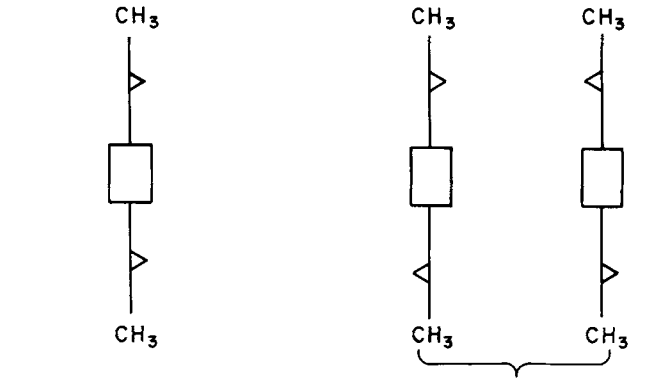

FIG. 1.

2C presumably are the expected *meso* isomer and the racemic mixture, but further work is required to make a definite configurational assignment.

Ethylene glycol *bis*-9,10-dihydroxystearate was also obtained as two solid forms. One solid, crystallized from acetone, melted at 101.7–102.0C, while the other crystallized from toluene, melted diffusely at 101.5–106.5C. Since these two materials are interconvertible so that their melting point is a function of the crystallization solvent, they are believed to be polymorphic forms of the same optical isomers, either the *meso* form or the *dl*-mixture. The expected second isomer was not isolated.

ACKNOWLEDGMENTS

Helpful discussions with J. S. Showell. Elemental analyses by Mrs. R. B. Campbell; X-ray studies by D. A. Lutz.

REFERENCES

1. Maerker, G., W. C. Ault and W. S. Port, JAOCS *40*, 193–196 (1963).
2. Murai, K., G. Akazome and S. Nishiyama, J. Chem. Soc. Japan, Ind. Chem. Sec. *63*, 280–283 (1960).
3. Findley, T. W., D. Swern and J. T. Scanlan, J. Am. Chem. Soc. *67*, 412–414 (1945).
4. Durbetaki, A. J., Anal. Chem. *28*, 2000–2001 (1956).
5. Showell, J. S., JAOCS *36*, 343–345 (1959).
6. Haeberer, E. T., and G. Maerker, *Ibid. 40*, 274–275 (1963).
7. Gelb, L. L., W. C. Ault, W. E. Palm, L. P. Witnauer and W. S. Port, J. Chem. Eng. Data *5*, 226–230 (1960).
8. Port, W. S., and D. M. Komanowsky, J. Applied Polymer Sci. *7*, 281–285 (1963).
9. Hess, K., and H. Frahm, Ber. *71*, 2627–2636 (1938).
10. Swern, D., L. P. Witnauer and H. B. Knight, J. Am. Chem. Soc. *74*, 1655–1657 (1952).
11. Parker, R. E., and N. S. Isaacs, Chem. Revs. *59*, 737–799. (1959).

[Received March 3, 1964—Accepted May 1, 1964]

Analysis of Triglycerides by Consecutive Chromatographic Techniques. I. *Cuphea llavia* Seed Fat[1]

CARTER LITCHFIELD, MARGARET FARQUHAR and RAYMOND REISER, Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, College Station, Texas

Abstract

The triglycerides of *Cuphea llavia* var. *miniata* seed fat were separated according to the number of double bonds/molecule using preparative thin layer chromatography (TLC) on silicic acid impregnated with silver ion. The recovered fractions were quantitated by the chromotropic acid technique. Each fraction was then separated according to mol wt using gas-liquid chromatography (GLC). This multiple chromatography procedure resolved *C. llavia* triglycerides into 17 different components.

The triglyceride composition of *C. llavia* seed fat was calculated from the above results. Since the fat contains 91.2 mole % decanoic acid, it was expected that each triglyceride molecule would contain at least two molecules of decanoic acid. Results showed this to be generally true, but several minor component triglycerides not conforming to this pattern were found.

Introduction

THE PAST FIVE YEARS have been exciting times in the search for new analytical techniques for determining the triglyceride composition of natural fats. Techniques recently introduced in this field have included: pancreatic lipase hydrolysis (1,2), silver ion (Ag^+) chromatography (3,4), GLC (5,6), liquid-liquid partition chromatography (7,8,9), and various chromatographic methods for separating oxidized triglycerides (10,11).

