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Fatty Acids, Fatty Alcohols, Wax Esters, and Methyl Esters from *Crambe abyssinica* and *Lunaria annua* Seed Oils[1]

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Abstract

Crambe abyssinica and *Lunaria annua*, members of the Cruciferae family, have seed oil glycerides containing ca. 55–65% of C_{22} and C_{24} unsaturated fatty acids. Fatty acids were prepared by saponification; fatty alcohols, by sodium reduction of glycerides; liquid wax esters, by *p*-toluenesulfonic acid-catalyzed reaction of fatty acids with fatty alcohols; and methyl esters, by reaction of fatty acids with diazomethane. Solid hydrogenated glyceride oils and wax esters were compared with several commercial waxes. Chemical and physical constants were determined for the seed oils and their derivatives. Position of unsaturation in the *Crambe* fatty acids was determined by gas chromatographic analysis of the permanganate-periodate degradation products. The major dicarboxylic acid was brassylic (C_{13}), proving the docosenoic acid to be erucic.

Introduction

CRAMBE ABYSSINICA Hochst. ex R. E. Fries (Family: Cruciferae) is an annual herb, about 3 ft tall, that produces numerous spherical pods which are one-seeded and indehiscent. Chiefly distributed around the Mediterranean, through western Europe, and in central Asia, *Crambe* may be introduced into the U.S. as a new chemurgic crop because of its potential industrial and feed uses (1,17).

Lunaria annua L. (*L. biennis* Moench) (Family: Cruciferae), commonly called "honesty," is an annual or biannual herb, 2–3 ft tall. It has fragrant pink-purple flowers and is grown chiefly for the ornamental, thin, lustrous septa that are held in the pod margins, like spectacles in their rims (3,5).

Preliminary analyses for oil and fatty acids, reported earlier (9,12), showed that *Crambe* seed oil contains ca. 60% docosenoic among the derived fatty acids, and *Lunaria* ca. 40% docosenoic and 20% tetracosenoic acids. The major acids in *Lunaria* were later shown to be 13-docosenoic (erucic) and 15-tetracosenoic acids (16). The amino acid composition of *Crambe* seed meal was also reported (15).

The present study follows a recent investigation on derivatives of *Limnanthes douglasii* seed oil (11). Selected chemical and physical properties of oil, fatty acids, fatty alcohols, wax esters, and methyl esters derived from seeds of *Crambe* and *Lunaria* are reported. The major acid in *Crambe* is shown by infrared analysis, permanganate-periodate degradation, and gas chromatographic analysis to be *cis*-13-docosenoic (erucic) acid.

Procedure

Materials, Sample Preparation, and Analytical Methods

Crambe seed was obtained from Montana State College, Bozeman, Mont., and *Lunaria* seed from Herbst Brothers, New York, N.Y. Botanical identity was verified by botanists of the Crops Research Division, U.S.D.A., Washington, D.C.

Solvents, reagents, procedures on preparation of

TABLE I

Derivatives from Seeds of *Crambe abyssinica*[a] and *Lunaria annua*[b]

Sample	Yield, %	Acid value	Iodine value	Hydroxyl, %
Oil (dry basis):				
Crambe	28[c]	2.3	90	....
Lunaria	38	2.0	79	....
Fatty acids (from oil):				
Crambe	90	172	93	....
Lunaria	88	170	80	....
Fatty alcohols (from oil):				
Crambe	79	0.0	100	5.8
Lunaria	77	0.0	85	5.2
Wax esters (from acids and alcohols):				
Crambe	88	0.7	98	0.0
Lunaria	92	0.0	87	0.0
Methyl esters (from acids):				
Crambe	100	0.0	88	....
Lunaria	100	0.0	77	....

[a] Seed + pericarp.
[b] Seed + seed coat.
[c] Oil content in *Crambe* seed may vary 25–40%. The average of crops from 9 locations in the U.S. was 32%.

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[1] Presented in part at the AOCS meeting in New Orleans, La., 1962.
[2] A laboratory of the No. Utiliz. Res. & Dev. Div., ARS, U.S.D.A.

