Technical

Geometrical Isomerization of Linolenic Acid During Heat Treatment of Vegetable Oils[1]

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ABSTRACT

Heat treatment of rapeseed (primor) and soybean oils resulted in the geometrical isomerization of linolenic acid. The geometrical isomers were isolated from a rapeseed oil heated at 240 C for 10 hr by a combination of thin layer chromatography (TLC) of the methoxy bromomercuric adducts of the total methyl esters and $AgNO_3$-TLC. Three major isomers were identified after hydrazine reduction followed by ozonolysis in BF_3-MeOH as 18:3Δ9c, 12c, 15t, 18:3Δ9t, 12c, 15c and 18:3Δ9t, 12c, 15t. These were accompanied by minor amounts of 18:3Δ9c, 12t, 15c, 18:3Δ9c, 12t, 15t and 18:3Δ9t, 12t, 15c. The 18:3 isomers were detected in both soybean and rapeseed oil heated at 240 C for 10 hr. At this temperature, the increase in time of the heat treatment from 10 to 40 hr resulted in a relative increase of the di-*trans* and in a decrease of the mono-*trans* isomers. Only minor quantities of these isomers were detected in the oils heated at 200 C for 10 hr. At this temperature, the increase in time of the heat treatment resulted in an increase of both the mono- and di-*trans* isomers.

INTRODUCTION

In many countries, the consumption of salad and cooking oils has increased during the last 30 years (1). Furthermore, the deep fat frying procedure is used more and more by the food industry. Among all the heated vegetable oils used to study the physiopathological effects of heated fats (2-5), some showed the presence of other unknown components along with the cyclic fatty acid monomers usually found in frying oils (6-7). These could be detected by gas liquid chromatography (GLC). The unknowns were immediately preceding and following the methyl ester of linolenic acid on a Carbowax liquid phase. As early as 10 years ago, Ackman et al. (8) identified components in deodorized oils having similar chromatographic properties as being geometrical isomers of linolenic acid.

Linolenic acid is regarded as having important biological effects (9-10). Furthermore, some studies seem to indicate that linolenic acid is an essential fatty acid (11) and that some prostaglandins and analogs are formed from linolenic acid metabolites (12). It is therefore of interest to study the possible transformations of linolenic acid during heat treatment.

We have followed the isomerization of linolenic acid in soybean and rapeseed oils as a function of the time of the heat treatment and the temperature of the reaction. Three major 18:3 isomers were identified after ozonolysis reaction as being 18:3Δ9c, 12c, 15t, 18:3Δ9t, 12c, 15c and 18:3Δ9t, 12c, 15t. These were accompanied by smaller quantities of 18:3Δ9c, 12t, 15c and of 2 other di-*trans* isomers.

MATERIALS AND METHODS

Purification of solvents—All the solvents were redistilled before use.

Heating conditions—Refined primor rapeseed and soybean oils were purchased respectively from Precy and Lesieur Cotelle (France). The rapeseed oil was deodorized at 200 C for 5 hr, and the deodorization temperature for the soybean oil was 180 C.

These oils were heated in a commercial fryer (Calor 08). The volume of the oil in the aluminium coated tank was 4.5 l. The surface of the oil in contact with the air was 530 cm^2. These two oils were heated at 200 C and at 240 C for 10 and 40 hr.

Each sample was converted to the methyl esters by reaction with a 5% HCl/MeOH solution.

Gas liquid chromatography (GLC)—All the GLC analyses were carried out on a Becker-Packard 420 chromatograph fitted with a flame ionization detector (FID) and a ROS injector (13). These analyses were performed on glass capillary columns, coated with either Silar 10C (25 m in length and 0.25 mm ID) or Carbowax 20M-TA (42 m in length and 0.35 mm ID) using helium as the carrier gas. The temperature of the injector and of the FID detector was 240 C. All quantitative analyses were performed using a Vista CDS 401 (Varian) or an Autolab System 4 integrator.

The equivalent chain length (ECL values) of the unsaturated fatty acids was calculated according to the method described by Ackman (14) using stearic and eicosanoic acid methyl esters as internal standards.

Isolation of the 18:3 geometrical isomers—The isolation and the determination of the structure of the 18:3 isomers were carried out on the rapeseed oil which was heated at 240 C for 10 hr.

