7	1.5	1.9	0.4	75.8	0.8	0.34	0.22		0.76	0.44
L. recurvata	44505	1.2	1.2	5.9	3.2	3.0	1.3	2.1	0.3	80.2	0.8	0.35	0.19		0.76	0.48
L. tenella[a]	45462	3.7	3.0	13.8	3.1	2.9	2.6	1.7	0.4	65.3	2.1	0.45	0.30		0.81	0.78
L. tenella	64487	3.9	2.8	12.0	2.8	2.3	2.0	1.8	0.5	68.6	2.1	0.41	0.23		0.78	0.74
L. floribunda	52582	2.3	1.2	7.2	2.1	3.1	2.1	0.9	0.4	78.3	2.0	0.28	0.15		0.98	0.83
L. ludoviciana[b]	61380	2.5	0.4	9.3	3.3	1.3	6.3	1.0	18.3	36.4	5.3	0.41	0.23	0.95	0.89	0.88

[a]Originally identified as *L. palmeri* but is *L. tenella* (Dierig, D., private communication). See Table 1 for NU# and Class explanations. Abbreviations as in Table 1.
[b]Class IV; 15% 18:2-OH among acyl groups released *via* 1,3-specific lipolysis; 0.99 equals proportion of 18:2-OH acyl groups in 1-(3-) position.

TABLE 5
Triglyceride (TG) Composition and Assignments for Class III *Lesquerella* Oils

			T54[a,b]			18,18-OH,20-OH				Tetraacyl	Tetraacyl
Species	NU#	T48-53[a]	16,18,20-OH	T54[a]	18,18,20-OH	20,18,20-OH	20-OH,18,20-OH	20-OH,18-OH,20-OH[c]	20-OH,20-OH,20-OH	TG[d]	TG[e]
L. angustifolia	60665	0.4	0.0	1.2	4.7	3.3	75.0	0.0	14.7	0.0	0.0
L. argyraea	60692	0.6	0.9	1.8	12.3	0.0	74.6	0.0	7.0	0.0	0.0
L. engelmanii	40926	0.9	0.8	2.3	13.1	0.0	73.0	0.0	6.2	0.0	0.0
L. fendleri	64261	1.3	2.0	2.4	12.5	0.0	81.1	0.0	0.0	0.0	0.0
L. gordonii	40816	0.3	0.0	1.2	6.9	0.0	74.2	0.0	12.6	0.0	0.0
L. gracilis	49338	0.4	0.4	0.9	2.3	3.3	44.6	19.6	25.9	0.0	0.0
L. grandiflora	60659	0.0	0.0	2.3	18.5	0.0	64.2	0.0	7.3	1.4	0.0
L. inflata	49618	0.0	0.4	0.8	4.5	1.0	68.5	0.0	23.3	0.0	0.3
L. lasiocarpa	33090	1.4	2.4	4.2	19.5	7.1	56.9	2.1	0.8	3.5	0.0
L. lasiocarpa	40812	0.5	1.4	4.1	13.4	6.7	67.9	2.2	0.0	2.4	0.0
L. lindheimeri	33082	0.3	0.6	0.0	3.7	2.1	42.2	0.0	43.0	0.1	0.7
L. mirandiana	44760	2.7	2.9	1.7	9.3	0.0	45.8	0.0	4.8	0.0	0.0
L. pinetorum	64507	3.0	1.5	4.4	17.2	0.0	64.3	0.0	0.0	0.0	4.5
L. purpurea	64508	0.4	0.8	1.2	4.7	1.4	66.3	0.0	24.5	0.0	0.0
L. recurvata	44505	0.9	0.2	0.1	2.2	1.4	54.8	0.0	24.8	0.0	4.3
L. tenella	45462	2.4	0.9	4.0	12.0	0.0	77.1	0.0	1.6	0.0	0.0
L. tenella[f]	64487	1.6	1.1	3.6	10.7	0.0	76.4	0.0	2.1	0.0	0.0
L. floribunda	52582	0.6	0.7	2.7	17.8	0.0	73.6	0.0	0.0	0.0	0.0

