Chromatographic Studies on Fatty Acid Dimers: Gas-liquid Chromatography, High Performance Liquid Chromatography and Thin-Layer Chromatography

C.N. Christopoulou[a] and **E.G. Perkins**[b,*]

[a]Colgate-Palmolive Co., 909 River Road, Piscataway, NJ 08854, and [b]University of Illinois, Department of Food Science, 1208 W. Pennsylvania Avenue, Urbana, IL 61801

Methods for separation of various structural entities of dimers from heated fats and oils are not yet available. A series of dimers, representative of these, which may be formed in thermal oxidative reactions of fats and oils have recently been made available. In this study, the results of the analysis of a mixture of standard synthetic dimers, differing in polarity and structure, by packed column and capillary gas-liquid chromatography (GLC), high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) are presented. The HPLC method is based on the use of a octadecyl-bonded reverse phase column with acetone actonitrile (1:1, v/v) as the mobile phase with refractive index detection.

The analysis of the decomposition products from the thermal and oxidative treatment of fats and oils has been widely studied. However, systematic studies concerning separation, determination and elucidation of the chemical structure of the higher molecular weight materials such as dimers and higher polymers formed during thermal-oxidation reactions are far from complete.

Various liquid and thin-layer chromatographic methods have been used to separate dimers into polar and nonpolar fractions either from actual oxidized oils (1-5) or model lipid systems (6-8). Methods have not yet been developed to fractionate the polar or nonpolar dimeric fraction into its individual component structural dimers.

In the present study, the gas-liquid (GLC), high performance liquid (HPLC) and thin-layer chromatographic (TLC) behavior of various standard dimers prepared in our laboratory was examined in order to develop methods to allow the separation of dimers formed in fats via thermal-oxidative reactions into their component structures.

EXPERIMENTAL

Standards. The dimers used as standards in the various chromatographic studies were the monocyclic thermal dimer, monocyclic dehydrodimer and the bicyclic dimer of methyl linoleate prepared according to Wheeler and White (9), the dehydrodimer of methyl oleate prepared according to Paschke *et al.* (10), and the tetrahydroxystearate, dihydroxystearate and diketostearate dimers prepared according to Christopoulou (11).

Packed column GLC. Packed column GLC was carried out with an HP 5840-A Programmable Gas Chromatograph (Hewlett Packard Company, Avondale, PA). The effect of column polarity on dimer separation was studied with the use of three columns: each 6 ft × 2 mm i.d., glass, packed with 3% OV-1, 3% OV-17 and 3% OV-25 respectively, and coated on 80/100 Supelcoport (Supelco, Bellefonte, PA). Chromatographic conditions were as in Table 1.

Capillary column GLC. Analytical separations of the standard dimers were also carried out with a Hewlett Packard 5792A capillary gas chromatograph (Hewlett Packard, Avondale, PA) equipped with a flame ionization detector, all glass inlet splitter system, and electronic integrator HP-3390A. Two different columns were used. Column I: SPB-1 (bonded dimethyl polysiloxane), 15 m × 0.25 mm i.d.; film thickness—0.25 μm (Supelco Inc., Bellefonte, PA); and Column II: HP-5 (crosslinked 5% phenyl methyl silicone), 25 m × 0.31 mm i.d.; film thickness—0.17 μm from Hewlett Packard (Avondale, PA). The operating conditions for each column were as in Table 2.

Calculation of equivalent hydrocarbon length (EHL) and relative retention time (RRT). EHL was determined in both packed and capillary GLC studies according to

TABLE 1

Packed Column GLC Chromatographic Conditions

Chromatographic conditions	Column I (3% OV-1)	Column II (3% OV-17)	Column III (3% OV-25)
Initial temp. 1 (T_1), °C	270	300	280
Time at T_1 (t_1), min	0	0	0
Rate 1, °C/min	8	4	4
Temp. 2 (T_2), °C	330	330	330
Time at T_2 (t_2), min	25	20	20
Injector temp., °C	360	360	360
Detector FID temp., °C	360	360	360
Carrier gas	N_2	N_2	N_2
Flow rate, ml/min	35	35	35
Concentration range, mg/ml	5-10	5-10	5-10
Volume injected, μl	1-2	1-1	1-2

*To whom correspondence should be addressed.