The total fatty acid methyl esters (5 g) were converted to the methoxy bromomercuric adducts (MBM) by addition of an excess of mercuric acetate in methanol followed by reaction of sodium bromide in methanol as described by Sebedio and Ackman (15). The resulting methoxy bromomercuric adducts were fractionated by thin layer chromatography (TLC) using a mixture of hexane:dioxane (60:40, v/v) as the solvent system. The resulting bands were visualized by spraying the plate with a solution (0.4%) of diphenyl carbazone in ethanol. The triene band was removed into a 50 ml centrifuge tube and 5 ml of a mixture of HCl/MeOH (1:2, v/v) was added to destroy the MBM. After addition of H_2O, the methyl esters were extracted with hexane.

Hydrogenation—The hydrogenation of the isolated triene fraction was effected using platinum oxide as catalyst in 10 ml of a mixture of chloroform/methanol (2:1, v/v) as solvent and a hydrogen pressure of 2 bars.

Fractionation of the 18:3 geometrical isomers—The 18:3 isomers were further fractionated by $AgNO_3$-TLC (30% by weight) using silica gel G plates (0.5 mm thickness), according to De Vries and Jurriens (16). Development was in benzene/diethyl ether (90:10, v/v). The diethyl ether used was protected with 7 ppm of BHT in order to avoid the presence of peroxides. These are known to be involved in the formation of epoxides during $AgNO_3$-TLC (17). The resulting three bands (Rf = 0.12, 0.28 and 0.41) were visualized under UV light after spraying the plate with a solution of 2'7'-dichlorofluorescein in ethanol (0.2%). After scraping off the bands from the plates under nitrogen, the complex between the silver ion and ethylenic bonds was

[1] Presented in part at the I.S.F. Congress, October 1983, Budapest, Hungary.

destroyed according to the method described by Hill et al. (18). Modifications were made as follows: a solution of 1% sodium chloride in 90% methanol was added in portions until the red color of the silver-dichlorofluorescein complex disappeared. After the addition of H_2O, the methyl esters were extracted with hexane.

Structural determination of the 18:3 geometrical isomers—Each $AgNO_3$-TLC fraction was then submitted to the action of hydrazine (95%, Eastman Kodak) in ethanol according to Ratnayake (19-20). The reaction was stopped after 135 min and the resulting mixtures of saturates, monoenes, dienes and trienes were extracted with hexane and further submitted to the MBM adduct fractionation. The resulting monoene bands were extracted as previously described. The monoenes were further fractionated according to the geometry of the ethylenic bond on silica gel TLC plates (Merck 5721; 0.25 mm thickness) impregnated by dipping them in a 10% solution of $AgNO_3$ in acetonitrile for 30 min. The TLC plates were developed with a mixture of benzene:hexane (2:1, v/v) and the bands were detected by spraying with a solution of 2'7' dichlorofluorescein in ethanol. The resulting fractions were submitted to ozonolysis in BF_3-MeOH using a Supelco micro-Ozonizer. The method described by Ackman and Sebedio (21-22) for an ozonizer capable of producing important quantities of ozone was modified slightly as follows: in a typical reaction, 0.5 mg of fatty acid methyl esters were dissolved in 2 ml of BF_3-MeOH (7%) in a 10 ml screw-cap centrifuge tube and ozone in oxygen was bubbled through (total gas flow 10 ml/min) for 12 min. The tube was capped and inserted in an oven at 100 C for 2 hr. After cooling, distilled water (6 ml) was added and the ozonolysis products (mono- and di-esters) were extracted with chloroform.

The ozonolysis products (dimethylesters) were further analyzed by GLC on a Silar 10 C column and identified by comparison with authentic standards (Sigma, St. Louis, Missouri).

RESULTS AND DISCUSSION

The heat treatment of a primor rapeseed oil resulted in the decrease of linolenic acid (18:3ω3) and the formation of unknown components (Fig. 1) immediately preceding and following the methyl ester of linolenic acid on a Carbowax 20M TA liquid phase. Two unknown components also were detected in the linoleic acid region. Different compounds are known to be formed during the heat treatment of oils, such as polymers, conjugated acids and cyclic monomers (2). However, polymers cannot be detected under the GLC analytical conditions used and the C18 conjugated acids would have retention times which would be close to those of the C20 saturated or monoethylenic fatty acids (23). Furthermore, according to the work of Ackman et al. (8) on the deodorization of oils, these unknowns could be anticipated to be some 18:3 geometrical isomers.

