FA Composition and Regiospecific Analysis of *Acer saccharum* (sugar maple tree) and *Acer saccharinum* (silver maple tree) Seed Oils

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ABSTRACT: GC analysis was performed to determine regiospecific distribution and FA composition in seed oils of the Aceraceae species, *Acer saccharum* and *A. saccharinum*. The oil content in the seeds was low at 5.0% in *A. saccharum* and 5.8% in *A. saccharinum,* and the main FA were linoleic (30.8 and 29.4%), oleic (21.3 and 27.6%), palmitic (10.1 and 10.5%), and *cis*-vaccenic (9.4 and 7.9%) acids, respectively. In addition, both oils contained long-chain monoenes of the n-9 and n-7 groups, including 11-eicosenoic, 13-docosenoic, 15-tetracosenoic, 13-eicosenoic, and 15-docosenoic acids, whereas γ–linolenic acid accounted for 0.8% of total FA in *A. saccharum,* and 0.5% in *A. saccharinum*. Regiospecific analysis, performed using the methodology of dibutyroyl derivatives of MAG, indicated that linoleic, oleic, and linolenic acids were mainly esterified at the internal position of TAG in both seed oils, whereas long-chain monoenes of the n-7 group were almost exclusively esterified on the external positions.

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The genus *Acer* (Aceraceae) comprises around 100 species, of which about 20 grow in North America. These include *A. saccharum* (sugar maple tree, from which maple syrup is obtained) and *A. saccharinum* (silver maple tree), which are very common to eastern Canada. In spite of the widespread occurrence of this genus, the seed oil composition of *Acer* species has not been well documented (1,2). Hopkins *et al.* (1) reported the FA composition of 18 *Acer* species based on analyses using a packed column. Subsequent analysis has been performed on capillary columns for the determination of positional and configurational isomers of the unsaturated FA present in the oil, and the presence of both the n-9 and n-7 FA series has been confirmed (1,2). The oil also contains γ-linolenic acid at levels from 1 to 7% (2). In the present study, we report the FA composition of the seed oil of two *Acer* species, *A. saccharum* and *A. saccharinum* and the regiospecific distribution of the FA determined by using the method of dibutyrate derivatives of MAG (3). We also compare the regiospecific distribution of both n-7 and n-9 series of monounsaturated FA.

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MATERIALS AND METHODS

Samples and reagents. Seeds from *A. saccharum* and *A. saccharinum* were collected in Québec City region (Canada). Standard TAG (tripalmitin, tristearin, triolein, tri-*cis*-vaccenin, trilinolein, tri-α-linolenin, trigondoin, trieicosadienoin, and trieicosatrienoin) were purchased from Nu-Chek-Prep (Elysian, MN). Ethyl magnesium bromide, *n*-butyryl chloride, and triethylamine were obtained from Aldrich Chemicals (Milwaukee, WI).

Oil extraction. A seed sample of each species (*A. saccharum* and *A. saccharinum*) was pulverized with a household grinder, and the oil was extracted from the flour (10 g) with light petroleum ether using a Soxhlet apparatus. The extract was dried over anhydrous sodium sulfate, filtered, and the oil recovered by evaporation of the solvent under vacuum at 45°C in a rotary evaporator. The oil samples were stored under nitrogen at −35°C until further use.

Synthesis of dibutyrate derivatives of MAG. Dibutyrate derivatives of MAG were synthesized according to the method proposed by Angers and Arul (3), with minor modifications. To a stirred solution of seed oil (10 mg) in anhydrous diethyl ether (500 μL) contained in a flame-dried flask under inert atmosphere (N_2), a solution of ethyl magnesium bromide in the same solvent (3.0 M, 40 μL) was added. After stirring for 30 s at room temperature, glacial acetic acid (10 μL) was added, followed by boric acid (10% aqueous solution, 300 μL). Diethyl ether (0.5 mL) was added, phases were separated, the aqueous phase was further extracted twice with the same solvent (2 × 0.5 mL), and the extracts were combined, washed sequentially with Na_2CO_3 (10% aqueous solution) and then with saturated brine, dried over anhydrous Na_2SO_4, and finally concentrated under a stream of dry nitrogen. The residue was dissolved in dry chloroform (500 μL) to which triethylamine (100 μL) and *n*-butyryl chloride (50 μL) were added sequentially, and the reaction mixture was held at 60°C for 30 min in a closed vial with occasional stirring. After cooling to room temperature, the reaction mixture was diluted with *n*-octane (1 mL), washed sequentially with cold diluted hydrochloric acid (0.1 N), water, and saturated brine. The extract was filtered and dried over anhydrous Na_2SO_4. Aliquots (50 μL) were diluted with *n*-octane (200 μL) and analyzed by GC.

