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Glyceride Studies. Part III. The Component Glycerides of Five Seed Oils Containing Linolenic Acid

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Abstract

The component glycerides of linseed, wild rose, candlenut, soya and stillingia oils have been estimated by chromatography on thin layers of silica impregnated with silver nitrate. The separated glycerides are identified, qualitatively and quantitatively, by gas-liquid chromatography (GLC) of their methyl esters in presence of added methyl heptadecanoate as an internal standard. The results agree with those obtained by lipolysis or calculated directly from the component acids on the basis of the theory of positional distribution.

Introduction

THE COMPONENT GLYCERIDE analysis of drying oils containing linolenic acid has been undertaken by the classical crystallisation procedures of Hilditch and his colleagues (1-4), by the rather tedious countercurrent distribution technique of Dutton and Scholfield et al. (5,6) and by Youngs' oxidation procedure (7–9). This last method gives useful information about the distribution of saturated acyl groups but is less informative about the distribution of those unsaturated acyl groups which are oxidised to azelaic acid derivatives and thus become indistinguishable from one another. We find that separation on thin layers of silica impregnated with silver nitrate (10–13) provides a satisfactory basis for the quantitative determination of component glycerides and we have applied this to five seed oils: linseed, wild rose, candlenut, soya, and stillingia. Some workers have weighed the glycerides obtained (14,15), others have estimated the glycerol (12), or used densitometry (10) or the chromotropic acid colour reaction (13); we have added methyl heptadecanoate to the separated glycerides as an internal standard.

Experimental Procedure

Isolation of Neutral Glycerides

Seeds were obtained from J. Bibby and Sons (linseed and soya) and from the Tropical Products Institute [candlenut ex Uganda and stillingia (Sapium sebiferum) ex Bombay]. The wild rose seeds were obtained from hips collected locally. The outer shell was removed from the stillingia seeds and oil obtained from the kernel only. Crushed seeds were thoroughly extracted with boiling petrol ether (bp 40–60C) and neutral triglycerides were isolated by eluting the oil (1 gm) from a column of silica (30 gm) with benzene (200 ml) (16).

Separation of Glycerides by Thin-Layer Chromatography

The triglycerides (20–40 mg) are applied, in ether solution, in a band about 4 cm from one of the short edges of a glass plate (20 × 40 cm) layered (0.3–0.4 mm) with silica gel containing 15% of silver nitrate (10,11). These plates are developed by horizontal elution (17) for 2–3 hr using ether to separate the more unsaturated triglycerides and benzene containing 10% of ether to separate the less unsaturated triglycerides. Thereafter the plates are dried at room temperature in a current of nitrogen to remove all solvent and sprayed with a methanolic solution (0.2%) of 2′,7′-dichlorofluorescein. Six to ten bands appear and these are scraped from the plate with a sharp razor blade; the glycerides are thoroughly extracted with a mixture of methanol, ether, and water (5:5:1).

A known amount of methyl heptadecanoate in the form of a dilute methanolic solution is added to each glyceride extract which is then poured into water to remove silver nitrate and extracted three times with n-hexane. The triglycerides are converted to methyl esters by transesterification with methanol containing sodium methoxide (18). The esters are examined quantitatively by gas liquid chromatography (GLC) using a Perkin Elmer Fractometer fitted with a flame ionisation detector and 1 meter or 2 meter columns containing poly(ethylene glycol succinate) as stationary phase. The ratio of the area of the C_{17} ester peak to the total area of all other peaks is a measure of the amount of glyceride in each fraction.

Lipolysis

Our lipolysis procedure is based on that of Coleman (20), but the partial glycerides are separated on a thin layer (0.3-0.4 mm) of silica by developing with chloroform-acetone-ammonia (80:20:1, S.G. 0.88). The separated components are detected with 2′,7′-dichlorofluorescein and the monoglycerides (R_f about 0.3) are extracted three times with ether and then converted into methyl esters by transesterification (18).