TABLE 2

Capillary Column GLC Chromatographic Conditions

	Column I (SPB-1)	Column II (HP-5)
Gas chromatograph		
Inlet system	split mode	split mode
Split ratio	1:100	1:100
Column pressure, psi	10	7.5
Carrier gas	H_2 (99.997%)	H_2 (99.997%)
Gas flow, ml/min	30 ml/min	30 ml/min
Column temp. program		
Initial temp. 1 (T_1), °C	300	300
Time at T_1 (t_1), min	0	0
Rate 1, °C/min	1	1
Temp. 2 (T_2), °C	310	310
Time at T_2 (t_2), min	10	10
Injector temp., °C	330	330
Detector temp., °C	360	360
Concentration range, mg/ml	0.1-0.5	0.1-0.5
Volume injected, µl	1	1

the method of Miwa *et al.* (12). Relative retention times were calculated relative to tetratriacontane ($C_{34}H_{70}$) which was injected with each compound of interest.

High performance liquid chromatography. Separation studies on standard dimers. The high performance liquid chromatographic system used consisted of a Tracor 995 isochromatographic pump (Tracor, Inc., Austin, TX), and a Rheodyne 7120 loop injector equipped with a 20 µl sample loop (Rehodyn, Berkeley, CA). Chromatograms were recorded with an HP 3390-A Integrator (Hewlett-Packard, Avondale, PA). Three different types of detectors were employed: Detector I, Waters R401 Differential Refractometer (Waters Associates, Farmingham, MS); Dector II, Tracor 970 Variable Wavelength Detector (Tracor Instruments, Austin, TX) set at 205 or 232 nm; and Detector III, Dupont Liquid Chromatography Infrared Detector at 5.72 µm. Commercially-packed high performance liquid chromatographic columns were used (Supelco, Inc., Bellefonte, PA). Column I, LC-Si, 25 cm × 4.6 mm i.d., particle diameter, 5 µm; Column II, LC-18, 25 cm × 4.6 mm i.d., particle diameter, 5 µm; and Column III, LC-18, 15 cm × 4.6 mm i.d., particle diameter, 5 µm.

The various chromatographic solvents used in these studies wre distilled in glass: hexane (E.M. Science, Cherry Hill, NJ), isopropyl alcohol (A.C.S. grade, E.K. Industries, Inc., Addison, IL 60101), acetonitrile (nonspectro, Burdick & Jackson Laboratories Inc., Muskegon, MI), acetonitrile (spectro, E.M. Science, Cherry Hill, NJ), acetone (A.C.S. grade, Fisher Scientific, Fair Lawn, NJ) and methylene chloride (J.T. Baker Chemical Co., Phillsburg, NJ). The various mobile phases used were: System I, 1.5% isopropyl alcohol (IPA) in hexane. System II, acetonitrile-acetone (1:1, v/v), System III, acetonitrile (spectro) and System IV, acetonitrile-methylene chloride (3:1, v/v).

Column I and II were used with solvent systems I and II respectively and refractometry was the mode of detection. Column III was used with solvent System III and ultraviolet detection at 205 or 232 nm as well as with solvent System IV and infrared detection at 5.72 µm. The concentration of the various chromatographic samples was 20-40 mg/ml of solvent employed when refractometry or infrared spectroscopy was used as the mode of detection. When the UV detector was used, the concentration of the chromatographic samples was 1-5 mg/ml.

Thin-layer chromatography. TLC was carried out on analytical plates prepared using silica gel G (E. Merck, Darmstadt, Germany) as the adsorbent. The dr gel was mixed with water at a ratio of 1:2 and applied with a 250 µ fixed thickness applicator (Brinkman Instruments, Inc., Wetburg, NY) on 20 × 5 cm glass plates. The plates were air-dried overnight and activated prior to use for 30 minutes in an oven at 110-130°C.

Dimers to be chromatographed were diluted to a concentration of 50 mg/ml in tetrahydrofuran and spotted on the plate using a 10 µl microsyringe (Hamilton Company, Whittier, CA). The spots were developed with the appropriate solvent system. The solvent was then allowed to evaporate from the plates, and the spots were visualized by spraying with 50% sulfuric acid saturated with potassium dichromate followed by heating for 30 minutes at 120°C. The various chromatographic systems employed were: System I = hexane:ethyl ether:acetic acid (8:2:1, v/v/v), System II = isooctane:ethyl acetate (9:1, v/v), System III = acetonitrile:acetone (1:1, v/v), and System IV = hexane:ethyl ether (6:4, v/v).

