Preparative Chromatographic Isolation of Hydroxy Acids from *Lesquerella fendleri* and *L. gordonii* Seed Oils[1]

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To conduct product development research on *Lesquerella* seed oils, we explored methods to obtain >100 g quantities of lesquerolic (14-hydroxy-*cis*-11-eicosenoic) acid. Preliminary experiments with open-column silica gel chromatography showed that *L. fendleri* oil could be separated into 3 triglyceride (TG) fractions. The first (10%) contained nonhydroxy 16- (13%) and 18-carbon acids (65% 18:1,2,3). The second fraction (15%) contained monolesquerolins (39% lesquerolic acid). The major TG fraction (73%) was mainly dilesquerolins (66% lesquerolic acid) showing that a hydroxy acid-enriched TG oil was obtainable by this procedure. Silica gel chromatography easily separated *L. fendleri* fatty acid methyl esters (FAME) into a hydroxy-free ester fraction (40–44%) consisting largely of 18:1 (39%), 18:2 (19%) and 18:3 (31%), and a hydroxy ester fraction (56–60%) that was largely methyl lesquerolate (94%) with small amounts of auricolate (5%) (14-hydroxy-*cis*-11,*cis*-17-eicosadienoate) and traces of 18-carbon hydroxy esters. This process for isolating the hydroxy FAME of *Lesquerella* oil was scaled up 15-to 100-fold with a preparative high performance liquid chromatograph. Thirty-gram samples of *L. gordonii* FAME were dissolved in eluting solvent, pumped onto the high performance liquid chromatography (HPLC) silica column and eluted with 97:3 hexane/ethyl acetate. In an 8-hr period, up to 200 g of methyl lesquerolate could be obtained with a purity >98%, the only contaminants being methyl auricolate and methyl ricinoleate.

KEY WORDS: Chromatography, hydroxy acids, *L. gordonii*, *Lesquerella fendleri*, lesquerolic acid, NMR, preparative HPLC, seed oils.

Seed oils of *Lesquerella* species contain sizable quantities of three hydroxy fatty acids (1–5). One of these, lesquerolic acid, is homologous with ricinoleic acid currently obtained from imported castor oil. Castor oil is a nonedible strategic material used in the production of a variety of products such as lubricants, plastics and pharmaceuticals (6–8). The *Lesquerella* oils, which have longer chain-length acids than those in castor oil, may provide raw material for new industrial products in addition to directly substituting for castor oil. *Lesquerella fendleri* and *L. gordonii* seed oils contain 55–60% of 14-hydroxy-*cis*-11-eicosenoic (lesquerolic) acid, 2–4% of 14-hydroxy-*cis*-11-*cis*-17-eicosadienoic (auricolic) acid, and <1% of 12-hydroxy-*cis*-9-octadecenoic (ricinoleic) acid. To conduct product development research on *Lesquerella* seed oil, we needed significant quantities of lesquerolic acid. This paper describes methods explored to obtain lesquerolic acid in multi-gram quantities.

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MATERIALS AND METHODS

Lesquerella fendleri seed was obtained from Dr. Anson Thompson, U.S. Water Conservation Laboratory, Phoenix, AZ. Oil was extracted from the flaked seed by successive washes with hexane. *L. gordonii* oil was available from previous research.

L. fendleri oil was fractionated on an open gravity column (40 cm × 2 cm) packed with 70–230 mesh silica gel 60 (E. Merck, Darmstadt, Federal Republic of Germany). Mixtures of hexane and diethyl ether were used for elution, starting with hexane and then adding increasing amounts of ether to increase polarity of the eluant, and then eluting finally with ether.

L. gordonii oil was refined prior to preparing methyl esters by washing with 2% Na_2CO_3. Refined oil (25–100 g) was heated (60°C) with 0.5 M sodium methoxide for 1 hr under continuous stirring. Methanol was removed by rotary evaporator. The methyl esters were taken up in petroleum ether and then washed with water, dried over Na_2SO_4 and isolated by stripping the solvent.