Discussion and Results

Oils containing saturated acids (considered as a single group) along with oleic, linoleic, and linolenic acids may have twenty different glycerides, even when possibilities of isomerism are ignored, and we find it necessary to examine these oils on two chromatoplates. Development with ether separates the more unsaturated glycerides but not the less unsaturated ones which crowd together near the solvent front; development with a mixture of benzene and ether separates

TABLE I

Component Esters (% mol) of the Whole Oil, the 2-Monoglycerides, and the Sum of the Separated Glyceride Fractions [a]

	Number of fractions	16:0	18:0	16:1	18:1	18:2	18:3 [b]
Linseed							
Triglyceride		6.1	3.2	0.1	16.6	14.2	59.8
Plate A	(6)	6.5	3.3	0.5	16.4	14.4	58.9
Plate B	(10)	6.9	3.8	0.6	17.3	14.0	57.4
2-Monoglyceride		0.7	....	0.2	18.8	20.6	59.7
Wild rose							
Triglyceride		3.7	0.9	0.3	10.3	49.1	35.7
Plate A	(6)	4.0	1.4	0.4	10.7	46.8	36.7
Plate B	(10)	4.0	1.4	0.5	10.8	47.7	35.6
2-Monoglyceride		0.3	....	0.3	11.0	56.3	32.1
Candlenut							
Triglyceride		6.5	3.2	....	22.0	37.6	30.7
Plate A	(8)	7.2	3.1	....	22.0	38.7	29.0
Plate B	(14)	7.1	3.4	....	21.9	38.8	28.8
2-Monoglyceride		0.9	0.5	0.5	26.7	51.3	20.1
Soya							
Triglyceride		12.0	3.6	0.5	23.7	51.4	8.8
Plate A	(7)	12.4	3.8	0.8	23.3	51.1	8.6
Plate B	(8)	12.2	3.5	0.4	24.2	50.9	8.8
2-Monoglyceride		1.8	0.6	0.2	23.2	66.2	8.0

[a] Details of stillingia oil given in Table IV.
[b] These figures indicate the number of carbon atoms and double bonds per acid molecule; thus 18:2 represents octadecadienoic acid.

the less unsaturated glycerides leaving the more unsaturated members still near to the starting point.

We do not separate pure individual glycerides, and each glyceride fraction is treated as a ternary or quaternary mixture. In fact, one glyceride usually predominates and forms 80% or more of the fraction. For purposes of calculation it is important to know the order of elution of glycerides and we have found this by recognition (by GLC of the derived methyl esters) of the major glyceride components in the different oils examined. Under our experimental conditions the order of elution is given by the following sequence in which 3,2,1, and 0 indicate the number of double bonds in the three acyl groups of each glyceride:

333 332 331 330 322 321 320 311 222 310
221 300 220 211 210 111 200 110 100 000

It is apparent from this list that the two double bonds in a linoleic chain form a stronger complex with silver nitrate than two bonds in two oleic chains and, perhaps more surprisingly, the three double bonds in a linolenic chain form a stronger complex than the four double bonds in two linoleic chains. We have found it useful to assign arbitrary values for the complexing power of each chain *viz.* saturated (0), oleic (1), linoleic $(2 + a)$, and linolenic $(4 + 4a)$ where a is some fraction less than one. Thus the glyceride 330 with six double bonds has a complexing power of $8 + 8a$ which is greater than the value $8 + 6a$ for the glyceride 322 with seven double bonds. This holds for other pairs in the above series in which glycerides with n double bonds are held back on the thin layer plate more than glycerides with $n + 1$ double bonds.

Interpretation of Results

The glyceride composition of each fraction, in terms of a quaternary (or simpler) mixture, can be calculated from the molar composition of the esters and from a knowledge of the relative position of the glyceride fraction on the chromatoplate. Only the final results are given for all the oils (Tables III and IV) but one typical set of results is given in Table II for wild rose seed oil.

