Short Communication

Isolation of Methyl 5c,13c-docosadienoate from *Limnanthes alba* Benth. (Meadowfoam) Seed Oil Methyl Esters by Silver Resin Chromatography

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Multigram quantities of methyl 5c,13c-docosadienoate were isolated from Meadowfoam (*Limnanthes alba* Benth.) oil methyl esters by silver resin chromatography. The fractionation was accomplished on a 4.7 × 45 cm Michel-Miller column packed with silver ion-saturated XN1010 resin. Meadowfoam esters (ca. 14 g) were fractionated (acetone as eluent) to yield 2.3–2.6 g batches (98% pure) of the 5c,13c-22:2 isomer (>95% recovery).

Meadowfoam (*Limnanthes alba* Benth.) is an herbaceous, flowering annual plant native to Northern California and Southern Oregon. A winter-spring crop, it currently is being grown commercially in the Willamette Valley of Oregon. Meadowfoam oil is unusual in that >95% of its fatty acids are 20 and 22 carbon atoms in length and are unsaturated primarily at the Δ5 and Δ13 positions (60% 5c-20:1; 11% 13c-22:1; 19% 5c13c-22:2) (1). The combination of low unsaturation and long-chain length of the fatty acids is more stable toward oxidation than most unsaturated vegetable oils. Meadowfoam oil has applications in cosmetics, lubricants, surfactants and waxes (2,3).

As part of an ongoing study of the nutritive aspects of fatty acids, we required multigram quantities of the 5c,13c-22:2 fatty acid (FA) component of Meadowfoam oil. A silver resin chromatographic method was developed to isolate the 22:2 FA isomer (as the methyl ester) in >98% purity.

MATERIALS

Rohm and Haas XN1010 sulfonic acid resin (16/50 mesh) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Super-refined Meadowfoam oil (Croda, Japan) was obtained through the Oregon Meadowfoam Growers Association, 2140 Turner Road, S.E., Salem, Oregon and was transesterified (sodium metal in methanol) to prepare the fatty acid methyl esters (FAME). All other chemicals were used as received.

METHODS

Methods for preparation of the resin (4) and for use of mixed solvent systems (5,6) have been described previously. A 4.7 × 45 cm glass Michel-Miller column (Ace Glass, Vineland, New Jersey) was packed with ca. 750 ml (250 g) of 100/200 mesh, 100% Ag^+/Na^+ XN1010 resin. One hundred percent Ag^+/Na^+ means the sulfonic acid protons of the resin were replaced by sodium ions, which were then replaced with silver ions. Solvent composition was changed by use of two Model 510 HPLC pumps (Waters Associates, Milford, Massachusetts), and the eluent was monitored by a Model R403 refractive index detector (also Waters).

Rotary evaporation was used to remove the solvents from the various fractions, and the FAME were analyzed in a Packard 428 Gas Chromatograph equipped with a 100 m × 0.25 mm (0.2μ film thickness) SP2560 fused silica

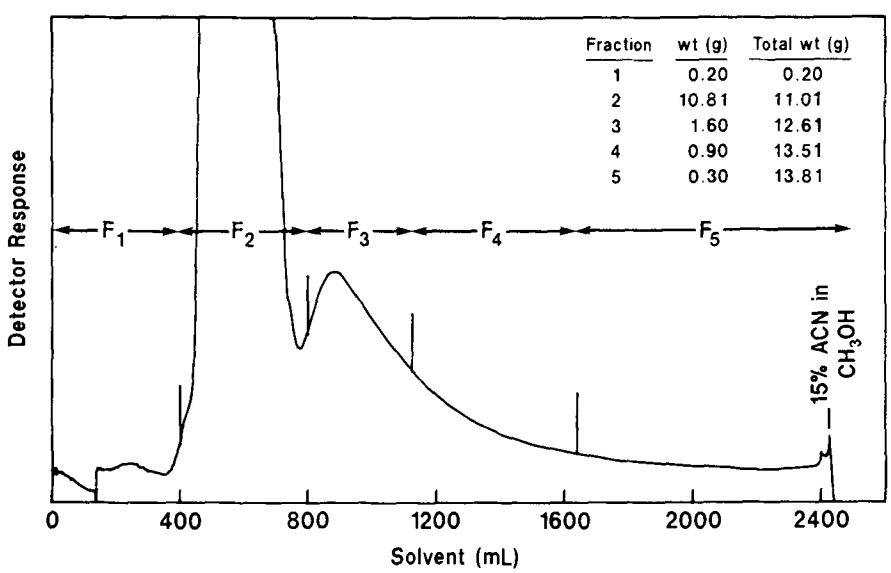

FIG. 1. Fractionation of Meadowfoam oil methyl esters on a 4.7 × 45 cm Michel-Miller Column. Flow rate, 7.0 ml acetone/min (15% acetonitrile in acetone wash for fraction 5); sample size, 13.70 g. See Table 1 for composition of fractions.

