Lipids of Maturing Grain of Corn (*Zea mays* L.): II. Changes in Polar Lipids[1]

EVELYN J. WEBER, Crops Research Division, ARS, USDA, University of Illinois, Urbana, Illinois 61801

Abstract

The polar lipids of a standard corn inbred, H51, were analyzed as the kernels developed. The concentrations of the glycolipids and phospholipids were highest at 30–45 days after pollination and then decreased. Digalactosyl diglyceride was the dominant glycolipid in the maturing grain. Monogalactosyl diglyceride and sulfolipid were also major sugar-containing lipids, but steryl glycoside ester, steryl glycoside and cerebrosides were relatively minor components. Phosphatidyl choline accounted for over 50% of the total phosphorus of the phospholipids at all stages of kernel development. Phosphatidyl ethanolamine and phosphatidyl inositol ranked second and third. Each individual lipid had its own characteristic fatty acid pattern, but the changes in fatty acid composition during development of the corn kernels were similar for all the lipids. The percentages of palmitic acid and linolenic acid decreased while those of oleic acid increased.

Introduction

Triglycerides are the preponderant lipids in most seeds, but the glycolipids and phospholipids, although present only in small quantities, are essential components of the seed membranes. Changes have been observed in the phospholipid and glycolipid classes of developing seeds of crambe (1), flax (2), rape (1), safflower (2) and corn (3). Individual polar lipids, such as phosphatidyl choline or digalactosyl diglyceride, have not been examined previously. This is the first report of changes in the individual polar lipids of a maturing seed.

Materials and Methods

Corn Samples

H51, a standard corn inbred, was chosen for these studies because its oil content and fatty acid composition resemble those of the common commercial hybrids. The corn was grown on the Agronomy farm of the University of Illinois at Urbana, Illinois during the summer of 1967. Samples were collected at intervals after the dates of hand-pollination.

Total lipids were extracted from the corn samples by a mixture of chloroform, methanol and water (3). Nonlipid contaminants, such as alcohol-soluble proteins, were removed by column chromatography on a dextran gel (4,5). The lipids were separated into classes on silicic acid columns as previously described (3) except that the polar lipids were divided into three fractions. Glycolipids were eluted by chloroform-acetone (1:1 v/v) and acetone (6). Phospholipids were eluted with methanol. Previous experience with similar silicic acid columns (3) indicated a good recovery of phospholipids from H51 total lipids (range 87–99%, mean 95%). The free fatty acid content of each of the samples was less than 1% of the total lipid extract.

Glycolipid Analysis

The two glycolipid fractions were chromatographed on silica gel thin layer plates in one dimension (Fig. 1). The solvent system was chloroform-acetone-methanol-acetic acid-water (65:20:10:10:3) (7). Duplicate plates of both glycolipid fractions were run for each sampling date. The plates were exposed to iodine vapor to visualize the glycolipid bands, and the bands were outlined. After the iodine had been vaporized from the plates with the aid of a high vacuum pump, the bands were scraped into test tubes.

The sugar content of each glycolipid band was determined by the orcinol procedure of Svennerholm (8) modified for direct digestion of the glycolipids in the presence of silica gel. To the silica gel from each band were added 0.5–1 ml of ethanol and 2 ml of 5 N sulfuric acid. Standards of 0, 40, 60, 80 and 100 μg of galactose were also run through all steps of the procedure. Silica gel from clear areas of the thin layer plates were added to the sugar standards before the digestion with sulfuric acid. All samples were hydrolyzed in a tube oven for 2 hr at 100 C. The tubes were cooled in running water, and the hydrolysates were centrifuged rather than filtered as in the original procedure. Triplicate determinations and a hydrolysis blank were run for each sample. In order to obtain low blank readings, careful recrystallization of the orcinol was essential. The orcinol was treated twice with activated charcoal and recrystallized four to five times from benzene.

For fatty acid analysis the glycolipids were visualized with dichlorofluorescein and eluted from the silica gel with 30 ml of chloroform-methanol (2:1) and 20 ml of absolute methanol. The extracts were transferred to a separatory funnel. Chloroform (10 ml) and water (12 ml) were added. The glycolipids were recovered from the lower layer. The fatty acid methyl esters were prepared by treatment of the lipids with boron trifluoride-methanol (9). The conditions for gas chromatography of the methyl esters have been described previously (3).

