47. *Glyceride Studies. Part II.*[1] *The Component Glycerides of Seed Oils Containing Saturated, Oleic, and Linoleic Acids.*

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The component glycerides of three *Jatropha* oils, containing only palmitic, stearic, oleic, and linoleic acid, have been determined by lipolysis and by a new method of crystallisation and column chromatography. The results differ from those previously obtained for seed oils of similar composition, but the two methods give values which agree with one another and with those calculated according to a theory previously postulated. Certain refinements of this theory are proposed. Low-temperature crystallisation of glycerides in presence of silver nitrate gives an improved separation of these compounds according to their degree of unsaturation.

In an earlier Paper [1] three theories concerning the distribution of acyl groups in natural triglycerides were examined and it was concluded that the results then available for seed oils were consistent with the view that the secondary glycerol hydroxyl group is preferentially acylated with unsaturated C_{18} acids and that the two primary hydroxyl groups are acylated subsequently with saturated acids and with unsaturated C_{18} acids not required at the 2-position. Mattson and Volpenhein [2] had previously shown that, in seed oils, monoethenoid C_{20} and C_{22} acids do not compete with the C_{18} unsaturated acids for the 2-position but are present almost entirely at positions 1 and 3. This theory of acyl-group distribution is accepted by Vander Wal [3] and by Coleman,[4] but it lacks experimental proof. We have now examined three *Jatropha* oils by two methods; the results confirm and extend the above theory of acyl-group distribution.

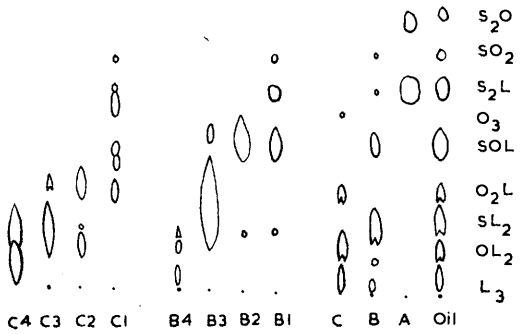
Methods.—Each oil has been hydrolysed with pork pancreatic lipase which preferentially removes acyl groups from positions 1 and 3, leaving the 2-monoglycerides. The 2-monoglycerides were isolated by adsorption chromatography and their component acids determined, as methyl esters, by gas–liquid chromatography. From the composition of the triglycerides and of the 2-monoglycerides derived from them it is possible to calculate glyceride composition if it is *assumed*, as Coleman [4] and Vander Wal [3] do, that the possible combinations of 1- and 3-acyl groups with those at C-2 are statistically arranged. Values are obtained for every possible triglyceride, including isomers, but these have been grouped together into the main categories shown in Table 5. The value shown against SOL, for example, is the total value for the six triglycerides containing one linoleic acid group, one oleic acid group, and one palmitic or stearic acid group.

A second method of glyceride analysis has been devised which gives information about component glycerides without any such assumption. Thin-layer chromatography on silica impregnated with silver nitrate gives very effective separation of triglycerides according to their unsaturation (see Figure).[5] The *Jatropha* oils show nine spots, corresponding to trilinolein (L_3), oleodilinolein (OL_2), saturated dilinoleins (SL_2), dioleolinolein (O_2L), saturated oleolinoleins (SOL), triolein (O_3), disaturated linoleins (S_2L), saturated dioleins (SO_2), and disaturated oleins (S_2O), and we have used this technique to monitor the efficacy of separations by other methods. Low-temperature crystallisation has been much used in the past for glyceride studies,[6] but some of the results are now suspect [1,7] and

[1] Part I, Gunstone, *Chem. and Ind.*, 1962, 1214.
[2] Mattson and Volpenhein, *J. Biol. Chem.*, 1961, **236**, 1891.
[3] Vander Wal, *J. Amer. Oil Chemists' Soc.*, 1960, **37**, 18; 1963, **40**, 242.
[4] Coleman, *J. Amer. Oil Chemists' Soc.*, 1961, **38**, 685.
[5] de Vries, *Chem. and Ind.*, 1962, 1049; de Vries and Jurriens, *Fette Seifen Anstrichm.*, 1963, **65**, 725; Barrett, Dallas, and Padley, *Chem. and Ind.*, 1962, 1050; *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 580; Gunstone, Padley, and Qureshi, *Chem. and Ind.*, 1964, 483.
[6] Hilditch, "The Chemical Constitution of Natural Fats," Chapman and Hall, London, 1956, p. 291.
[7] Dutton and Cannon, *J. Amer. Oil Chemists' Soc.*, 1956, **33**, 46; Scholfield and Hicks, *ibid.*, 1957, **34**, 77; Scholfield and Dutton, *ibid.*, 1958, **35**, 493; Scholfield, Nowakowska, and Dutton, *ibid.*, 1961, **38**, 175.

