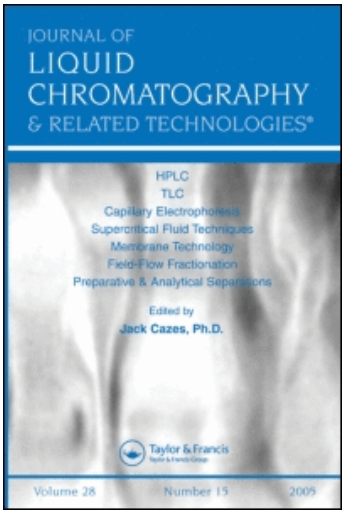

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ANALYSIS OF HYDROXY-CONTAINING SEED OILS USING ATMOSPHERIC PRESSURE CHEMICAL IONIZATION MASS SPECTROMETRY

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ABSTRACT

Atmospheric pressure chemical ionization mass spectrometry (APCI-MS) coupled to reversed-phase high performance liquid chromatography (RP-HPLC) was used for direct analysis of intact triacylglycerols (TAGs) from hydroxy-containing plant oils. Castor bean oil, *Lesquerella fendleri* and *Lesquerella gordonii* oils were separated into tri-hydroxy, di-hydroxy, mono-hydroxy, and non-hydroxy TAGs using the RP-HPLC method. The APCI-MS ionization source produced fragments representing loss of zero (protonated molecular ion), one, two, and three hydroxy groups. The primary fragments (base peaks) in the mass spectra resulted from loss of all hydroxy groups from the TAGs. Using the acetonitrile/methylene chloride solvent system, diagnostically important acetonitrile adducts were formed which allowed identification of the molecular weights of the hydroxy TAGs as well as confirmation of the number of hydroxy groups contained therein. A series of four adducts was formed: $[M+23]^+$, $[M+39]^+$, $[M+54]^+$, and $[M+59]^+$.

A mass difference of 76, 58, or 40 amu between the base peak and the $[M+23]^+$ peak allowed the TAGs to be identified as trihydroxy, dihydroxy, or monohydroxy, respectively. Additional fragmentation of the TAGs occurred with cleavage of the acyl chains next to the hydroxy group giving a net loss of 114 amu. This fragmentation occurred in combination with loss of hydroxy groups from the remaining acyl chains. Diacylglycerol fragments were formed which also exhibited sequential loss of the hydroxy groups, as well as the fragment produced by loss of 114 amu.

INTRODUCTION

Castor oil has been used since ancient times for such things as lamp oil and as skin and hair treatment.[1] The primary components contain a hydroxy fatty acid which imparts special properties, including increased viscosity, solubility in alcohols, and greater reactivity. In the modern era, castor oil has been directly used in pharmaceuticals, cosmetics, lacquers, adhesives, urethanes, sealants, caulks, pigments, dyes, coatings, hydraulic and brake fluids, machinery lubricants, casting lubricants, dielectrics, inks, waxes, polishes, soaps, as a laxative, and other applications.[2,3] After processing, its derivatives have found uses in Nylon-11, Nylon-610, surfactants, emulsifiers, polyurethanes (for use in electrical and communications casting resins and coatings), plasticizers, aircraft lubricants, and many more.

The primary component of castor oil is ricinoleic acid ($18:1^{9}$-OH^{12}), and alkaline fusion of the oil yields sebacic acid ($HOOC(CH_2)_8COOH$) and 2-octanol. The hydrogenated oil provides thickening properties in grease formulations, while the resultant sebacic acid acts as a lubricant for aircraft hydraulics. These latter two applications have caused the U.S. Department of Defense to put ricinoleic and sebacic acids on their list of strategic and critical materials. Since 1972 there has been no U.S. production of castor oil; therefore 100 percent of the oil used in the U.S. has been imported, primarily from India and Brazil.

These are among the reasons that substitutes for the castor crop, which could be domestically cultivated, have been sought. The primary candidates for crops to supplement the supply of hydroxy fatty acids are plants of the genus *Lesquerella*, which belong to the *Brassicaceae* (mustard) family. Over 85 species of lesquerella are currently known, all native to North America.[1,4] These contain lesquerolic acid ($20:1^{11}$ - OH^{14}), densipolic acid ($18:2^{9,15}$-OH^{12}), auricolic acid ($20:2^{11,17}$-OH^{14}), as well as small amounts of ricinoleic acid. In general, species from the western United States produce oils containing mostly lesquerolic acid, while densipolic acid predominates in those species from

Alabama and Tennessee, and one species from Oklahoma and eastern Texas contains significant amounts of auricolic acid.[4] After scientists at the U.S. Water Conservation Laboratory assembled and evaluated the lesquerella germplasm that had been gathered in the 1960s, they determined that the species with the greatest agronomic potential was *Lesquerella fendleri*.