TABLE 1

FAME Compositions

Fatty acid components[a]	Meadowfoam oil methyl esters[b]	Fractions[c]				
		1	2	3	4	5
14:0	0.5	13.3				
16:0	0.2	8.0				
16:1	0.1	10.9				
18:0	0.2	9.3				
18:1 Δ5	0.6		0.6			
18:1 Δ9	1.2		1.3			
18:2 Δ9, 12	0.8				0.8	39.2
18:3 Δ6, 9, 12	0.8					2.4
20:0	0.8	58.5	0.9			
20:1 Δ5	59.6		78.2			
20:1 Δ11[d]	0.4		0.8			
20:2[e]	0.4			0.4	1.0	11.2
22:1 Δ5	3.7		4.4			
22:1 Δ13	11.0		13.2			
22:2 Δ5, 13	18.9			97.9	98.2	28.3
22:3	tr[f]					13.9

[a]In order of elution from SP2560 capillary column, some peaks not defined, so total may not equal 100%. Δ's define double-bond position.

[b]Croda Super-Refined.

[c]See Fig. 1.

[d]Tentative assignment.

[e]tr < 0.1% – Molecular weight assignment by GC/MS.

[f]Four isomers.

capillary column (Supelco, Inc., Bellefonte, Pennsylvania). Helium carrier gas and flame ionization detection were used. The oven was maintained at 200°C. Gas chromatography/mass spectroscopy (GC/MS) was used to identify the various FAME. Analyses were made on a Finnigan GC/EI-CI MS system equipped with a 30 m × 0.32 mm (0.5μ film thickness) SP-10 column (Supelco, Inc.) programmed from 150°C to 265°C at 5°C/min with a 5-min final hold. Retention times were correlated with a standard mixture (68A, NuChek Prep, Elysian, Minnesota) (7), and with a variety of other standards obtained from various sources.

RESULTS

Separation of the 5c,13c-22:2 component was excellent when 20-μl samples of Meadowfoam methyl esters were fractionated on a 0.75 × 60 cm stainless steel column (100% Ag^+/Na^+) with 5% acetonitrile (ACN) in methanol. For the Michel-Miller column, acetone was used as eluting solvent (3.0 l at 7 ml/min) to enhance the retention capabilities of the resin.

ACN (15%) in acetone was used to remove those polyunsaturated FAME still on the column after the 5c, 13c-22:2 isomer had eluted (Fig. 1). Sample sizes of 12–14 g were fractionated. The fatty acid compositions of the starting material and the fractions collected from the Michel-Miller column are summarized in Table 1. Total run time per sample was seven hr. Fractions 3 and 4 (2.5 g total; 98% pure) were combined. Recovery of the desired FAME was 95–97% of the total amount present in the sample.

DISCUSSION

GC/MS was used to analyze the minor FA components of Meadowfoam oil. Good separation of positional fatty acid isomers was accomplished on the SP-10 capillary column. Although some standards were not available, an estimation of double-bond position could be made. These have been tabulated in Table 1. (Also, see refs. 9 and 10.)

Although Limnanthes methyl esters have been fractionated by low temperature crystallization (1,8) and mercuric acetate adduct formation (8), these procedures are tedious and time-consuming. Samples of the 5c,13c-22:2 component (>98% pure) were rapidly and reproducibly isolated by silver resin chromatography. Acetone, a weaker eluting solvent than methanol, was used so larger samples of Meadowfoam methyl esters could be fractionated on the Michel-Miller column.

The retention volume of the 5c, 13c-22:2 methyl ester was between that for a monoene and a methylene-interrupted diene. This characteristic made separation of the 5c,13c-22:2 ester from the 9c,12c-18:2 ester possible.

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S. Duval did the GC/MS analyses.

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