In order to identify these unknown components in the 18:3ω3 region, we have followed the analytical procedure outlined in Figure 2. Under the experimental conditions used, the MBM adducts were fractionated only according to the degree of unsaturation of the fatty acid with no influence of either the chain length or the position of the ethylenic bonds on the carbon chain. The GLC analysis of the triene fraction after destruction of the MBM adducts revealed the presence of four major peaks (Table I) with retention times identical to those of the components detected in Figure 1. It is important to note that other trienoic fatty acids such as 16:3ω3 which had been identified in Canadian rapeseed oils (24) were not detected in this particular oil. The hydrogenation of this band gave only

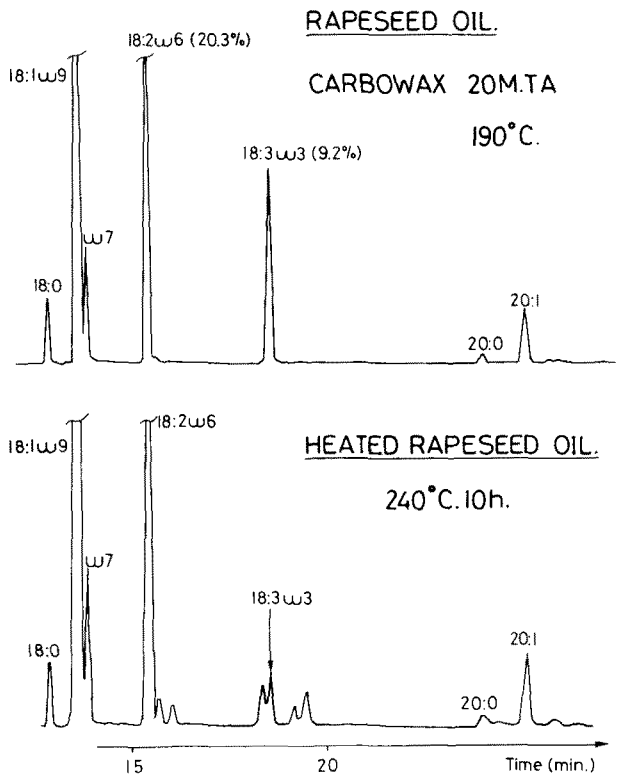

FIG. 1. Gas-liquid chromatographic analysis on a glass capillary column coated with Carbowax 20M TA, 42 m in length and 0.35 mm I.D. of a refined rapeseed oil and a rapeseed oil heated at 240 C for 10 hr.

stearic acid, which indicated that the unknowns are of C18 chain length and that the triene band does not contain any cyclic monomers. This confirms the experiment of Sebedio et al. (25) on heated linseed oil which showed that some of the MBM adducts of C18 cyclic fatty acid monomers migrate in the diene band and some in a band between the diene and the triene bands.

The TLC fractionation of the triene band on $AgNO_3$ plates (30%) gave three bands (A, B and C, Table II, Fig. 3). The Rf of these fractions (0.12, 0.28 and 0.41) were similar to those calculated from the data of De Vries and Jurriens (16) for the *cis, cis, cis* (Rf 0.15, band A), *trans, cis, cis* (Rf 0.30, band B) and *trans, trans, cis* (Rf 0.46, band C) octadecatrienoic acids. No compound was detected at an Rf corresponding to the *trans, trans, trans* isomer.

The GLC analyses of fraction A (Table II, Fig. 3) on Silar 10C and on Carbowax 20M TA showed one peak which had the same equivalent chain length (ECL) value as a standard of linolenic acid. In fraction B (Fig. 3), two isomers of similar quantities were detected on Carbowax 20M TA. However, an analysis on Silar 10C showed the presence of a small shoulder on the second peak which indicated the presence of a third component in this fraction. Considering the similar quantities of the two peaks, the fraction B was refractionated by $AgNO_3$-TLC and the band cut arbitrarily in two parts. The lower fraction, B_1 contained 40% of the isomer having the shorter ECL value (20.32 on Silar 10C) and 60% of the peak of ECL value 20.51. The upper fraction B_2 contained 54% of the first peak and 46% of the second. It is important to note that the relative proportions of the shoulder of ECL value 20.49 and the peak of ECL value 20.51 were similar in fractions B_1 and B_2. The fraction C (Fig. 3) contained a major peak accompanied with smaller quantities of two other components which were only detected on Carbowax 20M TA. Only one of these

