Participation of the *cis*-12 Ethylenic Bond to *cis-trans* Isomerization of the *cis*-9 and *cis*-15 Ethylenic Bonds in Heated α-Linolenic Acid

Robert L. Wolff[a,*], Mohamed Nour[b], and Corinne C. Bayard[a]

[a]ISTAB, Laboratoire de Lipochimie Alimentaire, Universite Bordeaux 1, Talence and [b]INRA, Unite de Nutrition Lipidique, Dijon, France

ABSTRACT: To understand the *cis-trans* isomerization reaction of ethylenic bonds in heated octadecatrienoic acids (occurring during industrial deodorization of oils), we have prepared a mixture of *cis*-9,*cis*-12, *cis*-12,*cis*-15, and *cis*-9,*cis*-15 18:2 acids by partial hydrazine reduction of *cis*-9,*cis*-12,*cis*-15 18:3 acid present in linseed oil. This mixture (as fatty acid methyl esters) was heated under vacuum at 270°C for 2.25 h. The two methylene-interrupted acids isomerize at a similar rate under such conditions, but the nonmethylene-interrupted *cis*-9,*cis*-15 18:2 acid remains unchanged. This means that the mechanism of isomerization does not involve a direct interaction between the two external ethylenic bonds as previously hypothesized. The central *cis*-12 ethylenic bond is apparently necessary for the isomerization of the two external *cis*-9 and *cis*-15 ethylenic bonds. However, this bond is itself rather protected against isomerization in the original *cis*-9,*cis*-12,*cis*-15 18:3 acid which is mainly isomerized to *trans*-9,*cis*-12,*trans*-15, *cis*-9,*cis*-12,*trans*-15, and *trans*-9,*cis*-12,*cis*-15 18:3 acids. The *cis*-9,*trans*-12,*cis*-15 18:3 isomer is less than 10% of total *trans* isomers of α-linolenic acid. As a general rule, only one of the two double bonds in a methylene-interrupted diethylenic system can undergo *cis-trans* isomerization when submitted to heat treatment, at least for temperatures equal to or less than 270°C. *JAOCS 73*, 327–332 (1996).

KEY WORDS: Geometrical isomerization, heat treatment, α-linolenic acid, octadecadienoic acids, *trans* fatty acids.

Trans isomers of polyunsaturated fatty acids are widespread components of deodorized oils and foods that contain such oils (1–6). It has been shown that *cis-trans* isomerization occurs at temperatures higher than *ca.* 200°C (7,8) and that α-linolenic acid is more prone to isomerization than linoleic acid (4,6), whereas monoenoic acids do not isomerize at all (1–8). In most commercial oils, which are deodorized at temperatures between 220 and 270°C for a few minutes to several hours, the ratio of the probability of isomerization of α-linolenic acid to that of linoleic acid is around 13–14 (3,4,6). Not only does α-linolenic acid isomerize when heated under vacuum; other octadecatrienoic acids, such as γ-linolenic (*cis*-6,*cis*-9,*cis*-12 18:3) (9) and pinolenic (*cis*-5,*cis*-9,*cis*-12 18:3) acids (10), also isomerize under these conditions. γ-Linolenic acid isomerizes in the same way as α-linolenic acid (9), but pinolenic acid shows distinct isomerization characteristics as compared to the two preceding octadecatrienoic acids (10). As a general rule, the external ethylenic bonds in octadecatrienoic acids isomerize to a greater extent than the central double bond (7,9,10).