RESULTS AND DISCUSSION

Gas liquid chromatography. Significant difficulties in developing satisfactory GLC separations of the standard dimeric mixture arise from the fact that the dimers prepared are high molecular weight components of different structure. Furthermore, each structure is associated with many geometrical and positional isomers. To separate these compounds by GLC, high temperatures must be employed which results in special demands on both the column and on its operation. A number of related factors, the type of stationary phase, its concentration on the support, the type of support used and its treatment, the dimensions of the column, the type of tubing used as well as the inlet and the septum temperatures, must be considered in order to carry out the separation. Thermally-stable packings, thin-walled and relatively short columns are necessary for this type of high temperature GLC.

In this study, columns packed with siloxane polymer-coated material of varying degrees of polarity and stabilized well above 300°C were used. Preliminary studies using 3% OV-1 packing and columns of varying lengths were important to determine the optimum column length for dimer separation. A column length of six feet was required to obtain at least partial separation within the polar and nonpolar dimer groups, and a 3% stationary phase was necessary to obtain some degree of separation between dimers of varying polarity.

The separation of standard dimers, on a 6 ft × 2 mm 1/4 SS column packed with 3% OV-1 on 80-100 mesh Supelcoport expressed as relative retention times, was compared with the results obtained when the dimers were also analyzed on columns of the same length but with increased polarity. The elution time relative to tetratriacontane for each dimer as well as the number of isomeric peaks obtained for all dimers on each column are given in Table 3. All three columns resulted in the separation

TABLE 3

Effect of Polarity of Stationary Phase on the Separation of the Standard Dimers[a]

Dimer	3% OV-1		3% OV-17		3% OV-25	
	RRTR[b]	P[b]	RRTR	P	RRTR	P
Methyl linoleate						
Thermal	1.29-1.98	1	1.75-3.60	1	2.50-4.45	1
Dehydro	1.26-2.08	2	1.66-3.23	2	2.40-1.19	2
Bicyclic	1.21-2.08	3	1.89-3.45	3	2.76-4.53	3
Methyl oleate						
Dehydro	1.22-1.92	3	1.66-2.58	2	2.38-2.93	2
Methyl stearate						
Tetrahydroxy	1.74-1.82	2	3.98-5.09	2	4.27-6.01	2
Dihydroxy	1.18-1.64	3	1.77-3.25	3	2.49-3.73	3
Diketo	1.43-1.98	1	2.74-4.25	3	2.79-3.89	2

[a] Dimers synthesized as described in experimental section. GLC conditions as described in Table 1.

[b] RRTR = Relative retention time range and P = number of isomeric peaks; relative retention time to tetratriacontane (RRT = 1.000).

TABLE 4

Equivalent Hydrocarbon Length (EHL) of Major Isomeric Peaks of the Standard Dimers (column 3% OV-17)[a]

Dimer	EHLR		Equivalent hydrocarbon length					
			Peak #					
			1	2	3	4	5	6
Methyl linoleate								
Thermal	38.15	43.04	40.90					
Dehydro	37.78	42.30	41.07	41.85				
Bicyclic	38.67	42.77	39.79	41.12	41.86			
Methyl oleate								
Dehydro	37.78	40.78	39.79	40.44				
Methyl stearate								
Tetrahydroxy	43.70	45.38	44.45	45.02				
Dihydroxy	38.15	42.30	40.53	40.89	41.75			
Diketo	41.02	44.01	42.45	43.10	43.49			

[a] GLC condition as described in Table 1. Calculation of EHL as described in experimental section.

of the dimers into two distinct groups: the first eluting nonpolar group of dimers and the latter polar group. However, complete separations of dimers within each polar or nonpolar group were not possible. The most satisfactory separations of the various polar and nonpolar dimers within themselves were obtained with the intermediate polarity OV-17 phase. An increase in polarity of the stationary phase above that of OV-17 resulted in inferior separation of the various dimers as indicated by the reduction in the range of elution of the standard dimers and the number of isomeric peaks separated. Significantly decreased separation of each dimer within its isomer group or in the elution behavior of the dimer mixture was not observed by increasing the column length to 10 ft.

