Search for New Industrial Oils. V. Oils of Cruciferae[1]

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Seeds from 37 species of plants in the family Cruciferae were analyzed for oil and protein, and the fatty acid composition of the oils was determined by gas-liquid chromatography. Erucic acid, generally considered characteristic of crucifer oils, occurs in about three-fourths of these species in amounts ranging from 3 to 59%. Some oils free of erucic acid contain up to 63% linolenic acid or up to 58% eicosenoic.

THE CRUCIFERAE (mustard family) includes some 300 genera and 2,500 species of herbs, widely distributed primarily in the cooler regions of the northern hemisphere. Well-known representatives are mustard, radish, rape, and cabbage. The only crucifer oils important in commerce are rapeseed and mustard seed oils, which are used primarily as edible oils but also to some extent in a number of industrial applications. Fatty acid composition has been reported for rapeseed and mustard seed oils (1,2,6,9) and for about 20 other species in the family (4,6–9, 14,15,16). Common constituents in addition to the usual C_{16} and C_{18} acids are arachidic, behenic, lignoceric, eicosenoic, erucic, and docosadienoic acids. Only two species have been reported to contain no acids longer than C_{18} (4,6,9).

Most crucifers were omitted from earlier papers of this series (3–5) because the analytical methods then used gave inadequate indication of the chain lengths of constituent acids. Reliance on such older methods is presumably the reason for reports in the literature of 60% oleic acid in *Crambe* species (11) and in *Lepidium sativum* (16), whereas our present study, utilizing gas-liquid chromatography, shows significant amounts of erucic acid in these oils.

The genus *Lesquerella* of this plant family is covered in a separate paper.

Materials and Methods

Seed procurement, analyses, and oil extraction were all as previously described (3).

Methyl esters were prepared from 1-g. samples of oil by methanolysis, with sodium methoxide or hydrogen chloride as catalyst. Yields of recovered ester ranged from 88 to 97%. Losses were probably partly mechanical, which would not alter the composition of the recovered portion, and partly chemical, e.g., as sodium soaps from the reaction of free fatty acids with sodium methoxide, which might cause preferential loss of some components. Any preferential loss from these samples is so small that it would have little effect on the analyses for major components, but might possibly prevent detection of some unusual minor constituents.

The gas chromatographic analysis was carried out with a Burrell Kromo-Tog, model K-5, equipped with a thermal conductivity detector. All esters were analyzed twice: Once in a 0.6 x 275 cm. column containing 20% of Apiezon L on the 100- to 150-mesh fraction from Celite 545 at 250°C. with a helium flow rate of 70 ml./min.; and once in a 0.6 x 200 cm. column containing 20% LAC-2-R 446 on the same support at 210°C. with a flow rate of 40 ml./min. Samples of 5 μl. were used with the Apiezon L and 1 or 2 μl. with the LAC-2-R 446. Data from the two columns were used to calculate the oil compositions shown in Table I. Acids present have been identified only by chromatographic characteristics (13). No oils showed unusual infrared absorption. Results on two different samples of seed are reported for five species: *Brassica carinata, B. napus, B. campestris, Crambe abyssinica,* and *Lunaria annua.* Curves in Figure 1 (A and B) indicate the order of elution and the degree of separation obtained in the analysis of methyl esters of *Crambe abyssinica* oil.

Results and Discussion

Seed analyses and oil characteristics are given in Table I along with the chromatographic analysis of the methyl esters from the oils.

Although erucic acid is generally considered to be a characteristic constituent of crucifer oils, it varies

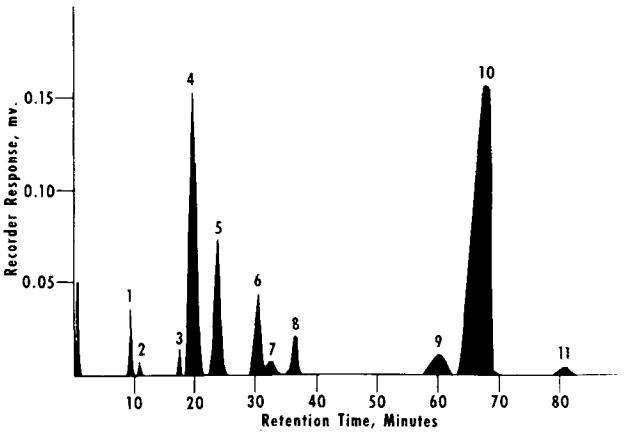

FIG. 1A.

