Lipid Changes in Maturing Oil-Bearing Plants. III. Changes in Lipid Classes in Flax and Safflower Oils[1,2]

MARY E. McKILLICAN and R. P. A. SIMS,[3] Genetics and Plant Breeding Research Institute, Research Branch, Department of Agriculture, Ottawa, Canada

Abstract

Seeds from Raja flax and Indian safflower were collected at increasing stages of maturity and the free lipid extracted from them with hexane. The true lipid material obtained in this manner was separated into lipid classes by silicic acid column chromatography using a diethyl ether–hexane gradient and methanol for the phospholipids. Thin-layer chromatography was used to establish the homogeneity of each lipid class. The composition of the lipid classes was examined by a combination of gas-liquid, silicic acid-impregnated paper and thin-layer chromatography.

With both flax and safflower, the relative amounts of the different lipid classes were shown to vary as the seed matured; the phospholipids showed the greatest degree of change. Free fatty acid, mono- and diglycerides were not encountered; acidity, when present, was not due to lipid material. Differences within lipid classes were also investigated.

Introduction

THE CHANGES in lipids that occur when a plant proceeds toward maturity continue to excite interest. Germinating seeds have been studied (1,2) as well as the developing seedling (3). However, maturing oil-bearing plants have received more attention. Simmons and Quackenbush studied the soybean plant (4) and Crombie, the West African palm (5). Vidal (6) followed changes in a variety of common oil-bearing plants and Kartha (7) measured changes in developing mustard seed.

In these laboratories, lipid changes in maturing flax and safflower plants have been studied. Gross changes in the seed, its acetate-incorporating ability and its oil content were described as functions of degree of maturity (8). In a second study, variation in fatty acid composition of the free lipid in the maturing seed was reported (9). The objective of the present work is to separate the free lipid of maturing seed into component lipid classes and study the changes within and between classes as functions of degree of maturity. Because stored tissue was used in these experiments and the complete absence of artifacts cannot be assumed, the results presented here are not intended to be a definitive description of any one class of lipids. Indeed, the complexity of composition revealed in this study suggests that each lipid class should be studied separately.

Materials and Methods

Raja flax and Indian safflower seed, grown in the summer of 1960, was collected as described in Part I of this series (8). Extent of maturity was expressed as "days after fertilization," abbreviated to DAF. The free lipid was removed from the freeze-dried seed by grinding a sample at 45000 RPM in deoxygenated hexane in a Virtis "45" tissue grinder. The air above the hexane was displaced with oxygen-free nitrogen and the Virtis flask was surrounded by an ice-water mixture during grinding. Centrifugation at +5C at 1700 x g for 30 min packed the finely-powdered seed dust and the supernatant hexane solution of oil was removed and concentrated on a rotating evaporator at 20C. Vacuum was always broken with nitrogen and lipid was always stored under nitrogen, in the dark, at −15C.

Silicic Acid Column Chromatography. Separation of the free lipid into its classes was accomplished in the apparatus described by Sahasrabudhe and Chapman (10) using 90 gm. silicic acid in a column 25 mm in diameter. The dehydrating procedure and the gradient from pure hexane to 60% diethyl ether in hexane were essentially those described by Hirsch and Ahrens (11); the phospholipids were eluted with absolute methanol. Because of the preponderance of glycerides in the lipid mixture from even the least mature seeds, a total charge of 200 mg was all that could be accommodated by the 90 gm. silicic acid. For quantitative chromatography, the 150 fractions were each taken to dryness under nitrogen at temperatures below 35C. For preparative work, an LKB Uvicord eluent analyzer was used with its filter removed to chart the peaks of the various fractions. The contents of the tubes corresponding to these fractions were pooled, the bulk of solvent removed on a rotating evaporator and the fraction stored in the dark at −15C. At no time was the solvent removed completely. Deoxygenated solvent was used in all steps and all operations, including filtration, were performed in a nitrogen atmosphere.

Chromatography on Silicic Acid-Impregnated Paper. Marinetti-type chromatography (12) was used to analyze the material eluted from the silicic acid column by methanol. Three papers were employed simultaneously: one treated with ninhydrin spray (13) and then dipped in Rhodamine 6 G solution (12); another treated with the Periodate-Schiff reagent of Hack and Ferrans (14) and then Rhodamine 6 G and the third paper treated with Rhodamine

[1] Presented at AOCS meeting in Chicago, Ill., 1961.

