Lipid Changes in Maturing Oil-Bearing Plants. IV. Changes in Lipid Classes in Rape and Crambe Oils[1]

MARY E. McKILLICAN, Food Research Institute, Department of Agriculture, Ottawa, Canada

Abstract

Seeds of *Crambe abyssinica* C.D. 6619 and the *Brassica napus* varieties Golden and Zero-erucic were collected at different stages of maturity and the free lipid extracted with hexane. The lipid thus obtained was separated into lipid classes by silicic acid column chromatography. The lipid classes were further examined by thin-layer chromatography and the component fatty acids and sterols by gas-liquid chromatography.

The relative amounts of the lipid classes in crambe and both rape varieties varied as the seed matured and a period of great change occurred about 10 days after fertilization. The greatest change was in triglycerides and phospholipids plus glycolipids. Free fatty acids, present in immature seeds, has almost disappeared at maturity. The lipid classes of crambe and both types of rape were in similar proportion at maturity. Differences in phospholipid and glycolipid composition were found between crambe and rape and between immature and mature rape. The fatty acid composition differed between lipid classes and changed with maturity. Changes in 18-carbon acids of Zero-erucic rape were concurrent with the development of erucic and eicosenoic acids in Golden rape.

Introduction

MANY WORKERS HAVE STUDIED the lipid changes in maturing oil-bearing plants such as soybean (20), mustard (10) and castor bean (2,3). In recent years, there has been an increase in interest in rapeseed oil (4,6,8) and in similar oil from the more recently introduced oil-bearing *Crambe abyssinica* (7, 17). The lipids of developing flax and safflower have been studied in this laboratory (15,23,24), and more recently rape and crambe (21) have been included as part of our investigation of lipid development in oil seeds. The object of the present work is to study the changes within and between lipid classes in the developing seed of crambe and of Golden and Zero-erucic (25,26) rape.

Experimental

Collection and Extraction

Crambe abyssinica C.D. 6619 was grown in the summer of 1963 and the *Brassica napus* varieties Golden and Zero-erucic in 1964 by the Genetics and Plant Breeding Research Institute in Ottawa (now Ottawa Research Station). The seed was harvested at 10, 20, 30 DAF (days after fertilization) and at maturity, as previously described (23), and freeze-dried immediately. The free lipid was extracted from the freeze-dried material first with 20 volumes, then twice with 10 volumes of cold deoxygenated hexane under nitrogen (15) and stored at −15C under nitrogen in the dark.

Silicic Acid Column Chromatography

The free lipid was separated into classes on a 1.8 cm I.D. column containing 20 g of 325 mesh silicic acid (Bio Rad Laboratories, 32nd and Griffin, Richmond, California). The silicic acid was activated by cycling with methanol, diethyl ether and hexane as described by Hirsch and Ahrens (9). The lipid classes were eluted by a multi-step increase of diethyl ether in hexane, from 0% to 60% ether in hexane, corresponding to the gradient used by Hirsch and Ahrens (9). The multi-step elution consisted of 24 fractions of 20 ml each and was followed by 120 ml of methanol to elute the phospholipids and glycolipids. The fractions were concentrated to 2 ml at below 35C under a stream of nitrogen; column separation was followed by TLC of 10 μl aliquots. Fractions were taken to dryness under nitrogen, weighed and those corresponding to each lipid class were pooled for GLC. Deoxygenated solvents were used throughout the procedure. A column load of 100 mg was used in 1963 for crambe but, under these conditions, free fatty acids were not completely separated from triglycerides. The load was therefore reduced to 50 mg for the rapeseed samples.

Thin-Layer Chromatography

Separations were made on chromatoplates with a 250 μ layer of silica gel G (Research Specialties), using the solvent systems a) hexane-diethyl ether-acetic acid, 90:10:1 (13) and b) diisobutyl ketone-acetic acid-water, 40:25:5 (14). Solvent system "a" was used for the separation of nonpolar lipid classes, while "b" was used to separate phospholipids and glycolipids. System "a" was also used to monitor elution from the column and to purify methyl esters for GLC.