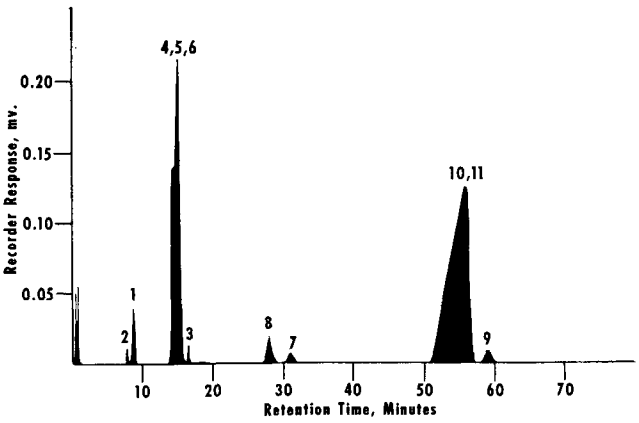

FIG. 1B.

FIG. 1. Illustration of gas chromatograms of fatty acid methyl esters from *Crambe abyssinica* oil. A. Analysis in LAC-2-R 446 column. B. Analysis in Apiezon L. column.

Identification of component peaks: (1) C_{16} sat'd, (2) C_{16} monoene, (3) C_{18} sat'd, (4) C_{18} monoene, (5) C_{18} diene, (6) C_{18} triene, (7) C_{20} sat'd, (8) C_{20} monoene, (9) C_{22} sat'd, (10) C_{22} monoene, (11) C_{22} diene.