[2] Contribution No. 84 from the Genetics and Plant Breeding Research Institute, Research Branch, Canada Department of Agriculture, Ottawa.

[3] Present address: Food Research Institute, Research Branch, Department of Agriculture, Ottawa, Canada.

6 G to locate the spots which were subsequently cut out and used for phosphorus analysis (15).

Thin-Layer Chromatography. The apparatus developed by Stahl (16) and distributed by C. A. Brinkman and Co. was used in this investigation. To test fractions for homogeneity, the hexane-ethyl ether-acetic acid system of Mangold and Malins (17) was used. The solvent systems described by Jatzkewitz and Mehl (18) were employed to separate hydrocarbons from sterols and to fractionate sterol esters and free sterols. In addition to the diisobutyl ketone-acetic acid-water system used to separate phospholipids on paper (12), Wagner's (19) methanol-acetic acid-water system was also used with thin-layer plates.

Iodine vapor proved to be a very useful general detecting chemical for all lipids and sugars on thin-layer plates (20). Where only part of the plate was to be exposed to chemical, as in preparative thin-layer work, 2,7-dichloro-fluorescein (17) was sprayed on the unmasked portion of the plate.

Gas-Liquid Chromatography. Methyl esters were prepared by heating 1 mg of esterified lipid dissolved in 1 ml hexane with 5 ml 0.2 M potassium methoxide in absolute methanol (21). Trials, using thin-layer chromatography to check completion of reaction, showed that 5 min refluxing in a bath at 85C was sufficient to convert the acyl groups of triglycerides and phospholipids to methyl esters and that a heating time of 10 min under the same conditions sufficed for sterol esters. The sterol esters were first proved, or made, homogeneous on a thin-layer plate, then the appropriate band of silicic acid was scraped off, the sterol esters eluted with chloroform and converted to methyl esters. The reaction products were then chromatographed on another thin-layer plate to separate the methyl esters from the freed sterols. Both lipid types were scraped off the second plate and used in gas chromatography. In both cases, the solvent was removed from the developed plates in a nitrogen atmosphere.

The methyl esters were fractionated on a 4 ft x $\frac{3}{16}$ in. column containing 3.92 gm of 60–80 mesh "Gas Chrom-P" (Applied Science Laboratories, State College, Pa.) bearing 6% (w/w) polyvinyl acetate (Union Carbide, type AYAC 8285) or on a 4 ft x $\frac{3}{16}$ in. Apiezon L column (10% w/w on "Gas Chrom-P" 80–100 mesh). The sterol separations were effected on a 20 in. x $\frac{1}{4}$ in. column containing 1.52 gm of 80–100 mesh "Gas Chrom-Z" bearing 2% (w/w) SE-52 silicone rubber (Applied Science Laboratories, State College, Pa.). A Research Specialties Series 600 gas chromatograph operating with its flash vaporizer 240C, column at 170C and Argon detector at 210C and 1.0 KV applied potential separated the methyl esters satisfactorily with a gas flow of 40 ml per min and a pressure drop of 56 cm Hg. The Apiezon L column was operated at 185C with the other conditions unchanged. Sterol analysis was performed through the courtesy of F. A. Vandenheuvel of the Animal Research Institute, Research Branch, Canada Department of Agriculture, on a Perkin-Elmer 154 D Vapor Fractometer using a hydrogen flame detector, a column temperature of 210C and a helium flow of 100 ml/min under a pressure of 15 psi.

The quantitative nature of the gas chromatographic analysis of the methyl esters was confirmed by reference to Test Mixture D distributed by the National Institutes of Health, Bethesda, Md.

Experimental and Results

Quantitative Column Chromatography. Quantitative column chromatography of the oil from both flax and safflower seed showed changes in all the lipid classes as maturity was approached. The general pattern of lipid classes was retained, but the relative proportions differed in oil from seeds of different degrees of maturity. The oil from 10 DAF flax seed exhibited additional peaks when chromatographed and these will be described in some detail later. The twin phospholipid peaks represented almost half the weight of material in the triglyceride peak and the free sterol peak almost one quarter that of the triglyceride peak. The changes in the shape of the triglyceride peaks as the oil became increasingly unsaturated is also noteworthy.