Iodine vapor (22) was used as a general detecting agent. Different types of lipids reacted with characteristic color when sprayed with 20% aq perchloric acid (11) and specific spray reagents were also used: Dragendorff reagent (1) to detect choline containing lipids; molybdate (5) to detect phospholipids, and ninhydrin (RSCo spray reagent) for the amino group.

Gas Liquid Chromatography

The methyl esters were prepared by heating with hydrogen chloride in absolute methanol (16), purified by TLC (18) and analyzed on a polyvinyl acetate column in a Research Specialties Series 600 gas chromatograph operating with a flame ionization detector. The sterols were converted to the trimethylsilyl ethers (19) prior to separation on a SE 30 column. Fatty acid methyl esters and sterols were identified by comparison with pure known standards and these standard compounds were also used to calibrate the gas chromatograph.

Results

In crambe and both types of rape, there was little change in the oil content of freeze-dried seeds after 20 DAF (Table I). The slightly lower weight and oil content of mature crambe relative to the 30 DAF sample appeared to be due to a higher content of poor,

[1] Contribution No. 36 of the Food Research Institute. Presented at the AOCS Meeting, Cincinnati, October 1965.

TABLE I

Lipid Content and Composition
Lipid Classes (% of Recovery)

Seed	Wt 100 seeds mg	Oil %	Hydrocarbon	Sterol ester	Triglyceride	Free fatty acid	Free sterols	Partial glyceride	Phospho- + glycolipid
Crambe									
7 DAF[a]	210.0	2.8	0.8	3.4	34.3	7.2	13.1	4.0	37.2
10 DAF	356.4	11.8	0.3	1.1	84.8	2.3	2.8	5.1	3.6
20 DAF	600.0	32.5	0.6	1.7	80.4	3.2	2.7	5.9	5.5
30 DAF	728.0	33.2	0.4	1.5	91.9	1.5	1.1	2.6	1.0
Mature	654.0	32.2	0.2	1.3	94.9	0.2	1.1	1.2	1.1
Zero-erucic rape									
10 DAF	71.6	1.4	2.9	4.0	23.1	15.8	9.7	12.3	32.2
20 DAF	201.0	27.7	1.1	1.6	87.0	1.1	1.7	3.7	3.8
30 DAF	375.8	37.9	0.6	0.9	89.8	0.8	0.7	4.3	2.9
Mature	430.0[b]	35.9	0.1	1.0	94.3	0.1	1.1	1.7	1.8
Golden rape									
10 DAF	83.0[b]	1.6	1.1	3.6	44.5	16.6	7.1	8.0	19.0
20 DAF	220.0	28.8	1.2	0.8	82.2	1.0	1.0	3.3	10.5
30 DAF	401.6	36.0	0.5	0.2	92.5	1.0	1.4	2.5	1.9
Mature	453.6	34.8	0.2	3.4	90.3	0.0	0.5	2.6	3.0

[a] 7 DAF crambe was grown in 1962.
[b] Weight of 100 seeds of mature Zero-erucic and of 10 DAF Golden is an estimate based on established graphs of oil % and seed weight for both varieties.

light seeds in the mature sample, which was harvested in bulk, than in the individually harvested 30 DAF sample.

Lipid Class Separation

The recovery from the silicic acid column was greater than 95% except for the 10 DAF Zero-erucic rapeseed. In the investigation of crambe in 1963, the resolution of triglycerides and free fatty acids was not complete. The load was thus reduced from 100 mg to 50 mg in 1964 and, in the rape samples, these two lipid classes were completely separated. The triglycerides and free fatty acids in crambe and the free sterols and partial glycerides in all varieties were resolved by TLC. The distribution of lipid classes (Table I) showed a marked change as the seeds matured. Triglyceride was by far the main component by 20 DAF and, at maturity, comprised over 90% of the oil. In very immature seed, however, there was nearly the same amount of phospholipid plus glycolipid as triglyceride. Also, there was a high content of free fatty acid in immature seed, but this disappeared almost completely by maturity, while the phospholipid plus glycolipid decreased to between 1 and 3%.