Triacylglycerols (TAGs) in the seed oils from the above-mentioned plants present special difficulties for analysis. Direct mass spectrometric analysis of hydroxy-containing TAGs has not been feasible because the molecules are not ionized intact, but rather lose all of their hydroxy groups through dehydration to form additional unsaturation,[5] giving no evidence of hydroxy-containing TAGs. Thus, no molecular ion has been observed which could be used for unambiguous identification of molecular species. Trimethyl silyl derivatization of castor oil lipids has been necessary in order to accomplish identification of molecular species of its hydroxy-containing TAGs.[5]

Thorough and complete characterization of numerous species of lesquerella seed oils has been accomplished by lipase hydrolysis and decanolysis,[4] but the methods do not directly measure individual triglyceride molecular species. No direct chromatography/mass spectrometry method has been demonstrated which is capable of identifying the molecular species of intact hydroxy-containing TAGs.

Here we report a method utilizing separation by reversed-phase high performance liquid chromatography (RP-HPLC) coupled with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) for direct detection of intact hydroxy-containing TAGs as well as normal TAGs. Distinct fragment ions, small molecular ions, and reproducible formation of adduct ions allow identification of intact molecular species of trihydroxy, dihydroxy, monohydroxy, and normal TAGs, as well as diacylglycerols.

MATERIALS

Refined Lesquerella seed oil samples from pilot plant studies were obtained courtesy of Dr. Ken Carlson (retired) of the New Crops Research Unit of the National Center for Agricultural Utilization Research in Peoria, Il. The castor oil (USP) was obtained from a local pharmacy. Solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or EM Science (Gibbstown, NJ). Solvents were HPLC grade or the highest available quality and were used without further purification.

METHODS

Liquid Chromatography

The HPLC pump was an LDC 4100 MS (Thermo Separation Products, Shaumburg, IL) quaternary pump system with membrane degasser. Two columns in series were used: Inertsil ODS-2 (GL Sciences, Keystone Scientific, Inc., Bellefonte, PA), 25 cm x 4.6 mm, 5μm. A gradient solvent program with acetonitrile (ACN) and dichloromethane (DCM) was used. The gradient was as follows: initial - 95% ACN, 5% DCM; linear from 20 to 40 min. to 20% ACN, 80% DCM, held until 50 min.; linear from 50 to 65 min. to 45% ACN, 55% DCM, held until 85 min. The flow rate throughout was 1.0 mL/min. The column effluent was split so that ~850 μL/min went to an evaporative light scattering detector (ELSD) and ~150 μL/min went to the APCI interface. 10 μL of each sample solution was injected. The ELSD was an ELSD MKIII (Varex, Burtonsville, MD). The drift tube was set at 140°C, the gas flow was 2.0 standard liters per minute. High purity N_2 was used as the nebulizer gas.

Mass Spectrometry

A Finnigan MAT (San Jose, CA) SSQ 710C mass spectrometer fitted with an atmospheric pressure chemical ionization source was used to acquire mass spectral data. The vaporizer was operated at 350°C and the capillary heater was operated at 250°C. The corona voltage was set at 6.0 μA throughout. High purity nitrogen was used for the sheath and auxiliary gases, which were set to 35 psi and 5 mL/min, respectively. Spectra were obtained from 400 amu to 1100 amu, with a scan time of 2.67 sec. All mass spectra shown represent an average of spectra over the breadth of a chromatographic peak.

RESULTS

The chromatogram in Figure 1 represents the RP-HPLC/APCI-MS separation of castor oil. The largest peak in the chromatogram arose from the triacylglycerol containing three ricinoleate (Ri, $18:1^{9}\text{-}OH^{12}$) acyl chains, triricinolein (RiRiRi), the major component of the oil. The other major components contained two ricinoleate acyl groups in combination with normal fatty acyl chains, linoleate (L, 18:2), oleate (O, 18:1), stearate (S, 18:0), palmitate (P, 16:0), eicosenoate (Ei, 20:1), and arachidate (A, 20:0).

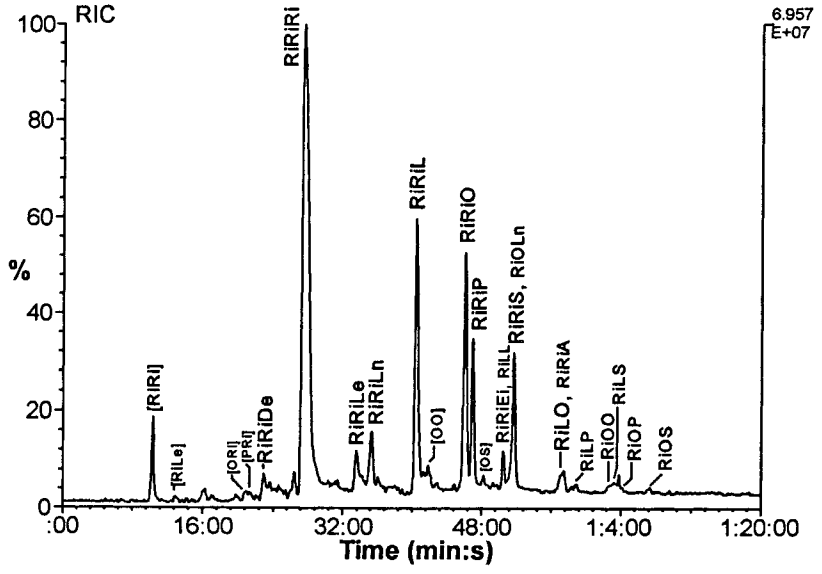

Figure 1. Reconstructed ion chromatogram of Castor Oil. Acyl chain abbreviations: Ri, ricinoleic acid (18:1^{9}-OH^{12}); De, densipolic acid (18:2^{9,15}-OH^{12}); Le, lesquerolic acid (20:1^{11}-OH^{14}); Au, auricolic acid (20:2^{11,17}-OH^{14}); Ln, linolenic acid; L, linoleic acid; O, oleic acid; S, stearic acid; Ei, eicosenoic acid; A, arachidic acid (20:0).