[a]48 Refers to TG containing carbon number 48, etc. See Table 1 for NU# and Class explanations.
[b]Contains large amount of saturated acyl groups.
[c]May be hidden in TG(20-OH,18,20-OH) peak. See Figure 2c.
[d]TG have molecular weights similar to Class I TG.
[e]TG have molecular weights similar to Class II TG.
[f]Originally identified as *L. palmeri* but is *L. tenella* (D. Dierig, private communication).

submitted for publication). In addition, polyunsaturated C_{18}s were less likely than $C_{18:1}$s to be located at the 1- and 3-positions (Table 4), as was true for Class I and II oils (discussed above) and for several Brassicaceae oils (33).

A few of the oils had a small percentage of tetraacyl TG (Table 5), with *L. pinetorum* and *L. recurvata* (Fig. 2c) having the largest percentage (4–5%). Oils from *L. pinetorum, L. recurvata, L. inflata,* and *L. lindheimeri* had tetraacyl TG with MW identical with those contained in *L. auriculata* oil (Class II). Indeed, small-to-trace amounts of E^1[20-OH,20-OH] decyl ester were detected in these oils after alcoholysis, as well as in oils from *L. gordonii, L. lasiocarpa* subsp. *lasiocarpa, L. mirandiana, L. purpurea,* and *L. tenella*. Also, tetraacyl TG of smaller MW, with retention times near those for Class I TG, were found in oils from *L. grandiflora, L. Lasiocarpa* (including subsp. *lasiocarpa*), and *L. linheimeri*. In agreement, these oils, as well as oils from *L. mirandiana, L. pinetorum,* and *L. tenella*, were found to have trace quantities of E^1[18-OH,18-OH].

Class IV (mixture of hydroxy acids) oil. Lesquerella ludoviciana oil is the most unusual oil examined here because it contains a mixture of ricinoleic (16.2%), densipolic (13.8%), lesquerolic (31.9%), and auricolic (5.0%) acids. However, the oil's structure is quite similar to those discussed above for Class III. For example, lipolysis results indicate the absence of estolides (Fig. 2d). In addition, trends discussed above concerning $C_{16/18}$ saturates and unsaturates and glyceride position for Class III oils are matched (Table 4). (Linolenic acid composes 45% of the C_{18} unsaturates.) Furthermore, like Class III oils, hydroxy acids are present mostly in the 1- and 3-positions (Table 4). The most common TG species, based on calculations and SFC results, are TG(18-OH,18,20-OH), followed by TG(18,18,20-OH).

Class V (lesquerolic and erucic acid-rich) oil. The oil from *H. amplexicaulis*, like Class III oils, has a large content (35.1%) of lesquerolic acid (and lesser amounts of 18:1, 20:2, and 22:1 hydroxy acids), but in contrast, it possesses large quantities of long-chain acyl groups, e.g., 7.2% 20:1 and 23.0% 22:1 acids (Table 1). The hydroxy acid content we determined was larger than that previously reported (12). The previous study (12) showed that the majority of the oil's TG contained five acyl groups (HPLC) and lacked free hydroxy groups (infrared spectroscopy). Our results, displayed in Figure 2e, agree with their findings. More specifically, we found that the oil was composed of 96% pentaacyl TG and 4% tetraacyl TG. Lipase-catalyzed 1-,3-specific alcoholysis yielded monoestolide decyl esters in the major product (Fig. 2e). The monoestolide composition was 75% E^1[20-OH,22], 22% E^1[20-OH,20], 3% E^1[20-OH,24]/E^1[22-OH,22], and 1% E^1[20-OH,18]. No monoestolide-containing MG were produced; only C_{18} unsaturate MG were detected. Thus, as encountered for Class I and II oils, estolides are bonded exclusively at the 1-(3-) TG positions. The apparent composition of DG produced agrees with these assignments. But unlike the Class I and II estolide-containing oils, *H. amplexicaulis* oil contained a small quantity of diestolide. Peak g of Figure 2E has been attributed to the species E^2[20-OH,20-OH,20] decyl ester, based on the SFC MW calibration. Of the small quantity of fatty acid decyl esters produced, *ca.* 9% were 16:0, 4% 18:0, 43% C_{18} unsaturate (mostly oleic), 2% C_{18}-OH, 2% 20:0, 9% C_{20} unsaturate, 29% C_{20}-OH, 2% 22:0, and 3% 22 unsaturate. Similar to the results encountered for Class I–IV oils and other Brassicaceae (33), the saturated acyl groups are encountered mostly at the 1-(3-) positions without being part of estolides. Based on our analysis, 75% of acyl groups belong to monoestolide and 3% to diestolide. In addition, >95% of C_{22} unsaturates and hydroxy acyl groups are present in the monoestolides.