The period at approximately 10 DAF appeared to be one of great change. For example, the rape grown in 1964 was very immature at 10 DAF whereas the 1963 crambe had already passed gross immaturity by 10 DAF. Therefore, a separation of classes was made, in 1965, of the 7 DAF crambe grown in 1962. Although the lipid had been stored for 3 years, there was no obvious change in fatty acid composition, thus this sample should give a reasonable picture of the lipid class distribution in immature crambe seed. The lipid from this immature seed had, like the 10 DAF rape, a high content of phospholipid plus glycolipid, and a higher free fatty acid and sterol content than the more mature crambe.

On a weight per seed basis (Table II), the minor lipid classes in crambe increased to a maximum at about 20 DAF, in the case of sterol esters, 30 DAF, then decreased at greater maturity. Most minor classes of both rape varieties reached a maximum at 30 DAF. Sterol esters, however, increased until maturity and phospholipids plus glycolipids in Golden rape showed no definite trend.

Separations of Phospholipids and Glycolipids

In crambe, the phospholipids plus glycolipid fraction was characterized by its simplicity. The main components were phosphatidyl ethanolamine, phosphatidyl choline, sterol glycoside, di-galactosyl glyceride and mono-galactosyl glyceride. Other phospholipids and glycolipids were present, if at all, in quantities too small to be detected. Little change in the phospholipids and glycolipids of crambe was observed with increasing maturity.

In both rape varieties, a more complex pattern was found (Fig. 1.). Esterified sterol glycoside (12) was

TABLE II

Lipid Content and Composition
Milligrams per 100 Seeds

Seed	Weight	Oil	Hydrocarbon	Sterol ester	Triglyceride	Free fatty acid	Free sterols	Partial glyceride	Phospho- + glycolipid
Crambe									
7 DAF[a]	210.0	5.9	0.1	0.2	2.0	0.4	0.8	0.2	2.2
10 DAF	356.4	42.1	0.1	0.5	35.7	1.0	1.2	2.2	1.5
20 DAF	600.0	195.0	1.2	2.3	156.8	6.2	5.5	11.5	10.7
30 DAF	728.0	241.7	1.2	3.6	222.1	3.6	2.7	6.3	2.4
Mature	654.0	210.6	0.4	2.7	199.9	0.4	2.3	2.5	2.3
Zero-erucic rape									
10 DAF	71.6	1.0	0.03	0.04	0.23	0.16	0.10	0.12	0.32
20 DAF	201.0	55.7	0.6	0.9	48.4	0.6	1.0	2.1	2.1
30 DAF	375.8	142.4	0.9	1.3	127.9	1.1	1.0	6.1	4.1
Mature[b]	430.0[b]	154.0[b]	0.2[b]	1.5[b]	145.2[b]	0.2[b]	1.7[b]	2.6[b]	2.8[b]
Golden rape									
10 DAF[b]	83.0[b]	1.3[b]	0.01[b]	0.05[b]	0.59[b]	0.22[b]	0.09[b]	0.10[b]	0.25[b]
20 DAF	220.0	63.4	0.8	0.5	52.1	0.6	0.6	2.1	6.7
30 DAF	401.6	144.6	0.7	0.3	133.7	1.5	2.0	3.6	2.8
Mature	453.6	157.9	0.3	5.4	142.5	0.0	0.8	4.1	4.7

[a] 7 DAF crambe was grown in 1962.
[b] Based on estimated seed weight as in Table I.

a main component at 10 DAF, but was minor at maturity. Phosphatidyl ethanolamine and mono- and digalactosyl glyceride were main components at both 10 DAF and maturity. Phosphatidyl choline, relatively minor at 10 DAF, increased and at maturity was one of the four major components. Sterol glycoside, like esterified sterol glycoside, decreased from a major to a minor component as the seed matured. Small amounts of phosphatidyl inositol and a phospholipid with some characteristics of phosphatidic acid and traces of lyso phosphatidyl ethanolamine and an unknown phospholipid were also present. By the use of iodine vapor, all samples were shown to contain 2 large, but very faint, low R_f spots. These were not made visible by other detecting agents, however.

