Composition of Triacylglycerols Containing Cyclopropene Fatty Acids in Seed Lipids of Munguba (*Bombax munguba* Mart.)[1]

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The seed lipids of Munguba (*Bombax munguba* Mart., Bombacaceae) were found to contain substantial proportions (ca. 30%) of cyclopropene acyl moieties (sterculoyl and malvaloyl). In a novel approach to the analysis of glycerolipids containing cyclopropene acyl moieties, the triacylglycerols of Munguba seed were first treated with silver nitrate in acetonitrile-acetone (1:1, v/v), in order to convert the cyclopropene groups to the corresponding α,β-unsaturated ketones. The resulting triacylglycerols were fractionated by thin layer chromatography into molecular species containing none, one and two keto (corresponding to none, one and two cyclopropene) acyl moieties per molecule. Each of these molecular species was further fractionated according to carbon numbers by gas chromatography, and the positional distribution of the acyl moieties in each species was determined. Both sterculoyl and malvaloyl moieties were found predominantly at the *sn*-2 position of the triacylglycerols. The major triacylglycerols of Munguba seed were found to be 1,3-dipalmitoyl-2-oleoylglycerol, 1,3-dipalmitoyl-2-linoleoylglycerol and 1,3-dipalmitoyl-2-sterculoylglycerol.

Bombax munguba Mart. (Bombacaceae) is a 20–30-m high tree that grows in different regions of Northeastern Brazil. The Munguba fruits contain yellowish-brown wool-like fiber that entraps about 150 small (5–8 mm diameter) seeds. The Munguba seed contains about 60% of a hard fat, which we found to be rich in cyclopropene acyl (CPEFA) moieties.

We report here the composition of fatty acids and triacylglycerols in lipids of Munguba seed. The cyclopropene groups in triacylglycerols containing CPEFA moieties were converted to α,β-unsaturated keto derivatives. These derivatized triacylglycerols were fractionated by adsorption thin layer chromatography (TLC) into molecular species varying in the number of keto (corresponding to cyclopropene) groups per molecule and analyzed by gas chromatography (GC).

EXPERIMENTAL

Materials. Munguba seeds, collected from the trees grown at the campus of the University of Paraiba, João Pessoa, Brazil, were provided by N. F. Miranda. Analytical grade reagents and adsorbents for TLC were from E. Merck, Darmstadt, Federal Republic of Germany. Distilled solvents were used throughout. Column packings for GC and lipid standards were purchased from Applied Science Laboratories, Inc., State College, Pennsylvania, and Nu-Chek-Prep, Elysian, Minnesota, respectively. Methyl esters of total lipids from *Sterculia foetida* seed served as reference standards for methyl sterculate and methyl malvalate.

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Lipid extraction and derivatizations. The seeds were finely ground, heated with isopropanol and the lipids extracted with a mixture of chloroform:methanol (2:1, v/v), according to an established procedure (1).

Total lipids and fractions thereof were converted to methyl esters by transmethylation using sodium methoxide (2). Methyl esters were purified by TLC on silica gel H using hexane:diethyl ether (90:10, v/v); the methyl esters were eluted from the adsorbent with diethyl ether, saturated with water.

Cyclopropene groups in methyl esters were converted to methoxy and α,β-unsaturated keto derivatives by treatment with $AgNO_3$ in methanol, according to Schneider et al. (3).

Cyclopropene groups in the acyl moieties of triacylglycerols were converted to α,β-unsaturated keto derivatives as follows (4). Triacylglycerols (up to 20 mg) were shaken with 1 ml of 0.1 N $AgNO_3$ in acetonitrile: acetone (1:1, v/v) for 24 hr at 24 C. Water was added to the reaction mixture, lipids were extracted with hexane and the hexane extract washed with 5% (by vol) HCl followed by 5% (wt/v) $NaHCO_3$.

Analysis of fatty acids. CPEFA moieties were detected in the total lipids by the Halphen test (5).

