A Detailed Triglyceride Analysis of *Lesquerella fendleri* Oil: Column Chromatographic Fractionation Followed by Supercritical Fluid Chromatography

Douglas G. Hayes* and Robert Kleiman

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

ABSTRACT: The triglyceride structure of oil from *Lesquerella fendleri*, a potential new U.S. crop, rich in C_{20} hydroxy fatty acids, was examined by silica gel column chromatographic fractionation followed by supercritical fluid chromatography. The analysis confirmed previous findings derived by our research group, but provided further detail. The analysis demonstrated the presence of trihydroxy triglyceride, which contained all of the oil's C_{18} hydroxy acyl groups (present at less than 0.5% in the oil). Lipolysis indicated that these groups were located solely at the 2-position. In addition, a strong correlation was detected between the presence of α-linolenic ($18:3^{9,12,15}$) and auricolic ($20:2^{11,17}$ OH^{14}) acids in triglycerides.
JAOCS 73, 267–269 (1996).

KEY WORDS: Lesquerella oil, supercritical fluid chromatography, triglyceride analysis.

Lesquerella fendleri is being examined by the U.S. Department of Agriculture and private-sector partners as a new crop (1). Its oil is rich (55–60%) in C_{20} hydroxy fatty acids—lesquerolic ($20:1^{11c}$-OH^{14}) and auricolic ($20:2^{11c,17c}$-OH^{14}) acids—which have several potential applications. Previous investigations from our research group indicated that the vast majority of hydroxy acyl groups are located at the outer (1- and 3-) glycerol positions along with the oil's saturated acyl groups, present at 4.1% (2). The 2-position of lesquerella oil triglycerides (TG) consists almost entirely of C_{18} unsaturated acyl groups (2). These trends are shared by oils from other members of the genus *Lesquerella*, family Brassicaceae (3). This communication provides detailed structured data of TG in lesquerella oil after silica gel column chromatographic separation and supercritical fluid chromatography (SFC).

EXPERIMENTAL PROCEDURES

Materials and methods. Refined lesquerella oil was available from pilot-plant studies (4). The oil (*ca.* 18 g) was fractionated on a column (16″ × 1″) of silica gel (SG 60, 6.0 nm, 230–400 mesh) from Aldrich Chemical Co. (Milwaukee, WI) with hexane/ethyl acetate in increasing polarity as eluent. Fractions of similar polarity, determined by thin-layer chromatography, were combined to yield nine overall fractions. Aliquots of lesquerella oil and its fractions were converted to fatty acid methyl esters (FAME) by combining five drops of oil with 1 mL 0.5 M sodium methoxide in methanol, extracted with hexane after addition of saturated salt solution. The isolated FAME were then analyzed by gas chromatography with a 25 m × 0.25 mm (0.21-μm film thickness) BPX70 column from SGE (Austin, TX). The conditions of the analysis are listed elsewhere (3). SFC of oil fractions was performed with a nonpolar 10 m × 50 μm SB-Methyl 100 column from Dionex (Salt Lake City, UT). A program that simultaneously increased column pressure and temperature was found to be superior in separating lesquerella oil TG based on molecular weight. This program and the equipment used are described elsewhere (5). To elucidate the location of C_{18} hydroxy acyl groups in oil fraction IX, a small sample was subjected to lipolysis with a 1,3-specific lipase, as described previously (2).

RESULTS AND DISCUSSION

Lesquerella oil was separated by column chromatography on silica gel into nine fractions based on relative polarity. SFC chromatograms of the major fractions are displayed in Figure 1, and the fatty acid (FA) analyses and relative weights are recorded in Table 1. The accuracy of the FA analysis is demonstrated by the strong agreement between the measured FA distribution for the overall oil and that calculated from the sum of the FA distribution for each fraction multiplied by its mass fraction (Table 1). The TG of Fractions I–IV do not contain hydroxy acids. Fraction V contains approximately one C_{20} hydroxyl acyl group per TG, while Fractions VII and VIII contain approximately two C_{20} hydroxy groups per TG. Fraction VI, a small fraction by weight, contains a mixture of mono- and dihydroxy acyl TG. Fraction IX contains a mixture of diglyceride and di- and trihydroxy acyl TG. The composition of fractions, along with analyte molecular weight, as determined by a calibration curve (5), allowed identification