Small amounts of TAGs made of two Ri chains in combination with the other hydroxy-containing fatty acids densipolic acid (De,18:2^{9,15}-OH^{12}), and lesquerolic acid (Le, 20:1^{11}-OH^{14}) were observed. Eluted after the di-ricinoleic TAGs were small amounts of mono-ricinoleic TAGs. Figure 2 shows spectra obtained for the tri-hydroxy, di-hydroxy, and mono-hydroxy TAGs. As was previously observed,[5] the primary fragment (base peak) in mass spectra of hydroxy-containing TAGs arose from loss of the hydroxy group(s) and a proton (dehydration) to form additional sites of unsaturation. Unlike previous results, however, fragment ions were observed for the complete series of protonated fragments representing loss of zero (protonated molecular ion) to three hydroxy groups. Thus, triricinoleate (Fig. 2A), for example, showed a base peak of *m/z* 879.8, resulting from loss of three hydroxy groups from the protonated molecular ion, and showed a fragment with good abundance at *m/z* 897.9, representing loss of two hydroxy groups, and also showed small peaks at *m/z* 915.8 and *m/z* 933.8 representing loss of one hydroxy group and the protonated molecular ion, respectively. Also found at high mass were a series of three important adduct ions formed in the APCI source which we found could be

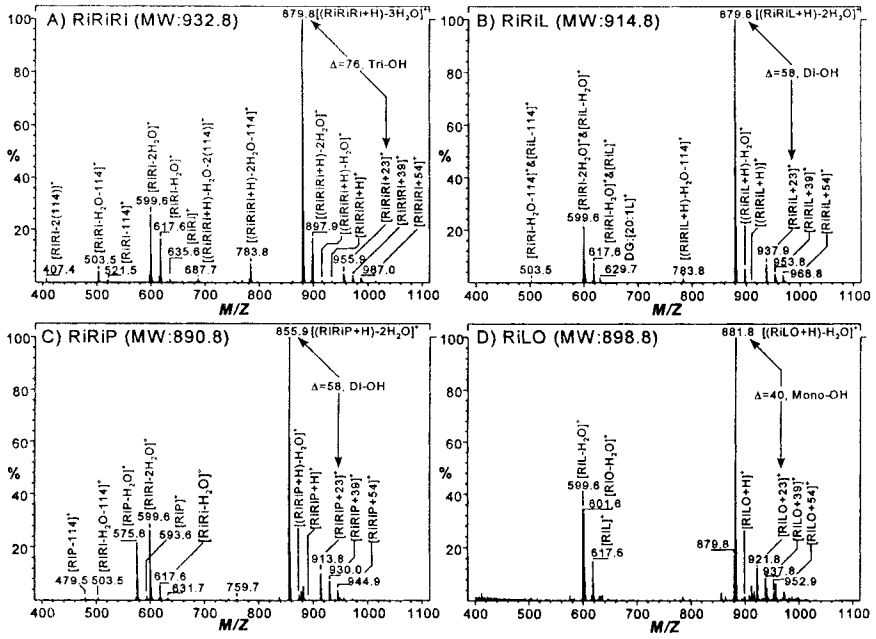

Figure 2. Mass spectra of castor oil hydroxy triacylglycerols. A) RiRiRi, B) RiRiL, C) RiRiP, D) RiLO. Abbreviations as in Figure 1.

used to clearly and reproducibly identify the molecular weights and number of hydroxy groups of all hydroxy-containing species. The first fragment/adduct ion arose from loss of the hydroxy group followed by addition of an acetonitrile-derived group as follows: - (-OH) + (-N=C=CH_2), for -17 + 40 = +23 amu added to the molecular ion, or $[M+23]^+$. Loss of one electron from the nitrogen allowed ionization without the normal protonation of the molecular ion. Similar mechanisms have long been observed for nitriles.[6,7]

The difference in mass between the base peak ($[M+H-n H_2O]^+$) and the first fragment/adduct formed was 76 amu for tri-hydroxy TAGs, the difference was 58 amu for di-hydroxy TAGs and it was 40 amu for mono-hydroxy TAGs. This difference, and the mass of the fragment/adduct, allowed identification of the molecular weight of the TAG and the number of hydroxy groups it possessed. The second fragment/adduct was produced by loss of the hydroxy proton followed by addition of the acetonitrile-derived group: -(H) + (-N=C=CH_2), for -1 + 40 = +39 amu added to the molecular ion, or $[M+39]^+$.