GC TAG. Dibutyrate derivatives of MAG were analyzed with a gas chromatograph (Model 6890, Series II; Hewlett-

Packard, Palo Alto, CA) equipped with an FID, and connected to a ChemStation (Hewlett-Packard). Sample volumes (1.0 μL) in *n*-octane were injected on a 65% phenyl methyl siloxane capillary column (Restek, Bellefonte, PA; 30 m × 0.25 mm i.d., 0.10 μm film thickness) with a split ratio of 1:50. Injector and detector temperatures were set at 400°C, whereas the oven temperature was programmed from 100 to 300°C at 5°C min^{-1}, and to 360°C at 20°C min^{-1}, and held for 5 min at this temperature. The inlet pressure of the carrier gas (H_2) was 84 kPa.

GC of FAME. Methylation of FA was carried out in a sealed tube with 0.4 N sodium methoxide in methanol. Analysis of the methyl esters was performed with a gas chromatograph (Hewlett-Packard model 5890 Series II), equipped with an FID and connected to a computer with a ChemStation (Hewlett-Packard). Sample volumes (1.0 μL) in hexane were injected on an open tubular DB-225 capillary column (J&W, Folsom, CA; 30 m × 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were maintained at 250°C, and isothermal oven temperature (190°C) prevailed. Hydrogen was the carrier gas, with a pressure of 140 kPa.

GC–MS. 4,4-Dimethyloxazoline (DMOX) derivatives were prepared directly from TAG fractions according to Garrido and Medina (6). The derivatives were analyzed by GC–MS (Hewlett-Packard model 5890 gas chromatograph attached to an HP model 5970 selective quadrupole mass detector) under an ionization voltage of 70 eV at 250°C. The chromatograph was equipped with a BPX-70 fused-silica capillary column (SGE, Melbourne, Australia; 25 m × 0.22 mm i.d., 0.25 μm film thickness). Injection (split mode) and detection ports were maintained at 250°C, while oven temperature programming consisted of 60°C for 2 min, up to 180°C at 20°C min^{-1}, then to 200°C at 2°C min^{-1}, and isothermal for 10 min at 200°C. Helium was used as carrier gas (1 mL min^{-1}). Picolinyl ester derivatives were synthesized by base-catalyzed transesterification of TAG according to the procedure of Destaillats and Angers (7), using potassium picolinate in methylene chloride at 40°C for 15 min and analyzed by GC–MS in similar conditions, but at 230°C for 40 min, and on a 60 m BPX-70 capillary column.

RESULTS AND DISCUSSION

The oil content in the seeds was low, averaging 5.0 and 5.8% in *A. saccharum* and *A. saccharinum*, respectively, compared to literature values of 10.6–17% and 2.4% (1,2), thus showing variations between species (Table 1). The refractive indices (1.469 and 1.466, at 20°C), saponification values (195 and 188), and iodine values (117 and 99, Hanus) were all characteristic of a moderately unsaturated oil.

FA composition. The results for FA composition of the two *Acer* spp. seed oils (Table 1) agree for the most part with literature data (1,2), including γ-linolenic acid (GLA) and long-chain monoenoic FA, except for docosenoic acid contents. Hopkins *et al.* (1) reported 10% of 22:1 in *A. saccharum* whereas Bohannon and Kleiman (2) reported 10.3%. We found levels of 3.4 and 0.7% for 22:1n-9 (erucic acid) and 22:1n-7 FA, respectively. Although in those studies (1,2) the positional isomers, 22:1n-9 acid and 22:1n-7 acid, were not resolved under the GLC conditions used, the difference of about 6% in the level of 22:1 cannot be explained unless it is inherent. The presence of GLA (6,9,12-18:3) in the oils was confirmed by analysis of DMOX derivatives by GC–MS. The presence of prominent molecular ions of GLA at *m/z* = 361 in the DMOX spectrum confirmed both chain length and number of double bonds. Cleavages for the bis-allylic interrupted ethylenic bond system characterized by ions at *m/z* 196, 208, 236, 248, 276, and 288 allowed the confirmation of a 6,9,12-18:3 acid structure. The results compared well with those reported by Bohannon and Kleiman (2) obtained by GC–ozonolysis and by GC–MS of methoxy derivatives. In addition, the presence of 18:1n-5 FA in the seed oil of *A. saccharum* was confirmed by analysis of picolinyl ester derivatives. Indeed, the spectrum allowed confirmation of chain length (*m/z* = 373) as well as the position of the double bond by cleavages of both allylic bonds, characterized by ions at *m/z* 276 and 330 (Fig. 1).