Phospholipid Analysis

Two-dimensional thin layer chromatography (TLC) separated the phospholipids (Fig. 2). The solvent systems were chloroform-methanol water-acetic acid (65:25:3:1), first in the x-direction, and chloroform-acetone-methanol-acetic acid-water (65:20:10:10:3) (7) in the y-direction. The phospholipid spots were visualized with iodine and analyzed for phosphorus in the presence of the silica gel (10). Areas of blank silica gel corresponding in size to the phospholipid spots and from the same plate were analyzed as blanks.

For fatty acid analysis of the phospholipids the x-solvent system for TLC was chloroform-methanol-water (65:25:4). The y-system was the same as above. The phospholipids were eluted from the silica gel in the same manner as were the glycolipids.

[1] Contribution from the Department of Agronomy, University of Illinois, and Crops Research Division, ARS, USDA.

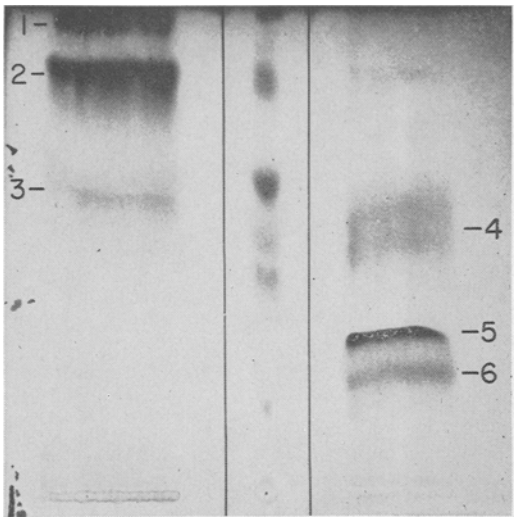

FIG. 1. Thin layer plate of 10 day H51 glycolipid fractions. The fraction to the left was eluted from a silicic acid column with chloroform-acetone (1:1 v/v); the fraction to the right with acetone. The TLC solvent system was chloroform - acetone - methanol - acetic acid - water (65:20:10:10:3). The bands were detected with iodine vapor. Standards in the center lane from bottom to top were phosphatidyl choline, phosphatidyl ethanolamine, cerebrosides (two spots) and monogalactosyl diglyceride. The glycolipid bands were identified as follows: 1, steryl glycoside ester; 2, monogalactosyl diglyceride; 3, steryl glycoside; 4, cerebrosides; 5, digalactosyl diglyceride; 6, sulfolipid.

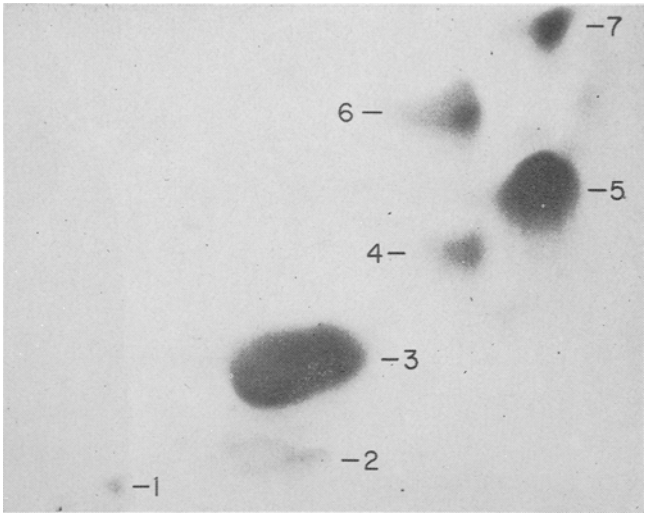

FIG. 2. Two-dimensional thin layer plate of 10 day H51 phospholipid fraction. The solvent systems were chloroform-methanol-water-acetic acid (65:25:3:1) in the x-direction and chloroform-acetone-methanol-acetic acid-water (65:20:10:10:3) in the y-direction. Spots were detected with iodine vapor and identified as follows: 1, lysophosphatidyl choline; 2, phosphatidyl inositol; 3, phosphatidyl choline; 4, phosphatidyl glycerol; 5, phosphatidyl ethanolamine; 6, diphosphatidyl glycerol; 7, phosphatidic acid.