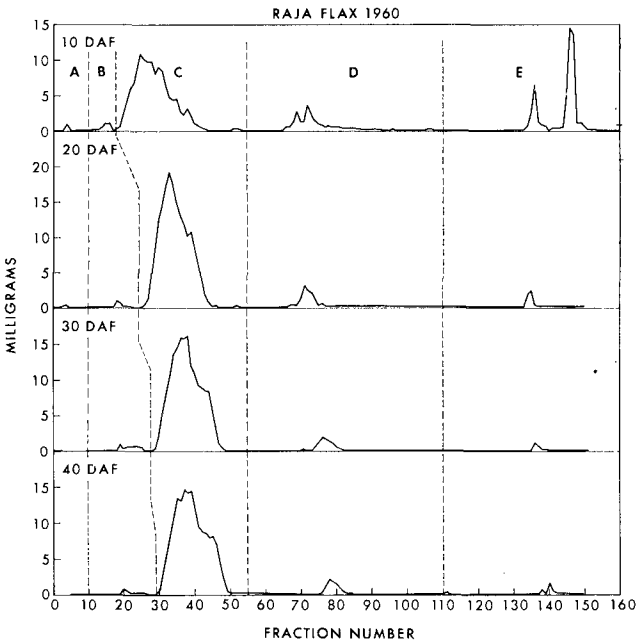

FIG. 1. Quantitative column chromatography of the oil from maturing Raja flax. A: hydrocarbons; B: sterol esters; C: triglycerides; D: free sterols; E: phospholipids.

In maturing flax, the hydrocarbons, sterol esters and triglyceride fractions increased as maturity was approached whereas the amounts of free sterol and phospholipid decreased. These latter components displayed the most pronounced changes (Table I).

TABLE I

Lipid Classes as % Total Recovery
1960 Raja Flax

Lipid class	Days after fertilization			
	10	20	30	40
Hydrocarbons	0.6	1.0	1.8	1.6
Sterol esters	1.2	1.2	2.5	1.3
Triglycerides	62.1	89.3	89.4	90.3
"Free fatty acids"	0.3	.....	.....	.....
Free sterols	11.4	6.9	4.8	4.9
"Partial glycerides"	.....	....	....	....
Phospholipids	24.5	2.2	1.1	1.4

The changes in lipid patterns in developing safflower resembled, in general, those of maturing flax. Here again, the shape of the triglyceride peaks changed as the oil became more unsaturated and the oil from 10 DAF safflower seed contained additional peaks, even more than the equivalent linseed oil. There appeared to be a closer resemblance between

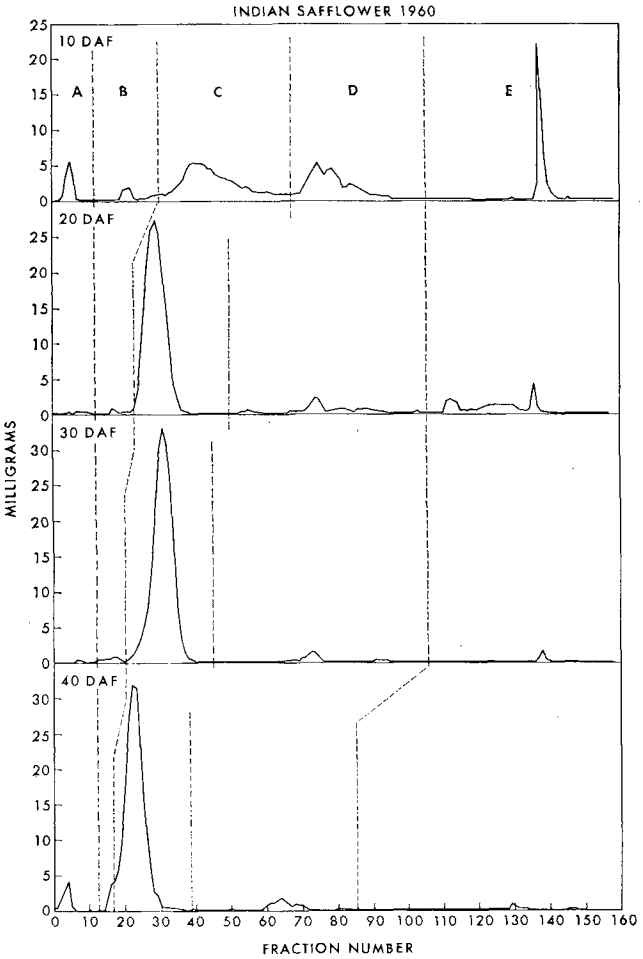

FIG. 2. Quantitative column chromatography of the oil from maturing Indian safflower. A: hydrocarbons; B: sterol esters; C: triglycerides; D: free sterols; E: phospholipids.

the oils from 10 and 20 DAF safflower seed than between those from equivalent flax seed. The free sterol content of the oil from 10 DAF safflower was 60% of the amount of triglyceride present and the phospholipids amounted to 35% of the triglycerides.