we have confirmed the incompleteness of these separations by thin-layer chromatography. In the presence of silver nitrate, however, we obtained much better separations. The neutralised seed oils, chromatographed on silica [8] to remove partial glycerides when necessary, are crystallised from dilute solutions in acetone and methanolic silver nitrate. At −10° to −20° the precipitate (fraction A) is mainly disaturated monounsaturated glycerides (S_2U) with some monosaturated glycerides (SU_2) [trisaturated glycerides (S_3) are absent from these oils]; cooling to −70° gives more crystals (fraction B), mainly SU_2 with a little U_3; the mother-liquor is almost entirely U_3. This extension of the use of silver nitrate in chromatography and counter-current distribution [9] can be carried out on any scale and provides a useful separation technique. A single crystallisation at −70°, for example, readily affords, in high yield, a fraction which is almost entirely triunsaturated glycerides.

Attempts to isolate binary or ternary mixtures of glycerides by column chromatography on silica impregnated with silver nitrate failed with the seed oils but were successful with the separated fractions A, B, and C; this was demonstrated by thin-layer chromatography (see Figure). By these means the oil is divided into 9—11 fractions, and the component



Thin-layer chromatogram of *Jatropha multifida* seed oil and glyceride fractions.

acids of each are determined, after transesterification, by gas–liquid chromatography. The sum of the acid increments from the analysed fractions shows a small loss of linoleic acid and we have adjusted the linoleic acid figures upwards, and all other values correspondingly downwards, to correct for this. Similar losses, often more serious, have commonly been encountered in glyceride studies.[10] The composition of each fraction, after adjustment, is then converted from a weight-percentage basis into a molar-percentage basis. Qualitative evidence of the glycerides present, from the thin-layer chromatograms, is combined with the quantitative information about the component acids and in almost all cases the amounts of component glycerides can be calculated. Fractions which cannot be so handled are usually small and reasonable assumptions can be made without introducing serious error into the final results (see Tables 2—5).

Results.—The *Jatropha* genus belongs to the family Euphorbiaceæ and information about *Jatropha* seed oils, previously limited to the component acids of *J. curcas*,[11] *J. glandulifera*,[12] and *J. macrocarpa*,[13] is now extended to two further species, *J. multifida* and *J. gossypifolia*. The results are summarised in the annexed Table; the *J. gossypifolia* seed oil with its high content of linoleic acid is of particular interest.

[8] Quinlin and Weiser, *J. Amer. Oil Chemists' Soc.*, 1958, **35**, 325.
[9] Dutton, Scholfield, and Jones, *Chem. and Ind.*, 1961, 1874; Schofield, Jones, Butterfield, and Dutton, *Analyt. Chem.*, 1963, **35**, 1588.
[10] Privett and Blank, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 70.
[11] Gunstone and Sykes, *J. Sci. Food Agric.*, 1961, **12**, 115.
[12] Sheth and Desai, *J. Indian Chem. Soc.*, 1954, **31**, 407.
[13] Rique, Diaz, and Figuero, *Rev. Argentina de Grasas y Aceites*, 1962, **5**, 31.

Jatropha oils.

Component acids (wt.-%)	*J. curcas*	*J. glandulifera*	*J. multifida*	*J. macrocarpa*	*J. gossypifolia*
16 : 0 + 18 : 0 *	21·9	22·8	24·6	15·2	12·8
16 : 1 + 18 : 1	41·5	34·2	25·0	33·4	16·6
18 : 2	36·6	43·0	50·4	51·4	70·6

* The symbols used here and in the Tables indicate the number of carbon atoms and double bonds in each acid molecule. Thus 18 : 1 and 18 : 2 are used for oleic acid and linoleic acid, respectively.

Before discussing the consequences of our results for any theory of acyl-group distribution we note that they differ from those of seed oils of similar composition previously examined by the normal low-temperature crystallisation. The major glycerides for *J. multifida* seed oil are compared in our next Table with those of *Chorozophora plicata* [14] and cottonseed oil [15] and those for *J. gossypifolia* are compared with results for tobacco-seed oil and sunflower-seed oil.