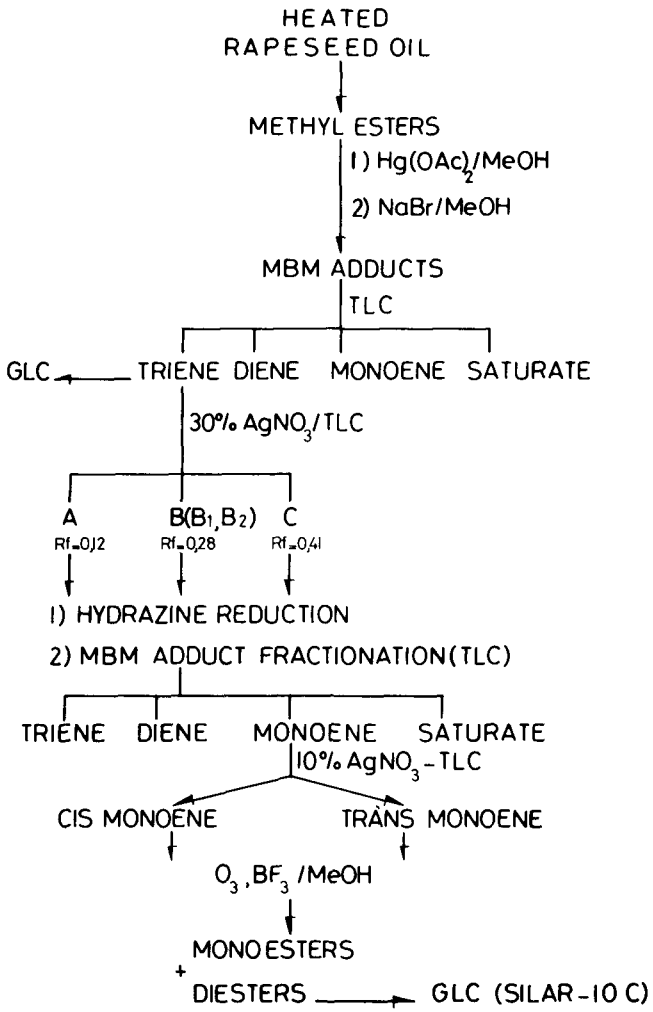

FIG. 2. Flow chart for the isolation and the identification of the 18:3 geometrical isomers in a heated rapeseed oil.

TABLE I

Retention Data on Silar 10C at 170 C and on Carbowax 20M TA at 190 C for Some 18:3 Geometrical Isomers in Rapeseed Oil (Primor) Heated at 240 C for 10 hr and Proportions in Whole Oil and in the Isolated MBM Trienoic Fraction

Observed ECL			
Silar 10C	Carbowax 20M	Whole oil[a]	MBM fraction
20.18	19.29	0.7	14.1
20.32	19.15	1.5	27.8
20.51[b]	19.34	1.6	25.7
20.56	19.18	2.0	32.5

[a]From analysis on Carbowax 20M TA (polymers not included).
[b]This peak presents a shoulder at ECL 20.49.

two minor isomers was detected on Silar 10C.

The hydrazine reduction of these fractions gave a mixture of saturates, monoenes, dienes and trienes. The major advantage of this reaction is that neither the position nor the geometry of ethylenic bonds is modified (26-28) during the reduction. Each of the monoenes obtained represents the position and the geometry of the ethylenic bonds in the parent molecule. The monoenes separated by MBM adduct fractionation are presented in Figure 4. The GLC analyses of fraction A on a Silar 10C column revealed the presence of three peaks of ECL values of 18.61, 18.77 and 18.99.

TABLE II

Percentages of the 18:3 Geometrical Isomers in the $AgNO_3$-TLC Bands of the Isolated Trienoic MBM Fraction (Fig. 2)

ECL (Silar 10C) 170 C	A (0.12)	B (0.28)	Rf B_1	B_2	C (0.41)
20.18	–	–	–	–	86.3
20.29	–	–	–	–	13.7
20.32	–	48.4	40.2	54	–
20.51[a]	–	51.6	59.8	46	–
20.56	100	–	–	–	–

[a]This peak also contains a shoulder at ECL 20.49.

