SYMPOSIUM: THE PLANT GENETICIST'S CONTRIBUTION TOWARD CHANGING THE LIPID AND AMINO ACID COMPOSITION OF OILSEEDS

presented at the AOCS Meeting in Houston, Texas

G.C. CAVANAGH, Program Chairman

Improving High-Erucic Oilseeds: Chemically or Genetically?[1]

W.H. TALLENT, Northern Regional Research Laboratory,[2] Peoria, Illinois 61604

ABSTRACT

Because of evidence that erucic acid may be undesirable in edible products, genetically developed low-erucic varieties of *Brassica campestris* and *Brassica napus* are expected to become a major part of the Canadian and European rapeseed crops within the next few years. In contrast the objective of work on crambe and related oilseeds in the U.S. is a reliable domestic source of oil high in erucic acid for industrial purposes. Whether the oil is produced for edible or nonfood uses, however, the glucosinolates that are characteristically present in oilseeds of the mustard family unfavorably influence the use of the byproduct meals as feed. These glucosinolates are the subject of current intensive research, both chemical and genetic. Development of convenient, accurate and sensitive analytical methods has markedly facilitated this research. To achieve optimum meal quality, procedures involving aqueous extraction of unreacted glucosinolates are under study. Genetically, a Polish variety of *B. napus* called Bronowski has been found to produce seed having very low glucosinolate content, and individual low-glucosinolate plants of *B. campestris* have been discovered. Crambe exhibits significantly less observable variability than rapeseed. Consequently the approaches based on chemical research seem more promising, but the possibility of developing a low-glucosinolate, high-erucic *Brassica* adapted to agricultural production in the U.S. to complement or even compete with crambe is not being overlooked.

[1]One of seven papers presented at the Symposium, "The Plant Geneticist's Contribution Toward Changing the Lipid and Amino Acid Composition of Oilseeds," AOCS Meeting, Houston, May 1971.

[2]No. Market. Nutr. Res. Div., ARS, USDA.

INDEX

INTRODUCTION

Traditionally the major high-erucic oilseeds have been *Brassica napus* and *Brassica campestris*, the two species to which the familiar cultivated varieties of rapeseed belong. Most of the oil from these is used in edible products, and the erucic (*cis*-13-docosenoic) acid content has been largely of incidental significance. Though rapeseed oil has not penetrated the U.S. food market, some is imported for industrial applications, and substantial opportunity for expansion of such nonfood usage exists (1). For these industrial purposes a continuing and reliable source of oil

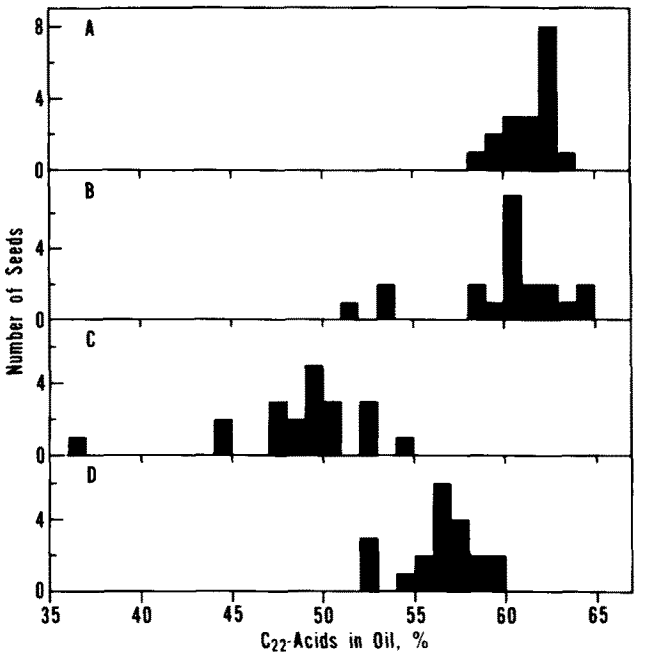

FIG. 1. Variation in C_{22}-acid content of oil from individual seeds. The C_{22}-acid is mainly (generally more than 95%) erucic acid. Twenty seeds of each species were analyzed (16). A, *Crambe abyssinica*; B, *Brassica campestris* (Indian yellow sarson); C, *Brassica carinata*; D, *Brassica napus* cultivar Matador.

with a high and constant erucic acid content is important, and fulfilling this need is the objective of research on crambe (*Crambe abyssinica*) and related oilseeds in the U.S. Department of Agriculture. Since recent research with experimental animals has suggested that erucic acid is a nutritionally undesirable constituent (2,3), "improvement" with regard to content of this acid has a different meaning depending on the use for which the oil is produced.