The third adduct in the series reproducibly produced an ion having a mass of $[M+54]^+$. This mass was consistent with an adduct formed from loss of the hydroxy proton with addition of the acetonitrile-derived group with an additional nitrogen and hydrogen as follows: (-H) + (-N=N-CH=CH_2), for -1 + 55 = +54. In addition to these adducts, most spectra also exhibited an $[M+59]^+$ adduct, which arose from $[M+H_2O+ACN]^+$ in some form. In some cases, for the tri-hydroxy TAGs, the $[M+59]^+$ adduct had a larger abundance than the $[M+54]^+$ adduct.

Similar to the molecular ion, the diacylglycerol fragments also exhibited dehydration to form additional unsaturation. They also showed fragments representing sequential loss of each hydroxy group, and usually a small fragment for the intact hydroxy-containing diacylglycerol (Fig. 2A). Another interesting fragmentation mechanism was observed in APCI-MS spectra. The molecules underwent cleavage next to the hydroxy group on the side containing the double bond (which was between C_{11} and C_{12}), for loss of the $C_7H_{14}OH$ group (=115 amu); however, one proton was picked up by the remaining acyl chain during fragmentation to produce a net loss of Δ=114 amu. This fragment was observed from both molecular ions and diacylglycerol groups. With molecular ions, this mechanism usually occurred along with loss of the maximum number of hydroxy groups possible, as in triricinoleate, which formed the fragment at *m/z* 783.8, exhibiting loss of two hydroxy groups along with loss of the $C_7H_{14}OH$ moiety. The diacyl groups formed this fragment with and without loss of other hydroxy groups (e.g. fragments having *m/z* 521.5, 503.5, and 407.4 in Fig. 2A for triricinoleate).

Because the number of fragments observed in spectra of hydroxy-containing TAGs was larger than for normal TAGs, the interpretation of spectra of co-eluting species could be complicated. To assist in identification of species, we used extracted ion chromatograms (EICs), in which a particular mass was extracted out of the total ion chromatogram, or reconstructed ion chromatogram. Examples of these EICs are shown in Figure 3. Figure 3A shows the EIC at m/z 599.5 which represents the fragment formed from $[RiRi-2H_2O]^+$, which is isobaric with $[RiL-H_2O]^+$.

Peaks in this chromatogram identify the elution times of [RiRi] diacylglycerol, RiRiRi, RiRiL, RiRiO, RiRiP, and RiRiS. Corresponding peaks occur at the same retention times in the EIC for *m/z* 617.6 ($[RiRi-H_2O]^+$, Fig. 3C), since these fragments arose from the same molecules. These dihydroxy TAGs were chromatographically quite distinct from the monohydroxy TAGs RiLL, RiLO, RiLP and RiLS, present in much smaller amounts and eluted at longer retention times, which gave isobaric fragments (Fig. 3A).

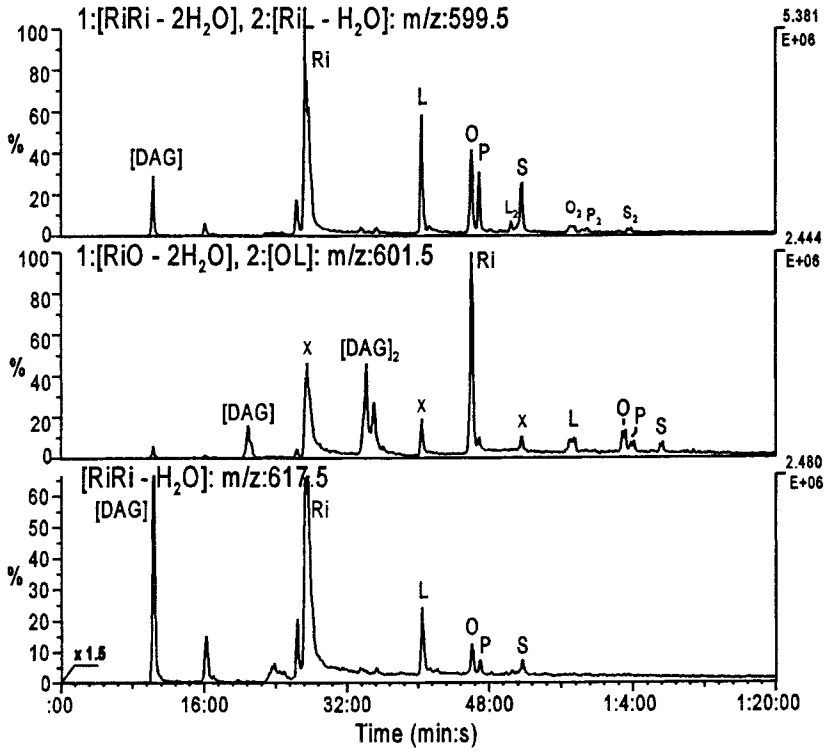

Figure 3. Extracted ion chromatograms of diacylglycerol fragments of castor oil. Abbreviations as in Figure 1. [DAG] = native diacylglycerol.