A Waters Associates Prep LC/System 500 was used for isolation of several hundred g of methyl lesquerolate. As many as 15 runs of 30 g each of esters could be made per day by using a single 500 g silica cartridge (Prep Pak-500/Silica). Mixtures of hexane and ethyl acetate (97:3 v/v) were used for elution. The flow rate was 0.25 L/min and the column chamber pressure was 25 atm. Fractions (0.5 L) were stripped on a rotary evaporator, weighed and analyzed for purity by gas chromatography (GC). Once conditions had been established, the normal ester peak was collected as one fraction and the hydroxy acids peak (lesquerolate, auricolate and ricinoleate) was collected as a second fraction. Remaining (tailing) hydroxy acids and minor polar components were eluted with ethyl acetate as a third fraction. Thin-layer chromatography (TLC) was performed on commercial precoated plates (Merck 0.25 mm silica gel 60 F-254). Developing solvent was hexane/diethyl ether/acetic acid (50:50:1, v/v/v). Visualization was by charring the plates at 110°C after spraying them with 50% H_2SO_4.

GC analyses of triglyceride fractions were carried out on a Hewlett Packard Model 5890A instrument. A fused silica SE-30 capillary column (1.6 m × 0.32 mm) was used with helium carrier set at 100 mL/min at 60 kPa. The oven was programmed from 150–350°C at 20°C/min. The injector and detector were set at 350°C. Actual helium flow through the short column was ca. 35 mL/min.

Methyl esters were analyzed in a Spectra Physics

SP-7100 GC equipped with a fused silica capillary column (60 m × 0.32 mm), SP2340 liquid phase (Supelco, Bellefonte, PA). Helium flow was set at 200 mL/min at 140 kPa. Actual flow through the column was ca. 1 mL/min. The oven temperature was programmed from 180–250°C at 3°C/min with a 1 min hold at 180°C and a 10 min hold at 250°C. Injector and detector were kept at 250°C. Both GCs were equipped with FIDs and electronic integration.

Mass spectra were obtained with a Hewlett Packard 5970 Series Mass Selective Detector (70 eV) coupled to a Hewlett Packard 5890 Gas Chromatograph fitted with a splitless injector and a 12 m × 0.25 mm DB-1 capillary column.

Nuclear magnetic resonance spectra (^{13}C, ^{1}H) were obtained with a Bruker WM-300 WB spectrometer and $CDCl_3$ as solvent.

RESULTS AND DISCUSSION

GC analyses of *L. fendleri* and *L. gordonii* oils (Fig. 1) were compared with a standard triglyceride mixture containing trilaurin (C36) to trieicosanoin (C60) with intermediate carbon numbers 42, 45, 48 and 54. The standard triglyceride mixture permitted calculation of equivalent carbon numbers (ECN) for the peaks in the *Lesquerella* oils. Of the 10 peaks (a–j) in the triglyceride region (Fig. 1), there are 3 major peaks (d, h, j) with ECN of 42.3, 57.7 and 61.6. Smaller peaks have ECN of 36.0, 38.2, 40.2, 42.1, 46.0, 50.4, 52.4 and 55.6. Given the fatty acid composition of the *Lesquerella* oils, all peaks cannot be triglycerides, e.g., peaks a–d may be diglycerides. The two species have similar oil contents and oil compositions, with *L. gordonii* having slightly higher lesquerolic acid content.