A new Canadian rapeseed oil named Canbra oil contains not more than 3.5% erucic acid (4). The impressive accomplishments of plant geneticists that led to development of practical "zero-erucic" varieties of rapeseed as sources of Canbra oil have already been excellently reviewed (5-7). Therefore little discussion of these accomplishments is needed here. They are referred to briefly in relation to more recent consequences and as background for information concerning improvement in the opposite sense, i.e., increasing the erucic acid content.

A major portion of this review is devoted to glucosinolates. Removal of these characteristic constituents of the plant family Cruciferae or minimization of their adverse effects on meal quality is sought by researchers in countries producing rapeseed oil for food uses as well as in the USDA high-erucic oilseed project.

ERUCIC ACID CONTENT OF OILS

Among oilseed crops rapeseed ranks fifth in world production (8). Canada has just become the leading producer of this oilseed in the world (9) as a result of increase in acreage from 0.8 million in the 1964-1965 crop year (10) to approximately 4 million in 1970-1971 (11). Research (5-7) conducted prior to the recent reports suggesting nutritional undesirability of erucic acid in edible oil (2,3) has enabled the Canadian Government to set a target date of 1972 for complete switchover to zero-erucic varieties of rapeseed (4). A similar change will undoubtedly also take place in Europe (7).

Since *B. campestris* makes up about 80% of the Canadian rapeseed crop (4), a new zero-erucic variety of this species named Span (12) will probably be grown more extensively than the zero-erucic *B. napus* varieties, Oro and Zephyr. To increase the seed supply of Span, approximately 2000 acres of it were reportedly grown under contract in the Imperial Valley of Southern California during the winter of 1970-1971 (4).

Though duly acknowledged in earlier reviews (5,6), the role of chemical methodology in research that led to development of Span, Oro and Zephyr bears reemphasizing, especially since the same procedures are useful in studies pertaining to increasing the erucic acid content of cruciferous oils for industrial uses. Downey and Harvey (13) found that oil extracted from a single cotyledon could be analyzed by gas liquid chromatography (GLC), leaving the other cotyledon from the same seed to be planted. Obviously special techniques and precautions are required in working with such small samples to avoid losses and contamination in the extraction of the oil, in preparation of methyl esters from it and in transfer of these esters from the vessel in which they are formed to the GLC column. Procedures by which these steps can be accomplished starting with as little as one-half of a seed have been described in some detail by Appelqvist (14). In another ingenious approach, Yermanos (15) reported that small portions of the oil can be extracted from single seeds of flax, safflower, soybeans, sunflower and sesame without preventing subsequent germination of the seeds. Preliminary experiments at the Oregon Agricultural Experiment Station suggest that this technique may be applicable to high-erucic oilseeds (W. Calhoun, unpublished). At the Northern Regional Research Laboratory (NRRL), the gentle extraction procedure employed in the technique was tested with small samples of rapeseed. The fractions of oil obtained had compositions representative of the total oil in the seed.

Appelqvist (16) has applied single-seed analysis to a number of species of crucifers. Selected results from his work are presented in Figure 1. In addition to crambe, data are shown for three representatives of the genus *Brassica* that may have potential as high-erucic oilseeds. If adaptation to U.S. agriculture could be achieved, selections might be made from the Indian yellow sarson variety of *B. campestris* that would rival crambe in erucic acid content of the seed oil. The *B. carinata* has already exhibited adaptation to growth in at least some areas of the U.S. (17), but the erucic acid content of its seed oil would have to be increased. Matador is intermediate between the yellow sarson and *B. carinata* in erucic acid production and is of interest as a member of the same species as the low-glucosinolate Polish variety Bronowski discussed below.

