Enrichment of Eicosenoic and Docosadienoic Acids from *Limnanthes* Oil

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ABSTRACT AND SUMMARY

The long chain ($>C_{18}$) monoene and diene acids of *Limnanthes* oil are useful in synthesizing diene and tetraene wax ester intermediates for prospective lubricant additives and PVC plasticizers. Low-temperature crystallization of the *L. alba* acids (61% eicosenoic, 19% docosadienoic, and 20% others) in acetone (initial concentration = 0.05 g/ml) at -50 C enriches eicosenoic acid (74%) in the precipitated fraction while concentrating docosadienoic (70%) in the supernatant fraction. This simple and efficient process is well suited for large-scale laboratory separations.

INTRODUCTION

The Limnanthaceae, a small family of North American flowering plants, includes a domesticated annual, *Limnanthes alba,* that has good potential for winter-spring crop production in the Pacific Northwest. A relatively novel oilseed, *Limnanthes* produces oil in which over 95% of the constituent acids have 20 or 22 carbon atoms and only a little of the polyunsaturation typical in commercial seed oils. An analysis of *L. douglasii* oil by Phillips et al. (1) provides the best detailed characterization. Briefly, *Limnanthes* oil is a glyceride in which the α- and α′-positions are most often occupied by Δ5 monoenoic acoids and the major fatty acids are: 5-eicosenoic, 11-eicosenoic, 5-docosenoic, 13-docosenoic, and 5,13-docosadienoic.

Recent interest in the oil stems from observations on the waxy character of its hydrogenation products and the similarity of liquid wax esters made from it to those that occur in sperm whale oil (2,3). Uses for solid waxes and liquid wax esters represent significant potential markets for products from this new crop, which might also provide a quality protein meal if glucosinolate levels are controlled (4). Further hypothetical applications for the oil and novel reaction products are suggested by the unusual location of unsaturated bonds in the *Limnanthes* acids.

In preparing and evaluating prospective sperm oil replacements made from *Limnanthes* oil, we encountered need for a simple nondestructive method to segregate hundred-gram quantities of the mono and dienoic acids. Distillation of methyl esters, which in our experience resulted in poor recoveries of docosadienoic acid, proved unsatisfactory for our purposes. Conditions for an alternative low-temperature fractionation that enriches docosadienoic acid fourfold are described herein.

EXPERIMENTAL PROCEDURES

Limnanthes acids were from laboratory-scale hydrolyses of hexane-extracted oils. Acid concentrations were determined as methyl esters by gas liquid chromatography (GLC) in a Packard 7401 gas chromatograph (Packard Instrument Co., Downers Grove, IL) equipped with a flame ionization detector and a glass column, 1/8 in. x 6 ft, packed with 60/80 mesh Chromosorb W (AW-DMCS) coated with 5% Apiezon L. (Applied Science Laboratory, State College, PA). Column temperature was 215 C. Methyl esters of saturated fatty acids (C_6-C_{22}) served as standards in establishing equivalent chain lengths (ECLs) (5) and identifying component fatty acids. Positional isomers, 5- and 11-eicosenoic or 5- and 13-docosenoic, were not separated. Peak areas were measured electronically.

Small-scale, low-temperature crystallizations used 5 g *L. alba* acids and 100 ml solvent, except for one experiment with 7.5 g acids. The solution was cooled for 1.5 hr before filtration, and the separated precipitate was not washed.

Large-scale, low-temperature crystallizations were convenient in a 2-liter Erlenmeyer flask with 90 g of acids dissolved in 1800 ml of acetone. The 5% solution was cooled at the desired temperature in an 2-propanol-dry ice bath for 2.5 hr. At times, small pieces of dry ice were added to the bath to maintain temperatures within 1 C. A spoonful of the crushed dry ice was placed in a Buchner funnel and allowed to evaporate. The precipitate was filtered in the cooled funnel under vacuum. After the funnel was relocated atop another Erlenmeyer flask, the precipitate was allowed to melt, and the funnel was washed with acetone. Both the filtrate and precipitate fractions were dried over magnesium sulfate and filtered. The solvent was then removed in a rotary evaporator.

Routine preparation of enriched fractions was carried out at -50 C according to the foregoing large-scale crystallization except that the precipitate was washed with a total of 100 ml acetone cooled to -50 C.