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TABLE I

Analytical Data on Cruciferae Seeds and Oils

Source	Common name	References	Wt./1,000 g.	Seed analysis		Oil analysis			Composition of methyl esters, %												
				Oil content % DB	Protein content N x 6.25 %DB	Iodine value	Saponification value	Refractive index n_D^{40}	C_{14} sat'd	C_{16} sat'd	C_{16} mono-ene	C_{18} sat'd	C_{18} mono-ene	C_{18} diene	C_{18} triene	C_{20} sat'd	C_{20} mono-ene	C_{20} diene	C_{20} triene	C_{22} sat'd	C_{22} mono-ene	C_{22} diene	C_{24} mono-ene
Alyssum saxatile	Goldentuft alyssum	.....	1.0	18	27	194	188	1.4756	2	5	.4	1	12	20	58	0	0	0	0	0	0	0	0
Arabis alpina	Alpine rock cress	.....	.4	31	24	192	174	1.4748	Trace	6	.3	2	12	24	53	0	0	0	0	0	0	0	0
Arabis virginica	Virginia rock cress	.....	1.0	31	20	97	164	1.4655	0	6	Trace	.6	17	15	5	.4	12	.7	0	Trace	44	0	0
Brassica campestris	Rape, field mustard (Arlo)	1,2,6,9	2.5	39	29	107	.....	1.4660	Trace	2	.3	1	25	18	10	.6	10	.4	0	.3	31	0	.9
Brassica campestris	Rape, field mustard	1,2,6,9	2.0	43	23	101	164	1.4638	0	3	.5	1	14	14	9	1	8	.5	.4	.7	47	0	.7
Brassica carinata	Abyssinian mustard	.....	3.7	26	34	.....	.....	.....	0	4	.4	1	8	19	14	1	8	.5	0	.6	42	0	2
Brassica carinata	Abyssinian mustard	.....	2.7	30	33	118	179	1.4677	.2	4	.5	2	10	21	13	1	7	1	0	1	37	.8	2
Brassica juncea	Leaf mustard	1,6,9	2.4	34	29	114	164	1.4662	0	3	.4	2	22	24	10	.8	13	.8	0	.3	22	0	.5
Brassica napus	Rape (Matador)	1,6	4.8	47	21	100	164	1.4654	0	3	.5	.9	14	15	8	.6	8	.2	0	.2	50	0	0
Brassica napus	Rape (Duro)	.....	2.5	48	20	104	170	1.4657	0	2	.2	.9	16	16	8	.3	10	1	0	Trace	44	0	.6
Brassica nigra	Black mustard	9	2.0	32	36	113	159	1.4666	Trace	3	.4	1	8	14	18	1	7	1	0	.5	43	1	1
Brassica oleracea var. *capitata*	Cabbage	.....	2.3	26	34	117	175	1.4671	Trace	5	.4	2	8	20	15	.8	9	1	Trace	1	36	1	1
Cakile edentula[a]	American sea-rocket	.....	9.8	49	31	130	178	1.4685	0	4	.2	2	23	26	18	1	7	.6	0	1	17	Trace	0
Camelina sativa	Bigseed false flax	6–9	1.6	33	33	160	178	1.4702	Trace	6	0	3	9	19	38	2	12	4	2	1	3	0	1
Capsella bursa-pastoris	Shepherd's-purse	.....	.05	26	32	148	176	1.4704	0	9	.3	6	11	18	35	3	13	1	2	0	0	0	0
Cheiranthus cheiri	Wallflower	9	1.4	30	28	129	178	1.4690	0	3	.3	.8	10	17	23	.5	10	2	0	.7	31	Trace	.5
Crambe abyssinica	Colewort	7,10,12	4.2	36	32	92	164	1.4641	.1	2	.5	.5	18	11	4	1	2	0	0	1	59	1	0
Crambe abyssinica[b]	Colewort	7,10,12	5.3	34	25	89	165	1.4648	0	2	.3	.7	18	9	6	.7	3	0	0	1	59	0	.4
Descurainia sophia	Flixweed tansy-mustard	.....	.1	35	30	158	184	1.4712	.1	6	.5	2	14	17	37	1	9	1	2	.2	9	.5	0
Eruca sativa	Rocket salad	.....	2.4	22	37	102	173	1.4644	Trace	4	.2	1	18	10	12	.9	10	0	.5	.3	43	0	1
Erysimum perofskianum[a]	Afgan erysimum	.....	1.9	35	28	136	180	1.4690	Trace	3	.4	1	13	27	22	.8	8	.2	0	.5	22	Trace	3
Hesperis matronalis	Dame's rocket	4,6,9	2.8	32	25	191	178	1.4748	Trace	8	1	2	13	24	51	0	0	0	0	0	0	0	0
Iberis amara	Rocket candytuft	.....	4.0	27	28	110	164	1.4681	0	3	.3	0	19	18	12	0	6	.3	0	0	38	0	2
Iberis umbellata	Globe candytuft	.....	.3	26	34	102	165	1.4672	0	3	.2	.3	10	19	7	0	6	.5	0	0	50	Trace	3
Isatis tinctoria[b]	Dyers woad	9	4.2	13	12	136	.....	.....	0	6	0	2	16	12	28	2	13	0	0	Trace	20	0	1
Lepidium lasiocarpum	.....	.....	.4	24	26	149	.....	1.4700	0	6	.8	2	17	8	40	3	15	.2	.5	0	8	0	0
Lepidium montanum var. *angustifolium*	.....	.....	.5	28	28	173	179	1.4723	0	7	.5	3	25	14	50	0	.6	0	0	0	0	0	0
Lepidium sativum	Garden-cress	14,16	1.5	22	31	137	189	1.4681	.1	9	.3	2	21	10	32	3	12	.4	0	.8	9	0	0
Lepidium virginicum[a]	Poor man's pepper	.....	1.0	20	26	143	172	1.4701	.2	7	1	2	17	6	31	3	10	.8	.3	2	19	0	2
Lobularia maritima	Sweet alyssum	.....	.8	33	27	95	176	1.4643	Trace	4	Trace	6	30	7	10	.6	42	.3	.3	.5	0	0	0
Lunaria annua	Honesty	.....	16.4	33	25	81	178	1.4633	0	1	.3	.3	26	7	.6	.4	2	0	0	0	48	0	14
Lunaria annua[c]	Honesty	.....	14.0	48	28	81	160	1.4643	0	2	.2	.4	23	7	2	.1	2	0	0	0	42	0	21
Malcomia maritima	Virginian stock	.....	.4	20	24	127	172	1.4696	0	6	.4	2	6	15	21	3	23	6	2	0	15	.4	Trace
Matthiola bicornis	Evening stock	4	1.2	29	35	198	186	1.4752	.3	6	.8	3	14	12	63	0	0	0	0	0	0	0	0
Nasturtium officinale	Water-cress	.....	.1	28	29	94	.....	1.4633	0	9	Trace	2	34	23	.5	1	11	Trace	0	.5	18	0	0
Nerisyrenia camporum	.....	.....	.1	38	28	136	174	1.4697	0	6	.4	3	21	24	21	2	19	2	0	0	0	0	0
Raphanus sativus	Garden radish	6,9	10.0	42	31	104	172	1.4652	0	6	.4	2	26	13	11	2	9	0	0	0	30	0	1
Selenia grandis	.....	.....	2.2	18	21	86	177	1.4635	0	2	.3	1	28	4	2	Trace	58	0	0	Trace	3	0	0
Sisymbrium irio	London rocket	6,9	.05	13	40	129	173	1.4687	0	14	.8	3	19	13	33	3	8	0	0	.5	6	0	0
Sophia ochroleuca	Menzies tansy-mustard	.....	.1	36	27	133	.....	1.4683	0	9	.2	3	12	18	26	2	12	1	.7	.6	14	0	Trace
Stanleyella texana	.....	.....	.4	36	28	123	169	1.4677	0	7	.6	1	23	9	23	2	15	0	0	2	18	0	0
Thlaspi arvense	Fanweed	6,9	7.0	32	24	122	163	1.4674	.3	3	.2	.5	10	20	14	Trace	10	1	0	.1	38	0	2
Tropaeolum majus[b, d]	Nasturtium	6,9	136.9	10	26	74	144	1.4636	0	1	0	Trace	7	.2	0	.3	21	0	0	.2	72	0	0