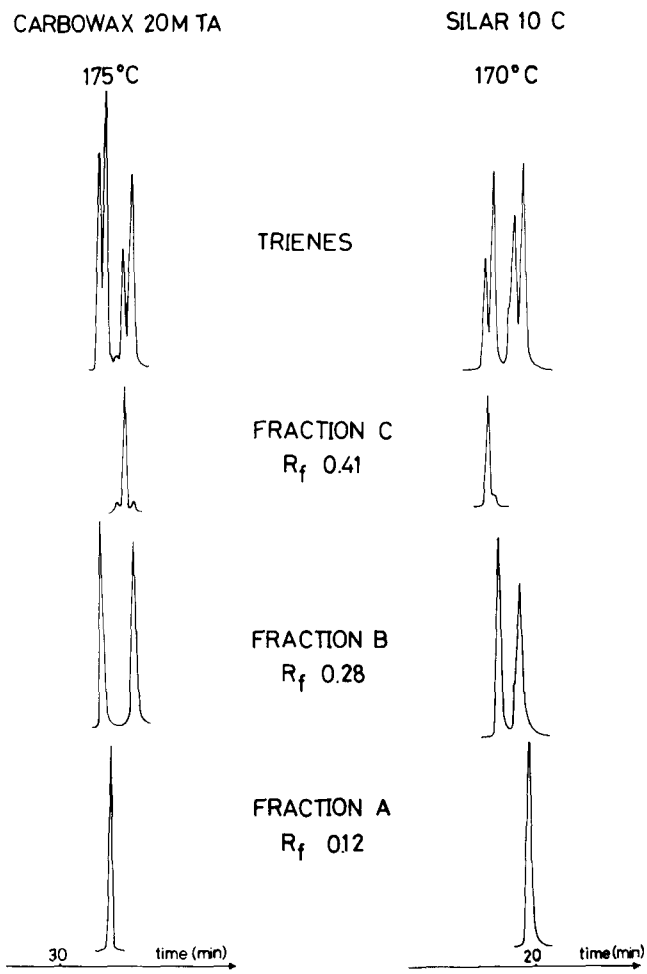

FIG. 3. Gas-liquid chromatographic analyses on glass capillary columns coated with Carbowax 20M TA (42 m in length and 0.35 mm I.D.) and Silar 10C (25 m in length and 0.25 mm I.D.) of the total 18:3 isomers and the 3 triene fractions obtained after MBM and $AgNO_3$-TLC fractionations.

The analyses of fractions B_1, B_2 and C showed the presence of a minimum of 5 to 6 components which were partially separated on this polar liquid phase. All three fractions had peaks having the same ECL values but differing in quantities. Due to the complexity of these fractions, it was impossible to identify the different monoethylenic fatty acids by comparison of their ECL values with those reported in the literature (23). We therefore submitted these fractions to a $AgNO_3$-TLC fractionation. This TLC fractionation of the monoenes revealed that the plates used were highly effective: the basic separation occurred according to the geometry of the unsaturation as is usually ac-

