Minor Components of *Lesquerella fendleri* Seed Oil

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Routine analysis of fatty ester fractions of *Lesquerella fendleri* oil suggested the presence of epoxy compounds and other minor components. By a combination of open silica column and high performance liquid chromatography (HPLC) fractionations of the methyl esters prepared from the oil, these constituents were isolated and then characterized by thin-layer chromatography (TLC), gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS—electron ionization, EI, and chemical ionization, CI) and nuclear magnetic resonance (NMR—^1H and ^{13}C). Three epoxy acids, 15,16-epoxy-9,12-octadecadienoic, 9,10-epoxy-12-octadecenoic and 9,10-epoxy-octadecanoic, were found. Hydroxy acids present included a C-22 homologue of lesquerolic acid (16-hydroxy-12-docosenoic acid) and 14,15-dihydroxy-tricosanoic acid. Other minor components included four sterols, brassicasterol, campesterol, β-sitosterol and stigmasterol, and a series of saturated and unsaturated fatty acids up to C30.

KEY WORDS: Epoxy compounds, GC-MS, hydroxy compounds, *Lesquerella fendleri*, minor constituents, NMR, seed oil.

The presence of large amounts of hydroxy fatty acids in the seed oils of *Lesquerella* species makes them attractive candidates for castor oil replacements or for other industrial use. The seed oil of *L. fendleri*, the species under breeding and agronomic development in the Phoenix, Arizona area, contains approximately 55% lesquerolic (14-hydroxy-11-eicosenoic) acid, the C20 homologue of ricinoleic acid, and small amounts of ricinoleic and auricolic acids. However, the minor seed oil constituents of this new crop have never been reported. During isolation of the major hydroxy acids for product development studies, we had opportunity to collect several potentially important lipid components which have rarely been found previously. We now report the presence of these compounds.

MATERIALS AND METHODS

L. fendleri seed was obtained from A.E. Thompson, U.S. Water Conservation Laboratory, ARS, USDA, Phoenix, AZ. Seed moisture was adjusted by adding 15% by weight water to the hot seed (80–100°C), which was tempered 1 hr at ≥80°C, and then was moisture equilibrated overnight. The next morning the seed was again stirred at ≥80°C for 90 min, after which its moisture content was 9.0%. The tempered seed was flaked on a Wolf flaking mill (12-in. smooth rolls set at 0.002–0.003 in.), which gave whole-seed flakes of uniform thickness.

Replicated batch extractions of oil were performed with 209 g of tempered and air-dried flakes by initial steeping in 350 mL of hot (50–60°C) hexane for 30 min followed by seven additional steeps, each with 150 mL of hot hexane for 30 min. The crude oil was isolated from the combined extracts by stripping the hexane with a rotary evaporator. The oil was analyzed for its various physicochemical properties (1).

The free fatty acid content of a 40-g sample of oil was determined according to AOCS method Ca 5a-40, and then free fatty acids were converted to methyl esters by passing diazomethane through the oil for 23 hr. Methyl esters of the oil triglycerides were prepared by refluxing the oil (38.6 g) with methanolic sodium methoxide (10 mL, 0.5 molar) and methanol (70 mL) for 1 hr. The excess methanol was distilled and the ester and glycerol layers were separated. The ester layer was dissolved in petroleum ether, washed with aqueous acetic acid and water to pH 7, and then dried and desolventized.

Methyl esters (18.2 g) were fractionated on a gravity column (L=64 cm, I.D.=4.8 cm) packed with 70–230 mesh silica gel (495 g). Mixtures of hexane and ether were used for elution, starting with 100% hexane followed by increasing amounts of ether in hexane and finally with 100% ether. The collected fractions, C-1 to C-7 (Fig. 1), were spotted on a precoated TLC plate (silica gel 60, without fluorescent indicator). A mixture of hexane, ether and acetic acid in 60:40:1 ratio was used as the developing solvent. Visualization was done with iodine as well as by spraying with 50% sulfuric acid followed by charring at 110°C. The GC analysis was done on a Spectra Physics Model SP 7100 equipped with a 10 m × 0.32 mm SE-30 capillary column programmed from 180–250°C at 3°C/min and with split mode of injection and flame ionization detector (FID). Fraction C-2 isolated by column chromatography was further fractionated in a 250 mm × 21.4 mm Dynamax Macro HPLC Si-Column by eluting with 90:10 hexane/ethyl acetate solvent mixture. A Waters 410 differential refractometer was used as the detector. These fractions, L-1 to L-5 (Fig. 1), were analyzed by GC (Hewlett-Packard 5890) on a 12 m SE-30 column temperature programmed from 175–300°C at 2°C/min. The peaks were integrated with a Hewlett-Packard 5895 A Chemstation.