In order to characterize the various dimers independently of experimental conditions, a modification of the method of Miwa *et al.* (12) was used and equivalent hydrocarbon lengths were calculated for each isomer within each dimer. The calculation of equivalent carbon lengths for the various dimers was not possible since all synthesized fatty acids dimers are composed of 36 carbons and are chemically different compounds not forming a homologous group. Thus, the hydrocarbons were selected for the construction of the reference curve due to the similarity of their retention characteristics to those of the various dimers. In Table 4, the EHL for the major isomeric peaks within each dimer for all standard dimers is presented. Results of the analysis of dimers on two different capillary columns are presented in Table 5. The elution pattern of the separation of the various dimers within themselves was similar to that obtained with packed columns. However, the high efficiency of the capillary columns resulted in an increased resolution within each dimer type as indicated by the number of the various dimeric peaks associated with each dimer with better resolution obtained with the 5% phenyl methyl silicone column. In Figures 1 and 2, the chromatograms of the separation of all dimers using the capillary HP-5 column

TABLE 5

Retention Data of the Standard Dimers on Capillary Columns SPB-1 and HP-5[a]

Dimer	SPB-1		HP-5	
	RRTR[b]	P[b]	RRTR	P
Methyl linoleate				
Thermal	1.50-2.56	4	1.57-2.58	9
Dehydro	1.56-2.39	8	1.57-2.42	9
Bicyclic	1.59-2.62	7	1.59-2.53	12
Methyl oleate				
Dehydro	1.55-1.97	4	1.59-1.94	4
Methyl stearate				
Tetrahydroxy	3.10-3.78	4	3.13-3.67	4
Dihydroxy	1.76-2.09	2	2.05-2.54	2
Diketo	2.21-2.95	4	2.29-2.95	4

[a] Dimers synthesized as described in experimental section. GLC conditions as described in Table 2.

[b] RRTR and P as described in experimental section; relative retention time to tetratriacontane (RRT = 1.000).

are shown; in Table 6 the EHL obtained with the capillary column for all isomeric peaks within each dimer for all synthesized dimers is presented.

The chromatographic separation of fatty acid dimers and corresponding isomers presents a formidable task. Factors which optimize chromatographic parameters are illustrated as follows:

Resolution and retention times are related to various column and operational parameters. Of the factors characteristic of the column, column length has the least impact on the resolution of dimers since resolution is proportional to the square root of the column length. Thus, the use of a 30 m SPB-1 column gave no better resolution for the standard dimer mixture. On the other hand,

