Fatty Acids and Triacylglycerols of Cherry Seed Oil

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Cherry seed oil, from the Rosaceae family, prunoid subfamily, is characterized by the existence of about 10% α-eleostearic acid. The structure of the acid was proven by H and ^{13}C nuclear magnetic resonance. The triacylglycerols of this oil were identified and quantitated by high-performance liquid chromatography by means of several types of detectors. α-Eleostearic acid was not found in the seeds of previously studied prunoids (almond, peach, apricot and plum). The main fatty acids found in the seeds of cherry and other prunoids were linoleic (L), oleic (O) and palmitic acids, and the major triacylglycerols were LLO, LOO and OOO. These chemical data support the botanical relationship within the prunoid subfamily and show the proximity of cherry to the Chrysobalanaceae family.

KEY WORDS: Cherry seed oil, α-eleostearic acid, NMR, *Prunus avium*, Rosaceae, triacylglycerols.

The cherry tree (*Prunus avium* L.) belongs to the Rosaceae family, subfamily of the prunoids. In an earlier article (1) we described the fatty acid and triacylglycerol composition of a few oils from prunoid Rosaceae seeds (almond, apricot, peach and plum). These oils have a common fatty acid composition with oleic, linoleic, palmitic and stearic acids. This is not the case with cherry seed oil which, in addition to the above fatty acids, also contains a rarely seen acid, whose precise identification requires the use of mass spectrometry, along with H and ^{13}C nuclear magnetic resonance (NMR). Given the existence of this acid, several types of detectors have to be used for qualitative and quantitative analysis of the triacylglycerols of this oil by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

The cherry stones used were of the morello and bigaroon types and, in the latter case, the coeur de pigeon and burlat varieties. After the harvesting of the stones, manual separation of the seeds, drying in an oven at 60°C and crushing, the seeds were hexane-extracted in a Soxhlet apparatus. The solvent was then evaporated under reduced pressure to leave the crude oil (yield = 18%, dry basis).

Oil (50 mg) was transesterified with methanol in the presence of sodium methylate (2). The fatty acid methyl esters (FAME) were analyzed by gas chromatography in a Girdel 3000 chromatograph (Delsi-Nermag, Argenteuil, France) fitted with a glass-needle evaporator injector (Ross type) and a flame ionization detector (FID). The column (25 m long, 0.32 mm i.d.) was impregnated with Carbowax 20M (film thickness, 0.25 μm). Analysis conditions were: injector and detector temperature at 250°C, oven temperature at 210°C, and the carrier gas was helium at a pressure of 1 bar.

The triacylglycerols in 100 mg of crude oil were separated from other materials by chromatography on a silica column (3). Analysis of the triacylglycerols according to the type of fatty acids was carried out on a Merck HPLC chromatograph (E. Merck, Darmstadt, Germany), fitted with a 25-cm RP (reversed phase) 18 column (Merck, Lichrosorb) with an internal diameter of 4 mm. Three types of detectors were used: i) A differential refractometer (Waters Associates, Milford, MA). The eluant was an acetone/acetonitrile mixture (2:1, vol/vol) with a flow of 0.8 mL/min. ii) A light scattering detector (or mass detector) (Cunow 10, Paris, France). Two solvents were used. **A** (acetone/isopropanol, 55:45, vol/vol) and **B** (isooctane). Gradient programming was as follows: 5 min of pure **A**, then an **A/B** mixture from 100:0 to 90:10 in 35 min. The flow rate was 1 mL/min. iii) A diode array ultraviolet (UV) detector (Waters 990 instrument). The elution and flow-rate conditions were the same as for the light scattering detector. The UV spectra were taken between 200 and 400 nm.

The UV spectrum of the oil was determined in solution in anhydrous cyclohexane at a concentration of 0.01 g/L on a Pye-Unicam SP8-300 spectrophotometer (Pye-Unicam Ltd., England). The spectra were studied from 220 to 400 nm, with 1-cm quartz cells.

The NMR spectra were measured in deuteriochloroform solution, on a Bruker AC360 instrument (Bruker Spectrospin S.A., Wissembourg, France) with an internal reference of tetramethylsilane.

The gas chromatography/mass spectrometry (GC/MS) spectrum was taken on a Hewlett-Packard instrument (Hewlett-Packard Corp., Palo Alto, CA). The chromatograph was equipped with an SE30 column (30 m long, 0.22 mm i.d.). The analysis conditions were identical to those given for the Girdel chromatograph.