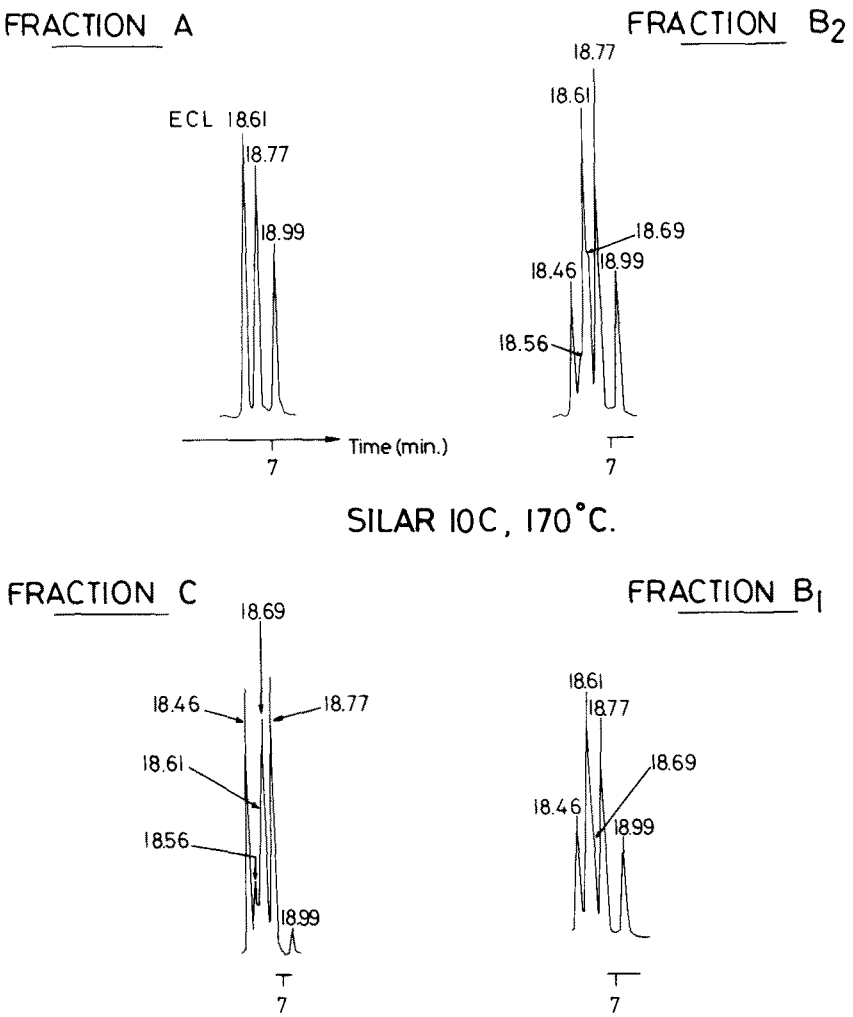

FIG. 4. Gas-liquid chromatographic analysis on a glass capillary column coated with Silar 10C (25 m in length and 0.25 mm I.D.) of the monoenes obtained after the hydrazine reduction of the isolated 18:3 fractions (A, B_1, B_2 and C).

cepted (29). The migration of the *cis* and *trans* monoenes also was influenced by the position of the ethylenic bond on the carbon chain.

This $AgNO_3$-TLC resulted in two bands for the 18:1 isomers of fraction A, three from those of fraction B_1 and four from fractions B_2 and C. Each band was then submitted to ozonolysis. The resulting dimethylesters were analyzed by GLC on Silar 10C at 170 C. For the four monoethylenic fractions, only dimethylesters with 9 (DMC_9), 12 (DMC_{12}) and 15 (DMC_{15}) carbons were observed (Table III). A good separation was obtained between the lower fractions (Rf 0.36, Rf 0.41) and the higher fractions (Rf 0.53, Rf 0.58). The separation between the bands of Rf 0.36 and Rf 0.41 as well as the bands of Rf 0.53 and 0.58 was not as good: for example, the resulting bands of Rf 0.53 and Rf 0.58 from fraction B_1 were not separated. The bands of Rf 0.36 and 0.41 corresponded to the Rf of a pure *cis* 18:1Δ9 (0.37) and those of Rf 0.53 and 0.58 to a pure *trans* 18:1Δ9 (0.54). From these data and those reported by Gunstone (30), it is obvious that the bands of Rf 0.36 and 0.41 correspond to the *cis* 18:1 isomers and those of Rf 0.53 and 0.58 to the *trans* isomers. This poor separation between the bands of Rf 0.53 and 0.58 resulted in the presence along with 18:1Δ9 of small quantities of 18:1Δ15 in the band of Rf 0.53 (Table III, DMC_{15} in fractions B_2 and C). The dimethylesters which resulted from the monoenes having an ethylenic bond of the same geometry were further pooled for quantitative analyses (Table IV). The data presented in this table are in mole % but do not include the correction factors for the flame ionization detector (FID). However, it was shown by Sebedio et al. (31) that the dimethylesters in C9, C10, C11 and C15 have similar correction factors, respectively 1.31, 1.32, 1.38 and 1.31. Important differences in FID correction factors were observed only for dimethylesters of shorter chain lengths (C3, C4). From fraction A, no *trans*

TABLE III

Quantitative Analysis of the Dimethylesters Obtained after Ozonolysis of the $AgNO_3$-TLC Bands of the Isolated 18:1 Isomers from Fractions A, B_1, B_2 and C

Bands Rf	Fractions A	B_1	B_2	C
0.58	–	9+12+15	12+15	12+15
0.53	–		9+15[b]	9+15[b]
0.41	12[a]+15	12+15	12+15	12+15
0.36	9	9	9	9

[a]Number of carbons of the dimethylester.
[b]Present in minor quantities.

TABLE IV

Position and Distribution (Mole %) of the Ethylenic Bond in the 18:1 Isomers Obtained after Hydrazine Reduction Followed by $AgNO_3$-TLC of the Fractions A, B_1, B_2 and C (Fig. 2)

Ethylenic position	Fractions							
	A		B_1		B_2		C	
	cis	*trans*	*cis*	*trans*	*cis*	*trans*	*cis*	*trans*
9	39	ND[a]	30	52	33	39	17	53
12	35	ND	41	13	45	9	76	10
15	26	ND	28	34	22	52	7	37

[a]ND = not detected.

monoenes were detected as described previously. The *cis* monoenoic isomers of this fraction gave dimethylesters in unequal quantities (DMC_9 > DMC_{12} > DMC_{15}). These differences probably are arising from the slight selectivity of the hydrazine reduction (19-20). In fraction A, the compound of ECL 20.56 was identified as linolenic acid (*cis*-9, *cis*-12, *cis*-15 octadecatrienoic acid).