Methyl esters were analyzed by GC using glass columns (1.8 m × 4 mm) in a Perkin-Elmer F-22 instrument equipped with flame ionization detectors (Perkin-Elmer & Co. GmbH, Überlingen, Federal Republic of Germany). Nitrogen (40 ml/min) was used as carrier gas, and the column temperature was programmed from 150 C to 230 C at 2 C/min. Peaks were identified by comparison of the retention times with those of authentic standards and the peak areas were calculated by triangulation. Figures reported are area % of the individual peaks.

Underivatized methyl esters were analyzed either on 10% (wt/wt) OV-101 on Gas-Chrom-Q (100–120 mesh) or on 10% (wt/wt) Silar-5CP on Gas-Chrom-Q (80–100 mesh).

Methyl esters, derivatized to methoxy and α,β-unsaturated ketones by treatment with $AgNO_3$/methanol, were analyzed on 10% (wt/wt) Silar-5CP on Gas-Chrom-Q (80–100 mesh).

Analysis of triacylglycerols. Triacylglycerols were isolated from the total lipids by TLC on silica gel H using hexane-diethyl ether-acetic acid (70:30:1, v/v/v), followed by elution with diethyl ether, saturated with water. Cyclopropene groups in the acyl moieties of triacylglycerols were converted to α,β-unsaturated keto derivatives as described. These derivatized triacylglycerols were fractionated by TLC on silica gel H with hexane-diethyl ether (70:30, v/v) into molecular species containing none, one and two α,β-unsaturated keto groups per molecule. Each fraction was isolated by elution with diethyl ether, saturated with water.

Aliquots of each molecular species of triacylglycerols were transmethylated and the resulting methyl esters analyzed by GC as described above.

Each of the three molecular species of triacylglycerols

was hydrolyzed with pancreatic lipase (Sigma Chemie, GmbH, München, Federal Republic of Germany) (6) and the resulting 2-acylglycerols isolated by TLC (7), transmethylated and the methyl esters analyzed by GC as described.

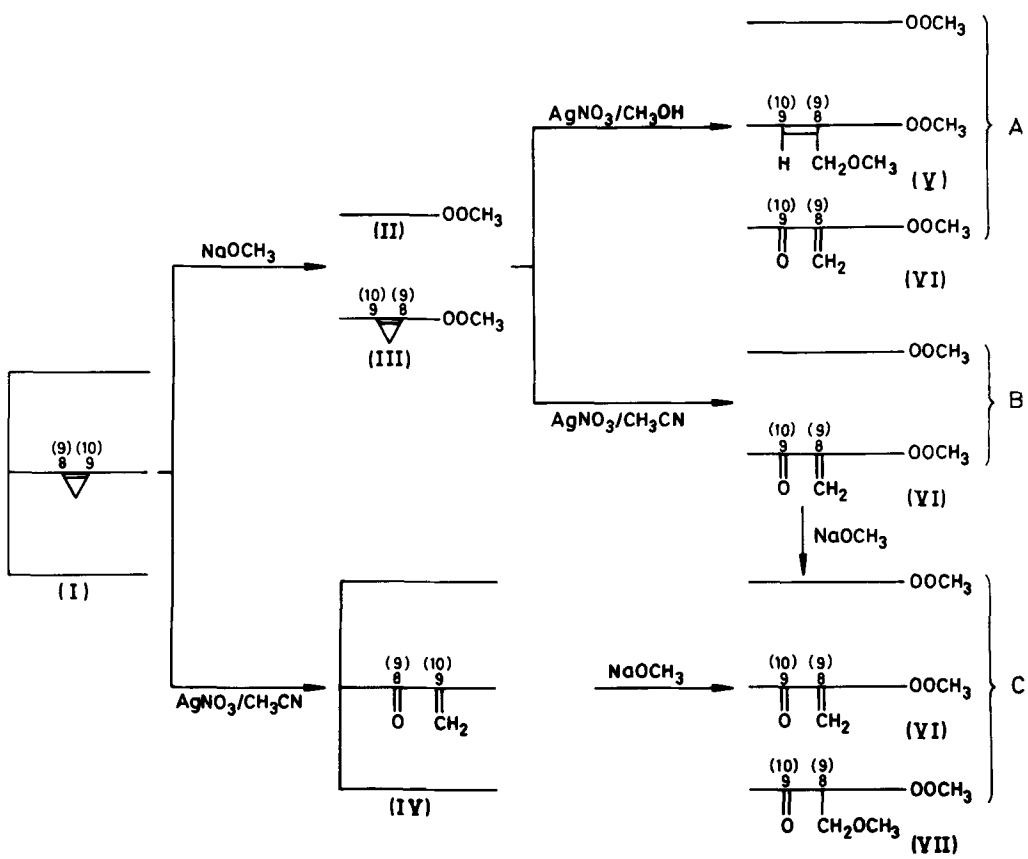
Aliquots of the molecular species of triacylglycerols were analyzed directly by GC on glass columns (50 cm × 4 mm) packed with 3% (wt/wt) OV-1 on Gas-Chrom-Q (100–120 mesh). Nitrogen (60 ml/min) was used as carrier gas, and the column temperature was programmed from 250 C to 350 C at 4 C/min. Peaks of triacylglycerols having various carbon numbers were identified and quantitated as described for methyl esters. The relative proportion of the individual molecular species in the total triacylglycerols was determined by GC of their methyl esters using methyl heptadecanoate as internal standard in a manner similar to that described for quantitation of lipid classes (8).