[a] Contains trace of C_{22}-triene.
[b] Seed + pericarp analyzed.
[c] Seed minus seed coat analyzed.
[d] Tropaeolaceae.

widely in amount and is absent from some (Table I). The richest sources of erucic acid among the 42 cruciferous samples analyzed here are *Crambe abyssinica, Iberis umbellata, Brassica campestris, B. napus, Lunaria annua, Arabis virginica,* and *Eruca sativa.* The variation noted by Craig and Wetter (2) within *Brassica campestris* is evident in the samples reported here since one sample agrees with his results and one with other published results (6,9). Many crucifer oils contain long-chain acids other than erucic: C_{20}-saturated in 35 samples, C_{20}-monoenoic in 38, C_{20}-dienoic in 27, C_{20}-trienoic in 11, C_{22}-saturated in 28, C_{22}-dienoic in 10, and C_{24}-monoenoic in 23. Only two of these long-chain acids occur in major amounts—C_{20}-monoenoic in numerous oils and C_{24}-monoenoic in *Lunaria annua.*

Our analysis of oil from *Tropaeolum majus* (garden nasturtium, family Tropaeolaceae) is included, although it is not a crucifer, because it is known as a rich source of erucic acid.

Eight species produce oils containing no erucic acid. Six of these however have some other acid in high enough concentration to be of potential industrial interest. Oils from five species (*Matthiola bicornis, Alyssum saxatile, Arabis alpina, Hesperis matronalis,* and *Lepidium montanum* var. *angustifolium*) are rich in linolenic acid and contain no acids longer than C_{18} except for a trace of C_{20} in the *Lepidium.* The *Hesperis* and *Matthiola* oils are from the same seed samples as the oils analyzed previously by other methods (4). When allowance is made for the difference in reporting basis (fatty acid in oil *vs.* ester in total esters), results are within the expected error of the methods. *Lobularia maritima* produces oil containing 42% of C_{20} monoene. The large proportion of C_{20} monoene does not necessarily preclude formation of erucic acid, since *Selenia grandis* oil contains 58% of C_{20} monoene and also 3% of erucic acid. The remaining erucic-free oils, *Capsella bursa-pastoris* and *Nerisyrenia camporum,* have no other unusual compositional feature.