Sterol Composition

The sterol composition of both sterol ester and free sterol classes of crambe changed little with increasing maturity and beta sitosterol was the main component amounting to over 60% of the crambe sterols. As the seed matured, free stigmasterol decreased slightly and an earlier-appearing, unnkown sterol (U1) increased slightly. In the sterol esters, campesterol decreased and U1 increased slightly with increasing maturity.

Both rape varieties showed more change with increasing maturity than crambe. Beta sitosterol, the major component, decreased from 76–80% at 10 DAF to 55–58% at maturity. Stigmasterol, which was present in 10 DAF seed, disappeared before 30 DAF. Campesterol and U1 increased with maturity in both sterol esters and free sterols of both rape varieties.

Fatty Acid Composition of Lipid Classes

Triglycerides were the main component of the oils after 10 DAF; thus, the fatty acid composition of triglyceride (Table III) was the determining factor in the fatty acid composition of all but the most immature oil. The main fatty acids in crambe were oleic, linoleic and erucic, with a decrease in oleic corresponding to the increase in erucic with maturity. The changes in Golden rape were similar to those of crambe although the proportions were different. The absence of erucic acid from Zero-erucic rape resulted in higher percentages of other acids and this variety was characterized by an increase in oleic paralleling the increase in erucic and eicosenoic acids in Golden rape and crambe.

On a weight per seed basis, the principal fatty acids of the triglycerides, oleic, linoleic, linolenic and erucic

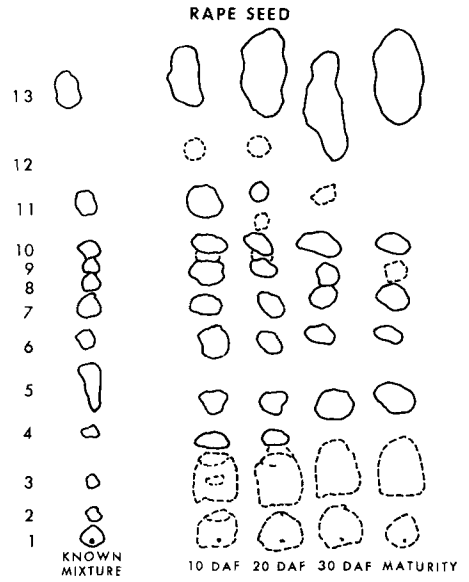

FIG. 1. Tracing of thin-layer chromatogram of rapeseed lipids. 1. Unresolved mixture. 2. Lyso phosphatidyl choline. 3. Lyso phosphatidyl ethanolamine. 4. Phosphatidyl inositol. 5. Phosphatidyl choline. 6. Digalactosyl glyceride. 7. Phosphatidyl ethanolamine. 8. Sterol glycoside. 9. Unknown phospholipid. 10. Monogalactosyl glyceride. 11. Esterified sterol glycoside. 12. Phospholipid, possibly phosphatidic acid. 13. Nonpolar lipids.

increased steadily to maturity in crambe. In Golden rape, however, erucic acid increased until maturity whereas the 18-carbon acids increased until 30 DAF then remained approximately constant. Oleic, linoleic and linolenic acids all increased steadily to maturity in Zero-erucic rape.

The minor lipid classes differed from triglycerides in fatty acid composition and, in general, were more saturated (Figs. 2–4). The partial glycerides and sterol esters of crambe and Golden rape contained less erucic than the triglycerides. The phospholipids and glycolipids in Golden rape were also low in erucic. All other lipids classes of crambe and Golden rape were higher in palmitic than the triglycerides, and there was a slight trend in this direction in the Zero-erucic rape. The free fatty acids showed the greatest similarity to the triglycerides. In all varieties, both partial glycerides and free fatty acids contained a higher percentage of palmitic and stearic acids than triglycerides and both these minor classes had the same or lower percentages of unsaturated fatty acids relative to triglycerides. In both crambe and Golden rape, the phospholipids plus glycolipids were char-