RESULTS

GC and FAME analysis of cherry seed oil revealed the presence of palmitic, stearic, oleic and linoleic acids, identified by co-injection with standard samples. An unusual chromatographic peak was also seen. This peak represented about 10% of the mixture and was characterized by an equivalent chain length of 21.6 on a Carbowax 20M polar column and 19.1 on a SE30 nonpolar column. It should be pointed out that this FAME was not detected when fatty acid (FA) esterification was carried out in an acidic medium (methanol/BF_3) (3) but was readily detected when transesterification was carried out in an alkaline medium. This suggested a conjugated polyene fatty acid (3). This hypothesis was confirmed by a study of the oil's UV spectrum, which had three absorption bands at 261 nm ($E^{1\%}_{1cm}$= 36,000), 271 nm ($E^{1\%}_{1cm}$= 46,100) and 281 nm ($E^{1\%}_{1cm}$= 35,500), attributable to the existence of a conjugated triene system (4). This intense absorption was linked to the existence of the unknown fatty acid, because there is no absorption in this region for the other fatty acids. We then carried out a GC/MS analysis of the FAME and an NMR study of the oil.

The GC/MS analysis of the cherry seed oil FAME gave a molecular ion at *m/z* 292 (47%) for the unknown ester,

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corresponding to an empirical formula $C_{19}H_{32}O_2$, which suggested an 18-carbon chain with three double bonds. Peaks at *m/z* 261 (M − OMe, 6%) and *m/z* 219 (M − CH_2CO_2 Me, 2%) are characteristic of the ester function. No cleavage attributable to the presence of double bonds was seen.

Confirmation of the existence of three double bonds and determination of their configurations was achieved through analysis of H, ^{13}C (proton decoupled then undecoupled), 2D H/H and 2D H/C NMR spectra obtained on the crude oil. An analysis of the ethylenic region of the undecoupled ^{13}C NMR spectrum revealed six signals attributable to the unknown acid (Table 1) in addition to the intense peaks due to the ethylenic carbon atoms of oleic (δ = 129.89 and 127.80) and linoleic acids (δ = 130.03, 129.86, 127.98 and 127.80) identified in the literature (5–7). These six signals appeared in the form of doublets (^1J = 150 Hz) in the undecoupled spectrum, which confirms the existence of six olefinic carbon atoms (no allenic compound). At the same time, the proton spectrum revealed five low-intensity signals; the sixth was probably mixed in with the intense pattern of ethylenic protons of the common fatty acids (δ = 5.3 to 5.4). The carbon (**A, B, C, D, E** and **F**) and hydrogen (**a, b, c, d, e,** and **f**) atoms of the conjugated system are identified in Table 1 along with the adjacent methylene atoms (**a′** and **f′**). The 2D H/H spectrum revealed that **a′** and **f′** were coupled with **a** and **f**, respectively. Starting from signal **a** (δ = 5.67), coupling was seen with **b** (δ = 6.14), **d** (δ = 6.34), **e** (δ = 5.96) and, finally, **f** (δ = 5.35). Analysis of the proton spectrum revealed coupling constants between the different protons. Coupling constants J > 13 Hz were attributed to an *E* configuration of the **AB** and **CD** double bonds, whereas J = 10.8 Hz was assigned to the **EF** double bond with a *Z* configuration. The unknown acid therefore had a *Z,E,E* or *E,E,Z* configuration. Analysis of the 2D H/C spectrum identified carbon atoms **A** to **F**, linked to protons **a** to **f**, respectively. Table 1 gives the chemical shifts for all the ethylenic carbon atoms, along with all the corresponding hydrogen atoms with their coupling constants.

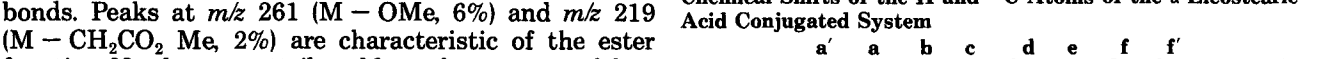
Comparing the values found to those given in the literature (5,8–11), we were able to conclude that the unknown fatty acid was octadeca-9,11,12-trienoic acid (9*Z*, 11*E*, 13*E*), also known as α-eleostearic acid.