The triglyceride compositions of most natural fats are so complex that no one analytical technique can completely resolve all components. Until recently, most workers have utilized only one separation method to study natural triglyceride mixtures. Considerably more information can be obtained, however, by the successive application of several separation techniques to natural triglycerides. For example, Youngs (10) has used permanganate-periodate oxidation on a number of fats and then analyzed the resultant triglyceride "cores" by liquid-liquid partition chromatography and lipase hydrolysis. Privett and Blank (11) have separated triglyceride ozonides by adsorption chromatography and then rechromatographed each hydrogenated fraction by the same method.

The present work was undertaken to demonstrate how a combination of Ag^+ chromatography and GLC can be used to characterize the triglyceride composition of *Cuphea llavia* seed fat. Triglycerides were first separated according to the number of double bonds/molecule using Ag^+ chromatography. The recovered fractions were then analyzed by GLC to determine the mol wt of the triglycerides present. The triglyceride composition of the total fat was then calculated from these results.

Cuphea llavia var. *miniata* (also known as *Cuphea miniata* var. *firefly*) is a subtropical ornamental shrub cultivated in the southern United States where it is commonly called the "Mexican cigar flower." The fatty acid composition of *C. llavia* seed fat has been investigated by Earle et al. (12) and found to contain 83 wt % decanoic acid. The triglyceride composition of this fat has not been previously reported.

Procedures

Materials

Cuphea llavia var. *miniata* seeds (Thompson & Morgan, Ipswich, England) were sorted to remove damaged seeds and foreign material. 11.58 g cleaned

[1] Presented at the AOCS Meeting in Minneapolis, 1963.

seed were ground with 50 ml distilled petroleum ether (bp 30–60C) for 3 min in a Waring Blendor. The resulting slurry was quantitatively transferred to a filter paper thimble and placed in a Soxhlet extraction apparatus where it was extracted with 500 ml distilled petroleum ether for 4 hr. The total extract was eluted over 20 g activated Florisil (magnesium silicate, Floridin Co., Tallahassee, Fla.) to which 7% water had been added (13). The column of Florisil was then flushed with 500 ml distilled benzene to remove all triglycerides but leave behind fatty acids, partial glycerides and other polar lipids. Evaporation of the total eluate yielded 2.67 g *C. llavia* triglycerides. A trace of hydrocarbon impurity (probably carotene) remained in these triglycerides, but this did not interfere with the subsequent analyses.

Methods

Thin Layer Chromatography. The procedure of Barrett et al. (4) for separating triglycerides by Ag^+ TLC was adapted for use on a preparative scale; 65 g Merck Silica Gel G was mixed with 130 ml of a 12.5% (w/v) aqueous solution of $AgNO_3$ and stirred in a mortar and pestle to remove air bubbles. The slurry was quickly applied in a 1.0-mm thick layer to three 20 x 20 cm glass plates. The plates (each containing ca. 20 g Silica Gel G) were air dried for 40 min and then activated in an atmosphere of nitrogen at 110C for 45 min. The inert nitrogen prevented darkening of the adsorbent layer at the higher temp. After activation, the plates were stored in a desiccator away from light.

Approximately 100 mg *C. llavia* triglyceride was diluted 1:1 in petroleum ether and applied as a band across the base of the TLC plate using a microsyringe. The plate was then placed in a stream of nitrogen to evaporate all of the solvent before development. Plates were developed by the usual ascending technique using a mixture of 1% ethanol in alcohol-free chloroform. (The 0.5–1.0% ethanol added to reagent $CHCl_3$ as a stabilizer was removed immediately prior to use by eluting 500 ml $CHCl_3$ over 20 g activated alumina, Brockman activity grade 1). Each plate was developed twice; i.e., after the solvent front had reached the desired height, the plate was removed from the solvent, dried under nitrogen, and then replaced in the same tank to develop again in the same direction. This double development procedure gave better resolution with large samples sizes. After the second development, the plate was dried with nitrogen, sprayed with a 0.02% solution of sodium fluorescein (Uranine), and viewed under long wave UV light. Four distinct bands were separated corresponding to triglycerides having 0,1,2 and 3 or more double bonds/molecule. The identity of these bands was checked by comparison with the R_f values of known triglycerides and by fatty acid analysis.

