

Lunaria Seed Oil—A Rich Source of C₂₄ Fatty Acids

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The seed oil of *Lunaria biennis* (family Cruciferae) contains 21% of *cis*-15-tetracosenoic acid. This proportion of C₂₄-monoethenoid acid is the highest yet reported in any seed oil. The oil also contains *cis*-13-docosenoic (erucic) acid (42%).

LUNARIA BIENNIS or honesty (family Cruciferae) is grown chiefly as an ornamental. The herbaceous plants grow to a height of 1½ to 3 ft. Their flowers are mostly pink-purple and fragrant; their seeds are flat and disk-shaped. The genus is native to Europe and western Asia (1).

A recent paper from this laboratory (2) indicated that the seed oil of *Lunaria biennis* contains 21% tetracosenoic acid. This acid has been isolated in 98% purity, and in at least 70% yield, by fractional distillation of methyl esters. Chemical characterization has now established that the acid is *cis*-15-tetracosenoic acid (trivial name: nervonic or selacholeic acid). *Lunaria* seed oil might become a practical source of this tetracosenoic acid or of the corresponding saturated fatty acid produced by hydrogenation if good seed yields of this species through cultivation are possible. Ligthelm and coworkers (3) reported that South African *Ximenia* kernel oils contained from 3 to 7% *cis*-15-tetracosenoic acid. It has long been known as a component of elasmobranch-liver oils (4,5) and also of brain cerebrosides (4,6). Bernard and Albrecht (7) found 2.9% of a Δ^{17} C₂₄-unsaturated acid in the lipids of *Phycomyces blakesleeianus*, a microorganism.

A monounsaturated C₂₂-acid was reported to be present in *Lunaria* seed, ca. 42%, (2) by gas chromatographic analysis (GLC) according to the procedure of Miwa and coworkers (8). Since then we have shown by the periodate-permanganate cleavage suggested by von Rudloff that this C₂₂ acid is the common *cis*-13-isomer, erucic acid.

Experimental

Oil Extraction. Coarsely ground seeds of *Lunaria biennis* (155.8 g.) were extracted with petroleum ether (b.p. 30–60°C.) using a Soxhlet extractor, and the solvent was removed in the usual manner. The yield of oil was 57.6 g.

Preparation of Methyl Esters. *Lunaria* seed oil (57.6 g.) was saponified by refluxing with alcoholic potassium hydroxide (23.0 g. of 87% KOH dissolved in 76 ml. of H₂O and 600 ml. of absolute ethanol) under nitrogen for 2 hr. The saponification mixture was processed as usual and gave 50.6 g. of acid. The fatty acids were esterified with 1% methanolic sulfuric acid. The yield of methyl esters was 51.6 g.

Distillation of Esters. The mixed methyl esters were distilled through a Podbielniak Model 4278 spinning-band column. Fractions 1–11 were distilled at 10 mm. pressure and fraction 12 was distilled at 9 mm. pressure (Tables I and II). Selected higher boiling fractions were analyzed by GLC as described by Miwa and coworkers (8). The infrared spectra of the selected fractions and of the original oil showed no *trans* C=C absorption (10.3 μ). Infrared spectra were measured as films on sodium chloride plates with a Perkin-Elmer Infracord spectrophotometer.

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TABLE I
Distillation of Methyl Esters of
Lunaria biennis Seed Oil

Fraction	Distillation temperature, °C./10 mm. ^a	Weight, g.
1.....	72–168	0.71
2.....	168–184	0.61
3.....	184–194	0.92
4.....	194–198	0.57
5.....	198–202	4.48
6.....	202–204	8.14
7.....	204–228	1.45
8.....	228–233	1.69
9.....	228–233 ^b	0.68
10.....	233–236	20.49
11.....	238–244	5.94
12.....	240–243	1.33 ^a

^a Fraction 12 was distilled at 9 mm.

^b Distillation was interrupted overnight between fractions 8 and 9.

Identification of the C₂₄-Monoene. Fraction 11 (5.9 g.) was saponified by the usual procedure and a white solid, m.p. 41–42°, was obtained. This solid was recrystallized from 65 ml. of acetone at 0° and yielded 4.5 g. of white crystals, m.p. 42.0–42.5°; lit. values 43.1–43.5° (3) and 42.5–43.0° (4,5).