TABLE I

Small-Scale Enrichment of Docosadienoic Acid from *Limnanthes douglasii* Acids

	Solvent		N-Hexane	Acetone				Methanol			
	Concentration, g/ml		0.05	0.05			0.075	0.05			
Crystallization conditions	Temperature, C Yield,[a] %		-50 37.8	-30 32.2	-50 19.3	-70 14.1	-70 10.6	-30 71.5	-50 23.4	-70 23.0	*L. douglasii* acids
Major components[b] in supernatant	20:1[c]	%	23.2	30.3	15.1	14.7	15.9	49.0	27.4	29.5	60.3
	22:1[d]	%	18.4	14.7	7.5	3.2	20.4	19.2	5.7	6.9	21.5
	22:2	%	38.5	29.3	51.2	34.0	19.6	12.2	43.5	42.3	10.9

[a]Weight % of supernatant fraction including all component acids.
[b]Determined by GLC of methyl esters.
[c]Includes Δ5 and Δ11.
[d]Includes Δ5 and Δ13.

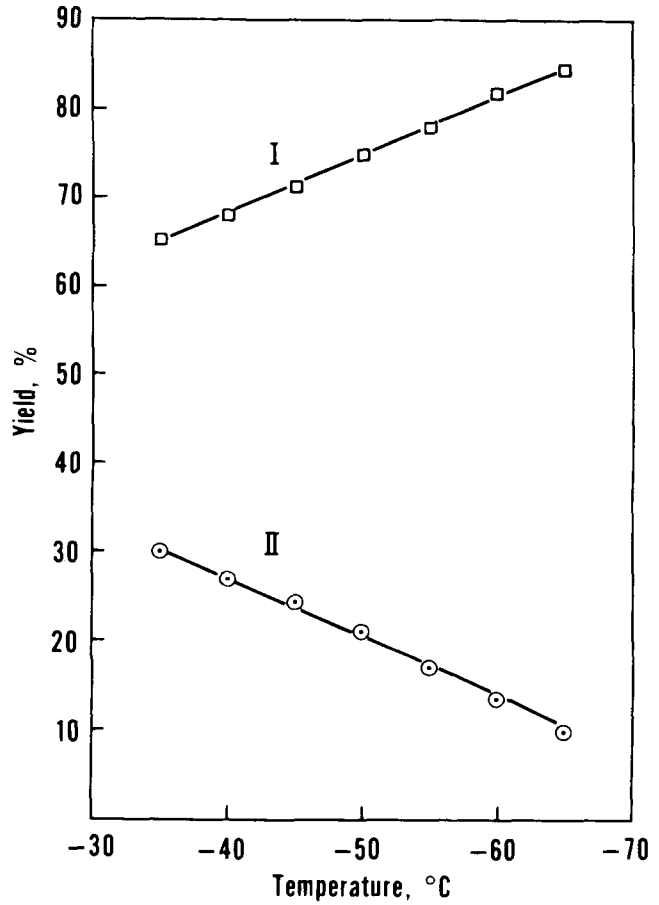

FIG. 1. Crystallization of *Limnanthes alba* acids in acetone (0.05 g/ml). I. Enriched eicosenoic acid fraction (precipitate). II. Enriched docosadienoic acid fraction (supernatant).

RESULTS AND DISCUSSION

The classic studies of Brown (6) illustrate amply the strengths and disadvantages of fatty acid enrichment through low-temperature crystallization. From his work, we anticipated that solubilities of the eicosenoic and docosadienoic acids might differ enough to allow resolution under carefully controlled conditions. The double bond at C-13 in the dienoic acid was expected to more than offset chain length effects, which would tend to minimize solubility differences between the two acids. Resolution of the 5-eicosenoic and 13-docosenoic acids appeared improbable from Brown's (6) studies. Individual trace acids (1% or less) were expected to have insignificant effects.

Selection of Solvent

Preliminary experiments with acids from *L. douglasii* (Table I) demonstrated that the docosadienoic acid could be concentrated more than fourfold and separated from the bulk of accompanying 20- and 22-carbon acids by crystallization from acetone at -50 C. Resolution was less satisfactory in methanol and much worse in hexane; the more polar solvent nearly excluded docosenoic acids but tended to dissolve eicosenoic acids; the less polar solvent retained relatively large amounts of both monoenoic contaminants. Crystals formed in hexane, acetone, and methanol were coarse, medium, and fine, respectively.