In two instances marked differences are evident between species of the same genus. Oils from *Arabis alpina* and *Lepidium montanum* are rich in linolenic acid and contain no erucic acid. *Arabis virginica* and three species of *Lepidium* however contain erucic acid as well as linolenic acid. An additional instance is indicated by a recent report (12) that *Matthiola incana* contains erucic acid as a major component, whereas our analyses show no erucic acid in *M. bicornis.*

Analysis of the oils for oxirane oxygen by A.O.C.S. Method Cd 9-57 indicated traces of apparent epoxy acid in about half of the samples, but even the highest amount found (1.3% in *Alyssum saxatile*) has no practical significance. Our sample of *Camelina sativa* oil showed no epoxy acid by titration with HBr and may differ from the sample in which Gunstone and Morris (8) found a small amount of epoxy linoleic acid. The precision and accuracy of the titration method in determining traces of epoxy compounds in varied natural oils have not been determined.

The inverse relation between linolenic and erucic acids, noted by Holmberg and Sellman (10), is also revealed in this group of crucifer oils but with increased variability. The relation between linolenic acid and the total content of acids longer than C_{18} is somewhat closer (Figure 2). Linolenic acid content and iodine value are well correlated (Figure 3). Figure 2 suggests that the maximum amount of acids longer than C_{18} to be found in Cruciferae oils may be about 70%. The parallelism between the upper edge of the scatter diagram and the $x + y = 100\%$ line suggests that the Cruciferae always synthesize at least about 30% of C_{16} and C_{18} acids other than linolenic. Analysis of additional species of crucifers may reveal exceptions. The *Tropaeolum* sample indicates that seed oils from plants in other families need not necessarily follow the same pattern as in Cruciferae.

Few of the species reported here have been grown as seed crops in the United States, and little is known about potential seed yields or optimum conditions of production. Development of a profitable domestic crop from among the species surveyed will require extensive study by plant breeders and agronomists. Three species rich in erucic acid—*Crambe abyssinica, Eruca sativa,* and *Raphanus sativus*—were grown at five State Agricultural Experiment Stations in 1959 in exploratory tests of adaptability and cultural methods. The compositions of the oils obtained showed

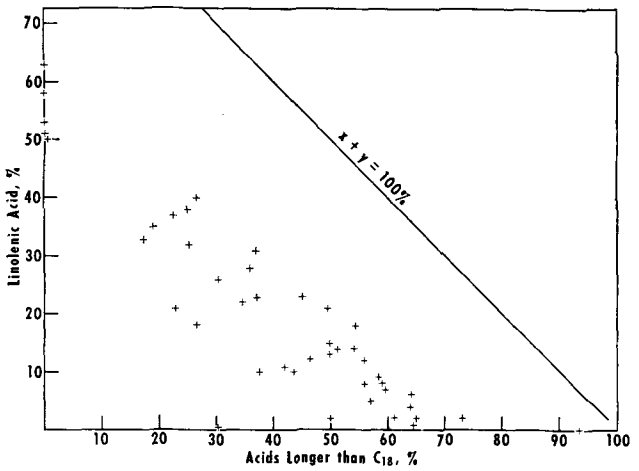

FIG. 2. Relation between linolenic acid and acids longer than C_{18} in oils from Cruciferae and *Tropaeolum majus.*

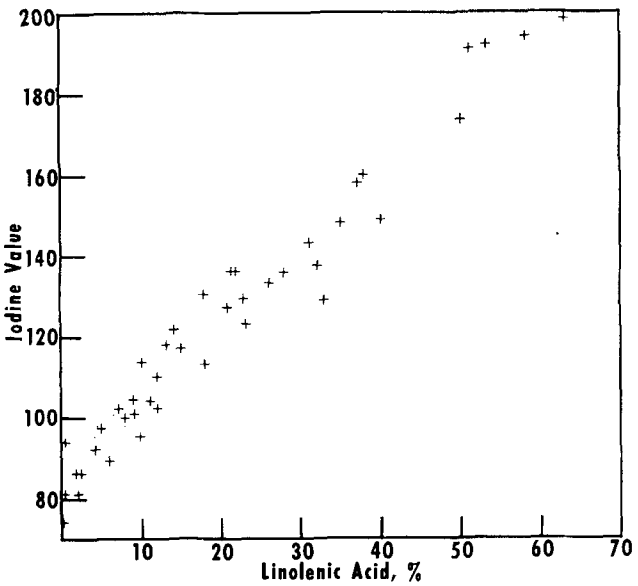

FIG. 3. Iodine value *vs.* linolenic acid in oils from Cruciferae and *Tropaeolum majus.*