To explain this fact, it was recently suggested (10) that the isomerization mechanism could involve some direct interaction (through a hexagonal folding of the molecule) between the external ethylenic bonds, with a rather limited participation of the central double bond. To check whether this hypothesis is right, it is necessary to have available a polyenoic fatty acid without this central ethylenic bond. For this purpose, we have partially reduced the α-linolenic acid, present in linseed oil, with hydrazine. This leads to the formation of *cis*-9,*cis*-15 18:2 acid, a good candidate to test the preceding hypothesis. If the *cis*-12 double bond is not necessary for the isomerization of the *cis*-9 and *cis*-15 double bonds, these bonds should readily isomerize in the nonmethylene-interrupted dienoic acid as in the original *cis*-9,*cis*-12,*cis*-15 18:3 acid molecule. If the hypothesis is not valid, each of the two double bonds should behave like an isolated double bond, as in monoenoic acids, and should not isomerize at all. In the present study, we demonstrate that the *cis*-9,*cis*-15 18:2 acid is insensitive to heat treatments and does not isomerize under experimental conditions that otherwise cause extensive isomerization of α-linolenic acid, and to a lesser extent of linoleic acid.

EXPERIMENTAL PROCEDURES

Hydrazine reduction of fatty acid methyl esters (FAME) prepared with linseed oil. Linseed oil (technical grade) was saponified, and the resulting free fatty acids were transformed into FAME by reacting them with 12% (wt/vol) methanolic BF_3. FAME were then submitted to hydrazine reduction according to Ratnayake (11) and Conway *et al.* (12). Briefly, FAME (3.5 g) were dissolved in 200 mL of 95% ethanol, maintained at 40°C. Hydrazine (2 mL) was added, and a stream of oxygen was applied to the surface of the solution,

*To whom correspondence should be addressed at Laboratoire de Lipochimie Alimentaire, ISTAB, Universite Bordeaux 1, Allee des Facultes, 33405, Talence Cedex, France.

which was continuously stirred. The progress of the reaction was monitored by gas–liquid chromatography (GLC) of FAME every hour. After 4 h, water was added, and FAME were extracted with hexane.

Heating of FAME. Approximately 2 g of FAME, treated with hydrazine, were sealed under vacuum in a glass ampoule and heated for 2.25 h in an oven held at 270 ± 5°C. The temperature was measured with an electronic thermometer. Linseed oil and FAME prepared with linseed oil, but not reduced with hydrazine, were heated under the same conditions, at the same time as hydrazine-treated FAME.

NO_2-isomerization of hydrazine-reduced FAME. Linseed oil-derived FAME that were partially reduced with hydrazine (400 µL) were added to a mixture of 6 M HNO_3 (400 µL) and 2M $NaNO_2$ (500 µL) aqueous solutions in a Teflon-lined screw-capped tube. Elaidination was allowed to proceed at 40°C for 20 min under continuous magnetic stirring. This duration is sufficient to obtain all geometrical isomers in sufficient proportions for accurate GLC analyses. Water was then added, and the modified FAME were extracted with hexane (13). An aliquot of these modified FAME was further purified by thin-layer chromatography (TLC).

Fractionation of FAME by argentation TLC (Ag-TLC). Heated hydrazine-reduced FAME and purified NO_2-isomerized hydrazine-reduced FAME were fractionated on $AgNO_3$-impregnated silicic acid-coated plates as described previously in detail (2) with hexane/diethyl ether (90:10, vol/vol) as migration solvent. Bands were detected by spraying a solution of 2′,7′-dichlorofluorescein in ethanol (0.2%, wt/vol) and observation under ultraviolet (UV) light.

GLC. Analyses of FAME were carried out on a Carlo Erba 4130 chromatograph equipped with a flame-ionization detector and a split injector (Carlo Erba, Milano, Italy). A fused-silica capillary column (CP-Sil 88, 50 m × 0.25 mm i.d., 0.20 µm film; Chrompack, Middelburg, The Netherlands) was used with helium as carrier gas (inlet pressure, 120 kPa). The oven was maintained at 160°C, and the injection port and the detector were held at 250°C. The chromatograph was coupled with an SP 4290 integrator (Spectra Physics, San Jose, CA).

Equivalent chainlength (ECL) determinations. Experimental ECL were determined according to Ackman (14) with 16:0, 18:0, and 20:0 acid methyl esters as reference compounds. All analyses were made twice. Calculated ECL were established according to Sebedio and Ackman (15) or Wolff (10).