As safflower seed matured, its hydrocarbon content decreased as did the content of sterol ester, free sterol and phospholipid. The triglyceride component doubled in amount between the 10th and 40th day whereas the material eluted from column under conditions appropriate to free fatty acids and partial glycerides was in negligible amount after the 20th day after fertilization (Table II).

TABLE II

Lipid Classes as % Total Recovery
1960 Safflower

Lipid class	Days after fertilization			
	10	20	30	40
Hydrocarbons	5.9	0.5	0.2	0.7
Sterol esters	2.7	0.9	1.7	4.0
Triglycerides	44.1	76.7	93.3	89.1
"Free fatty acids"	.....	0.8	.....	.....
Free sterols	28.6	7.6	3.0	4.9
"Partial glycerides"	2.9	10.7	0.4	....
Phospholipids	15.4	2.8	1.3	1.1

Examination of "Extra" Peaks in Oil from 10 DAF Seed. Samples of 10 DAF oil were titrated in the manner described by Crombie (1) in which the salts formed by neutralization are collected, acidified and separated on the basis of the solubility differences between acids composed of less than 6 carbon atoms and those containing more than 6 carbon atoms. Subsequent retitration gives the relative amounts of each type of acid. Data for flax and safflower oils are given in Table III. These data show that less than 25% of the titratable acidity in the oil from 10 DAF flax and less than 6% of the safflower acidity could be attributed to fatty acids of chain length greater than six carbon atoms.

TABLE III

Titratable Acidity by the Crombie Method (1)
Raja Flax

Extent of maturity DAF	% FFA as oleic acid	
	Before extraction (all acids)	After extraction (fatty acids only)
10	12.4	3.1
20	0.5	....
30	0.4	....
40	0.5	....
	Indian safflower	
20	21.5	1.2
10	0.6	....
30	0.8	....
40	0.4	....

The oil from 10 DAF seed had another unusual property. As a routine test in this laboratory, all extracted oil is tested for absorption at 233 mμ to detect possible conjugation, natural or the result of oxidation during extraction. In spite of careful treatment, 10 DAF oil indicated by absorption at this wavelength a conjugated diene content of almost 14%. The ultraviolet absorption spectra of oil from 10 DAF and 20 DAF flax and safflower seed were therefore recorded using a Beckman DK-2 Ratio Recording Spectrophotometer. The oil from 20 DAF flax and safflower seed gave the characteristic spectrum of a non-conjugated fatty substance whereas the oil from 10 DAF samples had a more detailed spectrum. Although a slow scanning speed (scan of 2) and a high concentration (0.25 gm per 1.) were used, however, absorption due to either conjugated diene or triene was not detected in the 10 DAF oils.

Changes in Fatty Acid Composition. Gas-liquid chromatography was used to measure changes in the fatty acid composition of the triglycerides, phospholipids and sterol esters in the oil from seeds of increasing maturity. The following changes were noted in the composition of the whole oil of maturing flax (Table IV). High palmitic and low linolenic acid

TABLE IV

Fatty Acid Composition, % by Weight, of the
Whole Oil from 1960 Raja Flax

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
16:0			15.7	8.1	6.4	7.2
16:1	16.3	19.7	0.1	0.5	0.6	0.9
18:0			4.3	4.9	4.9	3.8
18:1			22.5	18.0	19.4	19.8
18:2			23.0	15.2	16.4	16.6
18:3			29.7	52.2	51.2	51.2
20:0	20.0	19.9	1.4	0.6	1.1	0.4
20:1	20.4	19.7	1.1	0.6	....	....
20:2	20.9	19.4	1.7	....	....	....
Calc. I. V.			136.2	177.6	178.2	178.9

[a] Using PVA column.
[b] Using Apiezon L column.

contents and generally greater complexity characterized the oil from immature seed. The iodine values (I.V.) calculated from these data agreed within one unit of chemical measurements made by the Wijs method.