From fraction C, about two times more *trans* monoenes than *cis* isomers were obtained. This confirmed that the fraction contained the di-*trans*, mono-*cis* isomers. The major compound of this fraction (Table IV) was the *trans*-9, *cis*-12, *trans*-15 isomer. However, the presence of minor quantities of DMC_9 and DMC_{15} in the *cis* fraction showed that the two other di-*trans* trienes (*cis*-9, *trans*-12, *trans*-15 and *trans*-9, *trans*-12, *cis*-15) are also present. This is in agreement with the GLC analyses on Carbowax 20M of the fraction C before the hydrazine reduction.

In fraction B (the trienes with one *trans* ethylenic bond), two major isomers were present. The ozonolysis results showed that these isomers contain a *trans*-9 or a *trans*-15 unsaturation. In fraction B_1, the amount of *trans*-15 was reduced and the *trans*-9 increased in comparison to fraction B_2. Thus, the first peak of ECL value 20.32 on Silar 10C (Table II) is 18:3Δ9c, 12c, 15t and the second (ECL 20.51) (Table II) is 18:3Δ9t, 12c, 15c. A minor amount of a third mono-*trans* isomer also has been identified as 18:3Δ9c, 12t, 15c. This isomer is probably the compound with an ECL value of 20.49 on Silar 10C (Table II).

TABLE V

Equivalent Chain Length (ECL) Values for Some Octadecenoic Acid Methyl Esters, on Silar 10C at 170 C

	Scholfield (23)	This study
9 *cis*	18.61	18.61
12 *cis*	18.75	18.77
15 *cis*	18.96	18.99
9 *trans*	18.41	18.46
12 *trans*	18.54	18.56
15 *trans*	18.66	18.69

The ozonolysis results also permitted identification of the monoethylenic fatty acids obtained by hydrazine reduction of the triene bands (Fig. 4). The ECL values of these monoenes on Silar 10C are presented in Table V. Our ECL values and those obtained by Scholfield (23) are in good agreement.

Few studies have been carried out on these 18:3 geometrical isomers. Among them, two recent publications gave the ECL values of some 9, 12, 15 octadecatrienoic isomers on Silar 10C at 170 C (Table VI). It is important to note that the elution order of the 18:3 isomers found in this study agrees partially with the results of

TABLE VI

Equivalent Chain Length (ECL) Values for Some 18:3 Fatty Acid Methyl Esters on Silar 10C at 170 C

Fatty acids	Scholfield (23)	Rakoff & Emken (32)	This study
t_9, c_{12}, t_{15}	20.15	20.12	20.18
c_9, c_{12}, t_{15}	20.38	20.20	20.32
c_9, t_{12}, c_{15}	20.31	20.39	20.49
t_9, c_{12}, c_{15}	20.42	20.38	20.51
c_9, c_{12}, c_{15}	20.52	20.52	20.56