Identification of keto derivatives. The methyl esters of keto derivatives obtained by transmethylation of the triacylglycerols after treatment with $AgNO_3$/acetonitrile were isolated by preparative GC on 10% (wt/wt) OV-101 on Gas-Chrom-Q (100–120 mesh) at 230 C using helium (40 ml/min) as carrier gas. The fractions were monitored by a thermal conductivity detector and collected in glass tubes (25 cm × 6 mm) that were loosely packed with glass wool. The fractions subsequently were eluted with hexane and analyzed by mass spectrometry in a CH 7 instrument (Varian-MAT, Bremen, Federal Republic of Germany). Samples were introduced from the direct insertion probe at 70 eV.

RESULTS AND DISCUSSION

Considerable attention has been paid to the analysis of lipids containing CPEFA moieties, because of their unusual physiological properties (9). The most commonly used method of estimation of CPEFA moieties by GC is that described by Schneider et al. (3), which is based on derivatization of the cyclopropene groups with $AgNO_3$/methanol (4). The reaction steps carried out in this method (Scheme 1, A) involve the conversion of the cyclopropene groups of the methyl esters (III) into a mixture of methoxy ether (V) and α,β-unsaturated ketone (VI) followed by their separation and quantitation by GC. Recently Fisher and Schuller (10) demonstrated that even methyl esters containing cyclopropene groups can be reliably analyzed by GC without decomposition if well conditioned glass columns packed with a non-polar stationary phase are used.

We report here the acyl composition of Munguba seed lipids containing CPEFA moieties. The acyl composition was determined by GC analysis of derivatized methyl esters (3) on Silar-5CP columns and underivatized methyl esters using both polar (Silar-5CP) and non-polar (OV-101) columns. The results (Table 1) show a close agreement between the values obtained with derivatized and underivatized methyl esters. It appears that, under the conditions used, the decomposition of underivatized CPEFA moieties on both polar and non-polar packed glass columns are insignificantly small compared to the decomposition found with capillary columns (11). It should be noted that methyl malvalate is not well resolved



SCHEME 1. Reactions involved in the analysis of triacylglycerols containing CPEFA moieties.

from methyl linoleate on Silar-5CP column. On the other hand, unsaturated methyl esters are not separable on OV-101 column, but methyl esters containing cyclopropene groups are well resolved from the other methyl esters. Therefore, the acyl composition of lipids containing CPEFA moieties can be established from the data obtained by separations on both Silar-5CP and OV-101 columns without derivatizing the cyclopropene groups.