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

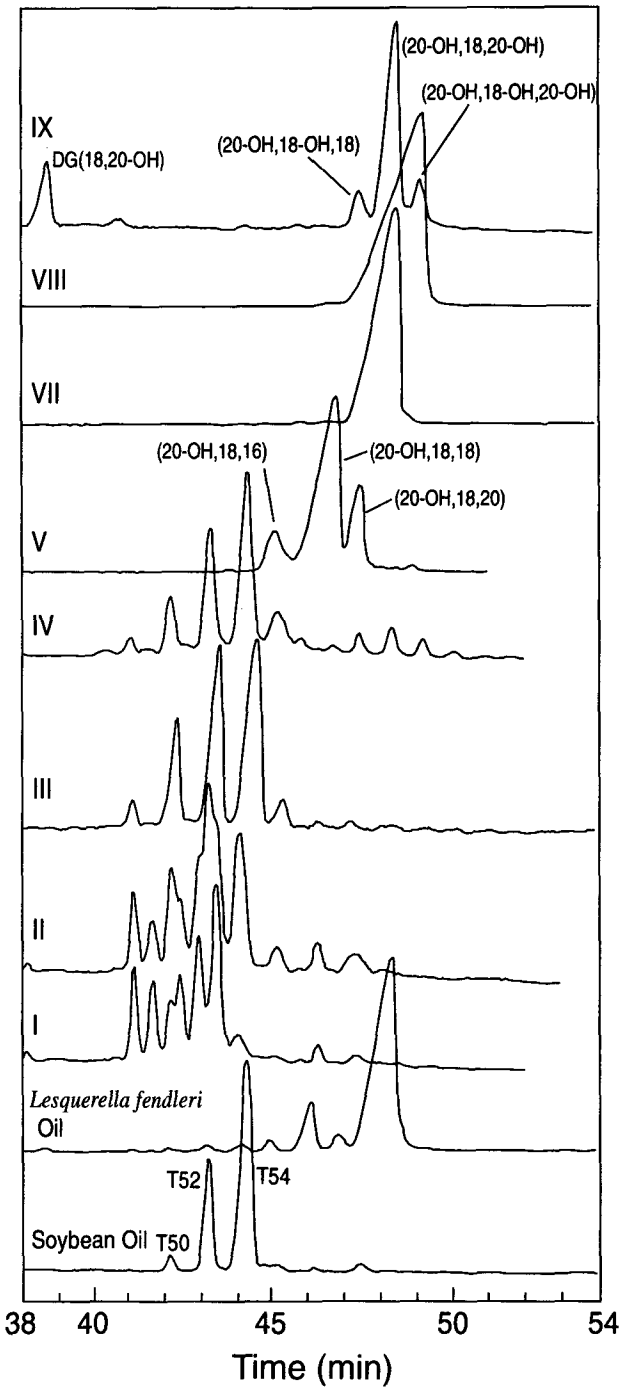

FIG. 1. Supercritical fluid chromatograms of fractions separated from silica gel column chromatography of lesquerella oil. The chromatogram of soybean oil is included as a reference. DG, diglyceride.

of SFC peaks (Fig. 1 and Table 2). Strong agreement exists between SFC results, those of Table 1, and those determined previously (6). For example, all three estimates indicate that 73–75% of TG contain two hydroxy acyl groups and one C_{18} unsaturated acyl group, and 16–17.5% is mono-C_{20} hydroxy TG.

The presence of multiple SFC peaks for TG of carbon numbers 50–54 is due to the presence of saturated fatty acyl groups. For example, the chromatograph of Fraction IV has fewer peaks in this region (40–45 min) than those of the less polar fractions (I–III). In addition, the peaks in the Fraction IV chromatograph align with those of soybean oil, which contains almost entirely unsaturated TG (Fig. 1). We also observed a partial separation of T54 molecules, based on degree of saturation, derived from the interesterification of stearic acid into triolein (data not shown).

The analysis presented here contains more detail than do previous investigations. The following new results have been obtained: First, the oil contains a detectable amount of trihydroxy acyl TG (Fraction IX). This fraction contains all of the oil's C_{18} hydroxy acyl groups, ricinoleic ($18{:}1^{9c}$-OH^{12}) and densipolic ($18{:}2^{9c,15c}$-OH^{12}) acid, which are present at less than 0.5% in the oil. Analysis of Fraction IX by 1,3-specific lipolysis indicates that all of the C_{18} hydroxy acyl groups are located in the 2-position (data not shown). A trihydroxy acyl TG of similar structure is present in oils from other species of the genus *Lesquerella* (3).

Second, almost all of the saturated and uncommon acyl groups (e.g., $18{:}1^{11}$ and $20{:}1^{11}$) are present in nonhydroxy or monohydroxy acyl TG. This supports our previous findings that such groups are located almost solely at the 1- and 3-glycerol positions (2). Moreover, in dihydroxy acyl TG, the 1- and 3-positions are occupied by C_{20} hydroxy groups and are unavailable for saturated groups. In addition, almost all of the palmitoleic (16:1) groups are present in the nonhydroxy acyl TG fractions. The presence of TG with carbon number 48, T48, in Fractions I–III (Fig. 1) demonstrates that at least some of the palmitoleic acyl groups must be present at the 2-position [as palmitic groups are located solely at the 1- or 3-position and no FA shorter than C_{16} are present at significant quantities (6,7)]. Furthermore, for a given family of FA, e.g., saturated or monounsaturated FA, a higher percentage of those with shorter chainlength is more likely to be present in the nonhydroxy acyl TG fractions (Table 1).