This work confirmed previous works (6,8,10) and provided more details about the study of α-eleostearic acid by NMR. The precise attribution of ethylenic proton chemical shifts by using 2D H/H NMR confirmed the attributions made by Suzuki *et al.* (10), who identified the six ethylenic protons' signals by studying spectra taken in the presence of lanthanides. Our work assigns coupling constants that determine the precise configurations of the three ethylenic bonds and yield the chemical shifts of the ethylenic carbon atoms. Previous work (6,8) utilized additivity rules, which require cautious interpretation and confirmation.

Table 2 gives the fatty acid composition of the kernel oil from three varieties of cherry. The proportion of α-eleostearic acid is similar in the three varieties, whereas oleic and linoleic acids predominate and are inversely related. Our results are similar to those reported by Funes and Cattaneo (12) for the Bing and Napolitane varieties. Based on a study of the oil's UV spectrum, these authors concluded that α-eleostearic acid was present. Lazos (13) and Kamel and Kakuda (14) did not report this fatty acid, probably because they esterified the fatty acids in acidic medium.

TABLE 1

Chemical Shifts of the H and ^{13}C Atoms of the α-Eleostearic Acid Conjugated System



H chemical shift (ppm)	H coupling constants (Hz)	^{13}C chemical shift (ppm)
(a′) = 2.08	J(aa′) = 6.5	(A′) = 32.6
(a) = 5.67	J(ab) = 13.5	(A = 134.9
(b) = 6.07	J(bc) = 9.8	(B) = 130.5
(c) = 6.14	J(cd) = 13.9	(C) = 132.8
(d) = 6.34	J(de) = 11.0	(D = 125.8
(e) = 5.96	J(ef) = 10.8	(E) = 128.7
(f) = 5.35	J(ff′) = ?	(F) = 131.5
(f′) = 2.15		(F′) = 27.9

TABLE 2

Fatty Acid Composition (mole%) of Three Cherry Seed Oils

Fatty acid		Cherry seed oil		
		1[a]	2[b]	3[c]
Lauric acid	C12:0	trace	trace	trace
Myristic acid	C14:0	trace	trace	trace
Palmitic acid	C16:0	7.6	9.4	6.8
Palmitoleic acid	C16:1	0.5	0.6	0.4
Stearic acid	C18:0	2.6	1.6	2.1
Oleic acid	C18:1 9*c*	37.5	23.9	32.8
Linoleic acid	C18:2 9*c*,12*c*	40.0	48.9	44.7
α-Linolenic acid	C18:3 9*c*,12*c*,15*c*	0.3	1.0	trace
α-Eleostearic acid	C18:3 9*c*,11*t*,13*t*	9.9	12.8	13.2
Arachidic acid	C20:0	1.2	1.3	trace
Eicosenoic acid	C20:1 11*c*	0.4	0.5	trace

[a]Type, bigaroon; variety; coeur de pigeon.
[b]Type, morello.
[c]Type, bigaroon; variety; burlat.

Comparing cherry seed oils to oils from other prunoid seeds (1), oleic acid is the main fatty acid (over 60%) in the other prunoids, and linoleic acid is much less abundant (14–30%); α-eleostearic acid is specific to cherry. These results show the peculiarity of cherry in the prunoid subfamily. The existence of α-eleostearic acid in Chrysobalanaceae lipids chemotaxonomically confirms cherry's genetic proximity to this family, as already indicated by morphological characteristics (15).

A study was conducted on triacylglycerols (TG) in cherry seed oil from the coeur de pigeon variety. The presence of α-eleostearic acid in this oil complicated qualitative and quantitative HPLC analysis of its TG when using a differential refractometer detector. We did not have a trieleostearin sample available, and we were unable to apply the rules set down by Goiffon *et al.* (16,17) and by Perrin and Naudet (18). Nevertheless, we were able to identify combinations of palmitic, stearic, oleic and linoleic acids, which had previously been identified during the study of the other prunoid oils (1). To detect the α-eleostearic acid containing TG, we used a diode array UV detector with two different wavelengths. At 210 nm, the n → π^* transition of the C=O bond of the TG ester function can be seen, and at 270 nm the $\pi \rightarrow \pi^*$ transition