RESULTS AND DISCUSSION

In all of our previous studies on the *cis-trans* isomerization of ethylenic bonds in octadecatrienoic acids (7,9,10), we worked on heated oils, i.e., on triglycerides. In the present study, we have used FAME instead of triglycerides. However, this modification does not affect, to a great extent, the isomerization reaction characteristics. This is supported by the comparison of linseed oil and of FAME derived from linseed oil that were heated under the same conditions. The degrees of isomerization (DI) of α-linolenic acid in the oil was 87%, and 78% in FAME after heating. In both cases, there was an overall loss of *ca.* 3–5% of α-linolenic acid, which can be attributed to side reactions other than *cis-trans* isomerization (cyclization, polymerization). Only slight differences occurred in the distribution pattern of individual α-linolenic acid geometrical isomers between heated FAME and triglycerides (results not shown). Consequently, FAME are as well suited as triglycerides for the study of the heat-induced isomerization of ethylenic bonds in polyunsaturated fatty acids.

Hydrazine reduction for 4 h of FAME, prepared from linseed oil, and initially containing 56.5% α-linolenic acid, resulted in the formation of 7.0% *cis*-9,*cis*-12 18:2 acid, 5.0% *cis*-9,*cis*-15 18:2 acid, and 3.2% *cis*-12,*cis*-15 18:2 acid, with 2.0% of residual *cis*-9,*cis*-12,*cis*-15 18:3 acid. Hydrazine-reduced FAME will be hereafter referred to in the text as H-FAME. Geometrical isomerization of this mixture with NO_2 led to the formation of all possible *trans* isomers of these acids, in sufficient proportions to allow accurate GLC data determinations. This complex mixture was fractionated by Ag-TLC. Silica gel from the whole plate was scraped in bands of approximately 1.5 cm width. These bands were those in which FAME were observed but also those where nothing could be seen under UV light. The distribution of FAME on the plate is summarized in Table 1 and illustrated in Figure 1. The identification of individual isomers was based on their migration characteristics after Ag-TLC fractionation (Table 1) and their ECL (Table 2). In some instances, this was done by comparison with authentic standards or with FAME prepared with NO_2-isomerized linseed oil (13). In fact, ECL are useful mainly for the identification of *cis*-9,*cis*-15 and *cis*-12,*cis*-15 18:2 acids and their geometrical isomers for which no standards are available. The accuracy in the determination

TABLE 1
Distribution of Individual Unsaturated Fatty Acid Methyl Esters (FAME) from Linseed Oil, Partially Reduced with Hydrazine and Isomerized with NO_2, After Fractionation by Argentation Thin-Layer Chromatography

R_f	FAME
0.70[a]	*Trans*-9 18:1, *trans*-12 18:1, *trans*-15 18:1
0.56	*Cis*-12 18:1, *cis*-15 18:1, (*cis*-9 18:1, *trans*-12,*trans*-15 18:2)[b]
0.50	*Cis*-9 18:1, *trans*-9,*trans*-12 18:2, *trans*-12,*trans*-15 18:2, (*cis*-12 18:1, *cis*-15 18:1)
0.43	*Trans*-9,*trans*-12 18:2, (*cis*-9 18:1)
0.36	*Trans*-9,*trans*-15 18:2, (*cis*-12,*trans*-15 18:2, *trans*-12,*cis*-15 18:2)
0.28	*Cis*-9,*trans*-12 18:2, *trans*-9,*cis*-12 18:2, *cis*-12,*trans*-15 18:2, *trans*-12,*cis*-15 18:2, (*trans*-9,*trans*-15 18:2)
0.25	*Cis*-9,*trans*-15 18:2, *trans*-9,*cis*-15 18:2, *trans*-9,*trans*-12, *trans*-15 18:3
0.11	*Cis*-9,*trans*-15 18:2, *trans*-9,*cis*-15 18:2, *cis*-9,*cis*-12 18:2, *cis*-12,*cis*-15 18:2, *cis*-9,*trans*-12,*trans*-15 18:3, *trans*-9,*cis*-12,*trans*-15 18:3, *trans*-9,*trans*-12,*cis*-15 18:3
0.07	*Cis*-9,*cis*-15 18:2

[a]Two close bands scraped together.
[b]Fatty acids in parentheses are minor components of the fraction.