Each of the four bands on the TLC plate was marked off, scraped off the glass plate and placed in a chromatography column containing 2–3 g activated silicic acid (Mallinkrodt, suitable for chromatographic analysis by the method of Ramsey and Patterson). The triglyceride sample was then eluted from the column with 50 ml dry distilled ether. The silicic acid at the bottom of the column prevented any sodium fluorescein from eluting, but did not hold back the triglycerides. Elution with benzene gave equivalent results.

Quantitation of Triglyceride Fractions. The amount of triglyceride in each TLC fraction was determined using the chromotropic acid color reaction of Van-Handel (14). Values reported in Figure 1 are an average of six determinations from three TLC separations.

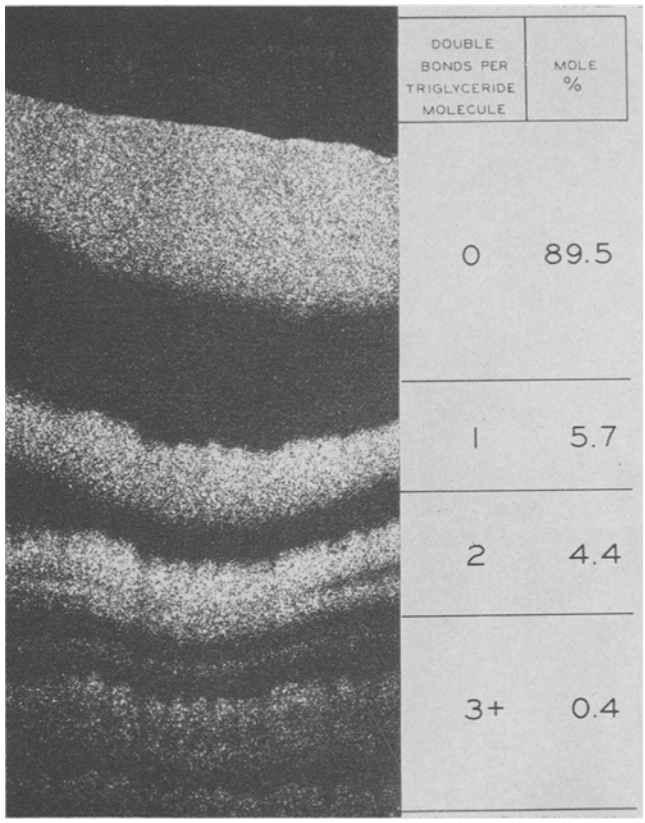

FIG. 1. TLC of 100 mg *Cuphea llavia* triglycerides on 1.0 mm thick Silica Gel G impregnated with 25% (w/w) $AgNO_3$. The plate was developed twice using 1% ethanol in alcohol-free chloroform, sprayed with sodium fluorescein solution and photographed under long wave UV light. Triglycerides were recovered from the four indicated bands and quantitated by the chromotropic acid technique.

Gas-Liquid Chromatography of Fatty Acids. 5–90 mg of each triglyceride sample was refluxed with 4 ml 2% (v/v) H_2SO_4 in methanol and 1 ml petroleum ether (bp 30–60C) for 4 hr; 10 ml water was then added, and the methyl esters were extracted with a minimum volume (2–4 ml) petroleum ether and injected directly into the gas chromatography. To avoid any loss of low mol wt esters, no solvent was evaporated at this point. The fatty acid methyl esters were analyzed on an Aerograph HyFi A-600-B gas chromatograph equipped with a flame detector and a 6 ft x ⅛ in. column containing 20% (w/w) diethylene glycol succinate polyester coated on acid washed 60/80 mesh Chromosorb W. The column was operated isothermally at 150–165C with 30 ml/min flow of nitrogen carrier gas. Peaks were identified by comparison with the elution times of known compounds on the packed polyester column, on a 100 ft x 0.01 in. Apiezon L capillary column (15), and on a 5 ft x ⅛ in. column containing 5% (w/w) SE-30 silicone coated on 60/80 mesh Chromosorb W, programed from 85–215C. Peak areas were measured by triangulation. Quantitative response factors for the various methyl esters were determined from mixtures of known composition, and peak areas were corrected accordingly. All fatty acid compositions are reported in mole per cent to better show their relationship to triglyceride composition.