The structure of triacylglycerols containing CPEFA moieties has received virtually no attention despite the biological significance of such lipids. Separation of triacylglycerols containing CPEFA moieties into individual molecular species differing in the number of olefinic bonds cannot be accomplished by argentation TLC, because the cyclopropene groups readily react with $AgNO_3$ (4,12). Direct separation of intact triacylglycerols containing CPEFA moieties by GC is also not feasible because of the instability of cyclopropene groups at high temperature. In an attempt to analyze the triacylglycerols containing CPEFA moieties in *S. foetida* seed, the cyclopropene groups were hydrogenated and the resulting triacylglycerols subjected to GC; however, the peaks were found to be poorly resolved for proper identification and quantitation (13).

Considering the above difficulties, we have used a novel approach for determining the structure of triacylglycerols in Munguba seed, which involves conversion of the cyclopropene groups into stable derivatives followed by fractionation with the aid of TLC and GC. The derivatization used is based on the experiments of Kircher (4), who found that the cyclopropene group reacts with $AgNO_3$ in

TABLE 1

Composition of Acyl Moieties of Total Lipids from Munguba Seed

Acyl moieties[a]	Composition % (equivalent chain length, ECL)		
	Underivatized methyl esters		Methyl esters derivatized with $AgNO_3$/methanol
	Silar-5CP	OV-101	Silar-5CP
16:0	53.8	52.9	51.8
18:0	3.4	2.9	3.1
18:1	7.0 (18.25)	12.9 (17.68)	6.7 (18.25)
18:2	6.9 (18.80)[b]		6.6 (18.80)
20:0	1.2	0.5	1.5
19:CE	27.4 (19.20)	27.6 (18.40)	27.5[c]
18:CE	(18.80)	1.1 (17.40)	0.9[c]
Unidentified	(19.20)	1.7 (18.78)	1.6 (19.30)

[a]Acyl moieties are designated by number of carbon atoms:number of double bonds; 19:CE, sterculoyl; 18:CE, malvaloyl.

[b]Includes 18:CE.

[c]Calculated as the sum of methoxy (ECL 21.05 and 22.20) and keto (ECL 23.25 and 24.35) derivatives of 19:CE and 18:CE, respectively.

TABLE 2

Composition (%) of Acyl Moieties of Molecular Species of Triacylglycerols in Munguba Seed Obtained by Derivatization with $AgNO_3$/Acetonitrile

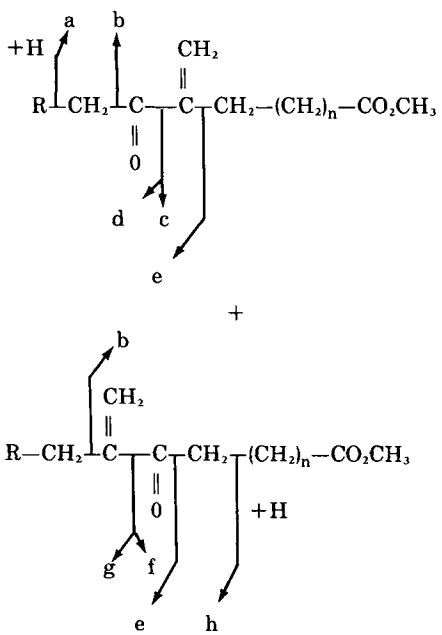
Acyl moieties[a]	Total triacylglycerols	Molecular species according to number of keto groups			
			0	1	2
		Relative proprotion (%)	41	50	9
16:0	59.7		62.7	58.1	35.3
18:0	3.3		3.6	3.0	2.3
18:1	7.0		14.5	1.9	2.0
18:2	6.1		12.5	1.7	1.3
20:0	1.3		2.1	0.7	0.5
19:CE	19.8[b]		1.0[b]	32.5[b]	53.0[b]
18:CE	1.3[b]			1.3[b]	4.7[b]
Unidentified	2.1		3.5	1.0	0.8

[a]See Table 1.