TABLE 1
FA Composition (wt%) of *Acer saccharum* (sugar maple) and *Acer saccharinum* (silver maple) Seed Oils

	A. saccharum		*A. saccharinum*	
FA	Experim.	Lit.[a]	Experim.	Lit.[a]
16:0	10.1	6.4[a]	10.5	17.0
16:1	0.6	—[b]	0.5	—
18:0	2.5	3.2	3.0	2.0
18:1n-9	21.3	28.2[c]	27.6	26.0[c]
18:1n-7	9.4		7.9	
18:1n-5	Trace[d]		—	
18:2n-6	30.8	36.6	29.4	32.0
18:3n-6	0.8	1.8	0.5	—
18:3n-3	9.0	0.5	5.2	19.0
20:0	0.3	0.6	0.3	—
20:1n-9	3.7	6.6[c]	4.0	—[c]
20:1n-7	0.6		0.5	
22:0	0.3	0.7	0.3	—
22:1n-9	3.4	10.3[c]	3.1	1.0[c]
22:1n-7	0.7		0.8	
22:2n-6	Trace	0.3	—	—
24:0	0.2	—	0.3	—
24:1n-9	2.7	3.8	2.4	1.0
Others	3.6	—	3.7	—

[a]References are *A. saccharum* (2) and *A. saccharinum* (1).
[b]Not reported/detected.
[c]Sum of all isomers.
[d]Trace values are lower than 0.1%.

Regiospecific analysis. Regiospecific profile study may be a satisfactory means to investigate structural composition of TAG, but it is not widely used because of the laboriousness of the protocols. We performed regiospecific analysis according to the procedure of Angers and Arul (3), which has been used previously for regiospecific distribution of petroselinic, oleic, and *cis*-vaccenic acids in basil (*Ocimum basilicum*) and coriander (*Coriandrum sativum*) seed oils (4), and for regiospecific distribution of Δ5-olefinic FA in conifer seed TAG (5). Regiospecific distribution for FA of the n-9 and n-7 groups present in the oils are shown in Table 2.

The major FA in the *sn*-2 position of TAG for both oil samples were linoleic and oleic acids. Levels of 52.6 and 55.9% were found for linoleic acid in *A. saccharum* and *A. saccharr-*