A skewed pattern as in Figure 1A also occurred in the corresponding histogram of another crambe accession. No single seed of crambe or any *Brassica* species was found to contain more than 65% of erucic acid in its oil. Self-fertilization and repeated selection of crambe and rapeseed did not yield seed with oils having more than about 60% erucic acid. These results suggest an upper limit to the amount of erucic acid in cruciferous seed oils that may be related to the intraglyceride distribution pattern of the acid (16). In oils from crambe, rapeseed and several related species, erucic acid is esterified almost exclusively to the 1 and 3 positions of glycerol (18-20). The small amount found in the 2 position is easily rationalized by the limitations of the experimental method, and the results support the view that the Cruciferae lack the capacity to attach erucic acid to this position.

On the other hand, in nasturtium (*Tropaeolum majus*, family Tropaeolaceae) seed oil approximately one-third of the 78 mole % of erucic acid present is attached to the middle hydroxyl group of glycerol (18). The distribution of the 14 mole % of erucic acid in meadowfoam (*Limnanthes douglasii*, family Limnanthaceae) seed oil indicates an actual preference for this 2 position over the 1 and 3

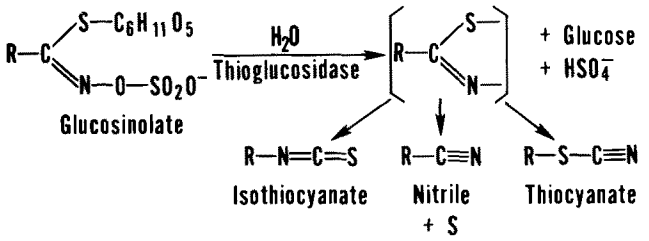

FIG. 2. Enzymatic hydrolysis of glucosinolates.

positions (21). Granted neither of these plants is a crucifer, and other unusually long chain acids in meadowfoam seed oil may have a stronger tendency to be attached to the 1 and 3 positions and in effect "crowd" erucic acid into the 2 position. Nevertheless the examples prove that the enzymatic machinery exists in the plant kingdom for placing erucic acid on the middle hydroxyl group of glycerol. The significance of the meadowfoam finding is increased if the production of glucosinolates by both Cruciferae and Limnanthaceae (22) can be taken as a suggestion that other biochemical similarities between the two families are apt to exist.

Finally the fact should not be overlooked that even if the hypothetical 67 mole % maximum exists, there is still room for substantial improvement. Because of the relatively high formula weight of the erucoyl moiety, GLC analysis of crambe oil containing this group in the 1 and 3 positions of all of the triglyceride molecules and none in the 2 position would show 70% of the desired acid on a weight basis. In comparison oil from 73 samples of crambe seed was found to have erucic acid contents ranging from 51-60% with the mode at 58% (23).

GLUCOSINOLATES AND DERIVED PRODUCTS IN MEALS

Biochemistry and Analytical Methods

The amino acid compositions of crambe and rapeseed indicate high protein quality (24), as do feeding studies with defatted glucosinolate-free meals (25,26). Unfortunately full exploitation of this high protein quality is currently limited by the presence of the glucosinolates, which may be transformed into toxic substances (25,27). Figure 2 shows in generalized form the hydrolysis of glucosinolates catalyzed by thioglucosidase (thioglucoside glucohydrolase, EC 3.2.3.1). This enzyme is present in the seed, and the hydrolysis may occur when they are crushed and moistened or thoroughly wetted without crushing. Glucose and inorganic sulfate are always formed in the reaction. The remainder of the glucosinolate molecule, the organic portion of the aglucon, is converted to an alkyl isothiocyanate, thiocyanate or nitrile and sulfur depending on reaction conditions and other factors (27).

When the moiety represented by R in the generalized formulas in Figure 2 contains an appropriately located hydroxyl group, the initially formed isothiocyanate may cyclize as shown in Figure 3. The trivial names goitrin [(*S*)-5-vinyloxazolidine-2-thione] and progoitrin (precursor of goitrin) are derived from the well-known goitrogenic activity of the former. Equally goitrogenic is (*R*)-5-vinyloxazolidine-2-thione from *epi*-progoitrin (29). More importantly the nitriles shown in Figure 3 have been found to possess more serious toxicity than the goitrins (25).