[b]Calculated as the sum of keto (ECL 23.25 and 24.35) and methoxy keto (ECL 25.35 and 26.50) derivatives of 19:CE and 18:CE, respectively.

the presence of nonhydroxylic solvents (CH_3CN, CH_3COCH_3) to produce only α,β-unsaturated ketone (VI), as shown in Scheme 1,B. We have treated the triacylglycerols from Munguba seed with $AgNO_3/CH_3CN$, in order to convert the CPEFA moieties into the corresponding acyl moieties containing α,β-unsaturated keto groups (IV, Scheme 1,C). The derivatized triacylglycerols are then fractionated by TLC into molecular species containing none, one and two keto (corresponding to none, one and two cyclopropene) acyl moieties per molecule (Fig. 1). The composition of the acyl moieties of each of these molecular species is determined by GC, after transmethylation (Table 2). It was observed, however, that the α,β-unsaturated keto group also reacts partially with $NaOCH_3$ during the transmethylation of the derivatized triacylglycerols (IV) to form about 50% of the product VII in addition to the expected methyl ester VI (Scheme 1,C). The structures of the compounds VI and VII were established as the expected α,β-unsaturated ketone and the methoxy keto compound, respectively, as described below. The products VI and VII are clearly separable by TLC (Rf 0.19 and 0.06, respectively) on silica gel H using hexane:diethyl ether (90:10, v/v), and they give distinctly separated peaks in GC. The percentage of CPEFA moieties in triacylglycerols is calculated as the sum of the peaks due to both methyl esters VI and VII.

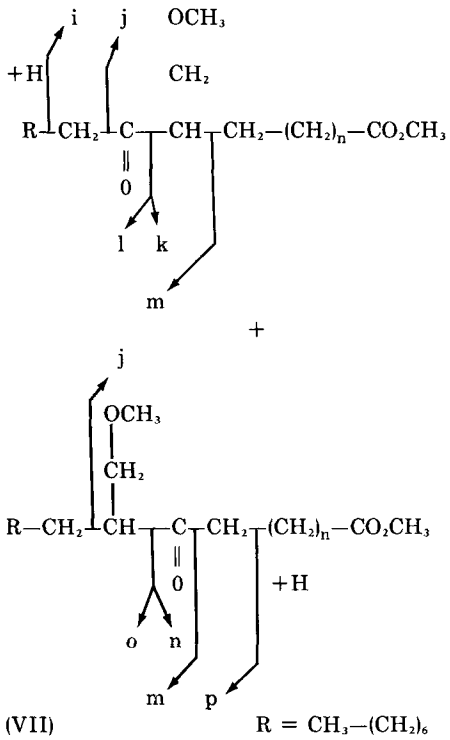
The identity of the compounds VI and VII was established by mass spectrometry. The pattern of fragmentation, the characteristic fragments and their percent of relative intensity (RI) in the mass spectra are given below:



(VI) R = CH_3—$(CH_2)_6$

Sterculate derivatives (n = 6). m/z 325 (M+1, RI 6), 324 (M^+, RI 26), 293 (M-31, RI 11), 292 (M-32, RI 12), 226 (a, RI 6), 212 (b+1, RI 2), 211 (b, RI 6), 195 (a-31, RI 9), 194 (a-32, RI 7), 185 (f, RI 2), 183 (c, RI 5), 182 (h, RI 18), 180 (b-31, RI 7), 179 (b-32, RI 8), 167 (e, RI 15), 154 (f-31 RI 2), 153 (f-32, RI 5), 151 (c-31, RI 19), 141 (d, RI 6), 139 (g, RI 6).