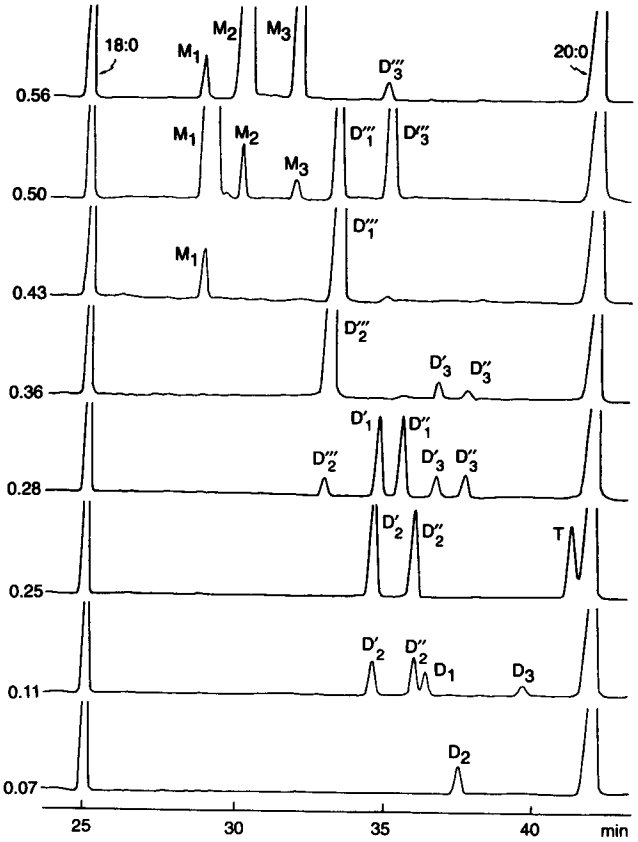


FIG. 1. Chromatograms of fractions that contain isomeric octadecadienoic acids isolated by argentation thin-layer chromatography (Ag-TLC) from fatty acid methyl esters prepared from linseed oil, partially reduced with hydrazine and isomerized with NO_2, with 18:0 and 20:0 acid methyl esters added to each fraction. Analyses on a CP-Sil 88 capillary column (50 m × 0.22 mm i.d.; Chrompack, Middelburg, The Netherlands), operated at 160°C with an inlet pressure of helium of 120 kPa. Peak identification: M_1, *cis*-9 18:1; M_2, *cis*-12 18:1; M_3, *cis*-15 18:1; D_1, *cis*-9,*cis*-12 18:2; D'_1, *cis*-9,*trans*-12 18:2; D''_1, *trans*-9,*cis*-12 18:2; D'''_1 *trans*-9,*trans*-12 18:2; D_2, *cis*-9,*cis*-15 18:2; D'_2, *cis*-9,*trans*-15 18:2; D''_2, *trans*-9,*cis*-15 18:2; D'''_2 *trans*-9,*trans*-15 18:2; D_3, *cis*-12,*cis*-15 18:2; D'_3, *cis*-12,*trans*-15 18:2; D''_3, *trans*-12,*cis*-15 18:2; D'''_3 *trans*-12,*trans*-15 18:2; T, *trans*-9,*trans*-12,*trans*-15 18:3. Values on the left of chromatograms are the R_f of the fractions isolated by Ag-TLC.