For this purpose the two saturated acids have been combined and the small proportion of hexadecenoic acid included with oleic acid. There is little difficulty in recognising the major glyceride in each fraction. For the first five fractions this is 333 (A1), 332 (A2), 331 and 330 (A3), 332 (A4), and 321 and 320 (A5); fraction A6 is a mixture of the less unsaturated glycerides. The figures in the final column show the concentration of the major component in each fraction.

In the second separation of this oil fraction B1 contained 34% of the oil and is therefore approximately equivalent to fractions A1-3(31%). Fractions B2 and B3 contained 29 and 17%, respectively, and the remaining 20% was divided into seven smaller fractions in which the minor glycerides are concentrated. For example, fraction B6, comprising only 2.3% of the total oil, and containing saturated (3%), oleic (61%), and linoleic acid (36%), is a mixture of two glycerides in which 211 (91%) predominates over 220 (9%). There is thus clear evidence for the presence of even the minor glycerides.

The sum of the esters obtained from each glyceride fraction compares favourably with results obtained from the original oil and provides a useful check on the recovery and quantitation of the separated fractions (see Table I). The more unsaturated acids show a small loss. Originally we thought this arose from oxidation but we now consider it to be due more to the difficulty of completely extracting the more unsaturated glycerides from the silica-silver nitrate mixture.

We have also examined the lipolysis of these oils and the component esters of the 2-monoglycerides are included in Table I. Using the assumption of Vander Wal (19) and Coleman (20) that the acids present in the 2-position are combined statistically with those at the 1- and 3-positions we have calculated the composition in terms of the glyceride categories used in our thin layer studies (Table III, column B). The calculation gives an estimate of the 75 possible glycerides in oils with five different acids but we have combined these into the 20 categories listed in Table III. Also in Table III (column C) we report the glyceride composition calculated on the basis of a theory of positional distribution propounded by one of us (21).

Linseed Oil

This is probably the first adequate analysis of the component glycerides of linseed oil. Hilditch and Seavell (2) examined two linseed oils by low-temperature crystallisation, a procedure now considered un-

TABLE II

Glyceride Fractions of Wild Rose Seed Oil

Fraction	Component esters (% mol)				Proportion (%)	Component glycerides (increments % mol)							Proportion of major glyceride in fraction
	16:0 and 18:0	18:1	18:2	18:3 [a]		333	332	331	330	322	321	320 [b]	
A_1	2	1	3	94	6.82	5.53	0.60	0.30	0.39	.....	.....	.....	81%
A_2		2	31	67	17.50	.....	16.18	0.99	0.24	0.09	.....	.....	92
A_3	11	18	8	63	6.64	.....	0.21	3.64	2.10	0.69	.....	.....	55
A_4	1	2	61	36	21.06	.....	.....	1.08	0.66	19.11	0.21	....	91
A_5	9	20	37	34	15.01	.....	.....	.....	0.36	1.93	8.82	3.90	59
A_6	9	18	68	5	32.97	....	.....	....	....	....	(32.97)		....
					total	5.53	16.99	6.01	3.75	21.82	(45.90)		

[a] See footnote (b) in Table I.
[b] These figures indicate the number of double bonds in the three acyl chains. Each glyceride category includes all positional isomers.

TABLE III

Component Glycerides (% mol) of Linseed, Wild Rose Seed, Candlenut, and Soybean Oils Determined by Thin-Layer Chromatography (A), by Lipolysis (B), and by Direct Calculation from the Component Acids (C)