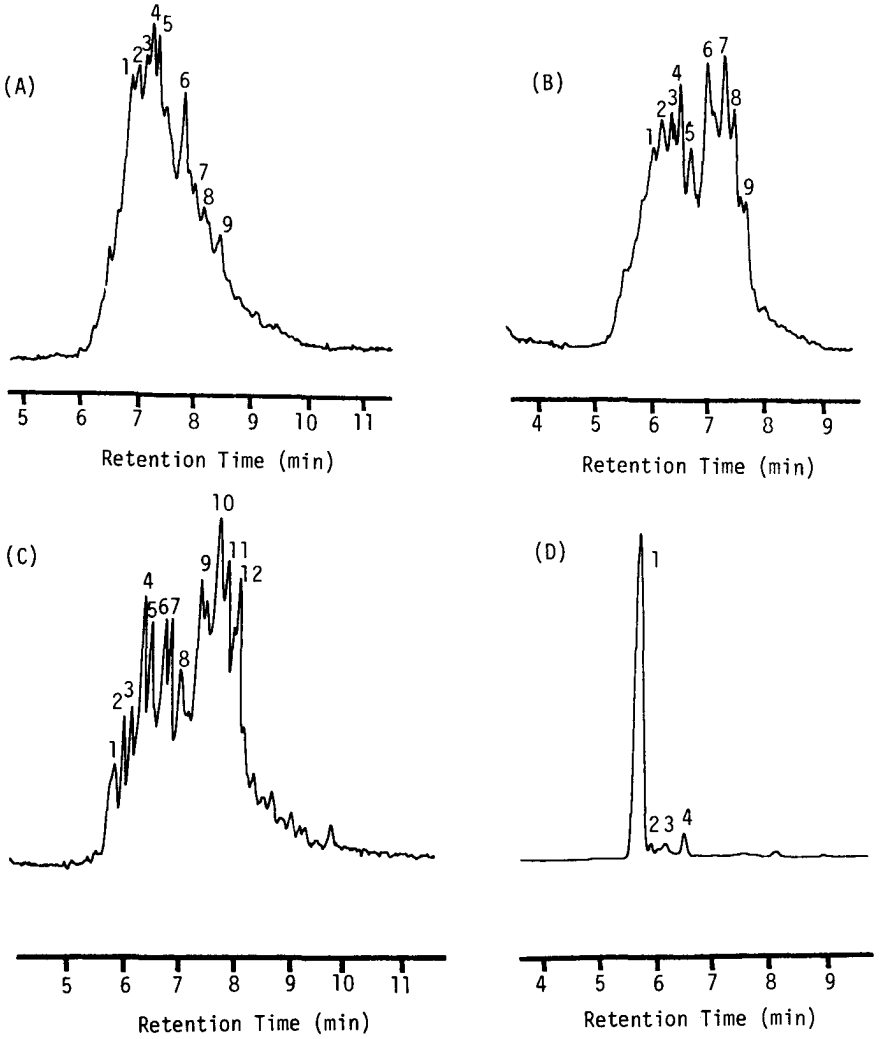

FIG. 1. Capillary GLC chromatograms of standard non-polar dimers: GLC conditions as described in experimental section/column HP-5. (A) Thermal, (B) dehydro-, (C) bicyclic dimers of Me-LN (Me-Linoleate) and (D) dehydrodimer of Me-OL (methyl oleate).

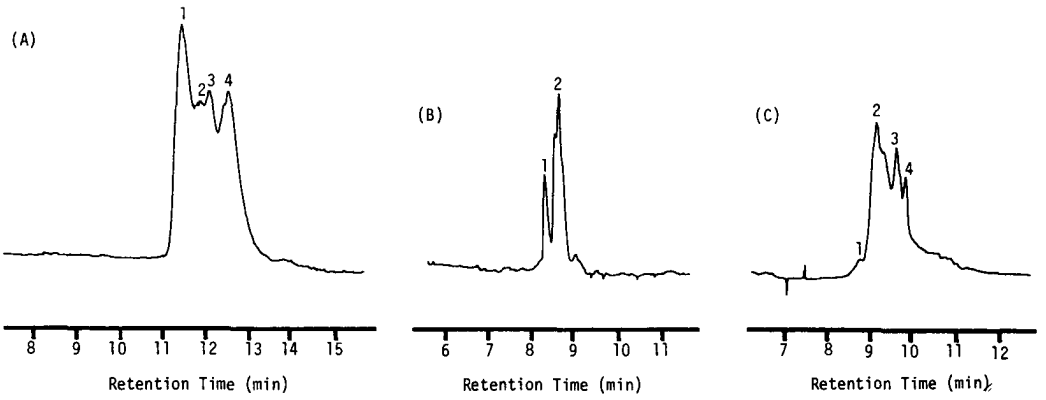

FIG. 2. Capillary GLC chromatograms of standard polar dimers: GLC conditions as described in experimental section/column HP-5. (A) Tetrahydroxy, (B) dihydroxy, and (C) diketodimers of methyl stearate.

the stationary phase film thickness and the column internal diameter can have a pronounced effect on resolution. Poor resolution was obtained when dimers were analyzed on a 50 m SPB-5 column of 1 μm film thickness. An increase in film thickness should result in an increase in the capacity ratio, which resulted in an increase in solute retention and resolution. This was not observed in the present study. A decrease in the column internal

TABLE 6

Equivalent Hydrocarbon Lengths (EHL) of Major Isomeric Peaks of the Standard Dimers (column HP-5)[a]