Equivalent data for safflower are also available (Table V). Like the oil from maturing flax seed, these oils increase in extent of unsaturation with the oil from 20 DAF seed resembling mature oil quite closely. The oil from 10 DAF safflower, however, appears to contain more minor components than the oil from 10 DAF flax. The linolenic acid content of the whole oil, although 4.6% of the unsaturated acids in the least mature oil, became negligible in the 30-DAF oil and nil by 40 days.

TABLE V

Fatty Acid Composition, % by Weight, of the Whole Oil from 1960 Indian Safflower

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
14:0			4.8	....	....	....
14:1	14.3	13.7	2.4	....	....	....
14:2	14.9	13.3	2.8	....	....	....
16:0			21.1	8.1	6.1	6.9
16:1			1.7	1.1	0.8	0.4
16:2	16.9	15.5	0.9	....	....	....
16:3	17.0		1.0	....	....	....
18:0			3.6	3.2	2.5	1.2
18:1			11.3	14.3	7.2	7.6
18:2			45.6	71.9	82.6	83.9
18:3			3.2	1.0	0.5	....
20:0			1.2	0.3	0.2	....
20:1	20.4	19.7	0.3	....	....	....
Calc. 1. V.			91.4	138.9	149.9	151.1

[a] Using PVA column.
[b] Using Apiezon L column.

The fatty acids in the phospholipids also changed in an interesting, although less regular, manner as the seeds matured (Table VI). In general, greater

TABLE VI

Fatty Acid Composition, % by Weight, of the Phospholipids in 1960 Raja Flax

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
12:0			0.4	0.3		0.4
13:1	13.4	12.7	0.3	0.3		0.3
14:0			1.2	1.5		1.1
15:0	15.1	15.0	0.3	0.2		....
15:1	15.5	14.8	0.3	0.2		....
16:0			24.0	19.1		7.1
16:1			1.4	1.6		1.7
17:0	17.1	16.9	0.2	0.3		0.4
18:0			2.1	5.5		3.5
18:1			7.7	17.3		21.0
18:2			19.7	18.8		20.8
18:3			41.7	34.2		42.4
20:0			0.7	.....		1.2
Calc. 1. V.			149.0	136.4		164.2

[a] Using PVA column.
[b] Using Apiezon L column.

variety and a higher proportion of saturated fatty acids were found in the phospholipids than in the triglycerides. In flax, linolenic acid comprised 50% of the unsaturated acids in the phospholipids compared to 60% in the triglycerides. Consequently, the I.V. merely changed from 149 in the 10 DAF seed to 164 in the 40 DAF seed. Corresponding changes in the I.V. of the triglycerides were from 136 to 179.

In the phospholipids of maturing safflower seed, the saturated fatty acids formed a higher proportion and the unsaturated fatty acids a lower proportion of the fatty acids than in triglycerides of equivalent maturity (Table VII). The linoleic acid content of the phospholipids of the 40 DAF seed, however, formed 90% of the unsaturated acids, a close approximation to the 84% value for the triglycerides.

The linolenic acid content of the safflower phospholipids was also interesting. It accounted for 11.75% of the fatty acids in the phospholipids of 10 DAF seed and more than 50% of all the linolenic acid in the 10 DAF whole oil. Even the phospholipids from

TABLE VII

Fatty Acid Composition, % by Weight, of the Phospholipids in 1960 Indian Safflower

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
10:0			0.6	....		....
12:0			0.5	....		0.1
13:1	13.4	12.4	1.2	0.7		0.5
14:0			1.8	0.7		0.8
15:0			0.3	1.0		....
15:1	15.2	14.6	0.2	....		0.4
16:0			40.7	27.7		7.8
16:1			1.8	1.0		1.1
17:0	17.0	17.0	1.7	0.6		0.3
	17.4	16.7	1.6	0.3		....
18:0			4.1	1.6		1.9
18:1			9.0	8.7		5.4
18:2			25.4	44.0		80.4
18:3			6.5	11.7		1.1
20:0			2.7	0.5		....
20:1	20.4	19.7	1.9	0.4		....
22:0			....	1.1		....
Calc. 1. V.			68.4	113.8		146.1

[a] Using PVA column.
[b] Using Apiezon L column.