TABLE III

Fatty Acid Composition
Triglycerides

Seed	Weight %										
	14:0	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:1
Crambe											
10 DAF	0	0	5.3	1.2	2.4	33.9	13.3	7.3	1.1	11.2	24.3
20 DAF	0	0	2.7	0.3	1.0	16.2	7.3	5.1	0.3	3.4	61.6
30 DAF	0	0	3.4	0.6	1.3	20.6	9.3	6.1	0.8	3.0	55.0
Mature	0	0	2.8	0.5	0.8	16.3	11.0	8.7	0.6	3.5	54.9
Zero-erucic rape											
10 DAF	0.8	0.4	13.2	0.8	6.4	9.3	51.6	16.8	0.4	0	0
20 DAF	0	0	7.5	0.9	4.3	61.1	19.5	3.6	1.1	1.1	0
30 DAF	0	0	5.7	0.4	3.3	56.2	23.5	8.8	0.7	1.3	0
Mature	0	0	4.3	0.3	2.2	58.3	22.6	10.9	0.6	0.9	0
Golden rape											
10 DAF	0.4	0	8.5	1.3	5.1	31.5	29.3	9.2	1.0	6.8	6.7
20 DAF	0	0	5.1	0.5	2.5	25.7	17.8	7.8	1.1	13.1	25.5
30 DAF	0.3	0	4.2	0.5	1.2	19.3	16.2	10.4	0	11.2	36.7
Mature	0	0	3.4	0	1.4	14.6	14.7	10.3	0.6	10.2	44.8

Plus traces in some samples of 15:1, 17:0 and 22:0.

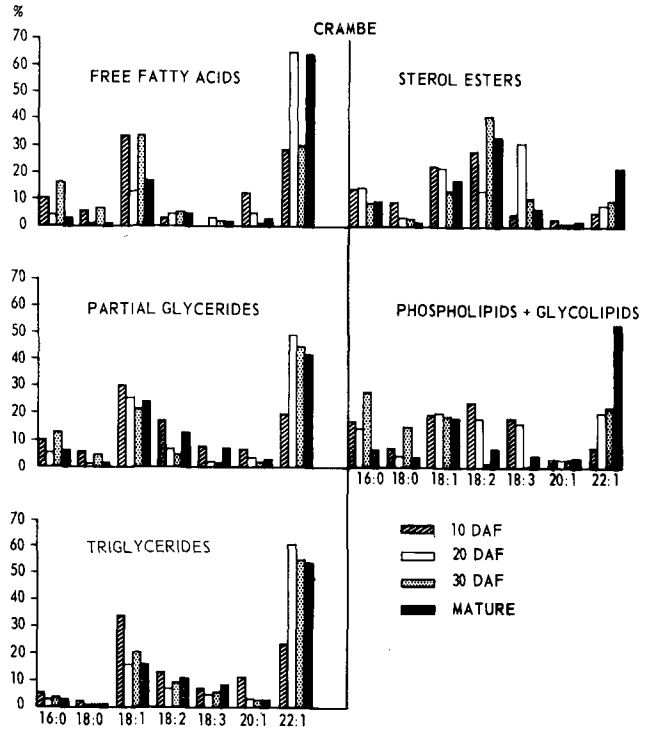

FIG. 2. Fatty acid composition of lipid classes of crambe 10 DAF to maturity.

acterized by greater change between 10 DAF and maturity than were the other lipid classes. Palmitic, stearic and linolenic acids were higher at 10 DAF, oleic and erucic lower, while at maturity all were similar to triglycerides in crambe, and all but erucic and oleic in Golden rape. The phospholipids and glycolipids of Zero-erucic showed less change with maturity. On a weight per seed basis, the phospholipids plus glycolipids reached a maximum at 20 DAF in crambe, 30 DAF in rape. This maximum occurred in the absolute amounts of fatty acids as well, and all fatty acids reached a maximum at 20 DAF in crambe, then decreased. A definite maximum occurred at 30 DAF in Zero-erucic rape followed by a decrease whereas there was a less definite maximum and greater variability in Golden rape. The absolute amounts of the fatty acids in the other minor classes also followed the development of the class and reached a maximum at 20 or 30 DAF. All minor fatty acid-containing lipid classes of Zero-erucic rape contained small amounts of erucic acid at maturity. These were not large enough to be detected in the whole oil.