Malvalate derivatives (n = 5). m/z 311 (M+1, RI 4), 310 (M^+, RI 13), 279 (M-31, RI 7), 278 (M-32, RI 7), 212 (a, RI 4), 198 (b+1, RI 2), 197 (b, RI 4), 182 (h, RI 12), 181 (a-31, RI 9), 180 (a-32, RI 7), 171 (f, RI 2), 169 (c, RI 4), 167 (e, RI 11), 166 (b-31, RI 6), 165 (b-32, RI 11), 141 (d, RI 6), 140 (f-31, RI 2), 139 (g and f-32, RI 8), 138 (c-31, RI 8), 137 (c-32, RI 19).



(VII) R = CH_3—$(CH_2)_6$

Sterculate derivatives (n = 6). m/z 357 (M+1, RI 1), 356 (M^+, RI 5), 325 (M-31, RI 7), 324 (M-32, RI 6), 293 (325-32, RI 4), 292 (324-32, RI 2), 258 (i, RI 11), 244 (j+1, RI 16), 243 (j, RI 5), 227 (i-31, RI 3), 226 (i-32, RI 16), 215 (k, RI 3), 214 (p, RI 13), 212 (j-31, RI 10), 211 (j-32, RI 15), 200 (m+1, RI 24), 199 (m, RI 18), 185 (n, RI 72), 184 (k-31, RI 4), 183 (p-31, RI 5), 182 (p-32, RI 9), 171 (o, RI 3), 154 (n-31, RI 2), 153 (n-32, RI 9), 141 (l, RI 18).

Malvalate derivatives (n = 5). m/z 343 (M+1, RI 1), 342 (M^+, RI 4), 311 (M-31, RI 5), 310 (M-32, RI 4), 279 (311-32, RI 4), 278 (310-32, RI 2), 244 (i, RI 8), 230 (j+1, RI 7), 229 (j, RI 3), 214 (p, RI 10), 213 (i-31, RI 4), 212 (i-32, RI 13), 201 (k, RI 6), 200 (m+1, RI 16), 199 (m, RI 16), 198 (j-31, RI 13), 197 (j-32, RI 10), 183 (p-31, RI 3), 182 (p-32, RI 12), 171 (n and o, RI 55), 170 (k-31, RI 3), 141 (l, RI 14), 140 (n-31, RI 3), 139 (n-32, RI 11).

It is interesting to note that the compound VII is not formed when cyclopropene methyl esters (III) are reacted with $AgNO_3/CH_3CN$ (Scheme 1, B), but a treatment of the α,β-unsaturated ketone (VI) with $NaOCH_3$ shows the formation of the methoxy keto compound (VII) (Scheme 1, B and C). The product VII probably is formed by a base catalyzed addition of methoxy ion to the α,β-unsaturated keto group.

It can be seen from the acyl composition and from the ratio of keto to normal acyl moieties (Table 2) that one predominant molecular species of triacylglycerols (41% of total triacylglycerols) in Munguba seed contains no CPEFA moieties. The most predominant molecular species of triacylglycerols (50% of total) carries one

cyclopropene group per molecule, since it contains approximately one-third (34%) of keto acyl moieties in the total acyl moieties. A minor molecular species (9% of total triacylglycerols) carries two cyclopropene groups per molecule, since it has about two-thirds (58%) keto acyl moieties in total acyl moieties.

The acyl composition at the *sn*-2 position of the individual molecular species of triacylglycerols of Munguba seed is given in Table 3. It can be seen that sterculoyl and malvaloyl moieties are preferentially located at the *sn*-2 position of the triacylglycerol molecule. In this context it should be noted that in triacylglycerols of *Bombacopsis glabra* oil most of the sterculoyl moieties are also present in the *sn*-2 position (14).

The two major molecular species of triacylglycerols of Munguba seed containing none and one α,β-unsaturated kept (corresponding to none and one cyclopropene) group per molecule were separated by GC into component triacylglycerols differing in carbon number (Fig. 1). The results obtained by GC of triacylglycerols are given in Table 4.

