Lipids in Cruciferae: VIII. The Fatty Acid Composition of Seeds of Some Wild or Partially Domesticated Species

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Abstract

A total of 75 seed samples of 29 species of Cruciferae have been analyzed for fatty acid composition by gas chromatography. All but three species contained erucic acid in the seed oils at levels ranging from 1% to 57%. Linolenic acid was present in all samples ; the levels ranged from 2% to 55%. A considerable variation in fatty acid patterns was observed at the intraspecific level for *Sinapis arvensis.* Species from five of the generas studied may have potential as new crops, namely *Barbarea, Conringia,* $Erysimum, Hesperis and Sisymbrium.$

Introduction

Several species of Cruciferae presently have great commercial value as oil crops, e.g., *Brassica napus, B. campestris* and *Sinapis alba* (1). Extensive investigations have recently been undertaken at our laboratory and elsewhere on the variability in fatty acid composition of seeds of these domesticated species (2-8), mainly to develop cultivars with the lowest erucic acid and linolenic acid contents and highest possible linoleic acid content.

In efforts to find species of potential value as new oil seed crops (9), Mickolajzcek et al. (10), Miller et al. (11) and Goering et al. (12) studied the fatty acid patterns of a large number of seed samples from the Cruciferae. The two former studies demonstrated that about three fourths of the species of this family contain erucic acid, 22:1, in their seed oils at levels from ca. 1% to ca. 60% . Since considerable interest has been shown in the development of seeds with highest possible erucic acid content (13), additional studies of cruciferous seeds were initiated.

The studies presented here were made on seeds collected from natural populations in Sweden or received from other institutes and studied in the nurseries at Svalöv. Although these studies were aimed at revealing seed sources of highest possible erucic acid content, the results obtained seem to have some significance also in the field of chemotaxonomy.

Materials and Methods

Seeds

Seed samples were obtained from the Oil Crops Division of the Swedish Seed Association, from the Institute of Systematic Botany, University of Lund, from Sven-Ake Johansson, Svalöv, from Bengt Lööf, Svalöv and from Bengt Mattsson, Svalöv.

Lipid Extraction and ratty Acid Methyl Ester Preparation

The procedures for extraction of seed oil and fatty acid methyl ester preparation have been described in detail elsewhere (14). When sufficient amounts of seed material were available, extraction was performed with hexane in the Svalöv steel tubes (15) . Very small samples were extracted in all-glass homogenizers and very hard seeds by mortar grinding

with sand as the grinding agent. The hexane solution of lipids was evaporated and methyl esters prepared as described previously (14). In certain cases the methyl esters were purified by preparative thin layer chromatography (TLC) before gas liquid chromatography (GLC) analysis (14); otherwise, the crude ester preparation was used directly.

Determination of ratty Acid Composition

The fatty acid composition was determined in duplicate by gas chromatography on polyester (BDS) columns in either an Aerograph A-350-B instrument or a Perkin-Elmer F-11 instrument as indicated in table heads. Operational details were presented in previous papers (4,7). Several samples were hydrogenated by a micro method (7) and rerun after hydrogenation to allow a more reliable peak assignment.

Results and Discussion

The samples of *Arabis* studied in the present investigation, one of *Arabis alpina* and three of A. *hirsuta,* are characterized by very small amounts of erucic acid and about 50% linelenic acid (Table I). Similar results have been recorded by other authors for single samples of these species (10,11). On the other hand, four other samples of this taxon had considerable amounts of erucic acid in the seed oils, 14% in *A. glabm* (11) and *A. Drummondi* (12), 15% in *A. laevigata* (11) and 44% in *A. virginica* (10). The low erucic acid species of this genus contain approximately twice as much linolenic, 50-55%, as linoleic acid, $25-30\%$, (Table I) $(10,11)$, whereas the linolenic acid content is lower than linoleic in species with substantial amounts of erucic acid in the oils (10,11). Thus, in the case of *Arabis,* there must be considerable intrageneric differences in the system controlling the elongation of oleic acid, as well as in the systems controlling its desaturation to linoleic and further to linolenie acid (16). None of the species investigated offer direct promise as a new crop, but the difference noted in fatty acid Spectra might be of taxonomic interest and thereby justify studies of additional samples.