Gas-Liquid Chromatography of Triglycerides. GLC

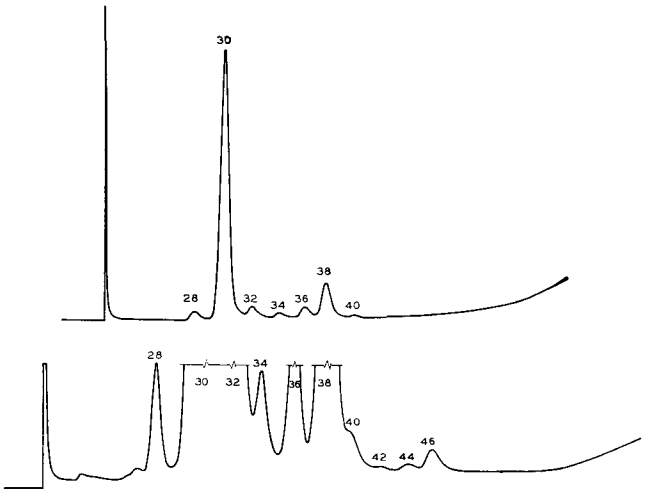

FIG. 2. Gas chromatograms of total triglycerides from *Cuphea llavia* seed fat. Peaks labeled by carbon number. GLC conditions: ⅛ in. x 18 in. column packed with 2.6% SE-30 silicone on acid washed 60/80 mesh Chromosorb W; 108 ml/min nitrogen carrier gas; column temp programed manually from 170–325C at 2–3C/min.

of triglycerides was accomplished using the general procedure described by Kuksis and McCarthy (6). Analyses were run on an Aerograph HyFi A-600-B gas chromatograph equipped with a flame detector and an 18 in. x ⅛ in. column packed with 2.6% (w/w) SE-30 silicone on acid washed 60/80 Chromosorb W. The column was manually programed from 170–325C at 2–3C/min with a nitrogen carrier gas flow of 108 ml/min. Because manual temp programing gives only approx programing speeds, chromatograms vary slightly in length (see Figs. 2 and 3). GLC on SE-30 separates triglycerides by mol wt, and each peak is referred to by its carbon number (the number of carbon atoms in the fatty acid moiety of the triglyceride). Peaks were identified by comparison with the elution times and temp of known compounds. Quantitative response factors for the various triglycerides were determined from known composition mixtures made from 99+% pure tridecanoin, trilaurin, trimyristin and tripalmitin (Applied Science Laboratories, State College, Pa.). All triglycerides of the same mol wt were assumed to have the same quantitative response factor. Peaks were quantitated by tracing them on vellum paper, cutting them out, erasing pencil marks and weighing them. This allowed accurate quantitation of the tail areas encountered in some GLC peaks. Quantitative results are reported in mole per cent values to better show the relationships between fatty acid and triglyceride composition.

Lipase Hydrolysis. Pancreatic lipase hydrolysis of triglycerides was carried out using the technique of Mattson and Volpenhein (1). The ether extracted hydrolysis products were separated by TLC on 1.0-mm thick Merck Silica Gel GF using 67% diethyl ether, 31% petroleum ether and 2% acetic acid (v/v/v) as the eluting solvent. The monoglyceride band was located by fluorescence under short wave UV light, scraped off the TLC plate, and eluted with ether and methanol. The monoglyceride was then converted to its corresponding methyl esters by H_2SO_4 catalyzed methanolysis, and their composition was analyzed by GLC as described above.

Experimental

The fatty acid composition of *Cuphea llavia* var. *miniata* seed fat triglycerides was analyzed by GLC (Table I). The triglycerides contained an unusually high amount of decanoic acid (91.2 mole %). Other even chain length saturated acids from octanoic through arachidic were present in small amounts. Traces of nonanoic and undecanoic acids were also found. The main unsaturated fatty acids were oleic (2.2%) and linoleic (2.6%), although a very small amount linolenic (0.1%) was also present.