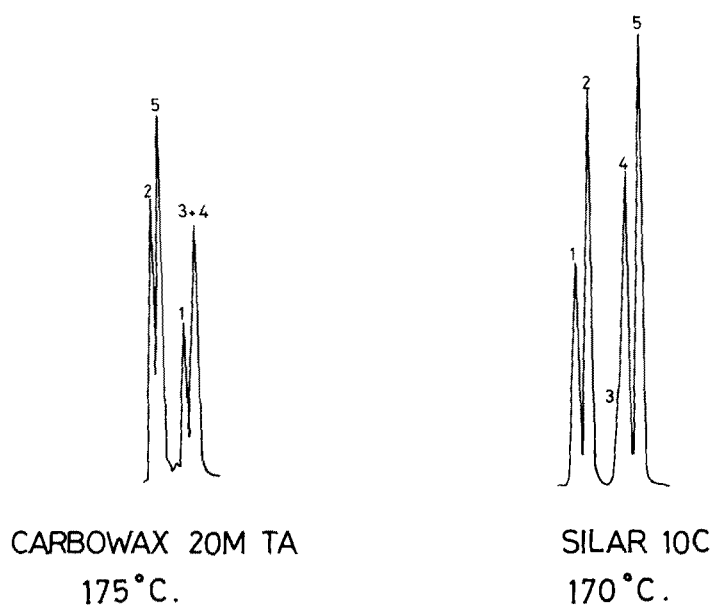

FIG. 5. Comparison of the gas liquid chromatographic analysis on glass capillary columns coated with either Carbowax 20 M TA, 42 m in length and 0.35 mm I.D. or Silar 10C, 25 m in length and 0.25 mm I.D. of the identified 18.3 geometrical isomers present in rapeseed oil heated at 240 C for 10 hr. (1) t_9, c_{12}, t_{15}; (2) c_9, c_{12}, t_{15}; (3) c_9, t_{12}, c_{15}; (4) t_9, c_{12}, c_{15}; (5) c_9, c_{12}, c_{15}.

TABLE VII

Percentages of Linolenic Acid Isomers in Heated Rapeseed and Soybean Oils[a]

	Rapeseed oil					Soybean oil				
		200 C		240 C			200 C		240 C	
	Fresh oil	10 hr	40 hr	10 hr	40 hr	Fresh oil	10 hr	40 hr	10 hr	40 hr
t_9, c_{12}, t_{15}	n.d.[c]	n.d.	0.1	0.7	0.6	n.d.	tr.	0.1	0.7	0.9
c_9, c_{12}, t_{15}	tr.[d]	0.2	0.6	1.5	0.3	tr.	0.2	0.8	1.4	0.5
t_9, c_{12}, c_{15}[b]	0.2	0.4	0.6	1.6	0.4	n.d.	0.3	0.9	1.5	0.7
c_9, c_{12}, c_{15}	9.2	7.5	3.7	2.0	0.1	7.6	6.5	4.6	1.9	0.2

[a]Polymers were not included in the quantitation.

[b]This peak also contains some c_9, t_{12}, c_{15}.

[c]n.d. = non-detected.

[d]tr. = trace (<0.1%).

Scholfield (23) which were based on the relative position of the 18:3 isomers in an argentation countercurrent distribution. There is a better agreement with the results published by Rakoff and Emken (32) except for the elution order of the *cis*-9, *trans*-12, *cis*-15 and the *trans*-9, *cis*-12, *cis*-15 which in both cases have similar ECL values (ΔECL = 0.01 and 0.02). However, the comparison of the ECL values (Table VI) indicated a difference in the polarity of the different Silar 10C columns.

The major 18:3 geometrical isomers identified in heated oils (Fig. 5) are identical to those identified by Ackman et al. (8) in deodorized oils, after hydrazine reduction and GLC analyses of the resulting mixtures. The heat treatment seems to induce a geometrical isomerization of the ethylenic bonds of linolenic acid especially at the Δ9 and Δ15 positions. The Δ12 position, a more internal position, seems to present a better resistance to geometrical isomerization. It is also important to note that no detectable positional isomerization is taking place during the heat process.

We have followed the formation and the evolution of these 18:3 geometrical isomers in primor rapeseed and soybean oils as a function of temperature and time of the reaction. The results obtained (Table VII) seem to indicate that the *mono-cis, di-trans* trienes are present in noticeable quantities in oils heated at 240 C. Furthermore, the largest quantities of 18:3 geometrical isomers were observed in oils heated at 240 C for 10 hr. For example, in the rapeseed oil heated under these conditions, 38% of the starting linolenic acid was transformed into geometrical isomers (33). After 40 hr of heat treatment, at the same temperature, the amount of these isomers decreased. This could indicate that these fatty acids are possibly precursors of other compounds such as cyclic or polymeric acids. Recent studies carried out by Devinat et al. (34) on oils containing linolenic acid indicated that the geometrical isomerization of 18:3ω3 did not seem to have any influence on the formation of polymers nor on the content of cyclic fatty acid monomers. However, the method used for the determination of cyclic acid contents (35) may not be suitable to detect small differences (36). At 200 C, a temperature which can be reached in deep fat frying, these isomers were also detected, mainly after a heat treatment of 40 hr. In this case, the quantities found were much smaller than those observed at 240 C. Eder (37) also observed during the deodorization process, which is performed without the presence of oxygen, that important quantities of geometrical 18:3 isomers were formed only when the temperature reached 240 C.

ACKNOWLEDGMENT

This work was supported in part by a research grant from the Ministère de la Recherche et de la Technologie, France.

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[Received March 1984]