Analysis. Calcd. for C₂₄H₄₆O₂: Neut. equiv., 367; iodine value, 69.2 (one C=C). Found: Neut. equiv., 367; iodine value, 70.3.

A 1.3-g. portion of this acid was oxidized for 24 hr. by the von Rudloff periodate-permanganate proce-

TABLE II
Fatty Acid Composition of *Lunaria biennis* Seed Oil and Distillates
(Area Percentage of Methyl Ester Peaks)

Acid	Original oil, % (2)	Distillates, %					
		Fraction number					
		5	6	7	10	11	12
Hexadecanoic	2.0	2.2	Trace	Trace
Hexadecenoic	0.2	0.3
Octadecanoic	0.4	1.3
Octadecenoic	23.0	76.3	80.4	57.9
Octadecadienoic	7.0	18.7	17.6	10.1
Octadecatrienoic	2.0	1.8	1.5	0.4
Eicosanoic	0.4	0.3
Eicosenoic	2.0	12.0
Docosenoic	42.0	0.6	0.5	18.0	92.0	2.2	1.2
Tetracosenoic	21.0	7.6	97.8	98.8

dures (9) using 60% *t*-butyl alcohol. After the product was worked up in the usual manner, it yielded 1.1 g. of a semisolid. This semisolid was steam distilled, and 0.35 g. of steam-volatile and 0.80 g. of steam nonvolatile acids with a melting point of 112.5–113° were obtained after recrystallization from ethyl-acetate-petroleum ether 1:1; lit. values 112.5° (3) and 114.6–114.8° (10).

Analysis. Calcd. for C₁₅H₂₈O₄: Neut. equiv., 136. Found: Neut. equiv., 137.

Methyl esters from each fraction were prepared by using diazomethane and analyzed by gas chromatography. The composition of each is listed in Table III.

The *p*-bromophenacyl ester of the steam-volatile acid was prepared according to the procedure of Shriner *et al.* (11). This ester had melting point 63–63.5°, undepressed on admixture with authentic *p*-bromophenacyl pelargonate, m.p. 63–63.5°; lit. value 68° (11).

The *bis-p*-bromophenacyl ester of pentadecanedioic acid was prepared essentially according to the procedure of Shriner *et al.* (11) using 0.081 g. of acid and 0.207 g. of *p*-bromophenacyl bromide with a 2-hr. reflux period in 170 ml. of ethanol. The yield was 0.114 g., m.p. 144–145°. This product was recrystal-

TABLE III
Gas Chromatographic Analysis of $\text{NaIO}_4\text{-KMnO}_4$
Oxidized C_{22} - and C_{24} -Monoene Fractions

Acid	C_{22} (Fraction 10)		C_{24} (Fraction 11)	
	Entire oxid. mixture		Steam-volatile	Steam-nonvolatile
	%		%	%
Octanoic.....		0.5
Nonanoic.....	41.0		99.5
Dodecanedioic.....	0.5	
Tridecanedioic.....	52.9	
Tetradecanedioic.....	2.6
Pentadecanedioic.....	5.6		97.4

lized from acetone and the yield was 0.083 g., m.p. 145–146°.

Anal. Calcd. for $\text{C}_{29}\text{H}_{38}\text{O}_6\text{Br}_2$: Br, 24.9. Found: Br, 24.6.

Pentadecanediamide was prepared essentially according to the procedure of Shriner *et al.* (11) using 0.137 g. of pentadecanedioic acid. A 0.079-g. portion of diamide, m.p. 172–173° was obtained after recrystallization from aqueous ethanol; lit. value is 173° (12).

Anal. Calcd. for $\text{C}_{15}\text{H}_{30}\text{O}_6\text{N}_2$: C, 66.6; H, 11.2. Found: C, 66.4; H, 11.0.

Identification of the C_{22} Monoene. A 1.2-g. portion of fraction 10 was oxidized by von Rudloff's periodate-permanganate procedure (9) using 60% *t*-butyl

alcohol for 24 hr. The product was worked up as usual and yielded 1.3 g. of semisolid acids. A portion of these acids was esterified, and their analysis by gas chromatography are given in Table III.