The TAGs in castor oil did not exhibit the large differences in the proportions of $[TAG+H]^+$ versus $[DG]^+$ fragments shown by normal TAGs (because they had similar degrees of unsaturation), so quantitation was performed using the base peaks ($[M+H-nH_2O]^+$) from all molecular species. The TAG composition thus obtained, and the fatty acid composition which was calculated from the TAG composition are not presented, because they are not useful data, but rather only demonstrate the need for response factors. The fatty acid composition showed that ricinoleic acid was the primary fatty acid present, but the percentage calculated from the TAG composition (77.8%) was less than that previously reported for castor oil (~90%),[3,5,8] which has been reported to be remarkably consistent.[3] Admittedly, in order to detect TAGs present in small amounts, the maximum amount of sample possible was used which still kept the triricinolein fragment abundances within the instrument's DAC dynamic

range. So the concentration of triricinolein was likely much higher than the linear range on a calibration curve. As confirmation of this, quantitation of the output from the ELSD detector was performed. Because of the split ratio and the flow rate into the ELSD, the detector was not overloaded, but fewer peaks were detected. For those peaks detected, the TAG composition resulted in a fatty acid composition which was much closer to that expected,[3,5,8] giving a composition for ricinoleic acid of 92.3 %. We concluded that a method for response factor calculation must be devised which would allow quantitative analysis using the APCI-MS data, which is beyond the scope of this initial report. For this reason, quantitation of TAG species in lesquerella seed oil is not discussed.

The fragmentation patterns of castor oil, which contained a fairly simple mixture of hydroxy TAGs, were presented in detail above because all of these mechanisms are observed in the more complex lesquerella seed oils. The reconstructed ion chromatograms (RICs) of *Lesquerella fendleri* and *Lesquerella gordonii* are shown in Figures 4A and 4B, respectively. These lesquerella seed oils contained tri-hydroxy, di-hydroxy, mono-hydroxy, and non-hydroxy TAGs, as well as some diacylglycerols. In spite of this complexity, the spectra could be interpreted in a straightforward manner according to the behavior described above. The use of EICs became increasingly important for identification of overlapped TAGs. Mass spectra for tri-hydroxy, di-hydroxy, and mono-hydroxy TAGs of *L. fendleri* are presented in Figure 5. As in castor oil, the base peaks in these mass spectra resulted from loss of all hydroxy groups by dehydration.

In addition to the base peak, the mass spectrum of LeLeRi (Figure 5A) clearly showed peaks representing loss of zero, one and two hydroxy groups from the protonated molecular ion. It further exhibited large fragment/adduct ions at $[M+23]^+$, $[M+39]^+$, $[M+54]^+$, and in this case the $[M+59]^+$ fragment/adduct was larger than that of $[M+54]^+$. As with the castor oil TAGs, the difference of 76 amu between the base peak and the $[M+23]^+$ fragment/adduct identified this as a trihydroxy TAG, while the *m/z* of the [M+23]+ peak established the molecular weight as being 989 (988.8) amu. The di-hydroxy diacylglycerol fragments also exhibited fragments showing loss of one and two hydroxy groups: $[LeRi\text{-}H_2O]^+$= *m/z* 645.6; $[LeRi - 2H_2O]^+$ = *m/z* 627.6; $[LeLe - H_2O]^+$ = *m/z* 673.8; $[LeLe - 2H_2O]^+$ = *m/z* 655.6. Also analogous to the castor oil, the LeLeRi exhibited $[(M+H)\text{-}2H_2O\text{-}114]^+$ and $[LeRi\text{-}H_2O\text{-}114]^+$ fragments at *m/z* 839.9 and 531.5, respectively. Similarly, the mass spectrum in Figure 5B, for LeLnAu, showed fragments and fragment/adducts analogous to those of LeLeRi. The difference of 58 amu from the base peak to the $[M+23]^+$ fragment/adduct indicated that this was a di-hydroxy TAG, while the mass of the $[M+23]^+$ ion gave the molecular weight as