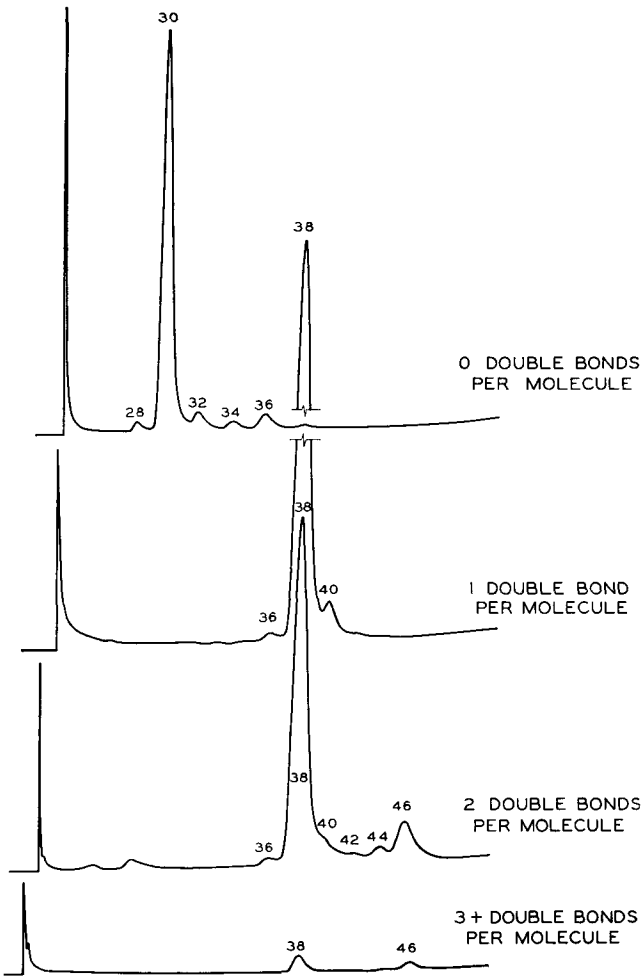

FIG. 3. Gas chromatograms of 0,1,2 and 3+ double bond *Cuphea llavia* triglyceride fractions separated by silver ion chromatography. Peaks labeled by carbon number. GLC conditions same as Figure 2.

100 mg *C. llavia* triglycerides was separated by preparative TLC on Ag^+ impregnated silicic acid. Four distinct bands were resolved corresponding to triglycerides having 0,1,2 or 3 or more double bonds/triglyceride molecule (Fig. 1). The 0 and 1 double bond bands appeared homogeneous, but the 2 double bond band showed partial resolution of two components. Barrett et al. (4) have reported the resolution of 1-stearo-2, 3-diolein from 1-linoleo-2,3-distearin and of 2-linoleo-1,3-distearin from 1-linoleo-2,3-distearin using analytical Ag^+ TLC. The 2 double bond band probably contained two similarly resolvable components. The 3+ double bond band was resolved into four different components indicating that this fraction was rather complex.

Each of the four bands was scraped off the glass TLC plate, and the triglycerides were eluted with ether or benzene. The amount of triglyceride in each eluted TLC fraction was then quantitated by the chromotropic acid technique. Results (Fig. 1) indicated that the 0 double bond fraction represented most

TABLE I

Fatty Acid Composition of *Cuphea llavia* Triglycerides (mole per cent)

Fatty acid	Total triglycerides	TLC fractions[a]		
		0	1	2
8:0	0.7	0.6	0.3	0.5
9:0	0.1	tr	tr	tr
10:0	91.2	94.4	63.4	58.3
11:0	tr	....	....	....
12:0	1.1	1.8	0.8	0.7
14:0	0.6	0.8	0.4	0.5
16:0	1.1	2.0	0.9	2.2
18:0	0.2	0.4	....	0.4
18:1	2.2	....	34.2	4.8
18:2	2.6	....	....	32.5
18:3	0.1	....	....	....
20:0	tr	tr	....	....
Unknown	0.1	....	....	....

[a] TLC fractions separated according to the number of double bonds/triglyceride molecule.