Dimer	Equivalent hydrocarbon length (EHL)					
	Peak #					
	1	2	3	4	5	6
	7	8	9	10	11	12
Methyl linoleate						
Thermal	37.77	37.90	38.06	38.18	38.28	38.77
	38.89	39.13	39.40			
Dehydro	37.52	37.73	37.91	38.27	38.44	38.73
	39.07	39.22	39.43			
Bicyclic	37.18	37.33	37.49	37.71	37.86	38.07
	38.13	38.41	38.74	39.04	39.18	39.31
Methyl stearate						
Tetrahydroxy	41.77	42.05	42.21	42.45		
Dihydroxy	39.66	39.98				
Diketo	39.70	40.37	40.53	40.74		

[a]Calculation of EHL as described in experimental section. Dimers synthesized as described in experimental section. GLC conditions as described in Table 2. Peak numbers according to Figs. 1 and 2.

TABLE 7

Relative Retention Times (RRT) of the Standard Dimers on Various High Performance Liquid Chromatographic Systems[a]

Dimer	Peak #	Relative retention time (RRT)		
		System I	System II	System III
Methyl linoleate				
Thermal	7	1.07	2.07	4.90
Dehydro	4	1.07	1.05	2.19, 2.27
Bicyclic	5	1.08	1.62	3.76
Methyl oleate				
Dehydro	6	1.05	1.93	4.60
Methyl stearate				
Tetrahydroxy	1	1.32	0.64	0.24
Dihydroxy	2	1.28	0.71	0.37
Diketo	3	1.26	0.89	0.49

[a]Dimers synthesized as described in experimental section. Relative retention time to methyl stearate (RRT = 1.000). Peak numbers for Systems II and III according to Figures 3 and 4. HPLC conditions as described in experimental section. System I: LC-Si/1.5% IPA in hexane/RI. System II: LC-18/acetonitrile-acetone (1:1, v/v)/RI. System III: LC-18/acetonitrile/UV at 205 nm.

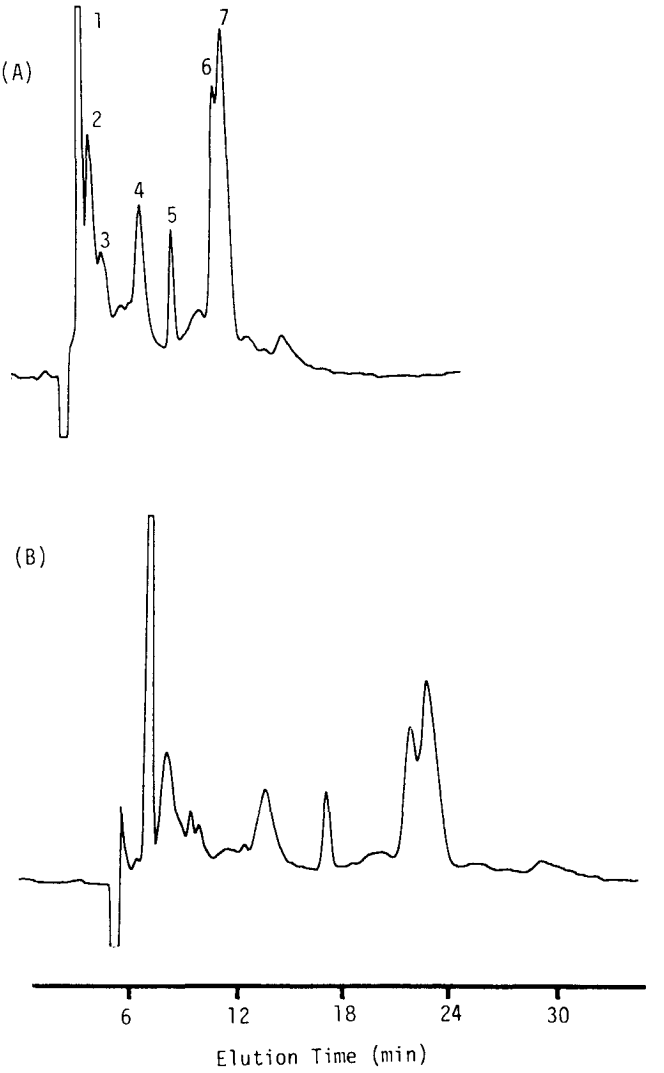

FIG. 3. HPLC of standard dimer mixture. Column: LC-18, 25 cm × 4.7 nm S.S., 5 μ diameter particle. Solvent: acetone:acetonitrile (1:1); flow rate, A = 1 ml/min, and B = 0.5 ml/min. infrared detector. Peak identification—(1) tetrahydroxy, (2) dihydroxy and (3) diketodimers of methyl stearate; (4) dehydro- and (5) bicyclic dimers of Me-LN; (6) dehydrodimer of Me-OL and (7) thermal dimer of Me-LN.

diameter would cause a decrease in the capacity ratio at a given film thickness which results in greater resolution in exchange for an increase in analysis time.