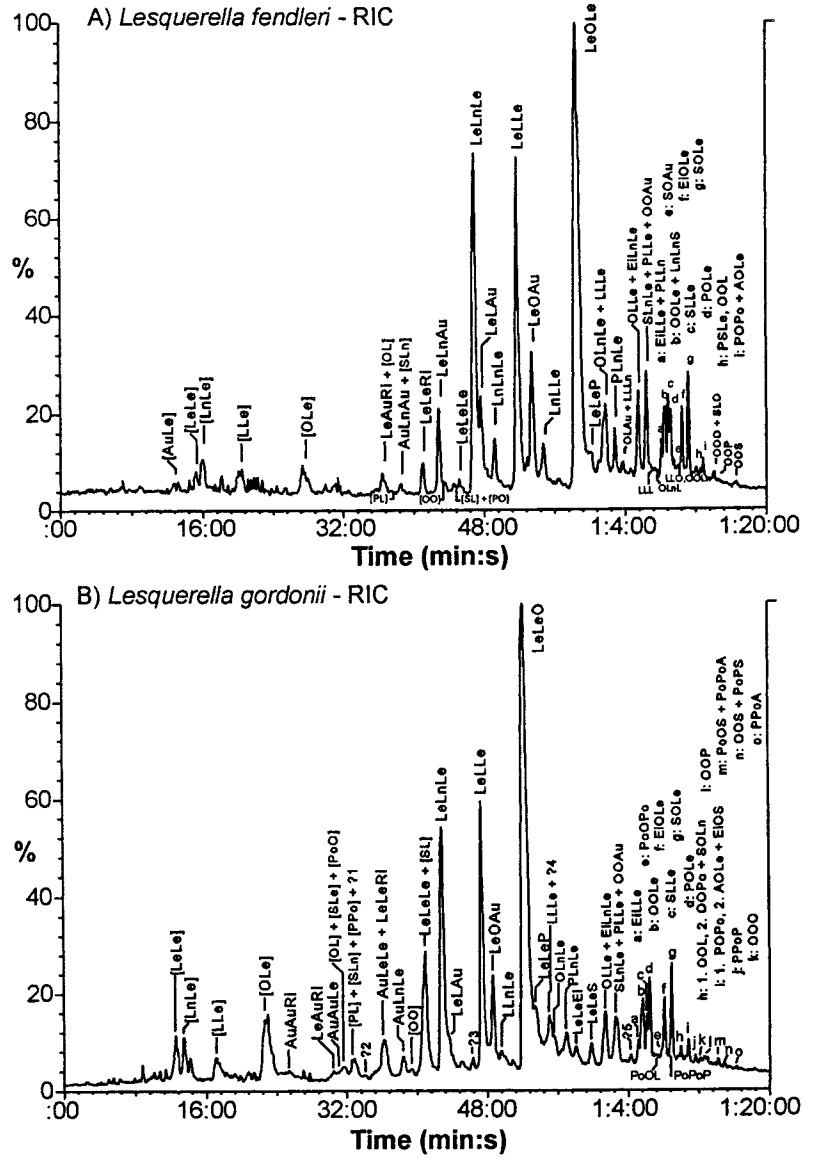

Figure 4. Reconstructed ion chromatograms of lesquerella seed oils. A) *Lesquerella fendleri*, B) *Lesquerella gordonii*. Abbreviations as in Figure 1.

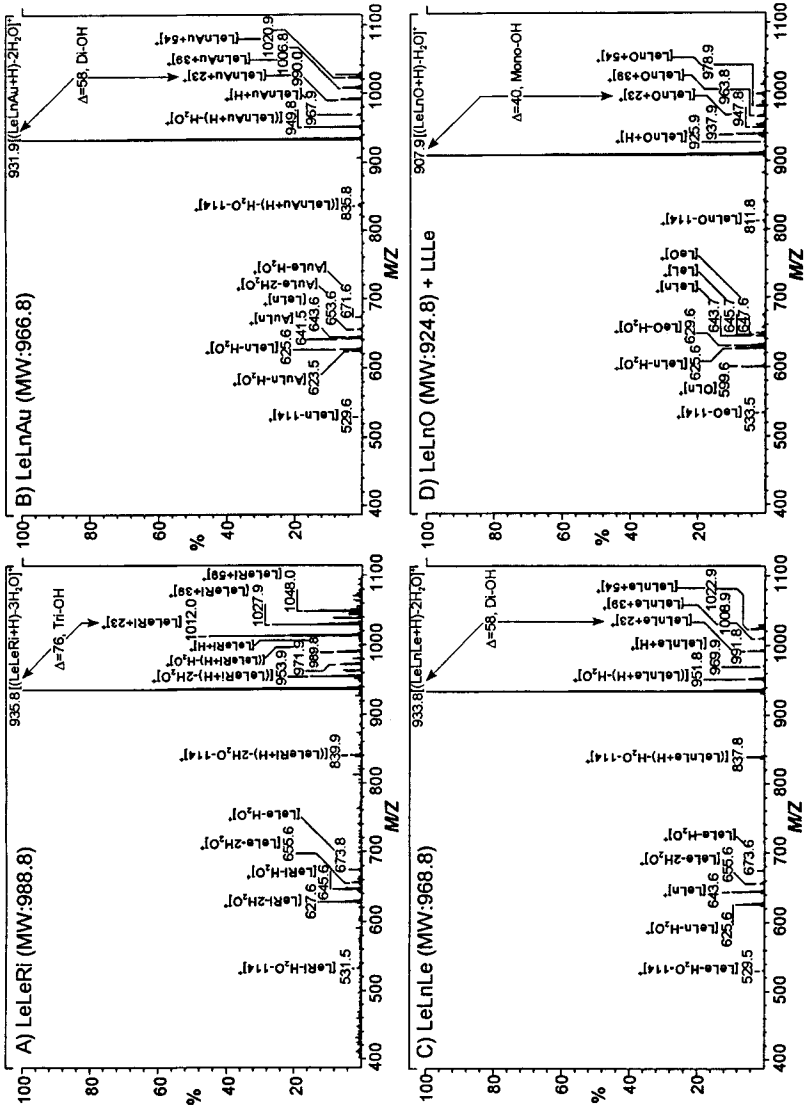

Figure 5. Mass spectra of *Lesquerella fendleri* seed oil hydroxy triacylglycerols. A) LeLeRi, B) LeLnAu, C) LeLnLe, D) LeLnO. Abbreviations as in Figure 1.