The major triacylglycerol species of *Bombax munguba* lipids are 1,3-dipalmitoyl-2-oleoylglycerol, 1,3-dipalmitoyl-2-linoleoylglycerol and 1,3-dipalmitoyl-2-sterculoylglycerol.

TABLE 3

Positional Distribution[a] of Acyl Moieties in Molecular Species of Triacylglycerols of Munguba Seed Obtained by Derivatization with $AgNO_3$/Acetonitrile

Acyl moieties[b]	Molecular species according to number of keto groups					
	0		1		2	
	sn-2	*sn*-1,3	*sn*-2	*sn*-1,3	*sn*-2	*sn*-1,3
16:0	14.2	87.0	3.4	85.5	4.7	50.3
18:0	2.2	4.3	0.8	4.1	1.0	3.0
18:1	37.7	2.9	8.0		5.3	0.4
18:2	33.8	2.5	6.9		4.0	
20:0	3.5		0.6	0.6	0.3	0.6
19:CE	2.9[c]		76.6[c]	10.5[c]	80.7[c]	39.2[c]
18:CE			2.6[c]	0.7[c]	3.0[c]	5.6[c]
Unidentified	5.6	1.4	1.1	1.0	0.3	0.7

[a]Composition of acyl moieties at the *sn*-2 position was determined by lipolysis with pancreatic lipase; composition of the acyl moieties at the *sn*-1,3 positions was calculated.
[b]See Table 1.
[c]Calculated as the sum of keto and methoxy keto derivatives as given in Table 2.

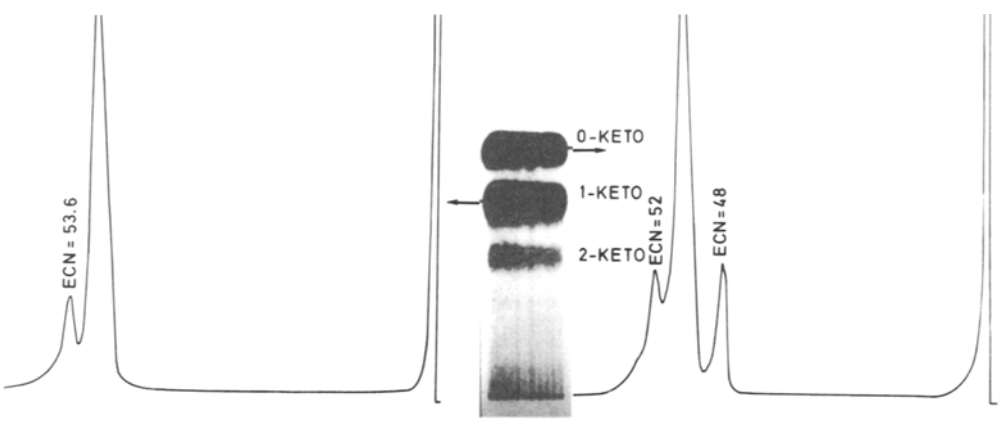


FIG. 1. Fractionation by TLC of triacylglycerols of Munguba seed after derivatization with $AgNO_3$/acetonitrile into molecular species according to the number of keto groups and subsequent GC analysis of the major molecular species.

TABLE 4

Triacylglycerol Composition of Munguba Seed

Molecular species according to number of cyclopropene groups per molecule	Carbon number	Major triacylglycerol species			Content %
0	48	16:0	16:0	16:0	12.3
	50	16:0	18:1	16:0	66.5
		16:0	18:2	16:0	
	52	16:0	18:1	18:0	20.5
		16:0	20:0	16:0	
		16:0	18:2	18:0	
1	51[a]	16:0	19:CE	16:0	87.0
	53[b]	16:0	19:CE	18:0	13.0

[a]Equivalent carbon number (ECN) = 52.40.
[b]ECN = 53.60.

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