Cooling a 5% acetone solution further, to -70 C, reduced the relative concentration of 22-carbon acids without substantial effect on the amount of eicosenoic acids remaining in solution. Curiously, when the initial concentration was raised to 7.5%, the docosenoic acids remaining

TABLE II

Enrichment of Eicosenoic and Docosadienoic Acids from *Limnanthes alba* Acids in Acetone (0.05 g/ml)[a]

Temperature, C	-35		-40		-45		-50		-55		-60		-65		Preparative[b]		*L. alba*
Fraction	I[c]	II[d]	I	II	I	II	I	II	I	II	I	II	I	II	I	II	acids
14:0[e]		0.1					0.3	0.2				0.2		0.2	0.1	0.2	0.1
16:0	0.1	0.5		0.4	0.2	0.4	0.2	0.6	0.1	0.5	0.1	0.5	0.2	0.5		0.6	0.3
16:1		0.7		0.6	0.1	0.7		1.0		1.2		1.5		1.5		0.9	0.2
18:0	0.1	0.1			0.1		0.3						0.2				0.1
18:1	0.5	3.7	0.8	3.5	0.5	3.7	0.4	5.3	0.4	5.8	0.4	7.4	0.6	7.1	0.3	5.3	1.5
18:2		2.1		2.1		2.2		3.1		3.9		4.8		4.9		2.3	0.5
19:1[f]					0.1		0.1		0.1				0.1		1.3		0.1
20:0	1.8		1.7		2.1	0.4	1.6		1.0		1.0		1.1				1.0
20:1	75.4	21.8	75.9	21.8	74.6	17.6	70.9	15.5	68.4	17.0	67.2	17.0	67.7	16.8	73.9	12.8	60.5
20:2		1.3		1.2		1.4		2.0		2.2		2.9		2.9		2.2	0.3
21:1[f]	0.4				0.3		0.5					0.5				0.5	0.1
22:1	19.7	14.0	19.9	9.9	19.9	7.9	18.6	5.0	18.9	5.0	18.1	4.9	18.7	5.3	19.5	4.9	16.7
22:2	2.1	56.0	1.5	60.6	2.2	65.7	7.1	67.3	11.0	64.5	13.2	60.1	13.3	60.8	4.9	70.1	18.8

[a]Area percent data from GLC.
[b]Average of 99 runs at -50 C. Precipitates were washed.
[c]Enriched eicosenoic acid fraction (precipitate). See Figure 1 for yield.
[d]Enriched docosadienoic acid fraction (supernatant). See Figure 1 for yield.
[e]Determined by GLC of methyl esters.
[f]Unidentified constituents with ECLs equivalent to 19:1 and 21:1.

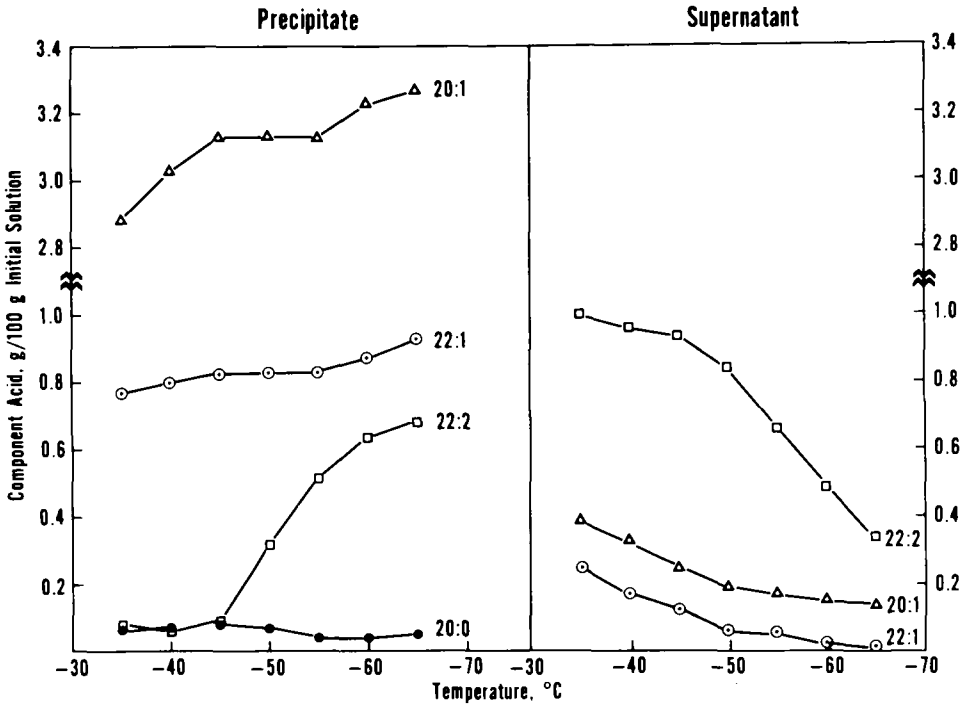

FIG. 2. Distrituion of major component acids.

in solution at -70 C increased sixfold even though the docosadienoic acid level decreased 42%.