L. fendleri oil was chromatographed on silica gel, and two oil fractions were obtained. The two fractions were converted to methyl esters and analyzed by GC. The more polar oil fraction (56% peak j, ECN = 61.7) (upper curve of Fig. 2) contained 66% lesquerolic acid, consistent with its identity as glyceride(s) containing two lesquerolic acid acyl groups (dilesquerolins). The methyl esters of the less polar oil fraction (55% peak h, ECN = 58.3) (lower curve Fig. 2) contained 39% lesquerolic acid, consistent with the peak being largely glyceride(s) with a single lesquerolic acid acyl group (monolesquerolins). It is interesting to note that the separation pattern shown in Figure 2 is similar to that obtained when castor oil was similarly chromatographed. The results obtained with *Lesquerella* and castor oils indicate that the hydroxy monoene moieties of ricinoleic and lesquerolic acyl chains add between 1.5 and 1.8 ECN units to the ECN of the respective C-18 and C-20 saturated chains.

Figure 3 shows the excellent open-column chromatographic separation of methyl esters prepared from

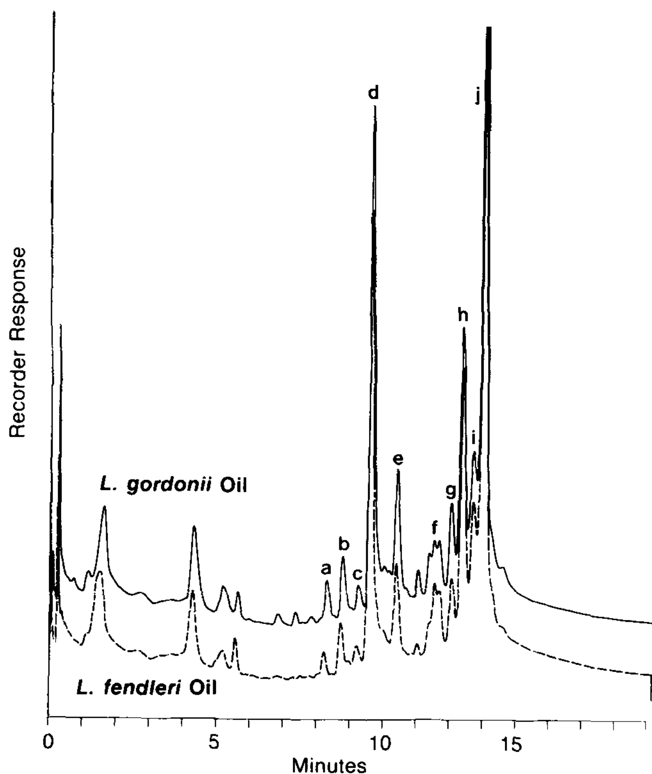

FIG. 1. Gas chromatography of *L. gordonii* and *L. fendleri* oils. Peak Equivalent Carbon Numbers (ECN): a (36.0), b (38.2), c (40.2), d (42.3), e (46.0), f (50.4), g (52.4), h (58.3), i (59.4), j (61.6).