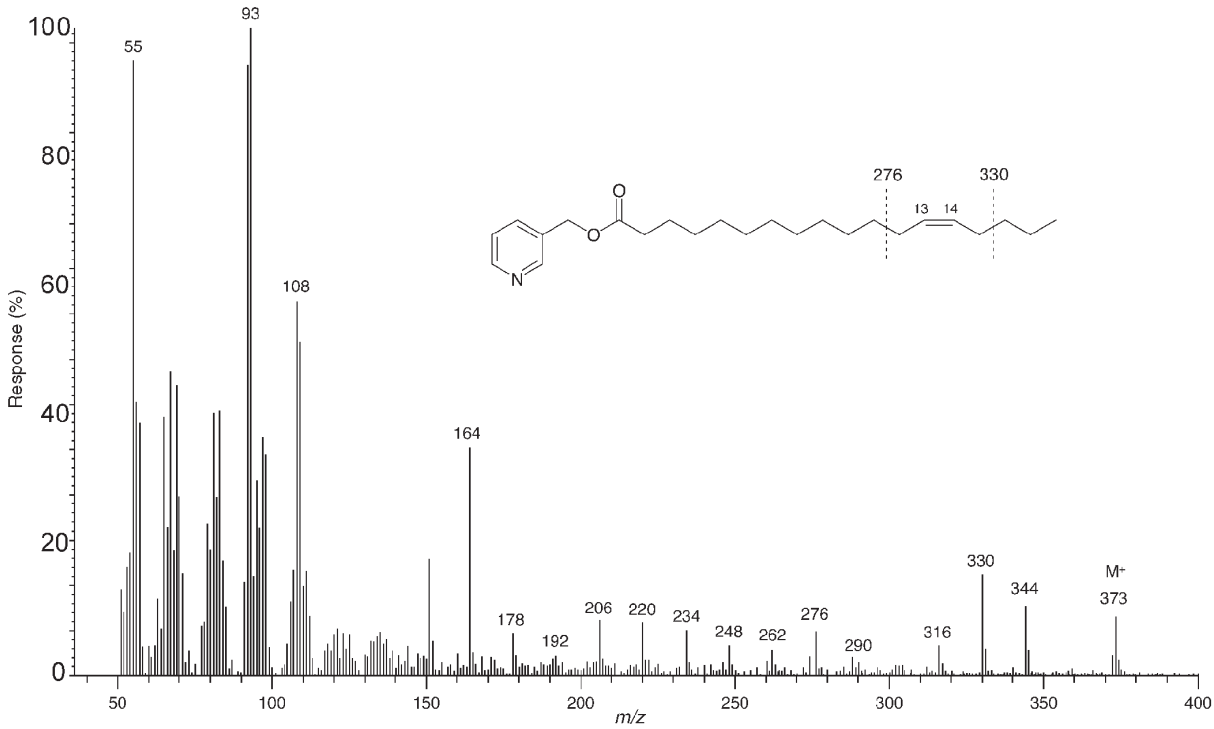

FIG. 1. Mass spectrum of picolinyl *cis*-13-octadecenoate from *Acer saccharum* seed oil.

inum, respectively, whereas the corresponding values for oleic acid were 25.9 and 33.3%, respectively. Linolenic acid was also mainly located at the *sn*-2 position, with values of 8.7% in *A. saccharum* and 7.1% in *A. saccharinum*.

FA of the n-7 group (16:1, 18:1, 20:1, and 22:1) were exclusively distributed at the *sn*-1(3) position except palmitoleic acid (16:1n-7), which was also located at the *sn*-2 position at a level of 0.2% in *A. saccharinum* seed TAG. These results and the previous observations made on coriander (*C. sativum*) seed oil (4) and seed oils from 18 species of conifer comprising five different families (5) lend support to the hypothesis that elongation of n-7 monoenoic FA from palmitoleic to docosenoic acids is highly regiospecific. Regiospecificity of *cis*-vaccenic acid preferentially at the *sn*-1(3) position in *A. saccharum* seed oil TAG is shown on a partial chromatogram of the C_{18} FA of dibutyrate derivatives of MAG (Fig. 2).

Among the monounsaturated FA of the n-9 group, oleic acid was mainly esterified on the internal positions of TAG in both species, whereas other n-9 FA, particularly 11-eicosenoic, 13-docosenoic, and 15-tetracosenoic acids, were mostly located at the *sn*-1(3) positions of the glycerol backbone. Saturated FA palmitic, stearic, arachidic, behenic, and nervonic acids were preferentially esterified at the *sn*-1(3) positions of TAG in both *A. saccharum* and *A. saccharinum* seed oils.

We have determined regiospecific distribution of FA in seed oils of two *Acer* species, *A. saccharum* and *A. saccharinum*. The results showed that long-chain monenoic FA from the n-7 group were mainly esterified on the *sn*-1(3) positions of TAG in *A. saccharum* and *A. saccharinum* seed oils, and linoleic and oleic acids were the major FA at the *sn*-2 position. In addition, the regiospecific method based on dibutyrate derivatives of MAG was successfully applied to the resolution of positional (n-9 vs. n-7) regioisomers, as well as extended to FA of C_{20} to C_{24}.