TABLE II

Triglyceride Composition of *Cuphea llavia* Triglycerides Determined by Multiple Chromatography (mole per cent)

Triglyceride carbon number	Total triglycerides	TLC fractions[a]			
		0	1	2	3+
28	2.6	2.0	....	....	....
30	82.9	81.4	....	....	....
32	2.5	2.5	....	....	....
34	1.0	1.2	....	....	....
36	2.4	2.2	0.1	0.1	....
38	7.8	0.2	5.4	3.7	0.3
40	0.2	....	0.2	tr	....
42	tr	....	....	tr	....
44	0.1	....	....	0.1	....
46	0.5	....	....	0.5	0.1
Total	100.0	89.5	5.7	4.4	0.4

[a] TLC fractions separated according to the number of double bonds/triglyceride molecule.

of the triglycerides present (89.5%). The 1 double bond fraction (5.7%) and the 2 double bond fraction (4.4%) each contained about one-twentieth of the total triglyceride, while the amount of 3+ double bond material was very small (0.4%).

The fatty acid compositions of those TLC fractions where there was enough material were determined by GLC of the corresponding methyl esters. Results (Table I) indicated that TLC separation was quantitative. The 0 double bond fraction contained only saturated fatty acids. The 1 double bond fraction contained one-third oleic acid and two-thirds saturated acids. The 2 double bond fraction contained slightly less than one-third linoleic acid plus a small amount of oleic acid with the rest being saturated. The presence of oleic acid in the 2 double bond fraction apparently indicated that some dioleo-triglycerides were present in this material.

The carbon number distributions of the total *C. llavia* triglycerides and of each of the TLC fractions were determined by GLC on an SE-30 column. GLC of the total triglycerides (Fig. 2 and Table II) showed that triglycerides of even carbon number from 28 through 46 were present, although the amount of material of carbon number 40 and higher was less than one per cent. As expected, the major peak occurred at carbon number 30 corresponding to tridecanoin. GLC of the 0 double bond fraction (Fig. 3 and Table II) indicated that its composition resembled that of the total fat except that the 38 carbon number peak was greatly reduced in size. This would, of course, have been expected if the oleic and linoleic acids were present as oleo-didecanoin and linoleo-didecanoin, which both have a carbon number of 38. GLC of the 1 double bond fraction showed it to be mainly 38 carbon number material as expected. GLC of the 2 double bond fraction indicated that its major component had a carbon number of 38, but considerable carbon number 46 material was also present. Dioleo-decanoin would, of course, have a carbon number of 46, so this chromatogram provided further evidence for the presence of dioleo-triglycerides in the 2 double bond fraction. The 3+ double bond fraction contained both 38 and 46 carbon number triglycerides. No evidence of odd carbon number triglycerides attributable to nonanoic and undecanoic acids was found in these chromatograms. Resolution of GLC peaks, however, was probably not sufficient to detect very small amounts of odd carbon number triglycerides if they were present.

Combining the TLC and GLC data on *C. llavia* seed fat, one can calculate the triglyceride composition of the fat in terms of 17 different components. These results are shown in Table II.

The total *C. llavia* triglycerides were hydrolyzed with pancreatic lipase. The resultant monoglycerides were isolated by TLC, and their fatty acid composition was determined by GLC. The distribution of the eight major fatty acids between the 2- and 1,3-positions was then calculated using the formula of Mattson and Volpenhein (1). Results (Table III) indicated that only 8:0 and 10:0 were widely distributed between the 2- and 1,3-positions. The remaining acids were all predominantly located in the 1,3-positions.

Discussion

By using a combination of TLC and GLC, *C. llavia* seed fat triglycerides have been separated into 17 different components (Table II). But even with these 17 components, we have probably not completely resolved all the different triglycerides present. In most cases, however, we can make a good approximation of the identity of each component. For example, the 0 double bond component of carbon number 32 could be lauro-didecanoin, myristo-decano-octanoin, or palmitodioctanoin. When one considers that the 0 double bond fraction contains 94.4% decanoic acid, this material seems most likely to be lauro-didecanoin. Similarly, the 2 double bond component of carbon number 38 could be linoleo-lauro-octanoin or linoleo-didecanoin. Of these two possibilities, linoleo-didecanoin is more likely because of the high decanoic acid content of the 2 double bond fraction. Finally, the 2 double bond component of carbon number 46 could be dioleo-decanoin, linoleo-stearo-decanoin, linoleo-palmito-laurin, or linoleo-dimyristin. Of these four possibilities, dioleo-decanoin seems most probable since: a) one must account for some dioleo-triglycerides in this fraction; b) the 2 double bond fraction contains too little stearic acid to account for this component being mostly linoleo-stearo-decanoin; and c) the 2 double bond fraction contains 58.3% decanoic acid. Following this general line of reasoning, one can assume that each component is most likely to be that triglyceride containing the max amount of