Identification of the Glycolipids and Phospholipids

The lipids separated by TLC were identified by comparing R_f values with authentic compounds, by specific sprays, and from the products of alkaline hydrolysis. The authentic compounds available were phosphatidyl choline, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, phosphatidyl glycerol, diphosphatidyl glycerol, phosphatidic acid, cerebrosides, ceramides (Supelco, Inc., Bellefonte, Pa.), monogalactosyl diglyceride, lysophosphatidyl choline, phosphatidyl serine (Applied Science Laboratories, Inc., State College, Pa.) and phosphatidyl inositol (11). Lysophosphatidic acid was prepared from phosphatidic acid by the enzymatic action (12) of *Ophiophagus hannah* snake venom (Sigma Chemical Co., Ltd., St. Louis, Mo.) (13).

The aqueous 20% perchloric acid spray suggested by Lepage (14) gave characteristic color reactions with glycolipids. A molybdenum spray (15) was specific for phospholipids, and a ninhydrin spray (16) detected aminophospholipids. Periodate-Schiff reagents (17) located lipids that contained vicinal hydroxyl groups. Phosphatidyl glycerol gave a purple color very rapidly with the Schiff reagent and could be identified easily, even at low concentrations.

Identical spots from 19 TLC plates were combined, and the lipids were eluted for alkaline hydrolysis (18). Paper chromatography of the products was run in phenol-water (88:12) and *n*-butanol-propionic acid-water (151:75:100). The glycerophosphoryl esters were detected by a phosphate ester spray (19).

Results

Changes in Glycolipids

As the H51 corn kernels developed, the amount of glycolipids increased to a peak at 45 days after pollination (Table I). This increase paralleled the sharp increase in all lipids of the grain during this period (3). After 45 days, the accumulation of the major lipid, triglyceride, was augmented slightly, but the glycolipids decreased rapidly.

At the sampling dates from 10 to 45 days after pollination, over 55% of the total sugar of the glycolipids was found in digalactosyl diglyceride. Although digalactosyl diglyceride would contain two moles of sugar compared to one in the other glycolipids, the digalactosyl diglyceride had a higher molar concentration than the others, and it was the dominant glycolipid in the grain. This contrasts with photosynthetic tissue, such as chloroplasts or *Chlorella,* where monogalactosyl diglyceride predominates (20). In grain monogalactosyl diglyceride ranked second at most of the harvesting dates.

In the last two samples the sulfolipid values were higher than those of the monogalactosyl diglycerides. However, as both digalactosyl diglyceride and sulfolipid increased with maturation of the grain, complete separation of these bands became more difficult. The sulfolipid values may be high due to contamination by digalactosyl diglyceride in the more mature samples.

The steryl glycosides and steryl glycoside esters increased slightly as the corn kernels developed but were relatively minor components of the glycolipids at all stages. In the cerebrosides band there were in-

TABLE I

Changes in Glycolipids of Developing Corn Kernels

	Micromoles of sugar per 100 kernels Days after pollination				
	10	15	20	45	60
Total glycolipids	4.2	11.9	32.1	52.6	22.0
Glycolipids bands					
1 Steryl glycoside ester	0.2	0.2	0.4	1.5	2.0
2 Monogalactosyl diglyceride	0.3	1.5	5.6	5.3	4.1
3 Steryl glycoside	0.3	0.5	0.6	0.8	1.1
4 Cerebroside[a]	0.9	1.9	2.1	2.2	2.4
5 Digalactosyl diglyceride	2.3	6.8	19.8	29.2	6.0
6 Sulfolipid[a]	0.1	0.6	3.6	13.5	5.4

[a] Band may contain other glycolipids. See text.

TABLE II

Changes in Phospholipids of Developing Corn Kernels

	Micromoles of phospholipids "P" per 100 kernels Days after pollination						
	10	15	20	30	45	60	75
Total phospholipids	9.0	27.4	57.1	66.8	56.4	32.3	36.1
Phospholipid spots							
1 Lysophosphatidyl choline	....	0.4	0.1	1.1	1.3	2.3	2.3
2 Phosphatidyl inositol	1.0	3.2	6.6	9.1	2.3	3.5	3.7
3 Phosphatidyl choline	4.6	13.7	32.8	39.9	40.4	20.6	18.6
4 Phosphatidyl glycerol	0.5	1.3	2.9	2.2	0.3	0.3	0.9
5 Phosphatidyl ethanolamine	2.1	5.9	11.3	8.7	9.1	2.4	2.8
6 (Diphosphatidyl glycerol)[a]	0.5	1.6	2.3	3.5	1.0	2.7	6.5
7 Phosphatidic acid	0.2	1.2	1.1	2.3	1.9	0.5	1.3

[a] Spot may contain other phospholipids. See text.

dications of additional sugar-containing components which have not been identified.