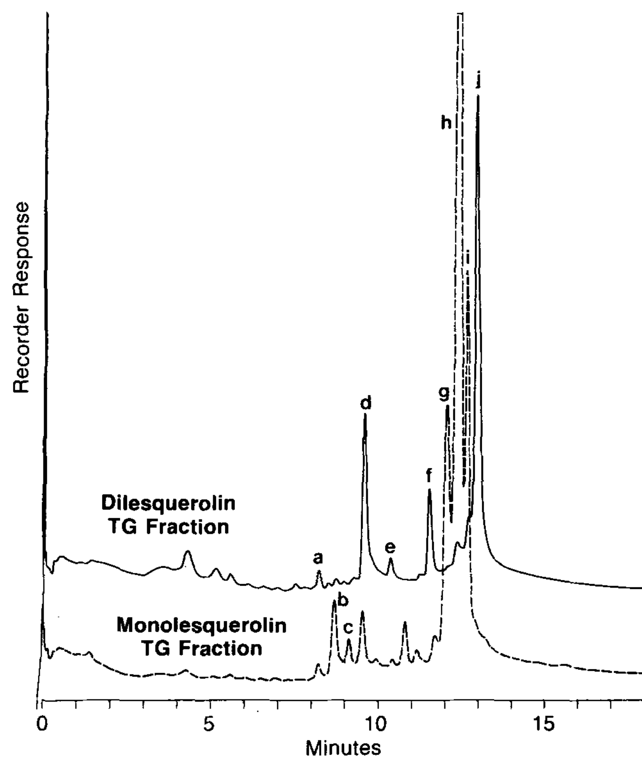

FIG. 2. Gas chromatography of *L. fendleri* oil fractions obtained by open-column chromatography on silica gel. Peak Equivalent Carbon Numbers (ECN): a (36.0), b (38.2), c (40.2), d (42.3), e (46.0), f (50.4), g (52.4), h (58.3), i (59.4), j (61.6).

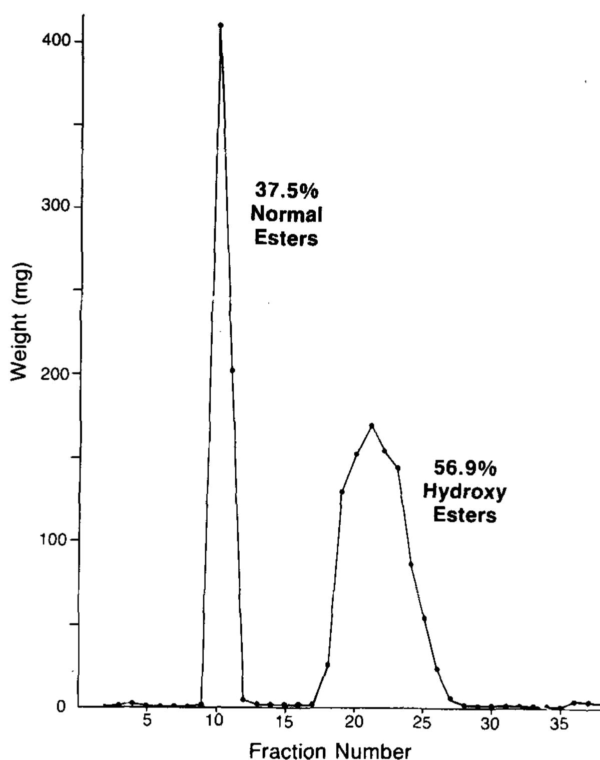

FIG. 3. Open-column chromatography of *L. fendleri* FAMEs.

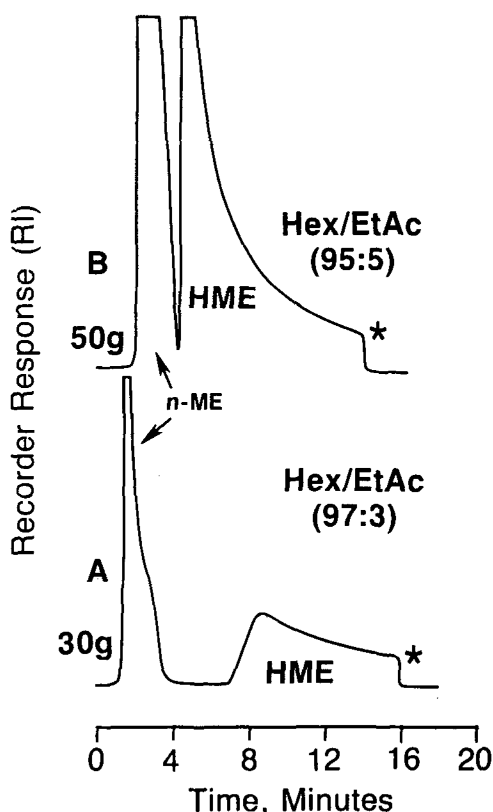

FIG. 4. Preparative HPLC of *L. gordonii* oil FAMEs. n-ME = normal methyl esters; HME = hydroxy methyl esters. (*EtAc wash).