Discussion

Crambe and both varieties of rape showed great similarity in the development of lipid classes. A period of great change was observed at about 10 DAF. The rape samples (1964) showed this immaturity at 10 DAF but the 10 DAF crambe (1963) had already passed this stage. However, 7 DAF crambe (1962) showed the same characteristics of immature seed observed in the 10 DAF rape. Most of the minor lipid classes in both crambe and rape increased in absolute quantity in the seed then decreased. It appears that these classes take part in the earlier stages of development.

Within the lipid classes differences in development were noted. A more complex pattern of phospholipids and glycolipids was found in rape than in crambe and there was more change with increasing maturity, especially in the esterified sterol glycoside. Also in rape, the changes with maturity in sterol-containing glycolipids paralleled those of the free sterols. The sterols, both free and esterified, were again more complex in rape than in crambe and showed greater change with maturity. This latter difference may well be due, at least in part, to the greater maturity of the 10 DAF crambe seed.

The development of erucic acid in Golden rape and crambe and the corresponding changes in Zero-erucic rape are of major interest in this type of seed. The

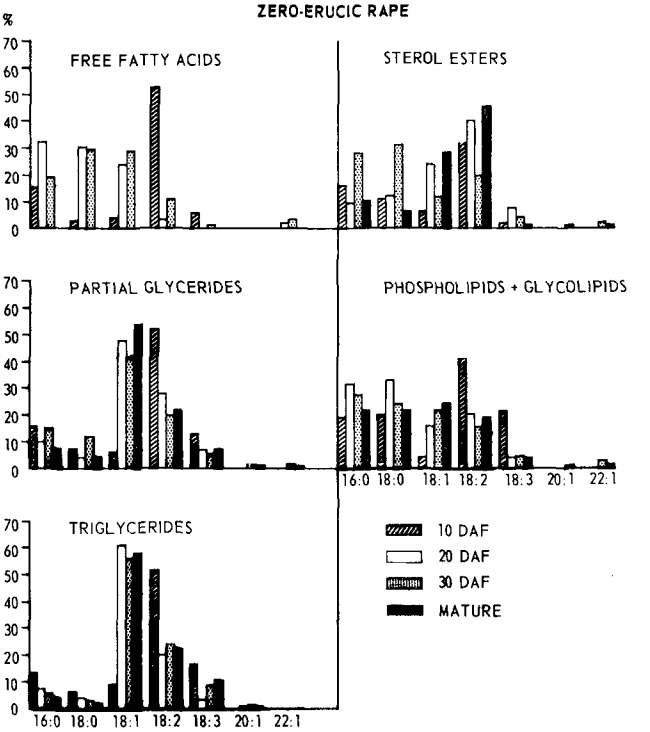

FIG. 3. Fatty acid composition of lipid classes of Zero-erucic rape 10 DAF to maturity.