	C. plicata [14]	Cotton-seed [15]	*J. multifida*	Sunflower seed [17]	Tobacco seed [16]	*J. gossypifolia*
			Component acids (mol.-%)			
Saturated (S) ...	23	29	26	15	11	14
Oleic (O)	25	24	25	18	17	17
Linoleic (L) ...	52	47	49	67	72	69
			Component glycerides (mol.-%)			
SOL	28	41	22	6	3	8
SL_2	19	18	23	39	23	26
O_2L	—	—	9	—	—	6
OL_2	37	28	15	47	48	22
L_3	3	—	10	8	23	33

The agreement between the results obtained by crystallisation and chromatography, by lipolysis, and by calculation according to our earlier theory [1] (Table 5), indicates the general correctness of this theory and provides, for the first time, justification for the assumptions underlying the calculation of component glycerides from lipolysis results. A more detailed examination of these results, however, shows that the theory requires refinement in two ways. Our lipolysis results and the larger number of results obtained by others show that the unsaturated C_{18} acids are *not equally distributed at position* 2 but that, in most cases, there is a stronger preference for linoleic acid in this position than for oleic or linolenic acid.

This general phenomenon can be discussed in terms of an " enrichment factor " which we define as the ratio of the molar concentration of an acid in the 2-monoglyceride resulting from lipolysis to its molar concentration in the triglyceride. The enrichment factor can have any value between 0 and 3; values <1 indicate a preference for positions 1 and 3, values >1 for position 2. The results given in Table 6 are typical of a much larger range of values (not reproduced here) calculated from the results of seed oils examined by Mattson and Volpenhein [2,18] and others.[4,5,19] Enrichment factors are $<0{\cdot}2$ for palmitic, stearic, eicosenoic, and docosenoic acid; amongst the C_{18} unsaturated acids, linoleic consistently has a higher value than has oleic or linolenic acid.

Minor differences in the distribution of oleic and linoleic acid are also apparent from our crystallisation and chromatography results. When the oleic : linoleic ratios in fractions A (mainly S_2U), B(mainly SU_2), and C (mainly U_3) are compared with those in the oils they are consistently higher in fractions A and C and lower in fraction B (Table 6).

In our results there is also some evidence, which we intend to examine further, that the

[14] Barker, personal communication reported by Hilditch (ref. 6, p. 368).
[15] Hilditch and Maddison, *J. Soc. Chem. Ind.*, 1940, **59**, 162.
[16] Crawford and Hilditch, *J. Sci. Food Agric.*, 1950, **1**, 230.
[17] Barker and Hilditch, *J. Oil Colour Chemists' Assoc.*, 1950, **33**, 6.
[18] Mattson and Volpenhein, *J. Lipid Res.*, 1963, **4**, 392.
[19] Mattson and Lutton, *J. Biol. Chem.*, 1958, **233**, 868; Savary and Desnuelle, *Biochim. Biophys. Acta*, 1961, **50**, 319.

saturated acids, palmitic and stearic, show minor differences in their behaviour. Comparison of the palmitic : stearic ratios in fractions A, B, and C, and in some of the chromatographic fractions, with those for the oils shows an enhanced ratio in the monosaturated glycerides (SU_2) and a depressed one in the disaturated glycerides (S_2U).

There remains, therefore, the following scheme of acyl group distribution in natural triglycerides of vegetable origin. Unsaturated C_{18} acids are esterified at position **2**, with linoleic acid taking slight precedence over oleic and linolenic acid; there is a tendency for palmitic acid and linoleic acid to be enriched in monosaturated glycerides (SU_2), for stearic acid and oleic acid to be enriched in disaturated glycerides (S_2U), and for oleic acid to be enriched in triunsaturated glycerides (U_3).

Experimental

Whenever possible operations were carried out under nitrogen. Glycerides, esters, and acids were stored under nitrogen at 0°. Light petroleum is the fraction of boiling range 40—60° unless otherwise designated.

Gas–Liquid Chromatography.—Quantitative gas–liquid chromatography was carried out with a Perkin-Elmer Fractometer and a 1-m. column of firebrick (60—80 mesh) coated with poly(ethylene glycol succinate) (20%). This was operated at 190° with a flame ionisation detector, and peak areas were obtained by multiplying the peak height by the width at half-height or, in some cases, by planimetry. Results are the mean of at least two chromatograms.