40 DAF seed contained 1% linolenic acid.

The composition of the fatty acid in the sterol esters did not resemble that of the phospholipids or the triglycerides (Table VIII). The oil was much

TABLE VIII

Fatty Acid Composition, % by Weight, of the Sterol Esters in 1960 Raja Flax

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
12:0			1.9	0.3	0.5	....
14:0			2.8	0.8	1.6	1.1
15:0			0.9	1.5	1.2	0.5
15:1	15.5	14.7	1.2	trace	....	....
16:0			31.1	16.2	16.0	10.8
16:1			2.8	2.5	1.9	0.4
17:0	17.1	16.8	2.8	2.1	1.8	0.2
18:0			7.8	13.4	12.8	13.0
18:1			16.1	29.1	31.3	52.0
18:2			16.8	18.1	18.0	12.4
18:3			9.7	12.3	11.9	8.2
20:0			3.0	3.1	3.0	1.0
20:1	20.3	19.7	1.5	0.5	....	0.5
Calc. 1. V.			68.0	88.1	88.8	87.3

[a] Using PVA column.
[b] Using Apiezon L column.

more saturated: linolenic acid was present in only nominal amounts, for linseed oil; oleic acid was the major unsaturated component, palmitic acid the dominant saturated acid. Only the oleic and palmitic acid contents were affected to any degree by increasing seed maturity.

The fatty acid composition of the safflower sterol esters is shown in Table IX. As with flax, these esters were more saturated than those in the triglyc-

TABLE IX

Fatty Acid Composition, % by Weight, of the Sterol Esters in 1960 Safflower

Acid type	Carbon number		Days after fertilization			
	PVA[a]	APL[b]	10	20	30	40
11:1	11.3	.....	4.9	....	....	....
11:2	11.6	10.4	....	0.5	....	0.7
12:2	12.7	11.2	....	0.4	....	....
13:1	13.3	12.6	9.6	0.7	2.2	0.7
14:1	14.4	13.7	1.7	....	....	....
15:0	15.0	15.0	0.4	trace	....	....
15 Br.			....	0.3	1.2	0.7
15:1	15.3	14.7	5.7	2.7	6.9	6.4
16:0			7.7	19.0	24.8	23.3
16:1			1.0	0.9	2.0	3.4
17:0	17.0	17.1	....	trace	....	....
17:1	17.4	16.9	1.0	trace	....	....
18:0			4.0	5.0	6.9	8.5
18:1			11.2	22.6	23.3	24.7
18:2			39.4	42.7	29.4	26.2
18:3			11.5	4.4	3.3	4.1
20:0			....	0.5	....	0.5
20:1	20.4	19.7	1.8	0.2	....	0.8
Calc. 1. V.			130.5	104.4	85.5	76.7

[a] Using PVA column.
[b] Using Apiezon L column.

erides or phospholipids. Although linoleic acid was the major component, it formed merely 40% of the total at its greatest concentration and decreased with increasing maturity, as did the small amount of linolenic acid. Palmitic acid was again present in abnormally high concentration which increased with maturity. The amount of oleic acid also became larger as the seeds matured.

Changes in Phospholipid Composition. These changes were determined by chromatography on silicic acid-impregnated paper, supplemented by thin-layer chromatography. Identification was on the basis of comparison of Rf values of known and unknown material chromatographed at the same time. The data for flax (Table X) and safflower (Table XI) were obtained with what were thought to be 100 μg. of each phospholipid mixture. Inadequate quantities were obviously used with the more mature samples which contained much less phospholipid. Nevertheless, the large amounts of glycolipid present in immature seed, and their positive reaction to the Schiff reagent, are clearly shown.

TABLE X

Phospholipids in 1960 Raja Flax

Tentative identification	Rf		Days after fertilization			
	Known	Unknown	10	20	30	40
Glycolipid		0.97	+++[b]			
		0.84		+[b]	+[b]	
		0.73	+[b]			
		0.60	+[b]	+[b]		
Kephalin	0.57					
		0.52	+[a]	+[a]		
Lecithin	0.48					
Lysokephalin	0.43	0.44	+[a]	+[a]		
Lysolecithin	0.39					
		0.37	+[b]	+[b]		+[b]
Inositol phosphatide		0.24	+		+	
Ninhydrin + ve		0.11				+
Inositol phosphatide		0.06	+[a]			

[a] Ninhydrin positive.
[b] Schiff positive.