Fraction L-5 was first subjected to preparative TLC with a mixture of hexane and ether (65:35) as developing solvent. Further purification and isolation were done with an SP 8800 HPLC on a silica column (L=250 mm, I.D.=4 mm) running isocratically with hexane and ethyl acetate in 95:5 ratio.

NMR spectra were obtained with a Bruker WM-300 WB instrument equipped with an Aspect 2000 computer. Deuterochloroform served as the solvent and the internal chemical shift references (δ7.25 for proton and 77.0 for carbon-13).

Fractions were silylated with Trisil reagent (Pierce Chemical, Rockford, IL) for GC-MS analysis on a Hewlett-Packard GC model 5890 connected to a Hewlett-Packard 5970 mass selective detector unit. A 12.5 m × 0.2 mm crosslinked dimethyl silicone fused silica column was used with splitless injection and programming from 120–200°C at 20°C/min and from 200–280°C at 2°C/min.

Chemical ionization mass spectra (isobutane, 0.3 torr) were taken at 70 eV in a Finnigan Model 4600 TSQ

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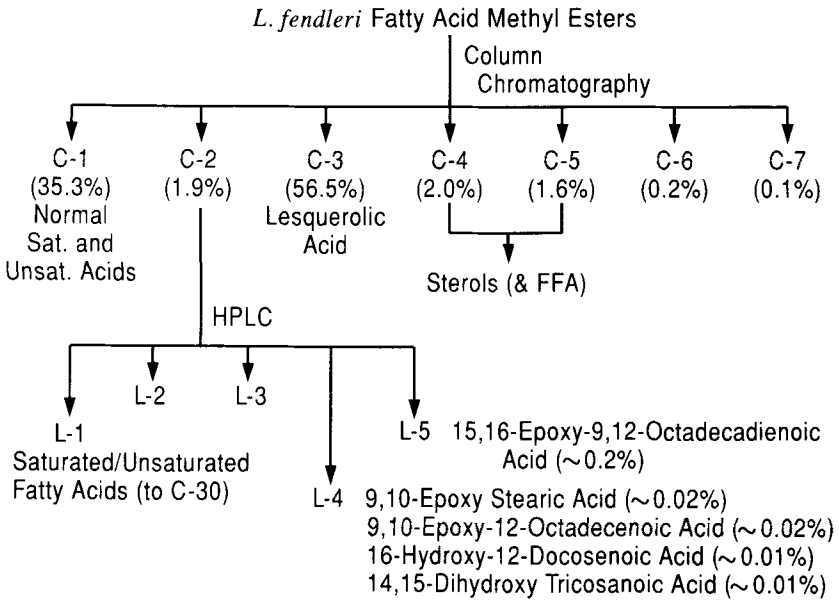


FIG. 1. Separation scheme for isolating minor components of *L. fendleri* oil.

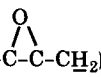
instrument from samples introduced through a gas chromatograph.

RESULTS AND DISCUSSION

Routine analysis of column fractions obtained from *Lesquerella fendleri* oil indicated the presence of some epoxy compounds. In order to facilitate the isolation of these minor components, the oil was first treated with diazomethane to convert free fatty acids present in the oil to their respective methyl esters, and then the oil was transesterified by treatment with sodium methoxide/methanol at ambient temperature.

The fatty acid methyl esters were fractionated on an open silica column (Fig. 1). Fractions C-1 to C-7 were then chromatographed on a TLC plate, which showed that fraction C-1 (35.3%) was a mixture of normal esters (16:0, 16:1, 18:0, 18:1, 18:2, 18:3 by capillary GC), and C-3 (56.5%) was a mixture of hydroxy esters (12-OH-18:1, 14-OH-20:1, 14-OH-20:2 by capillary GC). The compositions of fractions C-1 and C-3 were confirmed by GC-MS analysis. Of the remaining fractions, C-2 (1.9%) was further fractionated by HPLC into fractions L-1 to L-5, and TLC analysis showed fraction L-5 to be the prominent component of C-2. Fraction L-5 was further purified by HPLC. The purified compound (A) (0.2%) was characterized by TLC, GC, GC-MS (EI and CI) and NMR (^1H and ^{13}C). The TLC and GC analyses showed a single spot and a single peak, indicating that the compound was pure. NMR analysis of A had the following absorptions with proton integration, signal multiplicity and probable assignment in parentheses:

δ1.02 (3H, t, $C\underline{H}_3$-C-); δ1.30 (8H broad s, $[C\underline{H}_2]_4$);

δ1.6 (2H, m, $C\underline{H}_2$ β to $-C=O$); δ2.02 (2H, m, );