Third, it appears that auricolic acyl groups are more likely to be associated with linolenic ($18{:}3^{9c,13c,15c}$) acyl groups than with other unsaturated acyl groups. This is demonstrated by comparing the FA distribution of the two dihydroxy acyl TG fractions, VII and VIII (Table 1). Fraction VIII, the less abundant fraction, contains nearly 50% of the 18:3 and auricolic acyl groups for the two fractions combined. Moreover, the number of dihydroxy acyl TG containing both auricolic and linolenic acid is 2.75 times that predicted by random distribution. This agrees with observations of oils from the genus *Lesquerella*, where a correlation existed between relatively high linolenic acid content and high auricolic acid content (3). This correlation may indicate that linolenic acid is the precursor in the biosynthesis of auricolic acid, or that both molecules are derived from the same branch of the biosynthetic pathway.

TABLE 1
Fatty Acid Composition of Fractions Recovered from Silica Gel Column Chromatographic Separation of *Lesquerella fendleri* Oil

Fatty acid	Fraction I	II	III	IV	V	VI	VII	VIII	IX	Oil measured[a]	Oil calculated[b]
16:0	6.7	8.7	11.7	6.1	3.7	1.0	0.2		0.4	1.2	1.1
16:1	17.9	11.9	11.5	7.9	0.2	0.5				0.8	0.5
18:0	2.6	5.6	5.7	3.2	9.0	1.3	0.2		0.6	2.4	1.8
$18:1^{9}$	7.3	15.7	19.0	10.4	15.7	18.5	17.6	7.4	5.6	14.1	14.3
$18:1^{11}$	12.6	13.0	17.4	9.4	4.9	1.6	0.4	0.2	0.5	1.8	1.6
18:2	17.0	12.7	16.5	17.1	10.3	5.2	6.9	6.3	2.9	7.8	7.5
18:3	19.0	11.5	13.3	23.3	15.7	15.8	8.9	19.4	5.5	13.0	12.7
20:0	0.7	0.9	0.8	1.2	0.9					0.5	0.2
20:1	0.4	1.2	1.3	1.5	5.2	0.9			0.3	1.1	0.9
18:1-OH									10.5	0.4	0.4
18:2-OH									3.1	trace	0.1
20:1-OH				1.5	31.7	43.5	62.6	60.1	63.9	53.1	54.3
20:2-OH					1.0	1.1	3.2	6.3	4.4	3.5	3.4
wt%	0.7	0.4	1.4	1.5	16.2	1.0	51.9	23.0	3.8		

[a]Gas chromatographic analysis of lesquerella oil.
[b]Based on wt% and free fatty acid composition of each fraction.

TABLE 2
Triglyceride (TG) Structure of Lesquerella Oil Based on SFC Analysis[a]

TG species[b]	wt%
52	1.6
54	1.6
16,18,20-OH	2.0
18,18,20-OH	10.9
20,18,20-OH	4.4
20-OH,18,20-OH	73.3
20-OH,18-OH,20-OH	2.8[c]
Others	3.4

[a]Supercritical fluid chromatography (SFC) method described in the Experimental Procedures section.
[b]52 and 54 refer to carbon number of TG. "16," "18," and "20-OH" refer to C_{18}, $C_{18'}$ and C_{20} hydroxy acyl groups. The order in which the acyl groups are listed does not correspond to their position within TG molecules.
[c]Peak area estimated because baseline separation was not achieved.

ACKNOWLEDGMENT

Bliss S. Phillips performed the gas chromatography analysis of FAME.

REFERENCES

1. Roetheli, J.C., K.D. Carlson, A.E. Thompson, D.A. Dierig, L.D. Glaser, M.G. Blase, and J. Goodell, *USDA-CSRS Office of Agricultural Materials, Growing Industrial Materials Series*, Washington, D.C., 1991.
2. Hayes, D.G., and R. Kleiman, *J. Am. Oil Chem. Soc. 69*:1781 (1992).
3. Hayes, D.G., R. Kleiman, and B.S. Phillips, *Ibid. 72*:559 (1995).
4. Carlson, K.D., and R. Kleiman, *Proceedings of the First International Conference on New Industrial Crops and Products*, edited by H.H. Nagvi, L. Estilai, and I.P. Ting, Riverside, 1991, p. 169.
5. Hayes, D.G., and R. Kleiman, Supercritical Fluid Chromatographic Analysis of New Crop Seed Oils and Their Reactions, in *Supercritical Fluid Extraction and Chromatography in Lipid Analysis and Processing*, edited by T. Lee and J.M. Snyder, AOCS Press, Champaign, in press, 1996.
6. Carlson, K.D., A. Chaudhry, R.E. Peterson, and M.O. Bagby, *J. Am. Oil Chem. Soc. 67*:495 (1990).
7. Chaudhry, A., R. Kleiman, and K.D. Carlson, *Ibid. 67*:863 (1990).

[Received March 31, 1995; accepted November 17, 1995]