of ECL is remarkable: the differences in ECL for a given component are always less than 0.01 unit between two successive GLC runs. Incidentally, the mean dienoic adjustment (difference between calculated and experimental ECL) for 9,12-18:2 isomers (–0.16 ± 0.02) is higher than that for the 12,15-18:2 isomers (–0.09 ± 0.03), and the mean dienoic adjustment for 9,15-18:2 isomers is very small (–0.02 ± 0.02). Consequently, the agreement between experimental and calculated ECL for these last isomers is excellent, which is fortunate for our study. Geometrical isomers of 9,15-18:2 acid can thus be located on chromatograms with good accuracy. To calculate the ECL of octadecatrienoic acids, we have summed up the fractional chainlengths (FCL; FCL = ECL – base value) of the related methylene-interrupted dienoic acids and the base value, and subtracted the FCL of the monoenoic acid corresponding to the central ethylenic bond. For example, the ECL of *trans*-9,*cis*-12,*trans*-15 18:3 acid is the sum of 18.00 (base value), plus the FCL of *trans*-9,*cis*-12 18:2 (1.39) and *cis*-12,*trans*-15 18:2 (1.52) acids, minus the FCL of *cis*-12 18:1 acid (0.76), which equals to 20.15 (Table 2).

Under our chromatographic conditions, FAME that present a difference between their ECL equal to or less than 0.03 are not resolved. Thus, if heating of H-FAME produces mono-*trans* isomers of *cis*-9,*cis*-15 18:2 acid, the *cis*-9,*trans*-15 18:2 isomer will not be distinguishable from the *cis*-9,*trans*-12 18:2 acid, which is formed upon heating (16) (ECL of 19.29 and 19.30, respectively; Table 2). However, if the *trans*-9, *cis*-15 18:2 acid is formed, it should elute between the *trans*-9,*cis*-12 and the *cis*-9,*cis*-12 18:2 acids (Table 2) with sufficiently good resolution to be detected. When H-FAME are heated at 270°C for 2.25 h, no peak appears at this place (Fig. 2). This absence, and also that of the *cis*-9,*trans*-15 18:2 isomer, was confirmed by the analysis of fractions collected after Ag-TLC fractionation of heated H-FAME. Although the whole plate was scraped, no components with the ECL of these isomers could be detected, even after a fivefold concentration of the samples prior to GLC analysis. This also holds true for the *trans*-9,*trans*-15 18:2 isomer. We can thus conclude that the *cis*-9,*cis*-15 18:2 acid does not isomerize when heated. On the other hand, heating induces a partial isomerization of *cis*-9,*cis*-12 18:2 acid to *cis*-9,*trans*-12 and *trans*-9,*cis*-12 18:2 acids, easily identifiable on chromatograms (Fig. 2). The two mono-*trans* isomers represent 9.5% of the total 9,12 18:2 acids (Table 3). The *cis*-12,*cis*-15 18:2 acid also geometrically isomerizes when heated. One of its mono-*trans* isomer (the *cis*-12,*trans*-15 18:2 acid) is visible as a shoulder on the trailing edge of the main *cis*-9,*cis*-12 18:2 acid (Fig. 2), but the second isomer, if present, is entirely masked by the *cis*-9,*cis*-15 18:2 acid (Table 2 and Fig. 2). However, the two mono-*trans* isomers of *cis*-12,*cis*-15 18:2 acid could be isolated by Ag-TLC, together with the mono-*trans* isomers of *cis*-9,*cis*-12 18:2 acid (Fig. 2). This also allowed their quantitation: the mono-*trans* isomers of *cis*-12,*cis*-15 18:2 acid represent 9.5% of the total 12,15-18:2 isomers, which is the same proportion as that of mono-*trans* isomers of *cis*-9,*cis*-12 18:2 acid (Table 3). In both cases, it is the ethylenic bond nearest to the methyl end of the fatty acid molecule that isomerizes to the highest rate (Table 3). No di-*trans* isomers of these two methylene-interrupted dienoic acids could be detected. Despite the high DI of α-linolenic acid observed in either heated linseed oil or heated FAME derived from linseed oil, we did not detect the *trans*-9,*trans*-12,*trans*-15 18:3 isomer. The *trans*-9,*trans*-12,*cis*-15 and *cis*-9,*trans*-12,*trans*-15 18:3 isomers were present in trace amounts only, although the *trans*-9,*cis*-12,*trans*-15 18:3 acid accounted for as much as 30% of total α-linolenic acid *trans* isomers. The *cis*-9,*trans*-12,*cis*-15 18:3 acid was only 10% of total *trans* 18:3 isomers.