The growth habit and seed yield of *Barbarea stricta* and *Barbarea vulgaris* are indeed favorable for further improvement by plant breeding provided a strain with suitable fatty acid composition can be found. It seems to be unsettled, whether *B. stricta* and *B. vulgaris* are two distinct species or only one. They yield better than spring-sown but less than fall-sown *Brassica napus* and *B. campestris* and are annual to quadrannual (Lööf, unpublished data). Our samples, which had been subjected to some selection work, had favorable erucic (26%) and linoleic (25%) acid contents for use as an edible oil, but were obviously not suitable for nonfood industrial uses requiring a high content of erucic acid. The similarity of the fatty acid spectra of two samples of *Barbarea* (Table I) to that reported by Miller et al, (11) is striking and indicates less promise for developing either low or high erucic acid strains.

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Thlaspi arvense L.

TABLE I The Fatty Acid Composition of Seeds of Various Cruciferae^{4, h}

Samples marked ISBL and SAJ were from natural populations in Sweden, except those of Brassica cretica, which were collected in the Mediterranean area. Samples marked BL, BM and SSA came from the nurseries of this institute

 0.1

8.3

23.4

 1.9

Association.
"IND, not determined. These samples were analyzed in a Perkin-Elmer Mod. F-11 instrument (7).

Goering et al. (12) reported only 16% erucic acid

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for a sample of Barbarea orthoceras. A single collection of Berteroa incana was found to have no detectable amount of erucic acid but contained 48% linolenic acid. This species is reported to be an oil crop in India and Iran (17). Goering et al. (18), who consider this species as having some potential as a new crop in the U.S., reported a very similar fatty acid composition of seeds collected in Montana, U.S.A. Their finding that the second generation seeds obtained from plants grown in a nursery had 6% erucic acid as compared with only a trace in the field-collected seeds, represents a much greater change in content of the acid than is normally encountered as a result of environment (8). From a taxonomic point of view, the high content of myristic acid is noteworthy, since there is generally only a trace of this fatty acid in cruciferous seeds $(10,11)$.

TSBL

Seed of several interesting noncultivated Brassica species were available for analysis. Data on fatty acid patterns of B. Barrelieri and B. fruticulosa do not seem to have been reported earlier. None of them differ markedly from cultivated brassicas with respect to the fatty acid spectrum, and are thus of no apparent commercial interest (Table II). The fatty acid patterns for B. oxyrrhina and B. Tournefortii were presented earlier with data on intrastrain or intraplant variability (7). A remarkably small difference is noted for the different samples of B.

Tournefortii investigated (Table II) in view of the great differences in geographic origin and the phenotype (Mattsson, unpublished data). Also the sample reported by Miller et al. (11) was similar
in fatty acid composition. The close similarily in fatty acid spectra of the diploid and derived tetraploid genotypes add significance to the earlier observation of fatty acid spectra of diploid and autotetraploid seeds of other Cruciferae $(6,8)$. Since B. Tournefortii is a self-fertilizer, very little intrastrain variability is expected. This small inter- and intrastrain variability means that it may not be possible to develop a high erucic acid crop in this species.

 9.3

35.3

 1.8

 2.5

17.4

Although closely related to the cultivated Brassica *oleracea*, which is treated in another paper (8), the results of analyses of seeds of B. cretica are included in this paper, since the samples were collected from natural populations. As seen from Table II, there are considerable differences in oleic $(9-24\%)$ and
erucic acid contents $(31-57\%)$ among the samples studied. This variation would merit analysis of additional samples to find germ plasm for still lower or higher erucic acid contents. If found, such germ plasm could be merged with that of high yielding \bar{B} . campestris to form the allotetraploid \bar{B} , napus $(19).$

A sample of *Brassicella erucastrum*, also regrown at Svalöv, had a rather ordinary fatty acid spectrum for a crucifer (Table I) and was not thought to merit further study. No other data seem available

TABLE II The Fetty Acid Composition Soods of Some Because Species

a Analysis of fatty acid methyl esters in an Aerograph or a Perkin-Elmer gas chromatograph (4 and 7).

« ND, not determined.

in literature on this species (also named Rhynchosinapis cheiranthos (Vill) Dandy).