Thin-layer Chromatography.—To separate mono-, di-, and tri-glycerides a 2% light petroleum solution (1 μl.) was applied to glass plates covered with a layer of Merck's silica gel G (270 μ thick). These were developed with benzene–ether (17 : 3) and spots became visible when the plate was sprayed with 50% sulphuric acid and charred at 200°.

Glycerides of varying unsaturation were separated on layers of silica gel impregnated with silver nitrate (17%). The glycerides were applied in 1% light petroleum solution (0·5—1·0 μl.) and the plates were developed with benzene–ether (9 : 1) for about 40 min. After the solvent had evaporated from the plate the glycerides were made visible as dark charred spots by drawing a small hot flame from a glassblower's torch across the plate.

Transesterification.—Triglycerides were converted into methyl esters by reaction with anhydrous methanolic hydrogen chloride [20] or by reaction with sodium in dry methanol.[21]

Preparation of Neutral Triglycerides.—Samples of *J. curcas* seeds (Ibadan), *J. multifida* seeds (Achimoto), and *J. gossypifolia* seeds (Achimoto) were crushed and extracted with light petroleum in a Soxhlet extractor. The extracted oils, dissolved in chloroform, were percolated through a column of alumina (Peter Spence, Type H, 100—200 mesh) to remove free fatty acids. When thin-layer chromatography showed the presence of mono- and di-glycerides in the neutralised oil, a chloroform solution (1 g. in 15 ml.) was percolated through a column (33 × 1·8 cm.) of silica gel (30 g.).[8] Triglycerides (benzene), diglycerides (benzene–ether, 9 : 1), and monoglycerides (ether) are eluted in that order by the solvents shown. The complete removal of partial glycerides from triglycerides and the complete recovery of the latter were checked by thin-layer chromatography.

J. curcas kernels contain 49% of oil (30% based on seeds) of which 99% was recovered after neutralisation with alumina. The *J. multifida* kernels contain 52% of oil (40% based on seeds); 99% of this was recovered as neutral oil and separated into triglycerides (80%), diglycerides (19%) and monoglycerides (1%). The *J. gossypifolia* oil (27% from kernels) gave 95% of neutral oil separated into triglycerides 92%, diglycerides 6%, and monoglycerides 2%.

Lipolysis.[4]—The triglycerides (1·30 g.) were stirred in a double-walled vessel, kept at 37° by circulating water between the two walls, along with a 1·2M-ammonium chloride–aqueous ammonia buffer (30 ml.; pH 8·5), 22% calcium chloride solution (2·0 ml.), and 25% sodium taurocholate solution (0·1 ml.). A preparation of pork pancreatic lipase (100 mg., purified by homogenising it with acetone, centrifuging, and drying in a vacuum-desiccator) was added and the pH was held at 8·5 by continual addition of aqueous ammonia (*d* 0·880) whilst hydrolysis proceeded for 10 min. The solution was then adjusted to pH 1 with 2N-hydrochloric acid and extracted with ether (6 × 30 ml.). The ethereal solution was passed through a column of Amberlite IRA-400 resin (30 g.), previously treated with sodium hydroxide; the eluted

[20] Stoffel, Chu, and Ahrens, *Analyt. Chem.*, 1959, **31**, 307.
[21] Gauglitz and Lehman, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 197.

glycerides (0·93 g.), chromatographed on silica gel (Davidson Grade 923, 100—200 mesh), gave triglycerides (0·20 g., eluted with benzene), diglycerides (0·33 g., eluted with benzene–ether, 9 : 1), and monoglycerides (0·31 g., eluted with ether). The last fraction was examined by gas–liquid chromatography after transesterification.

Low-temperature Crystallisation.—A solution of *Jatropha* triglycerides (1·5—2·0 g.) in a saturated solution of methanolic silver nitrate (~2·5%; containing twice the amount of silver nitrate required to form complexes with all the olefinic centres) and acetone (3 ml. per 7 ml. of methanol) was kept at −10° for 24 hr., then quickly filtered through a sintered-glass filter cooled to −15°. The precipitate (fraction A) was twice washed with a little cold acetone–methanolic silver nitrate. The filtrate was held at −70° for 24 hr. and the precipitate (fraction B) was filtered from the mother-liquor (fraction C). The precipitated glycerides were dissolved in light petroleum (25 ml.) and washed with distilled water (3 × 50 ml.) to remove silver nitrate, and the aqueous washings were re-extracted with light petroleum (40 ml.). Solvent was removed from the petroleum solution after drying. The mother-liquor was concentrated to 20 ml., washed into a separatory funnel with petroleum (50 ml.), and treated as above.