L. fendleri oil. The ratio of isolated normal-to-hydroxy esters (40:60) confirms the ratio obtained by GC of the whole oil esters, and nearly quantitative recovery was obtained. GC analyses of the two fractions showed that the hydroxy esters consisted mostly of methyl lesquerolate (94%) with small quantities of methyl auricolate (5%) and traces of methyl ricinoleate and other hydroxy esters. The normal ester mixture consisted of unsaturated esters (92%), mostly 18:1 (39%), 18:2 (19%) and 18:3 (31%).

Separation of the esters on silica gel suggested that preparative HPLC could be used to obtain significant quantities of the hydroxy esters for our product development studies. Figure 4 shows two representative runs illustrating the separation of normal (n-ME) from hydroxy esters (HME) with 30 g (A) and 50 g (B) of ester per run. It was possible to reduce the run time by more than a factor of two while increasing the sample size threefold (15 g to 50 g) by increasing the level of ethyl acetate in the eluant from 3% to 5%. Baseline separation was essentially maintained. For example, of 339 g of esters chromatographed in 10 runs, 112.3 g (33.3%) of normal esters and 184.2 g (54.3%) of hydroxy esters were recovered. An intermediate cut contained a mixture of the two types of esters. We found that 30 g of sample and a hexane/ethyl acetate ratio of 97:3 (A) gave optimal results for repeated runs, and we made more than 30 runs with a single 500 g silica cartridge over a 3-day period (24 total hr). Progress of the HPLC runs was monitored by capillary GC analysis.

The GC curves of the HPLC methyl ester fractions are shown in Figure 5 along with the GC curve for the whole oil methyl esters. The hydroxy ester fraction was 98% methyl lesquerolate with 1.6% methyl auricolate and 0.3% methyl ricinoleate. The normal ester fraction consisted of 91% unsaturated esters, largely 18:1 (55.6%), 18:2 (14.8%) and 18:3 (12.9%). The level of 18:1 is significantly higher in *L. gordonii* oil (at the expense largely of 18:3) compared to *L. fendleri* oil (9).

Figure 6 shows the ^{13}C and ^{1}H magnetic resonance spectra of pure methyl lesquerolate and methyl ricinoleate. In the ^{13}C spectra, the terminal methyl carbons (18,20) and carbonyl carbons (1,1) are at the high and low field extremes of the spectra, respectively. The olefinic carbons are well separated between 125 and 135 ppm, and the hydroxylated carbons (12,14) near 72 ppm and the methoxyl carbons at 52 ppm are distinctly set apart in the spectra. All other carbons fall in identifiable order between 20 and 40 ppm. Except for the number of carbons, the spectra are similar. The ^{1}H spectra are also similar. The terminal methyl (18,20) and olefinic protons (9,10 and 11,12) fall at the field extremes, while the proton on the hydroxylated carbon (12,14) lies near mid-field along with the methoxyl protons. Protons adjacent to the carbonyl at C_2 and on either side of the olefinic carbons (8,11 and 10,13) lie

