Enrichment of Lesquerolic and Auricolic Acids from Hydrolyzed *Lesquerella fendleri* and *L. gordonii* Oil by Crystallization

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ABSTRACT: Lesquerolic and auricolic acids were obtained from hydrolyzed lesquerella oil by a low-temperature crystallization procedure. The lesquerolic and auricolic fatty acid fraction was enriched from 55–59% to 85–99% with high yields (94%). Washing the free fatty acids with pH 6.0 buffer provided reproducible crystallizations of those hydroxy fatty acids. In contrast, when hydrolyzed oil from *Lesquerella fendleri* was not buffer-washed, there was, in most cases, no separation of hydroxy fatty acids by crystallization. This crystallization procedure is suitable for a large-scale separation process of the hydroxy fatty acids from nonhydroxy fatty acids obtained from hydrolyzed lesquerella oil.

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KEY WORDS: Auricolic acid, buffer, crystallization, hydroxy fatty acids, lesquerella, lesquerolic acid, pH.

Lesquerella fendleri and *L. gordonii* are members of the genus *Lesquerella* of the Brassicaceae (mustard) family (1). Over 70 lesquerella species are known, and they are native to the southwestern United States, Texas, and northern Mexico (1). In recent years, there has been an effort to domesticate the wild species of *L. fendleri* (2). The oils derived from *L. fendleri* and *L. gordonii* are attractive due to their high content of hydroxy fatty acids (1,3,4). The major hydroxy fatty acid (53%) is 14-hydroxy-*cis*-11-eicosenoic acid (lesquerolic acid) (3), a homolog to ricinoleic acid. Furthermore, hydrolyzed lesquerella oil also contains a significant amount (3%) of 14-hydroxy-*cis*-11, *cis*-17-eicosadienoic acid or auricolic acid. Ricinoleic acid is a much sought-after hydroxy fatty acid from castor oil, which is imported to the United States at the 50,000 metric ton level every year (1). Therefore, there have been several attempts to isolate and purify the lesquerolic and auricolic acids from lesquerella oil by using lipases in a selective hydrolysis (5,6) or by preparative chromatography (7). There have also been attempts to synthesize lesquerolic acid by a Kolbe condensation (8). However, neither of these methods is suitable for large-scale production of lesquerolic and auricolic acids (9). We now report a method for increasing the purity of hydroxy acids from hydrolyzed lesquerella oil by crystallization.

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EXPERIMENTAL PROCEDURES

Materials. Lesquerella fendleri oil was supplied by International Flora Technologies, Ltd. (Apache Junction, AZ). *Lesquerella gordonii* oil was made available through earlier work of Carlson *et al*. (7). Methanol, hexane, and acetone were obtained from Fisher Scientific Co. (Fairlawn, NJ). Ethanol was obtained from Quantum Chemical Corp. (Tuscola, IL). Potassium hydroxide was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ), and monobasic phosphate and dibasic phosphate were obtained from EM Science (Gibbstown, NJ). Hydrochloric acid was obtained from Chemical General (Parsippany, NJ). Lipase sp435 was obtained from Novo-Nordisk Inc. (Danbury, CT). Filter paper was obtained from Whatman (Maidstone, England).

Instrumentation. High-pressure liquid chromatography (HPLC) analysis of fatty acid mixtures was carried out with a Thermo Separations (Fremont, CA) spectra system AS1000 autosampler/injector and a P2000 binary gradient pump, coupled to a Varex ELSD IIA light scattering detector (Alltech Associates, Inc., Deerfield, IL). A Phenomex silica (25 cm × 4.6 mm, 60Å, 8 μm) column from Rainin Instrument Co. (Woburn, MA) was used to separate the hydroxy fatty acids with an isocratic hexane/acetone (70:30) solvent system. Retention times for eluted peaks were 3.6 and 4.2 min for the unsaturated fatty acids and 4.9 min for the hydroxy fatty acids (all at 1 mL/min flow rate).

Gas chromatography (GC) of esterified hydroxy fatty acids was performed in a Hewlett-Packard 5890 Series II GC (Palo Alto, CA), equipped with a flame-ionization detector and an autosampler. A Hewlett-Packard Chemstation provided data collection and integration. A Supelco SP2380, 30 m × 0.25 mm column (Bellefonte, PA) was used to separate fatty acid ethyl esters (FAEE) by means of a programmed ramp of 150 to 175°C at 2°C/min, 175 to 220°C at 5°C/min, 220 to 265°C at 20°C/min and a hold of 2 min at 265°C; injector and detector temperatures were set at 250°C; head pressure was 20 psi

He. FAEE were made at room temperature overnight, by reacting 1 mmol free fatty acids with 20 mmol ethanol in 10 mL hexane with 35 mg lipase sp435, derived from *Candida antarctica* as catalyst. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained on a Bruker ARX-400 (Karlsruhe, Germany) with a 5-mm dual proton/carbon probe and $CDCl_3$ as a solvent.