TABLE XI

Phospholipids in 1960 Indian Safflower

Tentative identification	Rf		Days after fertilization			
	Known	Unknown	10	20	30	40
Glycolipid		0.94	+++[b]	+++[b]		
		0.84	+++[b]	+++[b]		
		0.79	+++[b]	+++[b]		
		0.61	+			
Kephalin	0.57	0.56	+[a]			
		0.52	+[a]			
Lecithin	0.48					
Phosphatidyl glycerol		0.47	+[b]			
Lysokephalin	0.43	0.42		+		++
		0.38			+	
Lysolecithin	0.37					
Inositol phosphatide		0.34	++[b]			
Ninhydrin + ve		0.24	+[a]	+		
Inositol phosphatide		0.13	++	+		
		0.11				
I.P. × Ninhydrin + ve		0.05	+	++		

[a] Ninhydrin positive.
[b] Schiff positive.

Larger quantities of the 30- and 40-DAF samples were used in the separation of phospholipids by thin-layer chromatography. In spite of the increased sample size and the greater resolving power of thin-layer plates, no phosphatidyl choline or phosphatidyl ethanolamine were found in flax after the 20th day after fertilization and none in the safflower samples of any stage of maturity. No phosphatidyl serine was found in any of the oils. Only traces of lysophosphatides were found in the flax phospholipids. These contained mostly glycolipid and inositol lipid; more glycolipid than inositol lipid was found in immature seed whereas the reverse situation obtained in mature seeds. Ninhydrin-positive material was found only in the kephalin fraction (sensitivity of 5 μg. = 5% of applied load) and choline (Dragendorff spray) (22) was located only in the lecithin spot (sensitivity of 10 μg. = 10% of applied load).

The safflower phospholipids, as revealed by thin-layer chromatography, differed from those of flax in the following respects. No ninhydrin- or Dragendorff-positive material was detected at any stage of development. Only traces (1% of the applied load) of glycerol or inositol phosphatides were revealed by iodine vapor. The bulk of the samples was composed of glycolipid.

Changes in Free Sterols. Thin-layer and gas-liquid chromatography were used to supplement each other in this investigation. The thin-layer plates of the sterol fractions, from both flax and safflower at all stages of maturity, displayed 3 spots, one above and one below that for β-sitosterol. The solvent system employed, however, could not resolve sitosterol-stigmasterol mixtures (Rf cholesterol = 0.23; Rf β-sitosterol = 0.20 = Rf stigmasterol).

Gas-liquid chromatography of the free sterols showed them to be less subject to variation with extent of maturity than the other lipid classes. Flax seed (Table XII) contained β-sitosterol and three

TABLE XII

Gas-Liquid Chromatography of Flax Sterols
(emergence distance of peaks in cm)

Peaks	Reference material	Days after fertilization			
		10	20	30	40
Preliminary peaks				1.1, S	1.1, S
			1.2, L		
		1.7, L		1.7, S	1.7, S
		2.4, S		2.3, S	
			2.8, S	2.6, S	2.6, S
Mid peaks		4.0, M	4.0, M	4.1, M	4.0, M
		5.2, M	5.4, L	5.3, M	5.3, M
	*	6.9, S	7.0, S	6.8, S	6.7, S
Sterol peaks	*	9.9, L	9.6, L	9.7, L	9.6, L
	*	14.4, L	14.8, M	14.5, L	14.5, L
Pro-β-sitosterol	14.5				
Stigmasterol	15.9				
		18.7, L	18.5, S	18.8, L	18.7, L
β-Sitosterol	18.6				
			22.6, L		22.7, M
				23.4, M	
α-Sitosterol	24.1	24.1, L			

L = large; M = medium; S = small peak.
* = also on β-sitosterol chromatogram.

compounds that accompanied the reference sample of this material. Stigmasterol, however, was absent and only the 10-DAF sample gave a peak with the same emergence as α-sitosterol. From the known weight of material injected and the measured detector response to the reference β-sitosterol, it was concluded that less than 50% of the sample was detected during the running of the chromatogram.

The sterols of maturing safflower seed (Table XIII) differed from those of flax in that the β-sitosterol and its nearest companion were found only in the 30- and 40-DAF samples. The two earlier companions, however, were present at all stages of development. The 30- and 40-DAF samples differed also by containing material that emerged at times intermediate to those of stigmasterol and β-sitosterol. Safflower sterols resembled those of flax in that, although apparently homogeneous by thin-layer chromatography, less than 50% of the injected sample was detected and recorded.