The type of carrier gas as well as the column temperature are important operational parameters affecting resolution and analysis speed. Hydrogen was chosen as the carrier gas since it generates more plates/second then helium or nitrogen at higher practical gas velocities. Column temperatures at 300°C and higher were used during analysis. Lower temperatures (~280°C) are adequate to elute dimers but resulted in increased retention times, decreased resolution and increased peak tailing. The requirement of using columns at high temperatures prevented the use of less stable polar stationary phases of the siloxane type such as OV-17 which would probably provide better resolution.

High performance liquid chromatography. Various liquid chromatographic systems have been developed to separate dimers into two major polar and nonpolar fractions (13-15). Attempts were first made in this study to separate dimers by normal phase liquid chromatography. Silica gel absorption chromatography was first used as a separation method. Hexane, as the mobile phase, gave very long retention times for the various dimers, and no separation was observed within either the polar or nonpolar dimers. The addition of 1-3% isopropyl alcohol as a polarity modifier to hexane resulted in a considerable

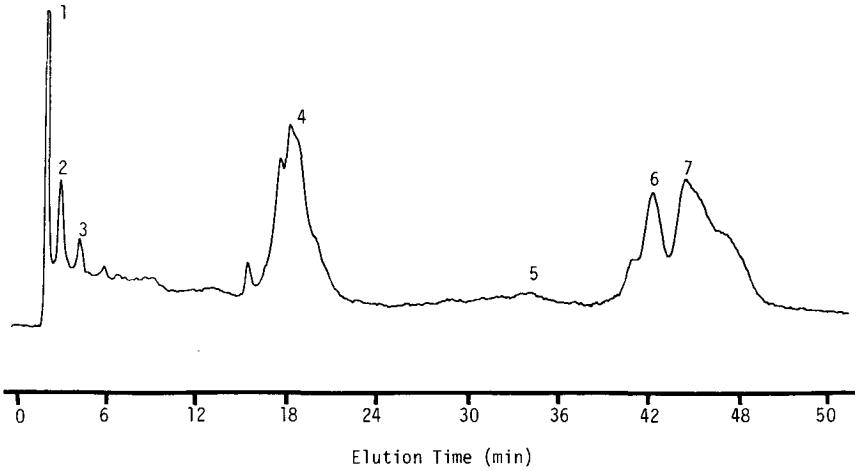

FIG. 4. HPLC chromatogram of standard dimer mixture: HPLC conditions as described in experimental section/LC-18/CH_3CN (F_c = 1 ml/min)/UV at 205 nm. Peak identification as in Fig. 3.

reduction of the retention times of all dimers—complete separation of polar and nonpolar dimers and partial separation within each dimer group with best results obtained when the concentration of isopropyl alcohol was 1.5% (Table 7).

More satisfactory separations were obtained when reverse phase liquid chromatography was used. Results of the separation of the standard dimeric mixture on an LC-18 column with refractometry as the mode of detection and acetonitrile-acetone (1:1, v/v) as the mobile phase (System II) are presented in Table 7 and Figure 3. Separation proceeded according to the polarity of the various dimers and complete separation of all dimers, except those of the thermal dimer of methyl linoleate and the dehydrodimer of methyl oleate were obtained at a flow rate of 0.5 ml/min. The resolution of the two unresolved peaks would be increased by using another LC-18 column in series, but a sacrifice in the analysis time would have to be made.

An increase in the polarity of the mobile phase produced by increasing the amount of acetone in the mobile phase resulted in a decrease in resolution within the polar and nonpolar dimers. On the other hand, a decrease in the polarity of the mobile phase by increasing the amount of acetonitrile resulted in the insolubility of the samples at concentrations required for refractive index detection, thus limiting the use of the mobile phase of polarity less than that of System II.