TG structure and chemotaxonomic relationships. In general, the prominence of saturated, long-chain, and unusual acids (hydroxy acids; those with non-Δ9 unsaturation, etc.) for the 1- and 3-positions in *Lesquerella* TG is similar to the already studied *L. fendleri* oil TG (7) as well as other Class I oil TG (33). Of the oils that were reported to contain naturally occurring estolides, most, like the oils investigated here, contained estolides almost solely in the 1-(3-) position (12–19), while only two oils had estolides in all positions (20,21) and one had estolides solely in the 2-position (22). Those that are members of the family Brassicaceae, *Cardamine impatiens* (16), *H. amplexicaulis* (12), and members of the genus *Lesquerella* (11 and above references), have estolides solely at the outer positions. However, the Class I and II *Lesquerella* oils are unique among all of the estolide-containing oils for having monoestolides mostly with a free hydroxyl group.

In general, Class I (densipolic acid-rich) species of *Lesquerella* are native to the southeastern U.S. (Tennessee, Alabama), while Class III species originate from the Southwest (Texas, Oklahoma, New Mexico, and Arizona). Rollins and Shaw (34) reported that *L. auriculata* (Class II) is "in a pivotal position between the western auriculate species and those of the most easterly area." *Lesquerella auriculata* shares the same chromosome number, 8, as its Class I relative, while only *L. argyrae* subsp. *argyraea* of the Class III species studied here shared the same chromosome number (1,34). But, the range of *L. auriculata* encompasses eastern Oklahoma and Texas, which comprises the eastern edge of the range for most Class III species (34). *Lesquerella ludoviciana* (Class IV) is native to the Great Plains states (e.g., eastern Colorado, Wyoming, Nebraska, and western North Dakota and South Dakota) above the 39th Parallel and has been found as far east as Illinois and Minnesota (34). Those *Lesquerella* spp. of Class III whose oils contain significant amounts of ricinoleic acid grow in the eastern range of the Class III species, e.g., southeast Oklahoma and eastern Texas (*L. angustifolia* and *L. gracilis*), or in Tennessee, Kentucky, or southern Indiana (*L. globosa*) (34). In general, those Class III species containing oils rich in auricolic acid correlate with species containing a chromosome number of 6 (oils from *L. argyraea* subsp. *argyraea, L. fendleri, L. globosa, L. gordonii,* and *L. ovalifolia* subsp. *ovalifolia*) (34). However, two exceptions to this rule exist for species containing a chromosome number of 6: *L. gracilis* subsp. *gracilis* and *L. lindheimeri* (34) (oils from

these two species have a larger proportion of lesquerolic acid than other oils of species containing a chromosome number of 6). In addition, the Class III *Lesquerella* species whose oils contain the highest amounts of tetraacyl TG are native to the southern tip of Texas (34).

The similarities of oil content and TG structure between *P. floribunda* and Class 3 members of *Lesquerella* are quite readily observed. In addition, *P. floribunda*, like Class III *Lesquerella* species, is native to the American Southwest (35). The taxonomic similarities between *Physaria* and *Lesquerella* have been well documented (36).

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