^1H NMR of lesquerolic/auricolic acids: δ 5.50-5.46 (*m*, 1H, $–CH_2–CH=CH–$), 5.36–5.33 (*m*, 1H, $–CH=CH–CH_2–$), 3.59–3.57 [*m*, 1H, $–CH_2–CH(OH)–CH_2$], 2.28 (*m*, 2H, $–CH_2–CH_2–CH–$), 2.17 (*m*, 2H, $=CH–CH_2–CH_2–$), 2.02–1.21 (*m*, 24H, $–CH_2–$) and 0.84 ppm (*t, J* = 6.33, 3H, $–CH_2–CH_3$). ^{13}C NMR of lesquerolic/auricolic acids: δ 179.2 (*s*), 133.0 (*d*), 125.0 (*d*), 71.6 (*d*), 36.5 (*t*), 35.0 (*t*), 34.0 (*t*), 31.7 (*t*), 29.6 (*t*) 29.5 (*t*), 29.3 (*t*), 29.2 (*t*), 29.1 (*t*), 29.1 (*t*), 29.1 (*t*), 29.0 (*t*), 27.3 (*t*), 25.5 (*t*), 24.6 (*t*), 22.5 (*t*), and 14.0 ppm (*q*).

Lesquerella oil (941 g, 1 mol) was hydrolyzed with 2.2 M KOH in ethanol (1.5 L) at reflux for 3 h. The conversion was measured by HPLC after acidifying with 2 M HCl and drying over $MgSO_4$. At complete conversion, the saponified mixture was acidified to pH 7 by 2 M HCl at 0°C and extracted with hexane. The free fatty acids from *L. fendleri* were then washed with saturated phosphate buffer (pH 6, 127 g Na_2HPO_4, 0.1 g NaH_2PO_4 in 1 L H_2O), dried over $MgSO_4$, and concentrated *in vacuo* to yield a yellow liquid.

Crystallization of hydroxy fatty acids from *L. fendleri*: Samples (5 g) of the washed free fatty acids were dissolved in 15–50 mL hexane and chilled to –25°C overnight. Crystallization of hydroxy acids from *L. gordonii*: Samples (5 g) of washed free fatty acids were dissolved in 15 mL hexane and chilled to –25°C overnight. Preparative-scale crystallization was accomplished by dissolving 100–150 g of *L. fendleri* free fatty acids in 500–750 mL hexane and placing in the freezer at –25°C overnight. Crystallization temperatures were obtained with dry ice/ethylene glycol (–10°C), a freezer (–25°C), or dry ice/isopropanol (–70°C).

The crystals were collected on a #1 Whatman filter paper in a Buchner funnel that had been cooled in the freezer at –25°C, and were washed one time with cold hexane (–25°C). The crystals were quickly transferred to a round-bottom flask and allowed to melt. The melted crystals were taken up in acetone and dried over magnesium sulfate and then filtered. The acetone was removed *in vacuo.*

RESULTS AND DISCUSSION

The difference in solubility and melting point has been used several times before (9–12) to separate mixtures of fatty acids by crystallization. It is possible to achieve a far more economical process by using crystallization techniques instead of column chromatography or distillation. In addition, distillation of lesquerella fatty acids easily forms estolides, or they dehydrate to form polymers (13–15). Therefore, separation of lesquerolic and auricolic fatty acids from the unsaturated fatty acids present in lesquerella oil is satisfactorily achieved by chromatographic methods by utilizing the difference in polarity, or by crystallization/extraction by utilizing the difference in solubility. Considering the options, from an equipment and cost perspective, our crystallization procedure likely will be favored on an industrial scale.

The fatty acid mixture, obtained by hydrolysis of lesquerella oil, was dissolved in hexane and chilled at –25°C overnight. The hydroxy fatty acids crystallize due to their limited solubility in hexane at low temperature (Table 1). The separation is excellent and provides pure hydroxy fatty acid crystals (85–99%) in high yields, with little hydroxy fatty acids in the mother liquor (1–17%). The amount of hexane used varied from 3 to 10 times (vol/wt) the hydrolyzed fatty acids, depending on desired crystal purity. In line with the fundamentals of crystallization, hexane is a good crystallizing solvent when crystallizing a more polar material from a nonpolar mix (Table 1).

Methanol and acetone are also extensively used as crystallization solvents. When acetone was used, lower yields and purity of crystals were obtained. The reduction in yield with acetone and methanol demonstrates the increased solubility of hydroxy fatty acids in solvents of high dielectric strength, even at low temperature (Table 2). In addition, the solubility of the less polar (unsaturated) fatty acids is diminished in these solvents, especially at low temperatures where cocrystallization can occur.

Of the temperatures tested, the optimal temperature for crystallization was –25°C (Table 1). The reason for this temperature dependence is the reduced solubility in hexane of unsaturated fatty acids below –25°C. Furthermore, slow chilling of the solution was less effective than fast chilling to the crystallization temperature (Tables 3 and 4).