Column Chromatography.[5]—Silver nitrate was deposited on silica gel (Whatman, Silica Gel, SG 31) by suspending the latter (100 g.) in water (100 ml.) containing silver nitrate (33 g.). The resulting slurry was heated in an oven at 100° until most of the water had evaporated and then at 120° for 16 hr. The cold dry adsorbent was finally passed through a 60-mesh sieve. A slurry of this adsorbent (30 g.) in dry benzene (150 ml.) made a column 32 × 1·6 cm. in a tube protected from light with black paper. A solution of each of the fractions A, B, and C (100—150 mg.) in benzene (5 ml.) was placed on the column and eluted with 200 ml. each of a range of solvents of increasing polarity. Each eluate was washed with distilled water (3 × 40 ml.) to remove silver nitrate, dried, and weighed after removal of solvent. Four to

TABLE 1.

Component acids (mol.-%)	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas						
Triglyceride	—	15·9	4·3	2·9	39·7	37·2
2-Monoglyceride	—	2·3	0·2	1·1	41·1	55·3
J. multifida						
Triglyceride	—	19·1	7·1	1·3	23·3	49·2
2-Monoglyceride	—	2·6	0·9	0·6	23·7	72·2
J. gossypifolia						
Triglyceride	0·3	7·7	5·8	0·4	16·5	69·3
2-Monoglyceride	0·3	1·0	0·2	0·2	18·0	80·3

TABLE 2.

Jatropha curcas oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas oil		Tr	15·4	6·5	1·3	40·2	36·6
Fraction A	20·4	1·8	32·6	17·7	1·4	28·3	18·2
B	37·1	0·1	24·1	8·8	2·0	30·1	34·9
C	42·5	—	0·5	—	0·9	54·0	44·6
A1 (PB50) *	3·8	0·4	39·8	20·3	2·3	32·0	5·2
A2 (PB70)	4·2	—	42·0	18·9	0·8	17·9	20·4
A3 (B)	9·0	—	27·9	17·0	1·5	34·6	19·0
A4 (E)	3·4	0·2	21·0	15·3	1·2	31·0	31·3
B1 (B)	17·2	0·2	22·4	8·4	2·2	46·2	20·6
B2 (BE5)	15·7	0·3	24·1	8·0	1·8	18·4	47·4
B3 (E)	4·2	—	20·0	7·0	1·7	14·6	56·7
C1 (BE2)	13·9	0·2	1·6	—	1·7	83·2	13·3
C2 (BE2)	10·8	0·1	1·0	—	2·0	59·3	37·6
C3 (BE5)	5·1	—	2·3	—	2·0	41·0	54·7
C4 (E)	12·7	0·1	0·9	—	1·4	26·4	71·2
[Total		0·1	15·5	6·6	1·8	41·0	35·0]

Iodine value of triglycerides = 97·8 (calc. from above results 99·4).

* Eluting solvents: P = light petroleum (b. p. 40—60°), B = benzene, E = ether; a number indicates the amount (v/v %) of the second solvent in the mixture.

TABLE 3.

Jatropha multifida oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. multifida oil		16·9	7·7	1·5	23·5	50·4
Fraction A †	12·9	38·4	21·5	1·8	12·9	25·4
B	52·7	22·5	8·7	1·1	20·5	47·2
C	34·4	1·6	—	1·5	32·2	64·7
B1 (B) *	18·7	26·4	11·4	2·2	37·1	22·9
B2 (B)	6·9	17·7	10·3	2·0	35·1	34·9
B3 (BE5)	23·2	24·8	9·3	1·6	4·8	59·5
B4 (E)	3·9	8·0	3·3	1·2	13·2	74·3
C1 (BE2)	6·7	7·3	—	2·5	64·4	25·8
C2 (BE5)	8·0	3·2	—	2·6	51·2	43·0
C3 (BE10)	4·1	1·0	—	1·8	35·3	61·9
C4 (E)	15·6	1·6	—	1·4	15·0	82·0
[Total		18·2	7·9	1·9	24·8	47·2]

Iodine value of triglycerides = 107·3 (calc. from above results 109·3).