Acetonitrile was used in the mobile phase when ultraviolet detection of the carbonyl group at 205 mm was used for the various dimers. Results on the separations obtained for all dimers are presented in Table 7 and Figure 4. Separation of the thermal dimer of methyl linoleate and the dehydrodimer of methyl oleate was again incomplete. The fact was that the sample at concentrations as low as those required for UV detection was sparingly soluble in the mobile phase resulting in increased retention times. Finally, incomplete separation was obtained when various systems of acetonitrile-methyl chloride were used as the mobile phase in HPLC with infrared detection of the carbonyl group at 5.72 μm.

Thin-layer chromatography. The application of thin-layer chromatography to dimer separation has been investigated by several workers (16–17). In the present study, various thin-layer chromatographic systems were used in an attempt to separate the various dimers within themselves. The R_f values for the most satisfactory systems are presented in Table 8. Complete separation within the various nonpolar or polar dimers was not possible. Polar dimers were visualized as broad tailing spots.

TABLE 8

R_f Values of the Standard Dimers on Various Thin Layer Chromatographic Systems[a]

	R_f		
Dimer	System I	System II	System IV
Methyl linoleate			
Thermal	0.65	0.33	0.80
Dehydro	0.62	0.31	0.81
Bicyclic	0.59	0.33	0.78
Methyl oleate			
Dehydro	0.66	0.37	0.80
Methyl stearate			
Tetrahydroxy	0.07	0.11	0.39
Dihydroxy	0.25	0.10	0.36
Diketo	0.27	0.03	0.24

[a]Dimers synthesized as described in experimental section. TLC conditions as presented in experimental section. System I: Hexane:ethyl ether:acetic acid (8:2:1, v/v/v). System II: Isooctane:ethyl acetate (9:1, v/v). System IV: Hexane:ethyl ether (6:4, v/v).

ACKNOWLEDGMENT

Partial support from the University of Illinois Agricultural Experimental Station is gratefully acknowledged.

REFERENCES

1. Perkins, E.G., and F.A. Kummerow, *J. Am. Oil Chem. Soc. 36*:371 (1959).
2. Firestone, D., W. Hozwitz, L. Friedman, and G.M. Shue, *J. Am. Oil Chem. Soc. 38*:253 (1961).
3. Ohfuji, T., and T. Kaneda, *Lipids 8*:353 (1973).

4. Zeman, A., and H. Scharmann, *Fette Seifen Anstrichm. 71*:957 (1969).
5. Gere, A., *Fette Seifen Anstrichm. 87*:359 (1985).
6. Michael, W.R., J.C. Alexander, and N.R. Artman, *Lipids 1*:353 (1966).
7. Perkins, E.G., and L.R. Wantland, *J. Am. Oil Chem. Soc. 50*:459 (1973).
8. Paulose, M.M., and S.S. Chang, *J. Am. Oil Chem. Soc. 50*:147 (1973).
9. Wheeler, D.H., and J. White, *J. Am. Oil Chem. Soc. 44*:298 (1967).
10. Paschke, R.F., L.E. Peterson, S.A. Harrison, and D.H. Wheeler, *J. Am. Oil Chem. Soc. 41*:56 (1964).
11. Christopoulou, C.N., Ph.D. Thesis, Chemical and Chromatographic Studies on Dimeric Fatty Acids, Univ. of Illinois, Urbana, IL (1986).
12. Miwa, T.K., K.L. Mikolajczak, I.R. Earle, and I.A. Wolff, *Anal. Chem. 32*:1739 (1960).
13. Frankel, E.N., C.D. Evans and H.A. Moser, D.G. McConnell, and J.C. Cowan, *J. Am. Oil Chem. Soc. 38*:139 (1961).
14. Evans, C.D., D.G. McConnel, E.N. Frankel, and J.C. Cowan, *J. Am. Oil Chem. Soc. 42*:764 (1965).
15. Aitzetmuller, K., *J. Chromatog. 83*:461 (1973).
16. Firestone, D., *J. Am. Oil Chem. Soc. 40*:247 (1963).
17. Zeman, I., M. Ranny, and L. Winterova, *J. Chromatogr. 354*:283 (1986).

[Received July 12, 1988; accepted April 3, 1989]
[J5510]