Changes in Phospholipids

The changes in the phospholipids of maturing H51 corn kernels are indicated by the phosphorus values in Table II. The total amount of phospholipids was highest at 30 days after pollination.

Throughout the harvesting periods over 50% of the total phosphorus was found in phosphatidyl choline. The total amount of phosphatidyl choline was highest at 45 days after pollination. In later samples, the phosphatidyl choline values decreased. There was a slight increase in lysophosphatidyl choline which may indicate some degeneration of the aging membranes of the kernel.

For 45 days after pollination phosphatidyl ethanolamine and phosphatidyl inositol ranked second and third, respectively, in phospholipid concentration. Expressed as per cent of total phosphorus, the fraction represented by phosphatidyl ethanolamine fell from 24% to 8% from 10 to 75 days after pollination while the phosphatidyl inositol remained nearly constant at 10%. Phosphatidyl glycerol and phosphatidic acid were minor components at all stages.

Spot 6 has been only tentatively identified as diphosphatidyl glycerol. This spot always appeared diffuse on the thin layer plates. Multiple salt forms, such as the calcium and sodium salts of diphosphatidyl glycerol (21), may contribute to the enlarged spot. Paper chromatography of the alkaline hydrolysis products suggested this spot might also contain a mixture of phospholipids.

Changes in Fatty Acid Compositions

Each individual lipid had a characteristic fatty acid pattern (Table III), but the changes in fatty acid compositions during development of the corn kernels were similar for all the lipids. The percentages of palmitic acid and linolenic acid decreased, while the percentage of oleic acid increased as the grain matured.

All the phospholipids had a higher proportion of saturated fatty acids than did the triglycerides or glycolipids. Within the phospholipids, lysophosphatidyl choline, phosphatidyl inositol and phosphatidyl glycerol showed the highest values for palmitic and stearic acid. The fatty acids of phosphatidyl glycerol and phosphatidyl inositol from *Narcissus* bulbs (22) and of phosphatidyl inositol from turnip root tissue (7), white potato tubers (23), cotton buds (24), soybean phosphatides (25) and rapeseed gum (26) were also highly saturated.

TABLE III

Fatty Acid Composition of Lipids of Developing Corn Kernels

	Days after pollination	Relative area per cent				
		16:0	18:0	18:1	18:2	18:3
Triglyceride	10	19.0	2.5	10.0	53.3	15.2
	20	18.6	1.7	30.8	46.7	2.2
	45	17.2	1.6	30.2	50.0	1.0
	60	16.7	1.6	31.5	48.9	1.3
Lysophosphatidyl choline	10	33.1	12.4	26.7	23.8	4.0
	20	35.6	16.2	23.7	23.0	1.5
	45	27.3	9.3	24.7	37.0	1.7
	60	28.8	2.5	27.0	40.0	1.7
Phosphatidyl inositol	10	38.7	4.4	7.6	43.1	6.2
	20	41.6	4.1	13.6	38.8	1.9
	45	38.5	4.3	15.1	40.3	1.8
	60	37.1	4.0	19.0	38.1	1.7
Phosphatidyl choline	10	25.5	2.3	7.6	57.9	6.7
	20	26.6	1.4	9.5	59.8	2.7
	45	21.4	1.6	23.8	51.6	1.5
	60	21.0	1.7	32.5	43.8	1.0
Phosphatidyl glycerol	10	48.2	4.0	6.5	36.0	5.2
	20	51.4	2.3	9.5	35.3	1.4
	45	40.7	6.1	18.4	32.9	1.9
	60	41.5	4.6	20.7	32.3	0.9
Phosphatidyl ethanolamine	10	30.5	1.6	3.6	58.7	5.6
	20	29.3	2.3	6.7	59.3	2.4
	45	27.1	1.3	9.6	60.8	1.2
	60	23.2	1.5	17.8	56.6	0.9
Diphosphatidyl glycerol	10	25.7	4.8	9.9	52.3	7.3
	20	22.7	3.0	12.7	58.0	3.5
	45	25.1	7.8	23.1	41.8	2.2
	60	21.5	3.0	30.3	43.5	1.6
Phosphatidic acid	10	28.6	4.4	9.4	52.9	4.6
	20	32.4	3.8	10.8	49.6	3.4
	45	28.3	9.3	23.3	37.8	1.3
	60	24.6	4.7	33.2	36.2	1.3
Monogalactosyl diglyceride	10	20.8	2.7	11.2	33.4	31.8
	20	7.6	1.3	11.8	65.1	14.2
	45	5.5	0.7	16.8	54.6	22.4
	60	12.9	3.6	20.9	36.4	26.2
Digalactosyl diglyceride	10	30.3	2.5	11.7	38.4	17.1
	20	16.3	2.1	10.5	60.0	11.1
	45	19.9	2.0	15.8	53.4	8.8
	60	19.8	3.5	18.7	44.0	14.0