TABLE 2
Experimental Values for FA Distribution Between *sn*-1(3) and *sn*-2 Positions in *Acer saccharum* (sugar maple) and *Acer saccharinum* (silver maple) Seed Oils

	Acer saccharum		*Acer saccharinum*	
FA	*sn*-1(3)	*sn*-2	*sn*-1(3)	*sn*-2
16:0	14.1	5.6	14.0	1.5
16:1n-7	0.9	—	0.6	0.2
18:0	3.8	1.4	4.0	0.7
18:1n-9	22.3	25.9	25.5	33.3
18:1n-7	13.5	—	12.2	—
18:2n-6	22.4	52.6	21.5	55.9
18:3n-3	6.6	8.7	3.6	7.1
20:0	0.4	—	2.1	0.6
20:1n-9	5.1	0.8	6.8	—
20:1n-7	0.7	—	0.7	—
22:0	0.4	0.3	0.7	—
22:1n-9	4.7	1.7	3.9	—
22:1n-7	1.0	—	0.5	—
24:0	0.8	—	0.8	0.2
24:1n-9	3.5	3.0	3.1	0.5

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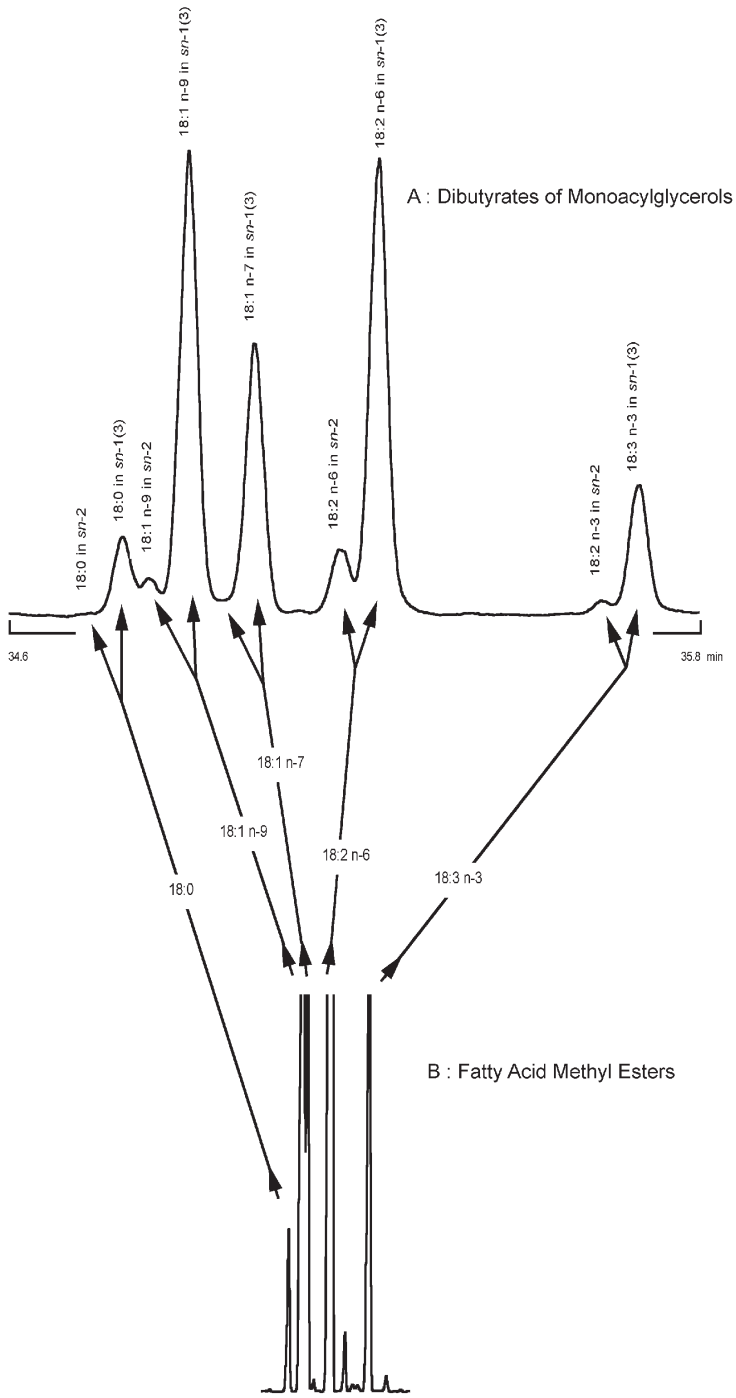

FIG. 2. GLC of C_{18} dibutyroyl derivatives of MAG of stearic (18:0), oleic (18:1n-9), *cis*-vaccenic (18:1n-7), linoleic (18:2n-6), and α-linolenic (18:3n-3) acids prepared from *Acer saccharum* on a 30 m RTX-65 TG (Restek, Bellefonte, PA) capillary column.

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