TABLE III

Hydrolysis of *Cuphea llavia* Triglycerides by Pancreatic Lipase

Fatty acid	Per cent in 2-position	Per cent in 1, 3-positions
8:0	37	63
10:0	36	64
12:0	4	96
14:0	2	98
16:0	4	96
18:0	5	95
18:1	6	94
18:2	10	90

TABLE IV

Estimated Triglyceride Composition of *Cuphea llavia* Seed Fat Based on Experimental Data in Table II and Assuming Maximum Decanoic Acid Content for Each Component

Mole %	Triglyceride
2.0	Octano-didecanoin
81.4	Tridecanoin
2.5	Lauro-didecanoin
1.2	Myristo-didecanoin
2.2	Palmito-didecanoin
0.2	Stearo-didecanoin
0.1	Oleo-octano-decanoin
5.4	Oleo-didecanoin
0.2	Oleo-lauro-decanoin
0.1	Linoleo-octano-decanoin
3.7	Linoleo-didecanoin
tr	Linoleo-lauro-decanoin
tr	Linoleo-myristo-decanoin
0.1	Linoleo-palmito-decanoin + dioleo-octanoin
0.5	Dioleo-decanoin
0.3	Linoleno-didecanoin
0.1	Oleo-linoleo-decanoin

decanoic acid. This assumption is probably not entirely justified, but it should not introduce excessive error into our calculations. Using this basic assumption, one can then estimate what each of the 17 separated components is most likely to be. The results of these calculations (see Table IV) represent a "best estimate" of the actual triglyceride composition of *C. llavia* seed fat.

The estimated triglyceride composition of *C. llavia* seed fat given in Table IV can then be used to calculate the fatty acid composition of the original fat and of each separate TLC fraction. Table V shows the results of this computation. Comparison of the calculated fatty acid compositions in Table V with those determined experimentally in Table I shows moderately good agreement, indicating that our estimated triglyceride composition is moderately accurate. However, the calculated decanoic acid contents are slightly higher than the experimental values, while the calculated values for lauric, myristic, palmitic, and stearic acids are too low. This indicates that a small amount of triglyceride material is present which does not contain the max amount of decanoic acid as we have assumed. For example, we have no triglycerides in our estimated composition to account for the myristic and palmitic acids found in the 1 double bond TLC fraction. For this reason, the triglyceride composition shown in Table IV is probably incomplete and represents only a best estimate value based on presently available information. When more refined techniques become available for triglyceride analysis, it seems likely that further minor component triglycerides may be found in *C. llavia* seed fat.

Pancreatic lipase is known to hydrolyze short chain fatty acids faster than long chain fatty acids (20), and this effect must be considered with *C. llavia* triglycerides. Jack et al. (21) have made a detailed investigation of this phenomenon using synthetic triglycerides and butter fat. They have concluded that lipase hydrolysis followed by analysis of the fatty acids in the resultant monoglycerides does show valid general relationships in fatty acid positional specificity, but not with absolute quantitative results Therefore, the lipase data in Table III may give a general indication of the positional specificities of the various fatty acids in *C. llavia* triglycerides, but any quantitative interpretation is probably not justified. This uncertainty about lipase data accuracy precludes any useful conclusions about the positional isomers present in *C. llavia* triglycerides.

Numerous statistical methods have been proposed for calculating the triglyceride composition of a natural fat from its fatty acid composition (16,17,18).

TABLE V

Fatty Acid Composition of *Cuphea llavia* Triglycerides Calculated from Estimated Triglyceride Composition of Table IV (mole per cent)

Fatty acid	Total triglycerides	TLC fractions[a]			
		0	1	2	3+
8:0	0.7	0.7	0.6	1.1	....
10:0	93.4	97.0	64.9	60.7	58.3
12:0	0.9	0.9	1.2	0.2	....
14:0	0.4	0.4	....	0.2	....
16:0	0.8	0.8	....	0.4	....
18:0	0.1	0.1	....	....	....
18:1	2.3	....	33.3	8.2	8.3
18:2	1.3	....	....	29.2	8.3
18:3	0.1	....	....	....	25.0

[a] TLC fractions separated according to the number of double bonds/triglyceride molecule.