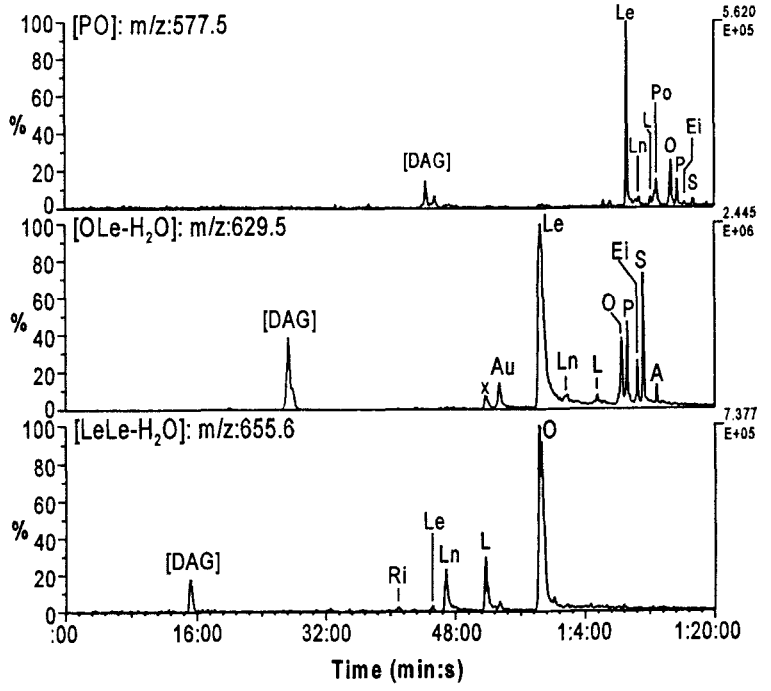

Figure 6. Extracted ion chromatograms of diacylglycerol fragments of *Lesquerella fendleri* seed oil hydroxy triglycerides. [PO] = non-hydroxy diacylglycerol fragment, [OLe-H_2O] = mono-hydroxy diacylglycerol fragment, [LeLe-H_2O] = di-hydroxy diacylglycerol fragment. Abbreviations as in Figure 1.

967 (966.8) amu. The other spectra in Figure 5 further demonstrate the formation of these diagnostic ions. In Figure 5D, the difference of 40 amu between the base peak and the $[M+23]^+$ ion established this as a mono-hydroxy TAG, while the $[M+23]^+$ ion gave the molecular weight as 924.8 amu (*m/z* 937.9 in this spectrum is from residual $[(LeLeO+H)-2H_2O]^+$).

Three EICs for TAGs of *L. fendleri* are shown in Figure 6. These were chosen to represent di-hydroxy, mono-hydroxy, and non-hydroxy diacylglycerol fragments. In the dihydroxy EIC (m/z:655.6, $[LeLe-2H_2O]^+$), the primary peaks were those of LeLeLn, LeLeL, and LeLeO, corresponding to the major components visible in the RIC, Figure 4A.

In the mono-hydroxy EIC (m/z:629.5, $[OLe\text{-}H_2O]^+$, peaks were seen for AuOLe, LeOLe, LnOLe, LOLe, OOLe, POLe, EiOLe, SOLe, and AOLe, as well as the native diacylglycerol. The $[PO]^+$ diacylglycerol likewise showed peaks for that fragment arising from combination with Le, L, O, P, 20:1, and S.

The RIC of *L. gordonii*, Figure 4B, is similar to that of *L. fendleri*. However, the relative proportions of such TAG species as AuLnLe, LeLeLe, and many others varied noticeably. Also, TAGs of *L. gordonii* contained much more palmitoleic acid, Po, than did the TAGs of *L. fendleri*. TAGs containing Po were mostly non-hydroxy TAGs. This preferential association of Po is clearly seen in the RIC in Figure 4B, and shows up clearly in the EICs for this seed oil. Thus, these seed oils clearly exhibited structural differences which were readily observed using LC/APCI-MS.

DISCUSSION

Because the seed oils studied contained TAGs of widely varying polarities, one chromatographic method was necessary which could accomplish the separation of all compounds from the trihydroxy TAGs to the normal, non-hydroxy TAGs, yet still remain under the limit of 99 minutes imparted by the LC/MS control software. Thus, the method we developed started with a much higher percentage of acetonitrile than was previously used, in order to elute the polar hydroxy compounds over a broad time period. Late in the run it was necessary to use a steep gradient to produce a solvent composition similar to the chromatographic systems reported previously for conventional TAG analysis.[9] The resultant run provided excellent separation of hydroxy TAGs, but produced less separation of normal TAGs than we have previously obtained. Nevertheless, sufficient resolution of normal TAGs was achieved so that virtually all molecular species could be clearly distinguished by mass, as exemplified by the EIC for *m/z* 577.5, Figure 6. As the mass spectra, which included peaks from several partially resolved TAGs, became more complex, increased emphasis was placed on the use of extracted ion chromatograms for data analysis and interpretation.