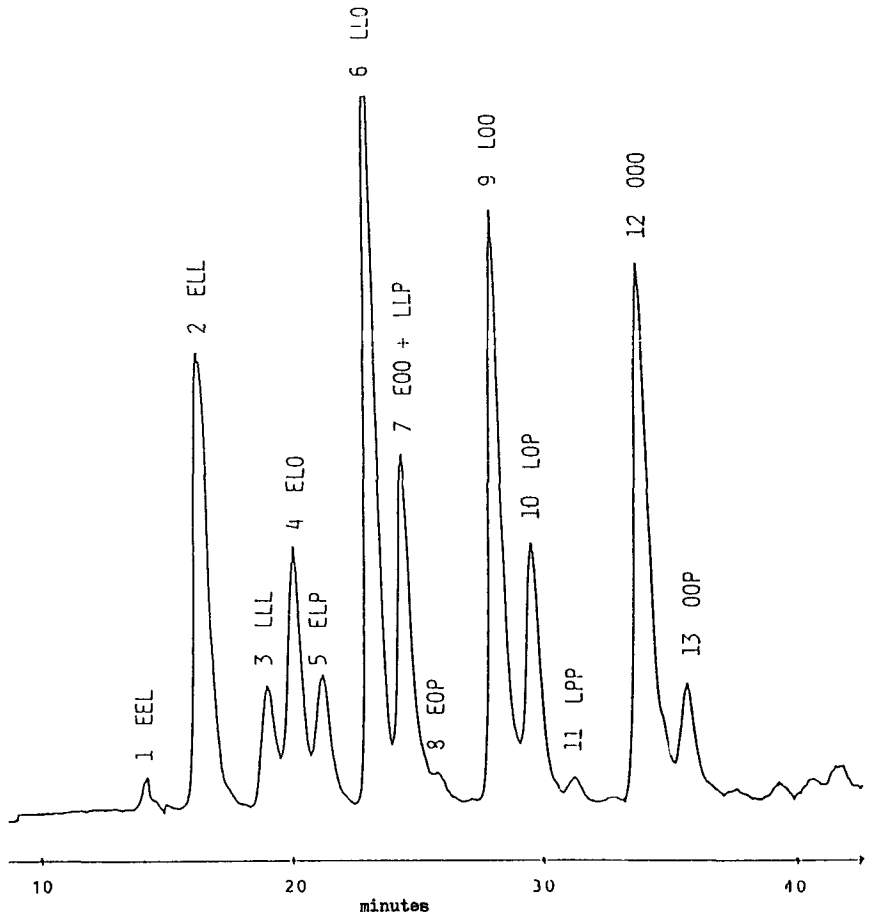


FIG. 1. High-performance liquid chromatography chromatogram of cherry seed oil triacylglycerols using a light scattering detector. P, O, L and E are palmitic, oleic, linoleic and α-eleostearic acids, respectively.

TABLE 3

Triacylglycerol Composition of Cherry Seed Oil[a]

HPLC peak	TG[b]	RT	%
1	EEL	14.55	0.5
2	ELL	16.77	12.9
3	LLL	19.39	3.3
4	ELO	20.53	6.9
5	ELP	21.61	3.9
6	LLO	23.63	18.5
7	LLP/EOO	24.90	9.8
8	EOP	26.05	trace
9	LOO	28.63	15.4
10	LOP	30.09	7.7
11	LPP	31.74	1.0
12	OOO	34.48	16.1
13	OOP	36.25	4.0

[a]Type, bigaroon; variety, coeur de pigeon. HPLC, high-performance liquid chromatography; TG, triacylglycerol; RT, retention time (min).

[b]P, O, L and E are palmitic, oleic, linoleic and α-eleostearic acids, respectively.

of the α-eleostearic acid's conjugated system can be observed. Comparison of chromatograms taken at these two wavelengths enabled us to identify the TG combinations containing α-eleostearic acid. The drawback of this method is that it is not quantifiable because the molar absorption coefficients of the various TG are different at 210 nm. Comparison of chromatograms obtained with refractive index and UV detectors enabled us to carry out a qualitative analysis of cherry seed oil TG.

We used a light scattering detector for quantitative HPLC analysis of the TG. We found that TG from oleic and linoleic acids combinations had identical response coefficients, as indicated by Stolywho *et al.* (19). We assumed the same response coefficients for TG combinations containing α-eleostearic acid (Fig. 1). This hypothesis was justified *a posteriori* because the fatty acid composition deduced in this way from the analysis of the TG fitted well with their direct analysis. By combination of these three detection methods, we were able to compile Table 3. Cherry seed oil is rich in LLO, LOO, OOO and EEL (L = linoleic, O = oleic, E = elaidic acid). Other TG are present in proportions under 10%.

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