Underlying all such methods is the basic assumption that one is dealing only with triglycerides synthesized by a single enzyme system. *C. llavia* seeds have a thin outer hull. It is impractical to remove this hull before fat extraction since the seeds are so small. It is quite possible that this hull may contain triglycerides synthesized by a different enzyme system than the endosperm fat. If this is the case, then the seed fat examined here is a "composite" rather than a "pure" fat, and statistical methods of calculating triglyceride composition would not necessarily apply. Until we know more about any triglycerides contributed by the hull (even in trace quantities), it does not seem worthwhile to compare the triglyceride composition of the total seed fat with the various distribution hypotheses.

Multiple chromatography of natural fat triglycerides using Ag^+ TLC followed by GLC has been shown to be a highly useful technique for the separation of complex triglyceride mixtures. *C. llavia* seed fat can be separated into 17 different component triglycerides by this technique. In addition to the Ag^+ chromatography and GLC techniques combined here, other methods such as liquid-liquid partition chromatography (7,8,9), GLC of oxidized triglycerides (19), TLC of ozonized triglycerides (11), etc. might usefully be integrated into a multiple chromatography procedure for the analysis of triglyceride mixtures.

ACKNOWLEDGMENTS

This investigation supported in part by grants from the National Institutes of Health (AH-6011), Cargill, Inc., and the Corn Products Co. Assistance with gas-liquid chromatography of triglycerides by Toru Takagi of Nagoya Univesrity.

REFERENCES

1. Mattson, F. H., and R. A. Volpenhein, J. Lipid Res. *2*, 58 (1961).
2. Savary, P., J. Flanzy and P. Desnuelle, Biochim. et Biophys. Acta *24*, 414 (1957).
3. de Vries, B., Chem. Ind. 1049 (1962); Fette, Seifen, Anstrichmittel *65*, 725 (1963).
4. Barrett, C. B., M. S. J. Dallas and F. B. Padley, Chem. Ind. 1050 (1962); JAOCS *40*, 580 (1963).
5. Huebner, V. R., JAOCS *38*, 628 (1961).
6. Kuksis, A., and M. J. McCarthy, Can. J. Biochem. Physiol. *40*, 679 (1962).
7. Hirsch, J., in P. Desnuelle, editor, "Digestion, Absorption Intestinale, et Transport des Glycerides chez les Animaux Superieurs." Editions du Centre National de la Recherche Scientifique, Paris, 1961, p. 11–33; J. Lipid Res. *4*, 1 (1963).
8. Kaufmann, H. P., and Z. Makus, Fette, Seifen, Anstrichmittel *63*, 125 (1961).
9. Black, B. C., and E. G. Hammond, JAOCS *40*, 575 (1963).
10. Youngs, C. G., *Ibid. 38*, 62 (1961).
11. Privett, O. S., and M. L. Blank, *Ibid. 40*, 70 (1963).
12. Earle, F. R., C. A. Glass, G. C. Geisinger, I. A. Wolff and Q. Jones, *Ibid. 37*, 440 (1960).
13. Carroll, K. K., J. Lipid Res. *2*, 135 (1961).
14. VanHandel, E., Clin. Chem. *7*, 249 (1961).
15. Litchfield, C., R. Reiser and A. F. Isbell, JAOCS *40*, 302 (1963).
16. VanderWal, R. J., *Ibid. 37*, 18 (1960).
17. Kartha, A. R. S., J. Sci. Ind. Res. *21A*, 577 (1962).
18. Gunstone, F. D. Chem. Ind. 1214 (1962).
19. Youngs, C. G., and M. R. Subbaram, JAOCS *41*, 218–221 (1964).
20. Desnuelle, P., and P. Savary, J. Lipid Res. *4*, 369 (1963).
21. Jack, E. L., C. P. Freeman, L. M. Smith and J. B. Mickle, J. Dairy Sci. *46*, 284 (1963).

[Received January 13, 1964—Accepted April 20, 1964]