Four collections of *Cakile marituma* were analysed (Table I) and found to be rather similar to one another and to the sample reported by Miller et al. (11) , as well as to the sample of *Cakile edentula* reported by Mikolajczak et al. (10). The higher linolenic acid content of the Swedish samples compared with those grown in the U.S. could be due to the well-known effects of climate during seed maturation on the linolenic acid content (6 and loc. cit.).

Considerable interest has been shown for the seed oils from various *Cardamine* species (11). Our samples of C. bellidifolia and C. pretensis are rather similar in fatty acid patterns to that of C. hirsuta reported by Miller et al. (11) with linoleic acid as the major C₁₈ fatty acid and with variable amounts of eicosenoic and erucic acids (Table I). On the other hand C. *impatiens* has been found to have large amounts of dihydroxy, longchain fatty acids in the seed oil (20) .

Conringia orientalis represented by two strains. 64-1027 and 64-1026, one of which grown in three
years in the nurseries $(64-1026, 66-1628$ and $67-$ 1664), had almost as much eicosenoic as erucic acid in the seed oil, ca. 24% and 29% , respectively (Table I). The high linoleic acid content, ca. 25% , and the low linolenic acid content, $2-3\%$, of C.

orientalis seem to offer the most favorable proportion of 18:2 to 18:3 content of all Cruciferae so far reported (Tables I-V) (4,10-12 and loc. cit.). Since the growth habit is favorable for further improvement (Lööf, unpublished data), this species might have potential as a new crop for edible oil production. The sample investigated by Miller et al. (11) had a fatty acid spectrum very similar to our samples,
whereas Goering et al. (12) reported a range in
erucic acid content from 22% to 34% among three samples. Therefore it might be possible to find collections of this interesting species with still wider ranges of variation in erucic acid content.

Because of the very high erucic acid content in the seed oil, Crambe abyssinica has come into the focus of interest as a new crop (13). Data from a large number of samples of this species have been reported in conjunction with results from selection work on other high-yielding Cruciferae (21). Results from analyses on four collections of C , maritima are, however, presented in Table I, as well as one sample received as C. hispanica. The latter had a pattern rather similar to that for C . hispanica reported by Miller et al. (11) . The four collections of C . maritima do not display any larger intraspecific variation and are rather similar to \bar{C} . cordifolia, C . orientalis and C. tatarica with 25-30% erucic acid, which is about one half of that in the other two *Crambe* species

TABLE III The Fatty Acid Composition of Seeds of Some Erysimum L. Species^a

Species	Sourceb	Identification number	Fatty acid composition. %								
			16:0	18:0	18:1	18:2	18:3	20:1	22:1	24:1	Other acids
E. cheiranthoides L.	ISBL	327	3.9	1.5	6.6	25.3	37.1	5.2	16.8	0.8	2.8
E. cheiranthoides L.	$_{\rm ISBL}$	328	4.0	1.3	6.0	26.4	36.7	4.6	17.7	0.7	$^{2.6}$
E. cheiranthoides L.	$_{\rm ISBL}$	329	4.8	$^{1.9}$	5.3	27.0	37.2	4.9	14.8	0.8	3.3
E. hieraciifolium L.	$_{\rm ISBL}$	330	3.7	1.0	6.4	21.5	39.2	5.8	17.7	1.8	2.9
E. hieraciifolium L.	вL	62 1536 II	3.7	1.0	5.3	24.6	29.2	6.0	22.1	2.5	5.3
E. hieraciifolium L.	вь	60 A 338	4.4	2.2	5.8	26.0	30.1	5.5	18.9	1.6	5.5
E. hieraciifolium L.	вL	60 A 339	4.0	1.0	7.6	23.7	25.5	7.4	22.5	1.5	6.8

⁴ Analysis of fatty acid methyl esters in an Aerograph gas chromatograph (4).

^a Analysis of fatty acid methyl esters in
^b Abbreviations: See Table I.
^c ND, not determined. a Perkin-Elmer gas chromutograph (7).

investigated. It should also be noted that the linolenic acid content is about the same in the high erucic and low erucic species, whereas the linoleic acid content of the latter is about three times as high as that of the former. This grouping of species as regards erucic acid content is similar to what has been observed for *Brassica juncea* (8) and could merit further studies into the inheritance of erucic acid content of other species than *Brassica napus* (22-24) and *B. campestris* (25).