The relative amounts formed of the several products shown in Figure 3 vary markedly with reaction conditions and meal or seed history (27,30). Recognition of this variability has been important in the development of analytical methods that are sufficiently accurate, rapid and sensitive for use in genetic selection and breeding work with

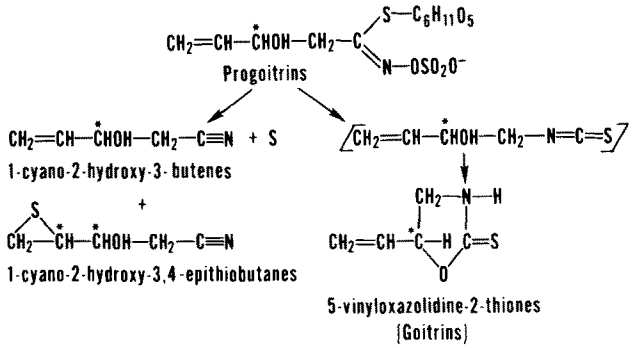

FIG. 3. Enzymatic hydrolysis of progoitrins. Asterisks indicate asymmetric centers. Products formed from *epi*-progoitrin [(*S*)-2-hydroxy-3-butenylglucosinolate] in crambe are enantiomorphs of the corresponding products from progoitrin [(*R*)-2-hydroxy-3-butenylglucosinolate] in rapeseed (28).

cruciferous oilseed species. In early analyses for progoitrins, involving conversion of them to goitrins and measurement of the latter by UV absorption, the hydrolysis of the glucosinolates was carried out with endogenous enzymes and at relatively low or uncontrolled pH. Under these conditions analytical results were often low because the progoitrins were not exclusively and quantitatively hydrolyzed to goitrin. By heating the seed or meal before analysis and carrying out the hydrolysis at pH 7 with added thioglucosidase, higher and more accurate results have been obtained (31). Two methods of microanalysis based on this discovery are now extensively used in genetic research on rapeseed (32,33).

Seed of the two rapeseed species normally contain both progoitrin and glucosinolate precursors of nonhydroxylated alkyl isothiocyanates. In the analytical procedure of

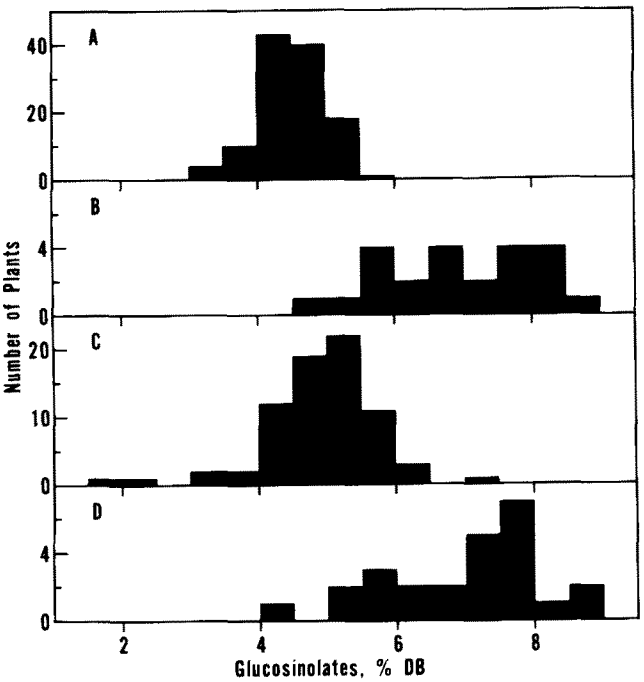

FIG. 4. Variation in glucosinolate content of defatted meal from seed produced by individual plants (34). Species, and number of individual plants, analyzed were: A, *C. abyssinica*–116; B, *B. campestris*–23; C, *B. carinata*–74; D, *B. napus*–24. *B. campestris* and *B. napus* plants were from lines previously selected for high-erucic acid content of their seed oils. Crambe seeds were not dehulled before analysis, so data represented in A are actually for defatted seed meal plus pericarp, the latter generally amounting to about 45% of the two by weight and containing negligible glucosinolate. The analysis of crambe samples was for *epi*-progoitrin only since this compound makes up approximately 95% of the total glucosinolates of this species.

Youngs and Wetter (32) the isothiocyanates are extracted into a methylene chloride layer as formed enzymatically. Nonhydroxylated alkyl isothiocyanates are determined by GLC of an aliquot of this methylene chloride solution. Then after addition of alcohol the 5-vinyloxazolidine-2-thione formed from progoitrin is determined by UV spectrophotometry.