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REFERENCES

1. Bailey, L. H., "Manual of Cultivated Plants," The Macmillan Co., New York, 1949.
2. Mikolajczak, K. L., Miwa, T. K., Earle, F. R., Wolff, I. A., and Jones, Q., Manuscript submitted to J. Am. Oil Chemists' Soc.
3. Lighthelm, S. P., Horn, D. H. S., Schwartz, H. M., and Holdt, M. M. von, J. Sci. Food Agr., 5, 281 (1954).
4. Ralston, A. W., "Fatty Acids and Their Derivatives," John Wiley and Sons, Inc., New York, 1948.
5. Tsujimoto, M., J. Soc. Chem. Ind. Japan, 30, 863 (1927).
6. Klenk, E., Z. Physiol. Chem., 166, 287 (1927).
7. Bernhard, K., and Albrecht, H., Helv. Chim. Acta., 31, 977 (1948).
8. Miwa, T. K., Mikolajczak, K. L., Earle, F. R., and Wolff, I. A., Anal. Chem., 32, 1739 (1960).
9. Rudloff, E. von, Can. J. Chem., 34, 1413 (1956).
10. Chuit, P., Helv. Chim. Acta., 9, 264 (1926); *ibid.*, 11, 1174 (1928).
11. Shriner, R. L., Fuson, R. C., and Curtin, D. Y., "The Systematic Identification of Organic Compounds," 4th ed., John Wiley and Sons, Inc., New York, 1957.
12. Canonica, L., and Bacchetti, T., Atti. acad. nazl. Lincei Rend., Classe sci. fis., mat. e nat., 15, 278 (1953); cf. C. A. 49, 8121f (1955).

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Dialkyl Esters of Alpha-Sulfofatty Acids

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Lower olefins, especially ethylene and propylene, have been found to add to alpha-sulfofatty acids in the presence of boron trifluoride or its etherate to give dialkyl alpha-sulfofatty esters. Some of the reaction variables studied include catalyst, catalyst concentration, and solvent. The esters were obtained as colorless, low melting solids after purification by low temperature crystallization.

UNTIL RECENTLY, esters of sulfonic acids have been available only by the action of sulfonyl halides on hydroxy compounds (1), by rearrangement of alkyl sulfites (2), or by a metathetical reaction of silver sulfonates with alkyl halides (3). None of these methods is satisfactory economically for the preparation of esters of sulfonated fatty acids. Recently however the esterification of several aromatic and short chain aliphatic sulfonic acids was reported, using boron trifluoride and olefins under pressure (4, 5). Also, boron trifluoride or its derivatives are useful catalysts for the esterification of carboxylic acids with olefins, and a number of simple carboxylate esters have been prepared in this way (6).

In the present work, reactions of ethylene with alpha-sulfofatty acids for the preparation of diethyl alpha-sulfofatty acid esters were run in a 1-l., stainless steel, stirred autoclave, boron trifluoride being used as a catalyst. In general, the boron trifluoride was added to the solid alpha-sulfofatty acid and ethylene, then charged to the vessel. The reaction starts almost immediately and is slightly exothermic. The reaction temperature generally ranged from 35 to 45°C. at 500–700 psig. ethylene pressure. Uptake

of ethylene usually ceased in 4 to 7 hr. at which time the reaction was essentially complete.

Some of the reaction variables such as catalyst, catalyst concentration, and solvent were investigated using alpha-sulfopalmitic acid. Table I illustrates the effect of catalyst concentration on the extent of reaction. It can be seen that a sharp break in per cent conversion occurs at a catalyst concentration of 33 mole-%. Concentrations above 33 mole-% do not affect the yield appreciably but catalyst concentrations below 33 mole-% result in markedly decreased yields.

The experiments in Table I were run in the absence of a solvent. A second series of experiments was run to determine the effect of solvent on the reaction. These are summarized in Table II which shows that a substantial lowering of yield occurs in the presence of solvents. Polymerization of ethylene is considerably higher as indicated by the polymer content of the crude ester.

Other Lewis acid catalysts which might catalyze the esterification of alpha-sulfopalmitic acid were

TABLE I
Catalyst Concentration

Run	BF_3 conc. mole-%	Conversion, %	Polymer, %	Acid value	Sap. equiv.
1	42.8	87.5	18.5	9.3	240
2	37.5	95.5	12.5	3.1	225
3	34.6	91.5	19.5	2.6	243
4	33.3	47.5	18.0	16.8	239
5	31.0	34.0	23.5	2.2	255
6	20.0	nil