Changes in Sterol Ester Composition. Gas-liquid

TABLE XIII

Gas-Liquid Chromatography of Safflower Sterols (emergence distance of peaks in cm)

Peaks	Reference material	Days after fertilization			
		10	20	30	40
Preliminary peaks			1.0, M		1.0, M
					1.3, M
		1.6, S	1.6, S		1.6, S
				1.9, M	
		2.4, L			
			2.6, L	2.9, M	
Mid peaks		4.1, M	3.9, L	4.2, M	3.9, L
		5.3, M	5.2, M	5.2, L	5.2, M
	*	6.8, S	6.5, S		6.7, S
				7.1, M	
Sterol peaks	*	9.5, L	9.3, L	9.7, L	9.4, L
	*			14.7, S	14.3, S
Stigmasterol	15.6			16.1, S	16.0, S
β-Sitosterol	18.6			19.0, L	18.8, L
		22.7, L	22.3, L	22.2, L	22.3, L
α-Sitosterol	24.1				
		53.5, M	53.3, L	53.5, S	53.5, S

L = large; M = medium; S = small peak.
* = also on β-sitosterol chromatogram.

chromatography of the intact sterol ester was not attempted. Thin-layer chromatography of the unhydrolyzed esters of both safflower and flax showed 5 spots under conditions that gave 10 spots for a corn oil hydrocarbon-sterol concentrate. Three of the 5 corresponded exactly in Rf to three in the corn oil concentrate. However, as shown in Tables VIII and IX, the sterol esters contain 5 major fatty acids and numerous minor acids and gas-liquid chromatography has shown the presence of at least 4 sterols in the free sterol fraction. Consequently, the 5 spots shown on the thin-layer plates must be considered to contain more than one sterol ester.

Discussion

During the course of an investigation of this type, attention must be given to all possible sources of artifacts. Autoxidation and adventitious hydrolyses leading to the production of free fatty acids, partial glycerides and lysophosphatides were considered to be the greatest sources of error. To prevent enzymatic hydrolysis, the seed was frozen in liquid nitrogen immediately after removal from the boll or head and, without intervening thawing, was freeze-dried. Extraction was subsequently performed at 0C using hexane to obviate lipase and phospholipase action. Indication of success in this direction is the absence of free fatty acid and partial glycerides from the extracted oil as revealed by thin-layer homogeneity tests. Admittedly, traces of lysophosphatides were found in some samples but they were present at no higher than the 1% level and, furthermore, might not be artifacts.

Strong absorbance at 233 mμ cast doubt, momentarily, on the efficacy of the precautions taken against autoxidation. The nature of the absorption spectra and the narrow, completely resolved peaks given by polyunsaturated esters on gas chromatography, however, support the view that efforts to frustrate autoxidation were successful. The presence of unusual chain lengths in the methyl esters prepared from sterol esters also suggested the possibility of degradation. The methylation procedure and the thin-layer preparative chromatography were tested with known mixtures of fresh and partially-oxidized safflower oil. The gas-liquid chromatograms of these esters agreed quantitatively with those obtained with shorter methylation times and in the absence of thin-layer chromatography. In addition, determinations were made of the infrared and ultraviolet absorption of the methyl esters prepared from sterol esters. No *trans* or conjugated double bonds were detected.

Maturing flax and safflower seed, although having characteristics peculiar to their families, displayed generally similar patterns of development. In fatty acid composition, the sterol esters were more saturated than the phospholipids which were, in turn, more saturated than the triglycerides. In phospholipids, the dominant fatty acid, which was also dominant in the triglyceride class, was in high proportion in 10 DAF seed whereas in triglycerides it developed more slowly. In sterol esters, however, a different fatty acid predominated and was present in high concentration even in the least mature seed.

Both safflower and flax displayed similar patterns of phospholipid synthesis. Immature seed contained large amounts of glycolipid which decreased in quantity with maturation and the phospholipids of mature seed contained mostly inositol phosphatides. The ready metabolism of glycolipids may possibly be the reason for this shift in dominance.

The aim of this investigation has been to measure changes within and between lipid classes in two very different types of oil seeds: a member of the Compositae family, producing an oil rich in linoleic acid and a member of the Geraniales family, with an oil rich in linolenic acid. Because the components of all the lipid classes are not known in detail and reference compounds were not always available, the present work is not offered as a definitive description of any one class but as an introduction to less well-known lipids which, it is hoped, will be studied in detail.

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