TABLE II
Physical Constants of Derivatives from Seed of *Crambe abyssinica*[a] and *Lunaria annua*[b]

Sample	Freezing point, C	Density, g/ml at 25C	Density, g/ml at 37.8C (100F)	Viscosity, centipoise at 25C	Viscosity, centipoise at 37.8C (100F)	Refractive index n_D^{25}	Refractive index n_D^{40}
Oil:							
Crambe	5 to −17	0.907	0.903	74	44	1.4707	1.4657
Lunaria	7 to − 8	.904	.898	90	52	1.4693	1.4644
Fatty acids:							
Crambe	30 to 17	......	.886	....	25	1.4630	
Lunaria	21 to 17	.886	.878	42	26	1.4613	
Fatty alcohols:							
Crambe	26 to 17	......	.845	....	20	1.4632	
Lunaria	20 to 12	.848	.843	37	22	1.4621	
Wax esters:							
Crambe	24 to 2	.874	.863	32	21	1.4668	1.4616
Lunaria	13 to 2	.864	.858	35	23	1.4653	1.4602
Methyl esters:							
Crambe	10 to −21	.871	.864	7	5	1.4552	
Lunaria	−1 to −14	0.874	0.864	8	6	1.4538	

[a] Seed + pericarp.
[b] Seed + seed coat.

samples, and analytical methods were the same as those described for *Limnanthes douglasii* (11). Degradation of *Crambe* fatty acid methyl esters was carried out according to von Rudloff (14), and the methyl esters of the derived dicarboxylic acids were identified by gas-liquid chromatography (10).

Results

Chemical and Physical Constants. Chemical analyses of the seed derivatives are listed in Table I. The yield of wax and methyl esters is given as the percentage of the calculated theoretical amount recovered after purification. The amount of unsaponifiable matter in the oil of *Crambe* was 2.7% and of *Lunaria,* 1.8%. Physical constants of the same derivatives are listed in Table II. Hydrogenated oil and wax esters showed no iodine absorption. Their melting points and Brinell hardness numbers (BHN) (2,7) compared with commercial beeswax, paraffin, and carnauba waxes are:

	Melting point, C	BHN[a]
Hydrogenated oil:		
Crambe	61–63	1.1
Lunaria	61–63	0.80
Hydrogenated wax esters:		
Crambe	66–68	0.28
Lunaria	66–68	0.28
Commercial waxes:		
Beeswax	62–65	0.38
Paraffin	51–58	0.24
Carnauba	76–84	2.6

[a] Determined at 25C with 4.0 kg load on a 10.0 mm diameter steel ball applied for 60 sec.

Gas-Liquid Chromatography of Fatty Acid Methyl Esters and Fatty Alcohols. The percentage of each fatty acid methyl ester obtained by calculation of area under the peaks in the gas chromatograms is listed below:

Parent acid	*Crambe abyssinica* %	*Lunaria annua* %
Tetradecanoic	0.1	....
Hexadecanoic	2	1
Hexadecenoic	0.6	0.2
Octadecanoic	2	0.2
Octadecenoic	17	25
Octadecadienoic	8	6
Octadecatrienoic	6	0.4
Eicosanoic	1	....
Eicosenoic	5	1
Docosanoic	2	....
Docosenoic	55	45
Tetracosenoic	1	21

The composition of the fatty alcohols from both species was similar to that of their methyl esters and was therefore not listed.

Characterization of Crambe abyssinica Fatty Acids. The *Crambe* methyl esters used in the degradative study differed slightly in composition from the sample above. Assuming the area percentage of the gas chromatographic peaks was equal to weight percentage of the components, the mole percentage of the dicarboxylic acids expected after degradation of the unsaturated acids was calculated. This calculated figure was compared with the mole percentage of the dicarboxylic methyl esters recovered and subsequently analyzed by gas-liquid chromatography.

Theoretical and experimental values are:

Acid	Theoretical mole %	Experimental mole %
Octanedioic	0	1
Nonanedioic	41	34
Decanedioic	0	0.3
Undecanedioic	5	6
Dodecanedioic	0	0.6
Tridecanedioic	53	56
Pentadecanedioic	1	2

Octanedioic, decanedioic, and dodecanedioic acids are probably oxidation products of nonanedioic, undecanedioic, and tridecanedioic acids, respectively. Mole ratio of the derived monocarboxylic acids—hexanoic, heptanoic, octanoic and nonanoic—was 2:1:3:94 when the methyl esters were analyzed by gas-liquid chromatography. Theoretical mole ratio is 12:1:0:87. The discrepancy was due primarily to low recovery of hexanoic acid.

Ultraviolet and infrared spectrometric analyses of Crambe seed oil showed no conjugated unsaturation, *trans* unsaturation, or hydroxyl substituent (8,13). Gas chromatographic equivalent chain lengths (10) of the C_{18} dienoic and trienoic acids were identical to those of linoleic (*cis,cis*-9,12) and linolenic (*cis,cis,cis*-9,12,15) acids. These results indicate that the major fatty acid *in Crambe seed oil* is *cis*-13-docosenoic (erucic) and that the other acids are similar to those commonly found in crucifer seed oils (4,6).