The genus *Erysimum,* which contains at least two species of promise as new farm crops (Lööf, unpublished data), demonstrates a considerable variation in fatty acid pattern. The three collections of *E. cheirantkoides* and the one collection of E. *hieraciifolium* analysed (Table III) had a rather similar fatty acid composition (ca. 6% of 18:1, 25% $18:2, 38\%$ 18:3, 5% 20:1 and 18% 22:1). This pattern is also analogous to that reported for E . *Perofskianum* (10), *E. linifolium* (11), *E. repandum* (11) and *E. silvestre* (11). The three samples from the nurseries of this institute also had fatty acid patterns of the previously mentioned nature although the linolenic acid content was lower and the erueic acid higher (Table III). A sample of *E. cuspidatum* is however reported to be rather different with only 14% linolenic acid but 46% erucic acid (11). Goering et al. (12) reported 32% erucic acid (no other details given) in *E. iconspicuum.* Obviously there is considerable heterogenety in the fatty acid spectra of various *Erysimum* species and therefore further studies may be warranted both from a practical agronomic and a chemotaxonomic point of view.

One sample of *Goldbachia laevigata,* grown in the nurseries at Svalöv for observation on its agronomic characters, had palmitic, linoleic and linolenic acid contents similar to those of the sample reported by Miller et al. (11). The marked differences in oleic, eicosenoic and erucic acid contents seem to indicate genetic variability in extent of oleic acid elongation (16) between various samples of this species. Although its fatty spectra and agronomic properties do not warrant plant breeding work at the present time, the variation observed is promising.

Hesperis matronalis, which also gives some hope as a new crop (Lööf, unpublished data), is known from earlier studies (10) to be a species lacking erucic acid. The sample studied at this laboratory, which bad been subjected to some selection work, presented a fatty acid pattern rather similar to that reported by Mickolajczak et al. (10), namely, 7% palmitic, 14% oleic, 22% linoleic and 55% linolenic acids. Minor amounts of $14:0$ and $16:2$ (?) were also present. Hydrogenated samples gave sizable peaks on polyester chromatograms for 14:0, 16:0 and 18:0 only. Since it could be of interest to distinguish between cruciferous species having small, but quite easily observable amounts of erucic acid, e.g., *Camelina sativa* (4) and *Arabis hirsuta* in this paper, and those having, at the most, traces of this fatty acid, overloaded isothermal chromatograms were recorded. Although our sample size was such that 0.5% erucic acid should easily have been seen, no peaks at the retention time of erucie acid could be observed. Therefore, at the most, only traces of eicosenoic and erucic acids can be present in *Hesperis matronalis* seed oil.

The sample of *Hirschfeldia incana* available, which had been regrown in the nurseries at Svalöv, differed rather much in the fatty acid spectrum from that reported by Miller et al. (11) notably with regard to the proportion of unsaturated C_{18} acids.

Samples of diploid and "derived" tetraploid Raphanus sativus studied at the nurseries at Svalöv, had fatty acid spectra (Table I) that did not differ markedly from those reported in the literature for *R. satiwes* (10) and *R. caudatus* (11). Goering et al. (12) reported a variation in erucic acid content among 17 samples of *R. sativa* from 17.5% to 30.3%. These results hardly merit further studies of this species for erucic acid content. The close similarity of the fatty acid spectra of the diploid and the corresponding autotetraploid seeds extend earlier results from diploid and autotetraploid seeds of *Brasxica campestris* (6), *B. nigra* (8), *B. Tournefortii* (Table II) and $Sinapis$ alba (6) .

ª Analysis of fatty acid methyl esters in an Aerograph gas chromatograph (4).
^b Abbreviations: See Table I.