Selection of Temperature

As illustrated in Figure 1, the quantity of *Limnanthes* acids crystallized from acetone increased linearly with decreasing temperature and did not reflect important qualitative differences between fractions taken at various temperatures. Conditions affording highest yields and optimum resolution of monoenoic and dienoic acids were identified by GLC analysis of crystal precipitates and the corresponding supernatants collected throughout the range -35 to -65 C. Table II lists results from these analyses.

Concentration of eicosenoic acids was highest (ca. 75%) in precipitates collected above -45 C. Below -45 C the solid contained docosadienoic acid, which precipitated in larger amounts after reaching maximum levels near 70% in supernatants collected at -50 C.

Figure 2 shows how the various *Limnanthes* acids distributed between solid and solution at different temperatures. The solution curve for eicosenoic acids compares favorably with data of Kolb and Brown (7), but levels of dissolved docosenoic acids were five to eight times higher than expected from their data on the solubility of erucic acid in acetone.

The docosadienoic acid distribution at higher temperatures is interesting because the rate at which its precipitation changes with temperature parallels that for arachidic acid, more closely than those for the other major acids. Although we have no proof, such behavior might be due to cocrystallization of these two rather different acids. Several years ago Singleton (8) found that oleic acid cocrystallizes in small amounts with stearic acid under conditions that should theoretically exclude oleic precipitation. The other *Limnanthes* acids, eicosenoic and docosenoic, also crystallize throughout the range above -45 C but in nonparallel fashion, as if independent of one another and the eicosenoic-docosadienoic mixture.

As anticipated, the monoenoic acids were not separated by low-temperature crystallization; they formed a eutectic

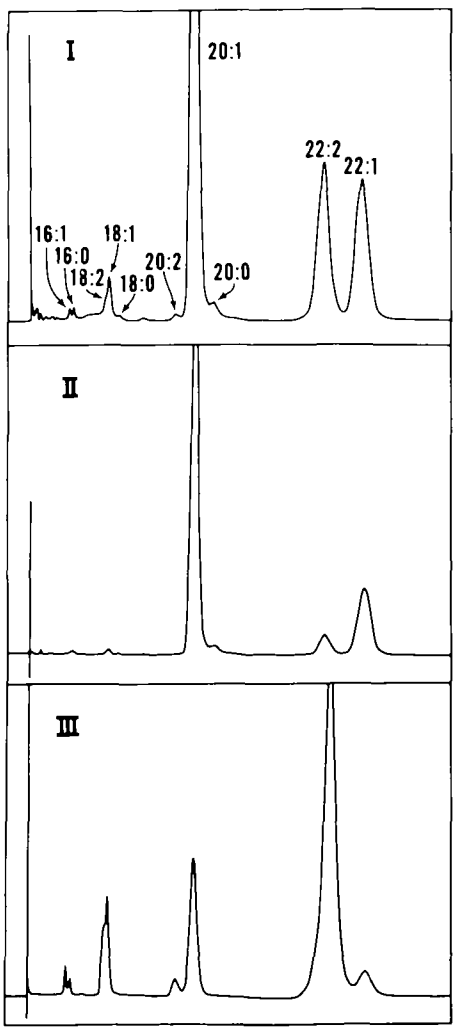

FIG. 3. Gas liquid chromatographic analyses of methyl esters. I. *Limnanthes alba* acids. II. Enriched eicosenoic acid fraction (precipitate). III. Enriched docosadienoic acid fraction (supernatant).

mixture (eicosenoic/docosenoic = 4/1) between -45 and -55 C. Fractional crystallization thus seems ill-suited for resolving these two acids directly from *Limnanthes* oil.

In comparison with other methods, low-temperature crystallization is a mild, efficient, and effective means for concentrating component acids in high purity. In 99 runs, we fractionated 8.4 kg of *Limnanthes* acids. Figure 3 shows the GLC analyses of the fractions as methyl esters. Purities and yields were 74% and 75.8% for the eicosenoic acid concentrate and 70% and 21.5% for the docosadienoic acid concentrate, respectively.

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