	Linseed			Wild rose			Candlenut			Soya		
	A	B	C	A	B	C	A	B	C	A	B	C
333[a]	23	22	22	5	5	5	2(3)[b]	3	3	....	....	....
332	14	15	15	17	19	19	10(11)	11	11	1	1	1
331	15	18	18	6	4	4	6(8)	6	6	....	1	1
330	12	10	10	4	2	2	3(4)	2	3	1	....	....
322	3	3	4	21	25	26	13(11)	13	13	7	7	7
321	5	8	8	10	11	12	14(13)	16	15	5	6	6
320	5	5	5	4	5	4	8(6)	7	7	4	4	5
311	5	5	5	1	1	1	5(5)	5	4	2	2	2
310	7	6	6	1	1	1	4(6)	4	4	3	2	2
300	1	1	1	....	....	....	.... ( )	....	1	2	1	1
222	1	....	....	13	12	12	6(7)	5	5	15	13	13
221	2	1	1	8	7	8	8(7)	9	9	16	19	19
220	....	1	1	4	4	3	6(5)	4	4	13	13	13
211	1	1	1	2	2	2	5(4)	5	5	8	9	9
210	3	2	1	2	2	1	5	5	5	12	12	12
200	....	....	....	1	....	....	1	1	1	4	4	3
111	1	1	1	1	....	....	1 (10)	1	1	2	1	1
110	2	1	1	....	....	....	2	2	2	5	3	3
100	....	....	....	....	....	....	1	1	1	....	2	2
U_3[c]	70(74, 74)[d]			84(86, 89)			70(74, 73)			56(61, 59)		
U_2S	29(24, 24)			15(14, 11)			28(24, 25)			38(35, 36)		
US_2	1(2, 2)			11(0, 0)			2(2, 2)			6(4, 5)		
Pe_3[e]	41			56			31			23		
Pe_2X	39			36			45			39		
PeX_2	17			7			20			31		
X_3	3			1			4			7		
Number of double bonds												
9	23			5			2			....		
8	14			17			10			1		
7	18			27			19			7		
6	18			27			23			21		
5	12			13			21			22		
4	8			7			15			24		
3	5			3			6			16		
2	2			1			3			9		
1	....			....			1			....		

[a] See footnote (b), Table II.
[b] Results of a second analysis (see text).
[c] U and S refer to unsaturated and saturated acyl chains.
[d] The figures in parenthesis are those obtained by lipolysis and by direct calculation, respectively.
[e] Pe and X refer to polyethenoid and other (monoethenoid and saturated) acyl chains, respectively.

suitable for highly unsaturated oils. Dutton and Cannon's (5) investigation of a linseed oil, containing 9% of saturated acids, revealed the proportion of four glycerides [333(18%), 332(12%), 331(20%), and 322 (4%)] but the nature of the remaining 46% was not detailed. Mattson and Volpenhein (22) have reported the lipolysis of a linseed oil but have not calculated glyceride composition from their results. Hirsch (23) separated linseed oil into eight fractions by factice chromatography. The first two fractions were 333 and 332. In our oil sample, glycerides containing three (23%) and two (41%) linolenic groups make up almost two thirds of the component glycerides; a further 26% of the glycerides contain one linolenic group and this acid is absent from only 10% of the triglycerides.

Our linseed oil, containing about 60% of linolenic acid, represents oil extracted from a large number of seeds which are unlikely to be identical in composition. To examine this point we determined the component acids of the oil extracted from six individual seeds (Table V). The unsaturated acids, particularly oleic and linolenic acids, but not the saturated acids, show considerable variation. Values for oleic acid vary between 11 and 22% and for linolenic acid between 54 and 68% and these two tend to vary inversely.

Using the theory of positional distribution (21) we calculated the glyceride composition of (i) oil present in seed A, (ii) oil present in seed F, and (iii) oil having fatty acid composition intermediate between the values for seeds A and F. We find no appreciable difference between (iii) and the mean of values (i) and (ii).

TABLE IV

Stillingia oil

	10:2	16:0	16:1	18:0	18:1	18:2	18:3[a]
Component acids (% mol)							
Whole oil	8.0[b]	6.4	0.4	1.3	10.8	25.9	47.2
Nonpolar fraction (69%)	....	6.2	0.3	1.8	11.1	26.3	54.3
(2 monoglyceride)		1.6			14.9	39.2	44.3
Polar fraction (31%)	33.3[c]	5.8	0.3	1.7	9.0	22.4	27.5
(2-monoglyceride)	3.3	1.3	0.2	0.1	11.1	36.1	47.9

Component glycerides (% mol)