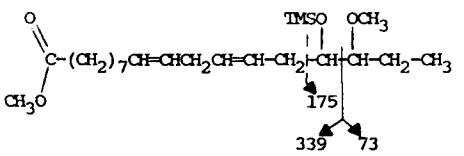
δ2.21 (4H, m, $O=C-C\underline{H}_2$, $C\underline{H}_2-C=C-$),

δ2.40 (2H, m, $-C=C-C\underline{H}_2-$ );

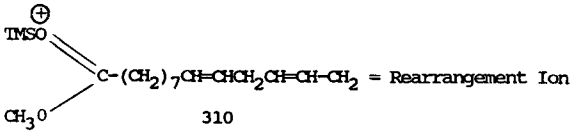
δ2.79 (2H, t, $C=C-C\underline{H}_2-C=$); δ2.94 (2H, m, $-C\underline{H}-C\underline{H}-$ );

δ3.67 (3H, s, $OC\underline{H}_3$); δ5.45 (4H, m, $-C\underline{H}=C\underline{H}-C-C\underline{H}=C\underline{H}-$).

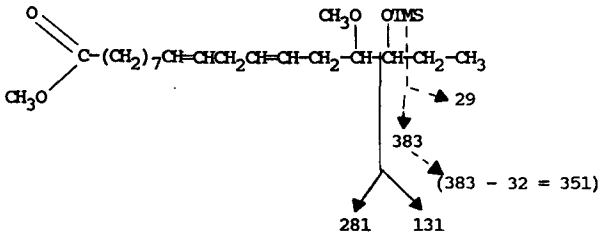
The mass spectra of unsaturated epoxy compounds are hard to interpret. Therefore compound A was derivatized to its corresponding hydroxy-methoxy derivative by treating with BF_3/MeOH and then silylated with Trisil (2-4). The EI-MS showed a molecular ion at m/e 412, and M-31 peak at 381. A rearrangement ion at 310 and other ions at m/e 339, 175 and 131 are characteristic fragments of Structures 1, 2, and 3, shown below.



STRUCTURE 1



STRUCTURE 2

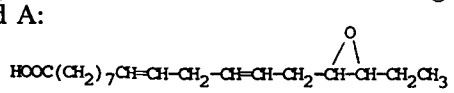


STRUCTURE 3

The ^{13}C NMR spectrum of compound A showed olefinic carbon chemical shifts at 130.74, 130.52, 127.24, and 124.15 consistent with C_9-C_{10}, C_{12}-C_{13} as the position of double bonds (5,6). The carbon chemical shifts were assigned with the help of previous work done in this field (7). The CI-MS (8) showed peaks at MH^+ m/e 413, MH-32 at 381 and MH-90 at 322.

The position of epoxy group was confirmed at C_{15}-C_{16} by MS analysis of the silylated methoxy derivative (9). NMR analysis showed that the double bonds were one methylene group separated from the epoxy function and from each other, therefore placing the double bonds at the Δ9 and Δ12 positions.

On the basis of NMR, EI-MS and CI-MS, the following structure—Structure 4—can be assigned to compound A:

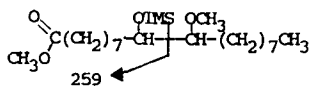


15,16-Epoxy-9,12-Octadecadienoic Acid

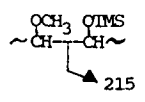
STRUCTURE 4

The TLC and GC analysis of fraction L-1 suggested a mixture of normal acid esters, i.e., straight chain unsaturated and saturated acid esters. The mass spectra of normal esters are similar, and the pattern for the homologous series is well defined and easily characterized. The presence of ions at m/e 74, ions of the series $CH_3OCO(CH_2)_n+$ at m/e (59 + 14n) (i.e., 87, 129, 143 . . . etc.). M-43 and M suggested the presence of saturated normal esters from C14 to C30. C15, C19, C23 were prominent compared to the other saturated odd carbon number acid esters. There is a marked difference in the mass spectra of unsaturated esters compared to their saturated counterparts (10,11). The presence of ions due to the loss of methoxyl radical (M-31), methanol (M-32), and of M-74 and M-116 showed the presence of unsaturated acid esters up to C-28.

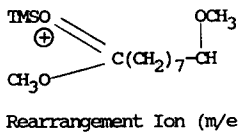
Because the TLC migration of fraction L-4 was close to the 15,16-epoxy-9,12-octadecadienoic acid, we suspected that more epoxy compounds were present in this fraction. Ions at m/e 155, 171, 199 and 281 (M-31) indeed suggested the presence of 9,10-epoxy stearate (2). Fraction L-4 was derivatized with BF_3/MeOH, and the hydroxy-methoxy derivatives were then silylated with Trisil. MS of the products showed ions at m/e 215, 259, and 274 to confirm the presence of 9,10 epoxy stearate (~0.02%)—Structures 5–7.