In lysophosphatidyl choline the percentage of linoleic acid increased with age of the kernel. This was in contrast to a decrease in linoleic acid in the other phospholipids. McKillican (27) studied the fatty acid compositions of phosphatidyl choline and lysophosphatidyl choline in wheat flour. Phospholipase A hydrolysis of the wheat phosphatidyl choline indicated that unsaturated fatty acids were preferentially esterified at the β-position and saturated acids at the α-position. Similar fatty acid distributions have been found in soybean and safflower phosphatidyl cholines (28). McKillican concluded that the lysophosphatidyl choline of wheat flour was made up of both alpha and beta species. Corn lysophosphatidyl choline also appeared to be composed of both species, but the proportions changed during kernel development.

In photosynthetic tissue the fatty acids of phosphatidyl glycerol are characterized by the presence of *trans*-3-hexadecenoic acid. This *trans* fatty acid was found almost exclusively in phosphatidyl glycerol and made up from 16–43% of its fatty acids (20,29). No such quantities of this fatty acid were found in the phosphatidyl glycerol of corn grain.

Phosphatidyl ethanolamine had the highest percentage of linoleic acid and the lowest percentage of oleic acid of all the lipids of the developing kernels.

Hydroxy-fatty acids were detected in the lower cerebroside band, but the total fatty acids of the cerebrosides have not been completely quantitated.

The galactosyl glycerides of the maturing corn grain were characterized by higher linolenic acid values than were found for any of the other lipids. Monogalactosyl diglyceride had a higher content of 18:3 than did digalactosyl diglyceride. The percentage of 18:3 was 26 for monogalactosyl diglyceride and

14 for digalactosyl diglyceride in mature corn kernels. In photosynthetic tissues the content of linolenic acid in the galactosyl lipids is very high. In alfalfa leaves, for example, linolenic acid represented 94% of the fatty acids of monogalactosyl diglyceride and 89% of the acids of digalactosyl diglyceride (30). Values intermediate between those for leaf and for corn grain glycolipids were noted for turnip root (monogalactosyl diglyceride-56% 18:3; digalactosyl diglyceride-55% 18:3) (7) and potato tubers (monogalactosyl diglyceride-50% 18:3; digalactosyl diglyceride-33% 18:3) (23). The concentration of glycolipids in potato tubers was highest in the skin region. Perhaps the galactolipids in this region become involved in the greening that occurs when potatoes are exposed to light. Linolenic acid and monogalactosyl diglyceride increase simultaneously with chloroplast development induced by light in dark-grown leaves (31). The location of the glycolipids in the corn kernel is not known.

Discussion

In maturing corn kernels the concentration of both phospholipids and glycolipids increased for 30–45 days after pollination and then began to decrease. Earley and DeTurk (32) observed that phosphorus for phytin synthesis in corn was supplied from outside the seed for the first four weeks after pollination, but during the fifth, sixth and seventh weeks phosphorus already present in the grain contributed to phytin synthesis. This conversion was greatest during the seventh week when the seed provided 40% of the phosphorus used in phytin synthesis. This is also the period when the phospholipids decrease. The phospholipids may be contributing their phosphorus to phytin synthesis. Perhaps the glycolipids are supplying sugar for starch synthesis.