Our experiment conclusively demonstrates that the two

TABLE 2
Experimental and Calculated Equivalent Chainlengths (ECL) of Octadecenoic, Octadecadienoic, and Octadecatrienoic Acid Geometrical Isomers Related to α-Linolenic Acid

Isomer[a]	ECL[b]			Presence[c]
	Experimental	Calculated	Difference	
Trans-9	18.44	—	—	No
Trans-12	18.55	—	—	No
Cis-9	18.59	—	—	Yes
Trans-15	18.67	—	—	No
Cis-12	18.76	—	—	Yes
Cis-15	18.99	—	—	Yes
Trans-9,*trans*-15	19.10	19.11	09.01	No
Trans-9,*trans*-12	19.14	18.99	−0.15	No
Cis-9,*trans*-15	19.29	19.26	−0.03	No
Cis-9,*trans*-12	19.30	19.14	−0.16	Yes
Trans-12,*trans*-15	19.34	19.22	−0.12	No
Trans-9,*cis*-12	19.39	19.20	−0.19	Yes
Trans-9,*cis*-15	19.44	19.43	−0.01	No
Cis-9,*cis*-12	19.49	19.35	−0.14	Yes
Cis-12,*trans*-15	19.52	19.43	−0.09	Yes
Cis-9,*cis*-15	19.60	19.58	−0.02	Yes
Trans-12,*cis*-15	19.62	19.54	−0.08	Yes
Cis-12,*cis*-15	19.81	19.75	−0.06	Yes
Trans-9,*cis*-12,*trans*-15	20.14	20.15	0.01	Yes
Cis-9,*cis*-12,*trans*-15	20.22	20.25	0.03	Yes
Cis-9,*trans*-12,*cis*-15	20.39	20.37	−0.02	Yes
Trans-9,*cis*-12,*cis*-15	20.43	20.44	0.01	Yes
Cis-9,*cis*-12,cis-15	20.50	20.54	0.04	Yes

[a]Octadecenoic and octadecadienoic acids from fatty acid methyl esters (FAME) prepared with linseed oil partially reduced with hydrazine and further geometrically isomerized with NO_2. Octadecatrienoic acids from FAME partially reduced with hydrazine and heated under vacuum at 270°C for 2.25 h. FAME are listed in descending order according to their elution order from the column.

[b]ECL experimentally determined (mean of two analyses) or calculated as described in the text, with a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands) operated as indicated in the legend of Figure 1. Differences are between calculated and experimental ECL.

[c]Presence (Yes) or absence (No) of a given isomer in FAME from linseed oil partially reduced with hydrazine and heated at 270°C for 2.25 h.

methylene-interrupted dienoic acids (*cis*-9,*cis*-12 and *cis*-12,*cis*-15 18:2 acids), derived from α-linolenic acid, isomerize to the corresponding mono-*trans* isomers at the same rate when heated. On the other hand, the nonmethylene-interrupted dienoic acid (*cis*-9,*cis*-15 18:2 acid) does not isomerize at all under the same conditions. In a previous study (10), it was hypothesized that isomerization of the external bonds in α-linolenic acid could result from some direct interactions between these bonds, following spatial folding of the molecule. If this were true, the *cis*-9,*cis*-15 should have isomerized like *cis*-9,*cis*-12,*cis*-15 18:3 acid, but this did not happen. Consequently, we must deduce that the *cis*-12 ethylenic bond is necessary for the isomerization of the *cis*-9 and *cis*-15 ethylenic bonds in α-linolenic acid, even if it does not itself isomerize to a great extent.