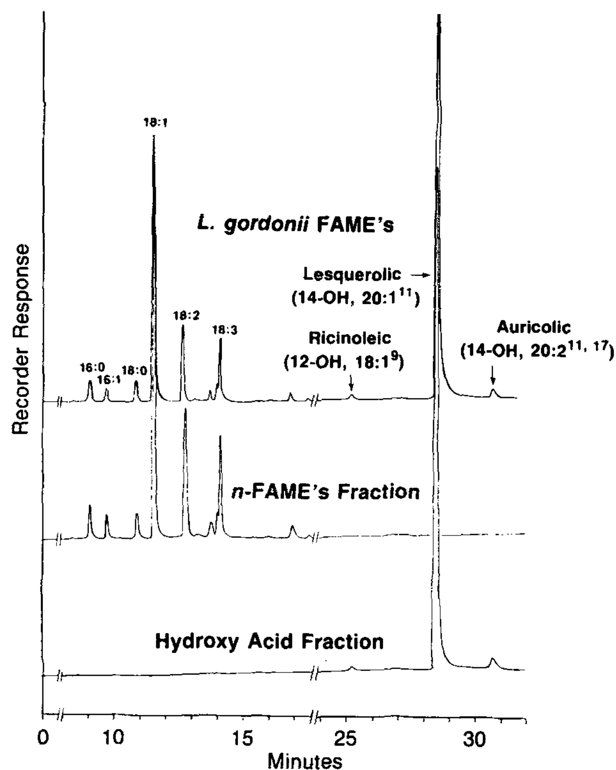

FIG. 5. Gas chromatography of *L. gordonii* FAMEs and fractions from HPLC of FAMEs.

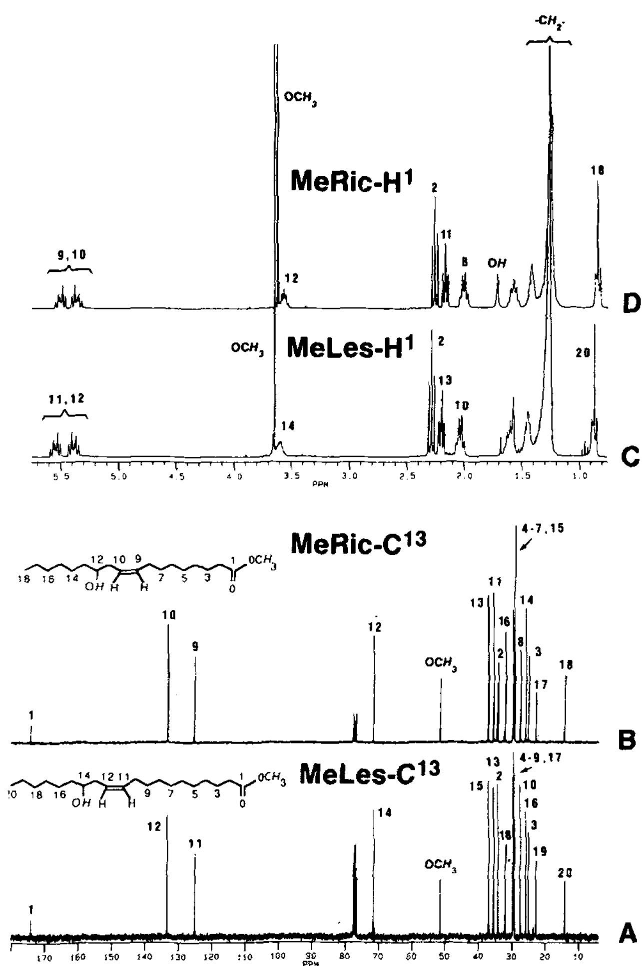

FIG. 6. ^{13}C and ^{1}H NMR spectra of methyl lesquerolate (A,C, respectively) and ricinoleate (B,D, respectively).

as distinct multiplets between 2.0 and 2.3 ppm. The remaining methylene protons fall in several groups in the range 1.2 to 1.7 ppm.

As expected, the mass spectra of the trimethylsilyl derivatives of methyl ricinoleate and methyl lesquerolate were similar. For each derivative, the base peak was at m/e 187, arising from cleavage adjacent to the trimethylsilyloxy group and loss of the hydrocarbon end of the esters ($C_{10}H_{23}SiO^+$). The same low mass ions 73, 103, 159 were found for each ester, and the high mass ions for ricinoleate at 245, 270, 299 and 369 were displaced 28 mass units for lesquerolate at 273, 298, 327, 365 and 397, respectively. Cleavage on the other side of the trimethylsilyloxy group (acyl side) is represented by m/e 299 (ricinoleate) and 327 (lesquerolate) ions.

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