* See footnote to Table 2. † Fraction A was not further separated by column chromatography.

TABLE 4.

Jatropha gossypifolia oil.

Component acids (wt.-%) of seed oil, fractions from low-temperature crystallisation, and fractions from column chromatography

	Wt.-%	14 : 0	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. gossypifolia oil		0·6	7·0	5·2	0·4	16·2	70·6
Fraction A †	2·8	0·4	23·5	36·3	0·7	9·7	29·4
B	45·9	0·3	13·4	9·8	0·6	12·3	63·6
C	51·3	0·1	0·4	—	0·3	19·8	79·4
B1 (BP30) *	1·5	3·8	42·5	10·2	3·1	24·2	16·2
B2 (B)	2·2	1·4	24·2	11·0	0·3	41·8	21·3
B3 (B)	5·3	0·5	19·4	10·1	0·4	35·4	34·2
B4 (BE5)	23·8	0·4	18·3	11·8	0·2	6·9	62·4
B5 (BE5)	4·5	0·3	10·9	4·0	0·1	17·8	66·9
B6 (E)	8·6	0·2	1·5	0·4	0·5	2·2	95·2
C1 (BE2)	5·2	1·0	7·8	1·5	2·5	57·9	29·3
C2 (BE5)	6·5	—	2·8	0·9	0·8	44·8	50·7
C3 (BE10)	26·6	—	0·4	—	0·6	17·5	81·5
C4 (E)	13·0	—	0·8	—	0·4	2·7	96·1
[Total		0·3	8·6	5·1	0·6	17·0	68·4]

Iodine value of triglycerides = 133·8 (calc. from above results 137·1).

* See footnote to Table 2. † See footnote to Table 3.

TABLE 5.

Component glycerides (mol.-%) of *Jatropha* oils.

	J. curcas			*J. multifida*			*J. gossypifolia*		
	A	B	C	A	B	C	A	B	C
S_2U	10	10	10	15	15	15	3	5	4
SU_2	47	41	44	50	47	48	35	32	33
U_3	43	49	46	35	38	37	62	63	63
S_3	—	0·2	—	—	0·5	—	—	0·1	—
S_2O †	3·5	4·2	5·6	4·2	4·1	5·0	1·1	0·8	0·8
S_2L	6·9	5·1	4·9	10·8	11·1	10·1	2·2	3·6	3·4
SO_2	11·6	11·0	12·4	4·6	4·7	5·2	1·1	1·2	1·2
SOL	23·8	21·2	21·8	23·1	21·0	21·1	7·6	10·1	10·1
SL_2	11·4	9·2	9·6	22·7	20·9	21·2	25·9	21·1	21·2
O_3	8·6	7·7	6·9	1·0	1·5	1·4	1·2	0·5	0·5
O_2L	14·7	20·3	18·1	9·1	9·0	8·3	6·1	5·9	5·7
OL_2	16·2	16·7	16·0	15·0	16·9	16·6	21·7	24·0	23·9
L_3	3·3	4·4	4·7	9·5	10·3	11·1	33·1	32·7	33·2

* A, Crystallisation and chromatography (M. I. Q.). B, Lipolysis (R. J. H.). C, Calc. according to theory I.[1] † O includes 16 : 1 and 18 : 1.

TABLE 6.

Palmitic : stearic and oleic : linoleic ratios (%.-wt.).

	J. curcas		*J. multifida*		*J. gossypifolia*	
	P : St	O : L	P : St	O : L	P : St	O : L
Oil	2·37	1·10	2·20	0·47	1·35	0·23
Fraction A	1·84	1·56	1·79	0·51	0·65	0·33
B	2·74	0·86	2·58	0·43	1·37	0·19
C	—	1·21	—	0·50	—	0·25

Enrichment factors (%.-mol.)

	16 : 0	18 : 0	16 : 1	18 : 1	18 : 2
J. curcas	0·14	0·05	0·38	1·04	1·49
J. multifida	0·14	0·13	0·46	1·02	1·47
J. gossypifolia	0·13	0·03	0·50	1·09	1·16

eight fractions were usually collected; these were subsequently examined by thin-layer chromatography on a silicic acid–silver nitrate plate, and fractions of similar composition combined.

Results.—Calculations are collected in the Tables.

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