The roles of the galactosyl diglycerides and sulfolipids must be important and unique in plants. In animals only trace amounts of monogalactosyl diglycerides have been found (33), and the sulfur-containing lipid of animals has a ceramide-type structure in contrast to the sulfolipid of plants which is a glyceride. The plant glycolipids appear to be intimately associated with photosynthesis in leaf chloroplasts, but the exact relationship is not clear.

In chloroplasts the molar ratio of glycolipids to phospholipids is approximately 4.5 (34). The phospholipid concentration is much higher in grain. The molar ratio of glycolipids to phospholipids in the developing corn kernel varied from 0.3 in the early samples to 0.7 at 45 days after pollination. The compositions of the phospholipids are also quite different. Chloroplasts are particularly rich in phosphatidyl glycerol, while phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl inositol are the major phospholipids of grain.

The functions of glycolipids and phospholipids in grain have not been investigated. We need to know the anatomical and subcellular locations of these lipids in order to obtain some clues as to their functions in grain.

ACKNOWLEDGMENT

Technical assistance by Lorraine Bollinger; Ian de la Roche planted and cultivated the corn.

REFERENCES

1. McKillican, M. E., JAOCS *43*, 461 (1966).
2. McKillican, M. E., and R. P. A. Sims, JAOCS *40*, 108 (1963).
3. Weber, E. J., JAOCS *46*, 485 (1969).
4. Rouser, G., G. Kritchevsky, G. Simon and G. J. Nelson, Lipids *2*, 37 (1967).
5. Nelson, G. J., Ibid. *2*, 323 (1967).
6. Vorbeck, M. L., and G. V. Marinetti, J. Lipid Res. *6*, 3 (1965).
7. Lepage, M., Lipids *2*, 244 (1967).
8. Svennerholm, L., J. Neurochem. *1*, 42 (1956).
9. Morrison, W. R., and L. M. Smith, J. Lipid Res. *5*, 600 (1964).
10. Parker, F., and N. F. Peterson, Ibid. *6*, 455 (1965).
11. Carter, H. E., and E. J. Weber, Lipids *1*, 16 (1966).
12. Christie, W. W., and J. H. Moore, Ibid. *4*, 345 (1969).
13. Nutter, L. J., and O. S. Privett, Ibid. *1*, 258 (1966).
14. Lepage, M., J. Chromatogr. *13*, 99 (1964).
15. Dittmer, J. C., and R. L. Lester, J. Lipid Res. *5*, 126 (1964).
16. Sambasivarao, K., and R. H. McCluer, Ibid. *4*, 106 (1963).
17. Randerath, K., "Thin Layer Chromatography," Academic Press, New York, 1966, p. 153.
18. Ferrari, R., and A. A. Benson, Arch. Biochem. Biophys. *93*, 185 (1961).
19. Bandurski, R. S., and B. Axelrod, J. Biol. Chem. *193*, 405 (1951).
20. Allen, C. F., P. Good, H. F. Davis, P. Chisum and S. D. Fowler, JAOCS *43*, 223 (1966).
21. Shimojo, T., and K. Ohno, J. Biochem. (Tokyo) *60*, 462 (1966).
22. Nichols, B. W., and A. T. James, Fette, Seifen, Anstrichm. *66*, 1003 (1964).
23. Lepage, M., Lipids *3*, 447 (1968).
24. Thompson, A. C., R. D. Henson, J. P. Minyard and P. A. Hedin, Ibid. *3*, 373 (1968).
25. Colacicco, G., and M. M. Rapport, J. Lipid Res. *8*, 513 (1967).
26. Weenink, R. O., and A. P. Tulloch, JAOCS *43*, 327 (1966).
27. McKillican, M. E., Ibid. *44*, 200 (1967).
28. Blank, M. L., L. J. Nutter and O. S. Privett, Ibid. *1*, 132 (1966).
29. Haverkate, F., and L. L. M. Van Deenen, Biochim. Biophys. Acta *106*, 78 (1965).
30. Van der Veen, J., K. Hirota and H. S. Olcott, Lipids *2*, 406 (1967).
31. Appelqvist, L. A., J. E. Boynton, P. K. Stumpf and D. von Wettstein, J. Lipid Res. *9*, 425 (1968).
32. Earley, E. B., and E. E. DeTurk, Agron. J. *36*, 803 (1944).
33. Steim, J. M., Biochim. Biophys. Acta *144*, 118 (1967).
34. Park, R. B., and J. Biggins, Science *144*, 1009 (1964).

[Received March 25, 1970]