Nonpolar fraction				Polar fraction	
333[d]	10.7	222	2.0	33D[e]	5.9
333	15.0	221	1.7	32D	7.2
331	7.7	220	1.6	31D	3.5
330	6.1	211	1.0	30D	3.2
322	7.0	210	1.0	22D	3.4
321	4.6	200	0.2	21D	2.8
320	5.2	111	0.1	20D	3.0
311	1.9	110	0.3	11D	0.5
310	2.1	100	0.5	10D	1.5
300	0.3	000		00D	

Glyceride categories (% mol)

				double bonds			
U_3[f]	75%	Pe_3[g]	54%	9	11%	5	15%
U_2S	24	Pe_2X	36	8	21	4	8
US_2	1	PeX_2	9	7	22	3	3
		X_3	1	6	19	1 and 2	1

[a] See footnote b, Table I.
[b] This includes some minor components eluted between 10:2 and 16:0 on g.l.c. This value is probably slightly low (approx. 10%) and the other values in this line correspondingly slightly high.
[c] This value, which includes the minor components referred to in footnote b, has been adjusted to 33.3% (see text).
[d] See footnote b, Table II.
[e] D refers to the C_{20} dienoic acid.
[f] See footnote c, Table III.
[g] See footnote d, Table III.

Wild Rose Seed Oil

The component acids of this oil have been reported by Rusch and Ivanova (24) and by Steger and van Loon (25), but there is no report of its component glycerides. Our sample is much more unsaturated than the Russian sample and slightly more unsaturated than the Dutch sample (Table VII). According to our analysis sixteen glycerides are present but five major components [332(17%), 322(21%), 321(10%), 222(13%), and 221 (8%)] comprise about 70% of the total. Over half (56%) of the oil consists of glycerides containing only the polyethenoid acids, linoleic and/or linolenic acid.

Candlenut

Hilditch et al. (2,3) have examined, by low-temperature crystallisation, the glyceride composition of three candlenut oils not very different in composition from that used in the present investigation, but the earlier results are less definitive and probably less satisfactory. According to our results this oil contains a wider spread of glycerides. Eleven glycerides, totalling 86%, are present to the extent of 5% or above whereas linseed has eight, wild rose seven, and soya eight glycerides at the 5% level or above. The major components [332(10%), 322(13%), 321(14%), 320(8%), and 221(8%)] are those containing at least one linolenic and linoleic chain.

We had occasion to repeat our analysis of candlenut oil on a larger scale, using about 100 mg of oil on four

TABLE V

Component Acids (% wt.) of Oil from Six Individual Linseeds and of a Composite Sample from a Large Number of Seeds

	16:0	18:0	18:1[a]	18:2	18:3[b]
seed A	6	3	11	12	68
B	7	3	12	11	67
C	6	4	12	12	66
D	7	3	12	15	63
E	6	4	20	14	56
F	6	4	22	14	54
composite oil	6	3	17	14	60

[a] Including a small amount (0.2–0.5%) of 16:1.
[b] See footnote (b), Table I.

TABLE VI
Component Acids (% wt.) of Wild Rose Seed Oil

Habitat	Sat.	18:1	18:2	18:3[a]
Russia	5	26	55	14
Holland	5	9	54	32
Scotland	4	11	49	36

[a] See footnote (b), Table I.

plates and dividing each into nine fractions. Corresponding fractions were combined and the glycerides recovered and *weighed* before conversion to esters for analysis by GLC. The results, given in parenthesis in Table III, show the reproducibility of results obtained by the thin-layer procedure.

Soya

The importance of soybean oil is reflected in the number of reports of the glyceride composition of this oil. Quantitative data have been published by Hilditch et al. (1,2) using low-temperature crystallisation, by Scholfield and Hicks (6) using counter-current distribution, and by Youngs et al. (8) using Young's oxidation procedure. Lipolysis results have been given by Mattson and Lutton (26), Coleman (20), and Mattson and Volpenhein (22). The relevant figures are compared with the present results in Table VII. Our results confirm those of Scholfield and Hicks (6) who first showed that soybean oil contains appreciable quantities of trilinolein. We find 51% of the glycerides to contain two or three linoleic groups, a value very similar to those of Hilditch et al. (52%, 48%) for glycerides having two linoleic groups; trilinolein was not detected in this early work. A further analysis has recently been reported (30).