Instead of the methylene chloride layer, ethanol is added in the procedure of Appelqvist and Josefsson (33) to increase the solubility of the enzymatic reaction products. After the reaction nonhydroxylated alkyl isothiocyanates are selectively extracted from the reaction mixture with isooctane and converted to alkyl thioureas by addition of ammonia. These thioureas are measured by their UV absorption. The 5-vinyloxazolidine-2-thione in the residual aqueous alcohol solution is also determined by UV spectrophotometry. Recommended for samples of about 10 mg of seed meal, this procedure is not quite sensitive enough for analysis of a single seed, but it has been used very effectively for seed from individual plants (34).

Daxenbichler et al. (35) have developed a procedure for simultaneously determining all of the progoitrin or *epi*-progoitrin hydrolysis products shown in Figure 3 by GLC. This procedure is very useful in studies of possible process modifications to eliminate or minimize the occurrence of these hydrolysis products in cruciferous meals.

Improvement by Process Modifications

Destruction of endogenous thioglucosidase activity renders crambe or rapeseed meal with a given glucosinolate content far less harmful to experimental animals than corresponding meals in which the active enzyme is present (5,25). Consequently cooking at carefully controlled moisture level and temperature to denature thioglucosidase is routinely practiced in Canada as the first step after crushing in processing rapeseed (5,36). Byproduct meal from this process is fed to cattle and sheep with impunity (37). However very high levels of it in the diets of rats and pigs resulted in growth depression and effects on reproduction (5), and rats fed crambe meal containing *epi*-progoitrin but no active thioglucosidase did not grow as well as the controls fed a standard laboratory ration (25). Mustakas et al. (38), have employed a sodium carbonate treatment to improve the quality of crambe meal. More recently Kirk et al. (39) have evaluated other metallic salts, particularly of iron and copper, as catalysts in a similar treatment.

Perhaps the explanation for the adverse effect of glucosinolates in meals without active thioglucosidases is slow hydrolysis of these compounds by intestinal microorganisms (40). In any event it is obvious that removal of the glucosinolates is necessary to achieve optimum meal quality. Procedures designed to achieve this removal by aqueous extraction are under study in at least two laboratories—one in Canada and one in the U.S. In the two such procedures that have been reported, the first step is a brief treatment of the seed with boiling or near-boiling water to denature the thioglucosidase before exploiting the high water solubility of unhydrolyzed glucosinolates. A light crushing precedes the steeping to take out these compounds in the Canadian process (41). At NRRL the surprising discovery was made that glucosinolates can be extracted from intact seed (mustard or dehulled crambe) with water (42). The diffusion of these compounds into the water is slower from intact than from crushed seed. However the NRRL approach is favored by ease of handling, rapid drainage of the steep water from the seed, and negligible loss of oil into the steep water.

Studies expressly or obviously aimed at making protein isolates from rapeseed have been reported from Chile (43) and Poland (44).

A final process that should be mentioned is the removal from Oriental mustard (*Brassica juncea*) meal of allylglucosinolate by enzymatically converting it to the volatile allyl isothiocyanate and steam stripping (45). This method should be applicable for a high-erucic oilseed in which only glucosinolates yielding volatile nonhydroxylated alkyl isothiocyanates are present.

Prospects for Improvement by Genetic Methods

Josefsson and Appelqvist (46) reported in 1968 that seed of the Polish *B. napus* variety Bronowski are very low in glucosinolate content. Downey et al. (6) reported the same observation concerning Bronowski as well as the isolation of individual *B. campestris* plants with low-glucosinolate seed. The latter discovery gains in importance because of the predominance of *B. campestris* in Canadian agriculture. Unfortunately this species is naturally cross-pollinated and self-incompatible, making breeding work with it very tedious. A 1970 estimate was that at least 5 more years would be required to develop a practical low-glucosinolate variety of *B. campestris* (26), and probably even more time will be needed to combine the low-glucosinolate and zero-erucic characteristics. Concurrent intensive research on process modifications to solve the glucosinolate problem is therefore quite justified.