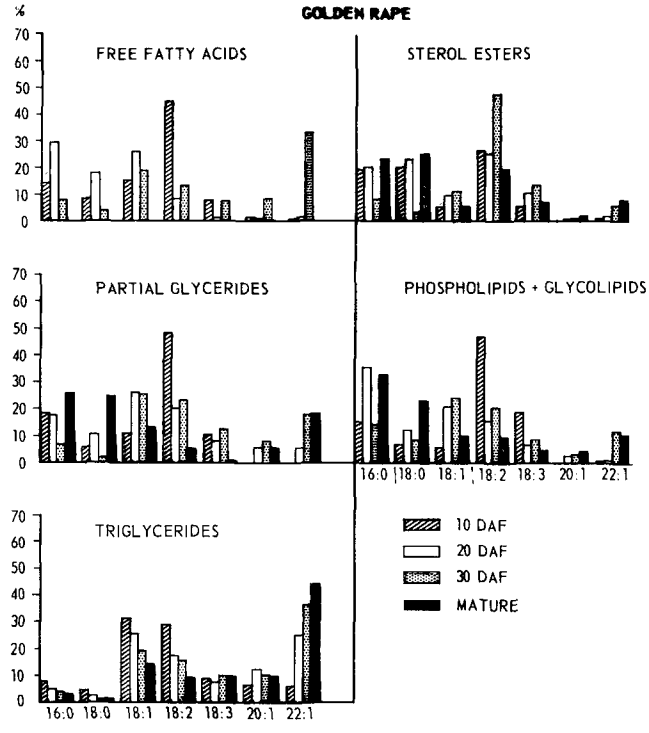

FIG. 4. Fatty acid composition of lipid classes of Golden rape 10 DAF to maturity.

erucic acid content was higher in the triglycerides and more evenly distributed in all lipid classes in crambe than in Golden rape. In these seeds, the erucic acid appeared to increase at the expense of the oleic acid. Moreover, the changes in the 18-carbon acids of Zero-erucic rape were concurrent with the development of erucic and eicosenoic acids in Golden rape. The partial glycerides and free fatty acids showed similar trends in fatty acid composition relative to the triglycerides.

Thus it is shown that crambe and Golden and Zero-erucic rape exhibit great similarity in the development of lipid classes, but also show characteristic differences within these classes.

REFERENCES

1. Bregoff, H. M., E. Roberts and C. C. Delwiche, J. Biol. Chem. *205*, 565 (1953).
2. Canvin, D. T., Can. J. Biochem. Physiol. *41*, 1879–1885. (1963).
3. Chandra, K. S. JAOCS *41*, 251–254 (1964).
4. Craig, B. M. Can. J. Plant Sci. *41*, 204–210 (1961).
5. Dittmer, J. C., and R. L. Lester, J. Lipid Res. *5*, 126–127 (1964).
6. Downey, R. K., and B. M. Craig, JAOCS *41*, 475–478 (1964).
7. Earle, F. R., J. E. Peters, I. A. Wolff and G. A. White, JAOCS, in press.
8. Harvey, B. L., and R. K. Downey, Can. J. Plant Sci. *44*, 104–111 (1964).
9. Hirsch, J., and E. H. Ahrens, J. Biol. Chem. *233*, 311–320 (1958).
10. Kartha, A. R. S. and R. Narayanan, J. Sci. Ind. Res. *18B*, 41–48 (1959).
11. Lepage, M., J. Chromatog. *13*, 99–103 (1964).
12. Lepage, M., J. Lipid Res. *5*, 587–592 (1964).
13. Mangold, H. K., and D. C. Malins, JAOCS *37*, 383–385 (1960).
14. Marinetti, G. V., J. Erbland and J. Kochen, Fed. Proc. *16*, 837–844 (1957).
15. McKillican, M. E., and R. P. A. Sims, JAOCS *40*, 108–113 (1963).
16. McKillican, M. E., and R. P. A. Sims, JAOCS *41*, 340–344 (1964).
17. Miwa, T. K., and I. A. Wolff, JAOCS *40*, 742–744 (1963).
18. Negishi, T., M. E. McKillican and M. Lepage, J. Lipid Res. *5*, 486 (1964).
19. Res. Spec. Co. Chromatofacts. Sept.-Oct. 1962.
20. Simmons, R. O., and F. W. Quackenbush, JAOCS *31*, 601–603 (1954).
21. Sims, R. P. A., Can. J. Plant Sci. *44*, 217–218 (1964).
22. Sims, R. P. A., and J. A. G. Larose, JAOCS *39*, 232 (1962).
23. Sims, R. P. A., W. G. McGregor, A. G. Plessers and J. C. Mes, JAOCS *38*, 273–276 (1961).
24. Sims, R. P. A., W. G. McGregor, A. C. Plessers and J. C. Mes, JAOCS *38*, 276–279 (1961).
25. Stefansson, B. R., F. W. Hougan and R. K. Downey, Can. J. Plant Sci. *41*, 218–219 (1961).
26. Stefansson, B. R. and F. W. Hougan, Can. J. Plant Sci. *44*, 359–364 (1964).

[Received November 17, 1965]