Stillingia Oil

In addition to saturated, oleic, linoleic, and linolenic acid, stillingia oil contains a conjugated C_{10}-dienoic acid (about 10% mol) and other more unusual acids (27). The glyceride composition of the oil has been examined by Crossley and Hilditch (4) and Maier and Holman (28). Crossley and Hilditch concluded that the C_{10} acid occurred only once in about 25% of the glycerides and that it was esterified with the secondary hydroxyl group. The major glycerides were 332(25%), 331, 330, and 33D(28%), and 321, 320, and 32D(42%). Maier and Holman also found the C_{10} acid to occur only once in 26% of the glycerides but they consider that the acid is attached to a primary hydroxyl group.

The American workers found the C_{10} diene-containing glycerides to be more polar than glycerides not containing this acid and therefore separable by chromatography on silica. We separated the polar (31% mol) from the less polar glycerides by chromatography on thin layers of silica using petrol ether (bp 40–60C)-ether (9:1) as solvent, and then examined each fraction on layers of silica impregnated with silver nitrate. The less polar fraction, containing only saturated acyl groups and unsaturated C_{18} acyl groups, behaved like the other oils we examined. The polar fraction also separated readily into fractions which showed the usual variation in the proportion of all acids except the C_{10} acid which was fairly constant at a value just below 33% (mol). (A slightly higher value was obtained by ultra violet spectroscopy than by GLC.) We interpret this as evidence that all the polar glycerides contain one C_{10} group and that fractionation depends on the two other acyl groups present in each glyceride. The results work out satisfactorily and are given (Table IV) to one place of decimals, not because of greater accuracy, but because of the greater number of minor components. We also examined the two fractions by lipolysis; the less polar fraction behaved in the usual way and in the polar fraction only a small proportion of the C_{10} acid was present in the 2-monoglyceride. This result, however, is inconclusive because short chain acids are known to behave abnormally during lipolysis. Comparing the polar and less-polar fractions it is interesting to note that the C_{10} acid takes the place of linolenic acid more than any other acid.

General Comments

The results obtained by thin-layer chromatography are in good agreement with those calculated from our lipolysis data by the method proposed by Vander Wal (19) and by Coleman (20), and also with those calculated directly from the component acids. They thus provide general support for the theory of positional distribution both in its empirical form (Vander Wal, Coleman) and in its more limiting form (Gunstone). Whilst the component glycerides can be calculated by this simple procedure the possibility remains that some oils, especially those containing unusual acids, might deviate from this pattern of distribution. An important example of this has recently been reported by Subbaram, Chakrabarty, Youngs, and Craig (29).

Since it is now possible to delineate the glyceride composition of linolenic acid-containing oils in terms of 20 glycerides it is useful to simplify this for easy comparison. This has been done (Table III) in terms of (i) glycerides containing saturated and unsaturated acyl groups (ii) glycerides containing polyethenoid and non-polyethenoid acyl groups and (iii) the number of double bonds present in each glyceride.

In these oils, containing only 5–16% of saturated acids, almost all glycerides contain two or three unsaturated acyl groups and a high proportion of them contain two or three polyethenoid acyl groups. It has been proposed that the proportion of glycerides containing two or three polyethenoid acyl groups, which we suggest could be called the polyethenoid index, is a useful index of the effectiveness of a drying oil. Linseed (80), wild rose seed (92), candlenut (76),