relatively little variation within species and were in excellent agreement with the data in Table I. For example, the range of erucic acid content in *Crambe abyssinica* (6 samples, 3 locations) was 56 to 59%, in *Eruca sativa* (6 samples, 3 locations) 42 to 45%, and in *Raphanus sativus* (5 samples, 4 locations) 31 to 34%.

Seeds of selected Cruciferae are shown by this survey to contain oils which, on the basis of chromatographic analyses, appear to be suitable industrial sources of linolenic acid and eicosenoic acid in addition to the usual erucic acid.

Acknowledgments

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Dilatometric Properties of Some Glycerides of Confectionery Fats[1]

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Two triglycerides, 1-oleodistearin and 2-palmito-oleostearin, which are components of some confectionery fats, were synthesized and their melting behavior and dilatometric properties were determined. Expansivities and melting dilations of the various polymorphic forms were measured.

1-Oleodistearin was found to have two melting points, 30.3 and 42.1°C., while 2-palmito-oleostearin was found to have melting points at 24, 37, and 40.5°C. The rates of transformation of the thermodynamically unstable polymorphs at temperatures below their melting points were much more rapid than those for the corresponding 2-oleo isomers previously reported.

Mixtures of 2-oleopalmitostearin with 2-palmito-oleostearin and 1-oleodistearin with 2-oleodistearin were examined dilatometrically. In each of these mixtures the components apparently do not temper at the same rate to similar polymorphic forms and thus there is some degree of incompatibility even though in each of these mixtures the components are positional isomers. The properties of the intermediate melting mixtures are dependent on the method of tempering.

COCOA BUTTER-LIKE fats derived from domestic vegetable oils by processes involving interesterification and from animal fats by processes involving fractionation should contain sizable proportions of both 1-oleodistearin and 2-palmito-oleostearin. One of these glycerides is the positional isomer of 2-oleodistearin, and the other is a positional isomer of 2-oleopalmitostearin. The 2-oleo glycerides comprise about 80% of cocoa butter. Unfortunately in neither pair of isomers do the members exhibit the same polymorphic behavior (11). The behavior in mixtures also should be different. Despite the common occurrence of the two glycerides, 1-oleodistearin and 2-palmito-oleostearin, in fat products, there have been only a few investigations with the pure compounds, and relatively little is known about their properties.

The synthesis and purification of 1-oleodistearin has been reported several times (1, 5, 6, 11). Lutton (11) made an investigation of polymorphic behavior and found three forms which he called sub-alpha, alpha-3, and beta prime-3. The sub-alpha was claimed to exist in modifications *A* and *B*, and it was presumed that the sub-alpha transformed reversibly to the alpha. The alpha-3 and beta prime-3 melted at 30.4 and 43.5°C., respectively. Apparently no one has made a dilatometric examination of 1-oleodistearin or of well-defined mixtures containing it.

2-Palmito-oleostearin also has been prepared and examined by several investigators (4, 9, 11, 13). Lutton (11) again is the only one who has made a thorough examination of polymorphic behavior. The polymorphs which he found to exhibit melting and their associated melting points were alpha-2, 25.3°C.; sub-beta prime-3, 37°C.; and beta prime-3, 40.2°C. The alpha form was found to transform reversibly to sub-alpha. The beta prime-3 form was obtained from solvent only. Apparently no dilatometric data on 2-palmito-oleostearin have been published heretofore.

The purpose of the present investigation was to determine dilatometric and other properties of 1-oleodistearin and 2-palmito-oleostearin and of a simple mixture of each of these compounds with its positional isomer occurring in cocoa butter.

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