As seen in the results above, the APCI source produced a complete set of fragments and adducts which allowed trihydroxy compounds to be clearly distinguished from the dihydroxy compounds which were then clearly differentiated from the mono-hydroxy compounds. The difference of 76 mass units between the base peak produced by loss of all hydroxy groups and the first acetonitrile adduct was characteristic of the trihydroxy TAGs, while a difference of 58 amu was observed for dihydroxy TAGs, and 40 amu was the difference observed for mono-hydroxy TAGs. More importantly, the ability to

see both a small molecular ion and a large fragment/adduct in the mass spectra, which could be used to identify the molecular weight of the hydroxy TAG, represents an advancement in the ability to analyze intact hydroxy TAG structures. This method allows for a rapid screening of a seed oil which identifies all of the disparate components therein. Other authors have used SFC-lipase analyses of lesquerella seed oils[4,10] to successfully characterize lesquerella oils. This method was very effective at elucidating the triglyceride structures of the oils, but was labor intensive, more complex, and time-consuming (requiring at least 12 hr for complete lipase reaction). However, that method did supply positional isomer information which is not readily available using LC/MS. Thus, it is useful to know that the hydroxy fatty acids prefer the 1 and 3 glycerol positions, whereas the 2-position is usually occupied by unsaturated normal fatty acids, and that saturates tend to be found on the 1 and 3 positions.[4,10,11] However, with that foundation established, it is desirable to be able to employ a single methodology for analysis which can identify all of the intact TAG structures in one chromatographic run. Thus, there is distinct potential utility for the use of APCI-MS in analysis of hydroxy TAGs.

Our results for *L. gordonii* confirmed previous analogous results for palmitoleic acid in *L. fendleri*[11] that this fatty acid is found primarily in the non-hydroxy TAG species. As mentioned in the results above (Fig. 4B and EICs not shown), our results showed distinct preference for incorporation of palmitoleic acid into non-hydroxy TAGs. Another tendency was for auricolic acid to be incorporated into TAGs containing lesquerolic acid. Upon further investigation into the steps necessary for reliable quantitation of all molecular species, we may be able to confirm the preference of auricolic acid to be associated with linolenic acid over other normal fatty acids which was previously reported.[11]

Quantitative analysis of hydroxy TAGs using APCI-MS will require method development and validation using calibrations curves or other approaches for producing response factors. As an alternative, given the demonstrated ability of APCI-MS to qualitatively identify the broad range of TAGs present, it may be possible to use another type of detector such as ELSD or FID in combination with the qualitative data to provide the necessary percentage composition data. We have demonstrated that a detector in tandem with the MS detector, which utilizes the same column effluent, has potential for providing better quantitation.

The tandem detector arrangement has the advantage that identical retention times are obtained in the MS data and the 2-D detector data, allowing facile qualitative identification of peaks. However, the optimal conditions necessary to provide sufficient sensitivity still need to be established, and the

nature of the best secondary detector to use (i.e. ELSD or FID) needs to be established. And again, validation of the quantitation method must be performed for whichever secondary detector is chosen.

Finally, despite the large number of components which could be conclusively identified in these oil samples, the *L. gordonii* sample contained some peaks which were not conclusively identified. The first three peaks, labeled ?1, ?2, and ?3 in Figure 4B, at retention times 32:45, 35:07 and 46:17, respectively, gave spectra which were very similar to the trihydroxy TAG mass spectra, but with important differences. In the spectra of all three of these peaks, the $[M+H-3H_2O]$ peaks were strong, but were not the base peaks. The $[M+59]^+$ peak was the largest of the high mass peaks for the TAG labeled ?1, while the $[M+23]^+$ was the base peak for ?2 and?3. The first two peaks both had a molecular weight = 984.8 amu (as given by the $[M+23]^+$, $[M+39]^+$, $[M+59]^+$ and $[M+H-nH_2O]^+$ ions), while the third had a molecular weight of 988.8 amu. The first two molecular weights are the same as AuAuRi, or AuLeDe, but these TAGs had shorter retention times.

One possibility is the presence of epoxy-containing molecules which are very similar in structure to hydroxy TAGs. Such epoxy TAGs have been reported in *L. fendleri*[12] (but not observed by us, as they were below the detection limit in our data). These epoxy fatty acids do give appropriate calculated masses for TAGs in combination with Au and Le fatty acids, but we cannot make identifications based on the present data. Tandem mass spectrometry of these peaks, on a triple quadrupole mass spectrometer, may yield definitive structures. Two other peaks, which eluted at 54:56 and 1:04:12 gave base peaks of 505.5 and 507.6, respectively. These fragments indicate structural features not observed in any of the other TAGs present. No other data is currently available for these components.

Overall, it was demonstrated that a single reversed-phase separation with APCI-MS detection was capable of identifying numerous TAG species of diverse polarities in hydroxy-containing oils. It had not been previously demonstrated that the APCI source produced fragments from hydroxy TAGs which could be used to unambiguously identify molecular species by the presence several confirming fragments, as well as a small protonated molecular ion. The fragmentation patterns of these hydroxy TAGs under APCI conditions have been described, falling into three general categories: 1) loss of hydroxy groups through dehydration to form additional unsaturation, 2) loss of a fragment giving a net loss of 114 amu, and 3) formation of a series of three or four important diagnostic fragment/adducts.

As with APCI-MS of normal TAGs, the diacylglycerol fragments of hydroxy TAGs provided further confirming evidence of the identity of the fatty acyl chains on the hydroxy TAG molecular species. Also, the necessity for continued work into the conditions required for reliable quantitation has been shown.

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