TABLE 1
Factors Affecting the Crystallization of Hydroxy Fatty Acids from Hydrolyzed *Lesquerella fendleri* Oil

Solvent (vol/wt)	Temperature (°C)	Lesquerolic and auricolic acids (%)	Yield (%)[a]	pH 6 Buffer washed
Hexane 5:1	–10	97	41	No
Hexane 5:1	–10	77	88	Yes
Hexane 10:1	–10	—	No crystals	No
Hexane 10:1	–10	94	58	Yes
Hexane 3:1	–25	98	89	No
Hexane 3:1	–25	99	85	Yes
Hexane 5:1	–25	94	96	No
Hexane 5:1	–25	94	99	Yes
Hexane 10:1	–25	98	91	No
Hexane 10:1	–25	88	86	Yes
Hexane 3:1	–80	77	84	No
Hexane 5:1	–80	74	90	No
Hexane 10:1	–80	97	58	No
Methanol 5:1	–10	40	0.2	No
Methanol 5:1	–10	13	0.1	Yes
Methanol 5:1	–25	82	6.8	Yes
Acetone 5:1	–10	58	1.4	No
Acetone 5:1	–10	—	No crystals	Yes
Acetone 5:1	–25	38	25	Yes

[a]Theoretical yield of hydroxy fatty acids based on isolated mass and purity.

TABLE 2
Yields and Purity of Hydroxy Fatty Acids from Hydrolyzed *Lesquerella fendleri* Oil When Crystallized at –67°C (for 2 h)

Solvent (vol/wt)	Lesquerolic and auricolic acids (%)	Yield (%)[a]	pH 6 Buffer washed
Hexane 5:1	97	78	No
Hexane 5:1	94	99	Yes
Hexane 10:1	96	40	No
Hexane 10:1	88	99	Yes
Acetone 5:1	83	39	No

[a]Theoretical yield of hydroxy fatty acids based on isolated mass and purity.

The optimal crystallization environment was obtained when the free fatty acid mix from *L. fendleri* oil was first washed with a buffer (pH 6). The reproducibility of the crystallization increased in conjunction with the purity and yield under buffer conditions (Tables 1–4). An additional effect of the pH buffer wash was the ability to crystallize samples that otherwise would not crystallize under normal hydrolysis work-up conditions. Often, when hydrolyzed oil from *L. fendleri* was not buffer-washed, no separation occurred in the subsequent crystallization. The reason for this phenomenon is the difficulty in determining the pH in organic solvents, and thus there is always a risk that some of the fatty acids remain as soap. Even small quantities of soap strongly inhibit the separation of hydroxy fatty acids by crystallization. Furthermore, small remaining quantities of monoglycerides from an incomplete hydrolysis can also have a negative effect on the separation. However, all these negative effects were circumvented by the buffer wash.

Free fatty acids from *L. gordonii* oil contain a higher percentage of lesquerolic acid than the oil from *L. fendleri*, and consequently, it is easier to crystallize the hydroxy fatty acids, even without the buffer wash (data not shown). Solely *L. fendleri* data are presented in this paper because much interest exists for its commercial cultivation.

The best crystallization conditions, as determined from the trials with 5-g samples of hydrolyzed lesquerella oil, were chosen for preparative crystallization. The optimal conditions were 5 equivalents of hexane (vol/wt) and crystallization at –25°C (Table 3).

TABLE 3
Preparative Scale Crystallization (100–150 g) of Hydroxy Fatty Acids from Hydrolyzed *Lesquerella fendleri* Oil (over 48 h)

Solvent (vol/wt)	Temperature	Lesquerolic and auricolic acids (%)	Yield (%)[a]	pH 6 Buffer washed
5:1	–25	96	89	No
5:1	–25	99	94	Yes
10:1	–25	99	89	No
5:1	–80	81	92	Yes

[a]Theoretical yield of hydroxy fatty acids based on isolated mass and purity.

TABLE 4
Effect of pH on Crystallization of Hydroxy Fatty Acids from Hydrolyzed *Lesquerella fendleri* Oil in 5:1 (vol/wt) Hexane[a]

pH of buffer wash[b]	Lesquerolic and auricolic acids (%)	Yield (%)[c]
5	70	92
6	80	86
7	80	88
8	76[d]	93[e]
9	No separation	99[f]

[a]The samples were cooled for 3 h at 4°C and then held at –25°C for 24 h.
[b]0.5 M phosphate buffers.
[c]Theoretical yield of hydroxy fatty acids based on isolated mass and purity.
[d]Mostly as soap.
[e]Combined yield of soap and oil.
[f]All as soap.

In summary, the crystallization of lesquerella free fatty acids is a viable process that might be used with great success to isolate hydroxy fatty acids in high yield and purity.

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