TABLE VII
Component Acids and Glycerides (% mol) of Soybean Oil

Ref.	Component acids				Major component glycerides								
	Sat.	18:1	18:2	18:3[a]	222	321	322	221	220	211	210[b]	U_3	U_2S
(1)	19	22	49	10	0	....		52		....	....	42	58
(2)	20	22	49	9	0	....		48		....	....	43	55
(6)	14	30	49	7	14	9	5	25	....	....	....	....	....
(8)	17	26	50	7	....	....	....	....	....	....	....	56	38
(30)	14	23	55	8		36		19	15	9	10	66	29
Present work	16	24	51	9	15	5	7	16	13	8	12	56	38

[a] See footnote (b), Table I.
[b] See footnote (b), Table II.

soya (62), and stillingia (90) have the values indicated in parentheses. It must be appreciated that the major polyethenoid glycerides are not the same in all these oils. In linseed oil most glycerides contain two or more linolenic groups, in dog rose and candlenut oil most contain at least one linolenic and one linoleic group, in soyabean oil most contain at least two linoleic groups, and in stillingia most contain at least two linolenic groups or at least one linolenic group and one C_{10} dienoic acid group.

The major glycerides of linseed oil contain 5–9 double bonds (85%) as do those of stillingia oil (88%), whilst those of rose oil have 5–8 double bonds (84%), those of candlenut have 4–8 double bonds (88%), and those of soyabean oil 3–6 double bonds (83%).

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Glyceride Studies. Part IV. The Component Glycerides of Ten Seed Oils Containing Linoleic Acid

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Abstract

The component glycerides of ten seed oils (safflower, tobacco, sunflower, *Argemone mexicana*, maize, cotton, groundnut, *Macadamia ternifolia*, *Gmelina asiatica*, and *Madhuca latifolia*) have been estimated by chromatographic procedures. The results agree with those obtained by lipolysis or calculated directly from the component acids on the basis of the theory of positional distribution.

Introduction

OLEIC AND LINOLEIC are the only unsaturated acids present in many seed oils, including some that are available in large quantities, but information about their component glycerides, apart from a few recent analyses, is based largely on the low temperature crystallisation procedure now considered to be inadequate for the more unsaturated seed oils. We have examined ten seed oils by lipolysis and by chromatographic separation on silica impregnated with silver nitrate. It is convenient to divide these oils into four groups:

(i) Oils with a very high proportion of linoleic acid (> 70%): safflower and tobacco.
(ii) Oils with a high proportion of linoleic acid (50–60%): sunflower, *Argemone mexicana*, maize, and cottonseed.
(iii) Oils with a high proportion of oleic acid (> 50%): groundnut and *Macadamia ternifolia*.
(iv) Oils with a high content of "saturated" acids (> 30%): *Madhuca latifolia* (Mowrah butter) and *Gmelina asiatica*.

Some of the oils fall into more than one category but we have chosen to include them in the group given above rather than in another.

Procedure

Seeds or extracted oils were obtained from J. Bibby and Sons (maize, cottonseed, and groundnut); from the Tropical Products Institute (safflower (var. U.S.A./P2) from Kenya, sunflower from Nigeria (var. Jupiter) and from Bulgaria, *Argemone mexicana* from Jamaica, *Macadamia ternifolia* from Tanganyika, *Madhuca latifolia* from Bombay, and *Gmelina asiatica* from Singapore); from Younghusband, Stephens, and Co. Ltd. (tobacco); and from Dr. C. Y. Hopkins (*M. ternifolia*).

Crushed seeds were thoroughly extracted with boiling petrol ether (bp 40–60C). The extracted oil was neutralised by percolation in chloroform solution through a column of alumina and the triglycerides were subsequently eluted from a column of silica (Whatman chromedia, SG31) with benzene; more polar solvents subsequently removed diglycerides and monoglycerides (1).

Lipolyses were carried out as described in our earlier papers (2,3). The two sunflower oils were examined by low-temperature crystallisation from acetone and methanolic silver nitrate followed by chromatography on columns of silica-silver nitrate (2). The remaining oils were examined by our thin-layer procedure (3,4), developing the plate (20 × 20 cm) with benzene containing 10% of ether. We find the latter procedure to be quicker and more satisfactory. The results are summarised in Tables I and III to V.