Josefsson and Jönsson (34) have analyzed seed from individual plants of several high-erucic oilseed species for glucosinolate content. Selected results from their report are shown in Figure 4. As in Figure 1A for the erucic acid content of crambe oil, comparatively little scatter is seen in Figure 4A for the glucosinolate content of the seed. A high degree of chromosomal polyploidy is probably responsible for this low level of observable variability (7). The relatively consistent high level of erucic acid in the oil is of course a desirable consequence, but carryover of similar constancy to the glucosinolate limits prospects for genetic removal of this undesirable constituent from the byproduct meal. Some improvement in other properties of crambe has been achieved by plant breeders. The new variety Indy has a shortened growing season, an increased test weight and promise of significantly boosted yields per acre (K.J. Lessman, unpublished).

Somewhat more scatter than in Figure 4A is apparent in Figures 4B and 4D, reflecting the variability also evidenced in the discovery of low-glucosinolate plants of *B. campestris* and the low-glucosinolate variety of *B. napus* (6,46). If the high erucic acid content of some of the Indian yellow sarson seed represented in Figure 1B could be combined with the low-glucosinolate property available in *B. campestris* and with adaptability to agricultural production in the U.S., the result would be a high-erucic oilseed with excellent prospects for commercialization. Some further work of Josefsson and Jönsson (34) with *B. napus* is relevant in this connection. Seed oil of the low-glucosinolate variety Bronowski contains only about 10% of erucic acid. They crossed this variety with one producing 48% of the acid in its oil. From analysis of seed from 373 F_2 plants, glucosinolate and erucic acid appeared to vary independently. The low-glucosinolate property seems to be governed by recessive polymeric factors and is determined by the genotype of the mother plant, while "high-erucicness" is determined by one (*B. campestris*) or two (*B. napus*) genes of the embryo (6,34).

Joseffson and Jönsson (34) found *B. carinata* plants to be somewhat more homogeneous in glucosinolate production than *B. campestris* and *B. napus*, but the two outliers at the low end of Figure 4C are encouraging. Moreover, developing a low-glucosinolate variety from this species might not be necessary. Only allylglucosinolate is present in the seed (27), so the process developed for mustard seed involving steam strippings (45) should be applicable. Some reservation is introduced by the possibility of reaction between the allyl isothiocyanate released during the process and meal protein with consequent adverse effect on

nutritional quality.

As a high-erucic oilseed crop for the U.S., *B. carinata* would probably complement crambe in geographic areas of adaptation. High-erucic, low-glucosinolate varieties of *B. campestris* or *B. napus* that might be developed could be similarly complementary, or they could prove to be competitive with the currently best high-erucic oilseed crop for the U.S. In any event the market opportunities for a domestic high-erucic oilseed crop (1) and the economic advantage to be gained by solving the glucosinolate problem are great enough to justify the dual research approaches currently being pursued. These are chemical investigations applied to crambe meal and genetic efforts to develop a suitable high-erucic *Brassica* for the U.S. Work is in progress on the former approach at NRRL and on the latter through a cooperative program involving NRRL, the Plant Science Research Division of the USDA and the Oregon Agricultural Experiment Station.