Discussion

Crambe and *Lunaria* seed oils are potential sources of erucic acid and other long-chain fatty derivatives, which may be utilized in the chemical industry. Their derivatives closely resemble corresponding derivatives from *Limnanthes* seed oil in physical and chemical properties. Preliminary determinations show the derivatives to boil at ca. 400C at atmospheric pressure. When hydrogenated, the seed oils form hard, glossy, white wax-like products, especially *Crambe* oil. The C_{13} dicarboxylic acid, brassylic, prepared by oxidative cleavage of erucic acid, is currently being investigated at the Northern Laboratory for industrial utilization.

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Identification of the Major Polyunsaturated C_{16} Acids of Marine Oils by GLC Separation Factors on Normal and Organosilicone Polyesters

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Abstract

The tentative identification of the unsaturated C_{16} acids of marine oils is facilitated through analysis on both normal and organosilicone polyester substrates. Two different separation factors can then be approximated from the more accessible separation factors appropriate to unsaturated acids of longer chain lengths.

Introduction

THE MAJOR unsaturated C_{16} acids of marine oils, such as menhaden and herring oils, have been identified as 9-hexadecenoic, 6,9- and 9,12-hexadecadienoic, 6,9,12-hexadecatrienoic and 6,9,12,15-hexadecatetraenoic (1–3). These are distinguished from the C_{18}, C_{20}, and C_{22} acids in that the end carbon chains (4–8) are 1,4 or 7, whereas in the other chain lengths the end carbon chains are normally 3,6 or 9 (3,9). Some minor C_{16} isomers do have the latter end carbon chains, but occur in such small proportions that they are not normally evident by GLC of esters from whole oils. Thus systematic separation factors based on end carbon chains which are interchangeable with acids of other chain lengths are, at first glance, of little use with the C_{16} acids.

The terms "separation" or "separation factor" have been used in a number of meanings in GLC by different authors (cf. 3). The "systematic separation factors" (6,8,10) referred to in the present study apply to ratios of adjusted relative retention times (the greater divided by the lesser) for particular pairs of monounsaturated or methylene interrupted polyunsaturated fatty acids of a given chain length, characterized by having in common either the same number of carbon atoms between the carboxyl group and the center of the first double bond (Type II), or between the ultimate double bond and the terminal carbon atom of the chain (Type I).

A further complication is that the normal chain length overlap of marine oil methyl esters on polyester substrates will result in one or more of the materials in question probably coinciding with heptadecanoate, heptadecenoate, or octadecanoate, which are present in roughly the same proportions, and on highly polar polyesters the trienoate and tetraenoate frequently are masked by the very large octadecenoate peak.

In a previous study of systematic separation factors applied to the identification of the peaks of a menhaden oil, analysis of the data for the C_{16} acids was inadequate for examination of the separation factors (3,8). By employing a concentrate of these acids obtained from seal oil (10), experiments have revealed certain properties of these acids which give significant variations in separation factors on normal polyester substrates as compared with organosilicone polyester substrates and enable comparisons to be made which greatly assist in the identification of these materials.

Experimental

Columns and operating conditions employed with either Wilkens Aerograph A-90 or Barber-Colman Model 10 gas chromatographs are given in Table I. The organosilicone polyester packing was from Applied Science Laboratories, Inc., while all other columns except No. 5 were prepared from commercial subtrates and support materials in this laboratory.

A high iodine value fraction obtained from urea complex fractionation of seal methyl esters (10) was employed and identifications previously obtained on

TABLE I

Type and Operating Data of Columns Used in Determining Separation Factors.

Column	% Poly-ester [a]	Support	Column		Temp °C
			Length	Diam	
1 [b]	20% EGS	100–120 Chromosorb W	6 ft	3 mm I.D.	200
2 [c]	20% EGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	205
3 [c]	20% DEGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	220
4 [c]	20% DEGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	220
5 [b]	18% EGA	100–120 Celite 545	5 ft	4 mm I.D.	197
6 [b]	10% EGSS-X	100–120 Sil. Gas-Chrom P	6 ft	3 mm I.D.	200
7 [b]	12% EGSS-Y	100–120 Sil. Gas-Chrom P	6 ft	3 mm I.D.	200

[a] Ethylene glycol succinate (EGS)
Diethylene glycol succinate (DGS)
Ethylene glycol adipate (EGA)
Ethylene glycol succinate silicone—X(EGSS-X)
Ethylene glycol succinate silicone—Y (EGSS-Y)

[b] Argon carrier gas, ionization detector, glass columns.

[c] Helium carrier gas, thermal conductivity detector, metal columns.