Three Swedish collections from natural populations and four other samples were available of *Sinapis arvensis.* A very marked difference in erucic acid content (6-39%) among the samples was noted (Table IV). Miller et al. (11) reported 35% erucic acid in one sample studied. Even the highest erucic acid content reported seems too low to make this species a candidate as a new crop for erucic acid production. Of considerable interest, however, is the proportions of oleic to linoleic to linolenic acids which resemble much more those of *Brassica nigra* (8) than they do those of *Sinapis alba* (4,8). The taxonomic status of *Sinapis alba* and *S. arvensis* seems to be disputable (Mattsson, unpublished data). Recent electrophoretic and serologic investigations of seed proteins of several brassicas and of *Sinapis alba* (26,27) have presented additional evidence classifying *Sinapis alba* in a genus separate from *Brassica.* (Thus, the alternative name for *Sinapis alba* L. namely *Brassica hirta* Moench., seems obsolete.) A comparison of the fatty acid spectra of *Sinapis alba* and *S. arvensis*, especially the difference in the unsaturation in the \bar{C}_{18} -series, provide additional evidence for the separate status of *Sinapis alba. Of* interest is also the high eicosenoic acid content of *S. arvensis,* which differs both from *S. alba* (4,8) and *Brassica nigra* (8). It should be remarked that the taxa of these samples were checked by several independant botanists. The considerable difference noted in erucic acid content of *Sinapis arvensis* may merit further fatty acid studies on this interesting species from a chemotaxonomic point of view.

Five collections of *Sisymbrium* from natural populations have been analysed and also three samples of *Sisymbrium,* that were subjected to a small scale selection work, because of their yield potential (Lööf, unpublished data). All the samples investigated had a rather similar fatty acid pattern with about 5%, 15% and 40% oleic, linoleic and linolenic acids, respectively, and about 8% eicosenoic and 20% erucic acid (Table V). The palmitic acid content varied comparatively more (between 4% and 8%). Most of the data reported for nine other species of *Sisymbrium* (11) also fall within this range of variation. There are, however, two rather dramatic deviations from this pattern, namely *Sisymbrium irio* (10) with only 6% erucic acid but 19% oleic and 14% palmitic acids, and *Sisymbrium alliaria* (11) with only 4% linolenic acid and 47% erucic acid. Miller et al. (11) also support the data on *S. alliaria* with figures from three additional accessions with about 45% erucic acid. Accordingly they placed *Sisymbrium alliaria* among the high erucic acid species. Hydrogenated samples of the total methyl esters of one of the samples gave only four sizable peaks namely for 16:0, 18:0, 20:0 and 22-0, besides minute amounts of 14:0, 15:0 and 17:0.

A collection of *Teesdalia nudicaulis* was characterized by the very high eicosenoie acid content, 56% (Table I). Only two other Cruciferae have been reported with such high levels of eicosenoic acid, namely *Selenia grandis* (10) and *Leavenworthia torulosa* (11). If a specific industrial need for eicosenoic acid arose, further studies on these three species should be undertaken.

One collection of each of two *Thlaspi* species, (T. *alpestre* and *T. arvense)* had fatty acid spectra (Table I) similar to those reported earlier in the literature (10-12).

The reliability of the data presented in this paper and elsewhere on the fatty acid composition of cruciferous seeds is dependent on two factors, provided the analytical techniques used are adequate. One is the frequent misidentification of cruciferous species. Kjaer, studying the glucosinolates of seeds of this family, reported that more than half of 300 seed samples of Cruciferae received from various sources bore incorrect labels, as revealed upon reexamination by botanists well trained in the taxonomy of the Cruciferae (28). Some examples of such misidentification are given in another paper in this series (8). From this point of view, the items marked 'Ac. No." here and in (8) might be dubious, since they represent seeds received from other sources and not regrown in the nurseries at Svalöv. The other factor to consider is the highly cross-fertilizing behavior of many Cruciferae coupled to the embryonic control of the erucic acid content of at least two species of this family, namely *Brassica campestris* (25) and *B. napus* (22-24). This means that if two crossfertilizing collections with different erucic acid contents are grown in a nursery in close proximity, the seeds harvested from both plots can have an intermediate fatty acid composition. A striking example of this effect is given in another publication from this institute (8). From this point of view, seeds regrown in a nursery together with other samples of the same species might be less representative than collections from natural populations. A simple, but costly, solution to this problem is the regrowing of all collections or samples of doubtful nature in isolation chambers.

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