STRUCTURE 5



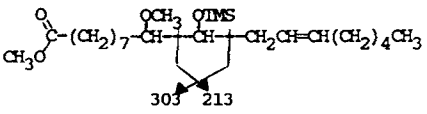
STRUCTURE 6



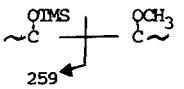
Rearrangement Ion (m/e 274)

STRUCTURE 7

Additional ions at m/e 213 and 303 suggested the presence of 9,10-epoxy,12-octadecenoate (~0.02%)—Structures 8 and 9 (2).

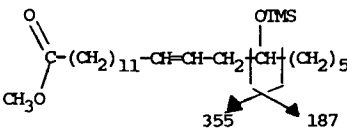


STRUCTURE 8

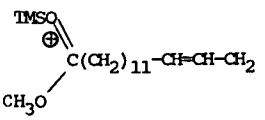


STRUCTURE 9

Evidence for a C22 homologue of lesquerolic acid, 16-hydroxy-12-docosenoic acid (~0.01%), was also found in fraction L-4. The mass spectrum revealed fragment ions at m/e 187 and 355 and the rearrangement ion m/e 326—Structures 10 and 11.



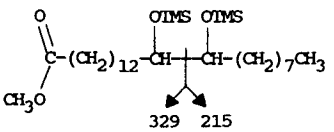
STRUCTURE 10



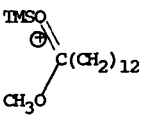
Rearrangement Ion (m/e 326)

STRUCTURE 11

Finally, mass spectra of dihydroxy esters are difficult to evaluate. Their silylated derivatives (2) are more readily characterized, because ions resulting from cleavage between the carbon atoms containing the trimethylsilyloxy (TMS) groups are usually prominent. Thus, ions at m/e 215, and 329 and at m/e 300 for a rearrangement ion are strong evidence for 14,15-dihydroxy tricosanoic acid in fraction L-4 (~0.01%)—Structures 12 and 13.



STRUCTURE 12



Rearrangement Ion (m/e 300)

STRUCTURE 13

GC-MS analyses of fractions C-4 and C-5 indicated the presence of some sterols. Some free acids, mainly lesquerolic, were also found in these fractions. The sterols were isolated with a reverse phase column, eluting with 100% acetone. The GC analyses of HPLC fractions were compared with some commonly available sterols and four, brassicasterol (~0.08%), campesterol (~0.11%), β-sitasterol (~0.15%) and stigmasterol (~0.02%), were identified on the basis of mass spectra of their TMS derivatives (12). Fractions C-6, C-7, L-2 and L-3 were not characterized because of the small amount of material available.

Thus, three epoxy compounds were identified as minor components in *L. fendleri* oil. Also present in detectable amounts were a C-22 homolog of lesquerolic acid, a C-23 dihydroxy acid, a series of saturated and unsaturated straight-chain fatty acids to C-30, and four sterols.

REFERENCES

1. Carlson, K.D., A. Chaudhry and M.O. Bagby, *J. Am. Oil Chem. Soc. 67*:438 (1990).
2. Kleiman, R., and G.F. Spencer, *Ibid. 50*:31 (1973).
3. Eglinton, G., D.H. Hunneman and A. McCormick, *Org. Mass Spectrom. 1*:593 (1968).
4. Kleiman, R., G.F. Spencer and F.R. Earle, *Lipids 4*:118 (1969).
5. Gunstone, F.D., M.R. Pollard, C.M. Scrimgeour and H.S. Vedanayagam, *Chem. and Phys. of Lipids 18*:115 (1977).
6. Bus, Jan, Izaäk Sies and Marcel S.F. Lie Ken Jie, *Ibid. 18*:130 (1977).
7. Bus, Jan, Izaäk Sies and Marcel S.F. Lie Ken Jie, *Ibid. 17*:501 (1976).
8. Plattner, R.D., H.W. Gardner and R. Kleiman, *J. Am. Oil Chem. Soc. 60*:1208 (1983).
9. Sessa, D.J., H.W. Gardner, R. Kleiman and D. Weisleder, *Lipids 12*:613 (1977).
10. McCloskey, J.A., *Topics in Lipid Chemistry,* Vol. 1, edited by F.D. Gunstone, Logos Press Ltd., Great Britain, 1969.
11. Ryhage, R., and E. Stenhagen, in *Mass Spectrometry of Organic Ions,* Chapter 9, edited by F.W. McLafferty, Academic Press, New York, 1963.
12. Knights, B.A., *J. Gas Chromatogr. 5*:273 (1967).

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