REFERENCES

1. Nieschlag, H.J., and I.A. Wolff, JAOCS, 48:723 (1971).
2. Vles, R.O., and A.M.M. Abdellatif, Program of the AOCS-ISF World Congress, Chicago, September 1970, Abstr. 210.
3. McAnsh, J., Rapeseed Digest (Rapeseed Association of Canada) 4(10):1 (1970).
4. Bolton, J.L., and C.H. Jefferson, Seed Scoop (Canadian Seed Growers' Association) 17(2):1,5,7 (1970).
5. Youngs, G.C., Oleagineux 20:679 (1965).
6. Downey, R.K., B.M. Craig and C.G. Youngs, JAOCS 46:121 (1969).
7. Downey, R.K., Ibid., in press.
8. U.S. Department of Agriculture, World Agricultural Production and Trade Statistical Report, January 1971, p. 2-3.
9. McAnsh, J., Rapeseed Digest (Rapeseed Association of Canada) 5(2):4 (1971).
10. Downey, R.K., S.H. Pawlowski and J. McAnsh, "Rapeseed, Canada's 'Cinderella Crop,'" Rapeseed Association of Canada, 1970, p. 2.
11. McAnsh, J., Rapeseed Digest (Rapeseed Association of Canada) 4(11):3 (1970); 5(3):1 (1971).
12. Downey, R.K., Ibid. 5(2):2 (1971).
13. Downey, R.K., and B.L. Harvey, Can. J. Plant Sci. 43:271 (1963).
14. Appelqvist, L.-Å., Ark. Kemi 28:551 (1968).
15. Yermanos, D.M., Calif. Agr. 22(12):15 (1968).
16. Appelqvist, L.-Å., Hereditas 61:9 (1969).
17. U.S. Department of Agriculture, Agricultural Research, January 1971, p. 13.
18. Mattson, F.H., and R.A. Volpenhein, J. Biol. Chem. 236:1891 (1961).
19. Grynberg, H., and H. Szczepanska, JAOCS 43:151 (1966).
20. Appelqvist, L.-Å., and R.J. Dowdell, Ark. Kemi 28:539 (1968).
21. Phillips, B.E., C.R. Smith, Jr. and W.H. Tallent, Lipids 6:93 (1971).
22. Kjaer, A., in "Comparative Phytochemistry," Edited by T. Swain, Academic Press, New York, 1966, p. 187.
23. Earle, F.R., J.E. Peters, I.A. Wolff and G.A. White, JAOCS 43:330 (1966).
24. Miller, R.W., C.H. VanEtten, C. McGrew, I.A. Wolff and Q. Jones, J. Agr. Food Chem. 10:426 (1962).
25. VanEtten, C.H., W.E. Gagne, D.J. Robbins, A.N. Booth, M.E. Daxenbichler and I.A. Wolff, Cereal Chem. 46:145 (1969).
26. Kondra, Z.P., Agriculture Bulletin (Alberta University Faculty of Agriculture) 11:13 (1970).
27. Van Etten, C.H., M.E. Daxenbichler and I.A. Wolff, J. Agr. Food Chem. 17:483 (1969).
28. Daxenbichler, M.E., C.H. VanEtten, W.H. Tallent and I.A. Wolff, Can. J. Chem. 45:1971 (1967).
29. Greer, M.A., Recent Prog. Horm. Res. 18:187 (1962).
30. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters and H.L. Tookey, J. Agr. Food. Chem. 14:426 (1966).
31. VanEtten, C.H., M.E. Daxenbichler, J.E. Peters, I.A. Wolff and A.N. Booth, Ibid. 13:24 (1965).
32. Youngs, C.G., and L.R. Wetter, JAOCS 44:551 (1967).
33. Appelqvist, L.-Å., and E. Josefsson, J. Sci. Food Agr. 18:510 (1967).
34. Josefsson, E., and R. Jönsson, Z. Pflanzenzuecht. 62:272 (1969).
35. Daxenbichler, M.E., G.F. Spencer, R. Kleiman, C.H. VanEtten and I.A. Wolff, Anal. Biochem. 38:373 (1970).
36. Youngs, C.G., in "Rapeseed Meal for Livestock and Poultry--A Review," Canada Department of Agriculture, Ottawa, 1965, p. 24.
37. Robblee, A.R., Ibid., p. 93.
38. Mustakas, G.C., L.D. Kirk, E.L. Griffin, Jr. and D.C. Clanton, JAOCS 45:53 (1968).
39. Kirk, L.D., G.C. Mustakas, A.N. Booth and E.L. Griffin, Jr., JAOCS, in press.
40. VanEtten, C.H., and I.A. Wolff, "Natural Sulfur Compounds," in *Toxicants Occurring Naturally in Foods,* National Academy of Sciences, National Research Council, in press.
41. Eapen, K.E., N.W. Tape and R.P.A. Sims, JAOCS 46:52 (1969).
42. VanEtten, C.H., M.E. Daxenbichler and I.A. Wolff, Abstracts of Papers, 158th National Meeting of the American Chemical Society, September 1969, Abstr. AGFD 53.
43. Owen, D.F., C.O. Chichester and A.N. Prater, Program of 55th Annual Meeting, American Association of Cereal Chemists, October 1970, Abstr. 92.
44. Korolczuk, J., and A. Rutkowski, JAOCS 48:398 (1971).
45. Mustakas, G.C., L.D. Kirk, V.E. Sohns and E.L. Griffin, Jr., JAOCS 42:33 (1965).
46. Josefsson, E., and L.-Å. Appelqvist, J. Sci. Food Agr. 19:564 (1968).

[Received May 5, 1971]