None of the three double bonds isomerize when they are isolated in monoenoic acids (Table 2). Isomerization begins when there are at least two ethylenic bonds in the same fatty acid molecule. These bonds can be methylene-interrupted (this study) or ethylene-interrupted (10). However, there is a difference, depending on the number of carbon atoms between the ethylenic bonds: an ethylene-interrupted dienoic acid, such as *cis*-5,*cis*-9 18:2 acid (present in *Pinus koraiensis* and *Taxus baccata* seed oils), gives rise to the corresponding di-*trans* isomer (10), whereas a methylene-interrupted dienoic acid generates the two corresponding mono-*trans* isomers (this study). When the two double bonds are separated by four methylenes, as in the present experiment, they do not isomerize. An experimental rule concerning the *cis-trans* isomerization of ethylenic bonds in methylene-interrupted diethylenic systems can be established. This rule applies to either octadecadienoic acids or to α-linolenic acid. When one of the ethylenic bonds in a methylene-interrupted dienoic system is isomerized to the *trans* configuration, the other bond does not isomerize, at least under our experimental conditions, and remains in the *cis* configuration. Methylene-interrupted dienoic acids can only give rise to the corresponding mono-*trans* isomers. In α-linolenic acid, two adjacent ethylenic bonds cannot both have the *trans* configuration. This acid does not give rise to significant amounts of the *trans*-9,*trans*-12,*cis*-15 and *cis*-9,*trans*-12,*trans*-15 18:3 acids upon heating, and even less so to the tri-*trans* isomer. A *cis-trans* methylene-interrupted dienoic system is apparently stabilized against any further effect of heating, provided the tempera-

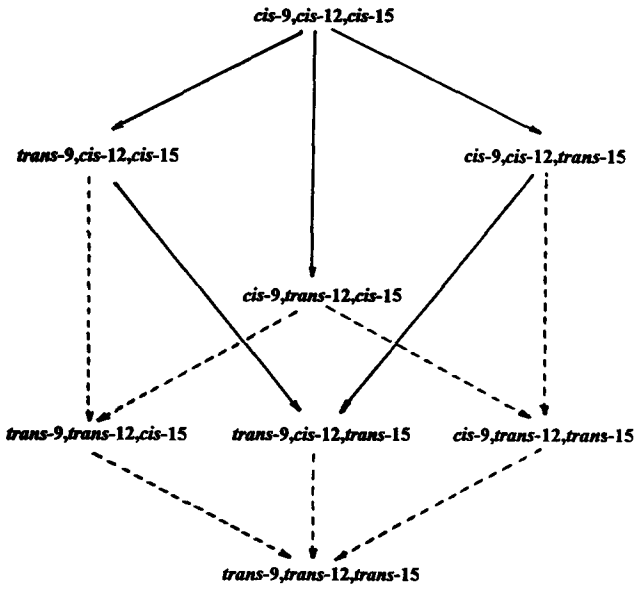


FIG. 2. Partial chromatograms of fatty acid methyl esters prepared from linseed oil and partially reduced with hydrazine, before (upper chromatogram) and after (middle chromatogram) heating at 270°C for 2.25 h in a glass ampoule sealed under vacuum. Lower chromatogram: methylene-interrupted mono-*trans* dienoic acid fraction isolated by argentation thin-layer chromatography, with 18:0 and 20:0 acid methyl esters added. Commercial source of the column and chromatographic conditions as in Figure 1. Identification of peaks: S_1, 18:0; S_2, 20:0; M_1, *cis*-9 18:1; M_2, *cis*-12 18:1; M_3, *cis*-15 18:1; D_1, *cis*-9,*cis*-12 18:2; D'_1, *cis*-9,*trans*-12 18:2; D''_1, *trans*-9,*cis*-12 18:2; D_2, *cis*-9,*cis*-15 18:2; D_3, *cis*-12,*cis*-15 18:2; D'_3, *cis*-12,*trans*-15 18:2; D''_3, *trans*-12,*cis*-15 18:2; T_1, *cis*-9,*cis*-12,*cis*-15 18:3; T_2, *trans*-9,*cis*-12,*trans*-15 18:3; T_3, *cis*-9,*cis*-12,*trans*-15 18:3; T_4, *cis*-9,*trans*-12,*cis*-15 18:3; T_5, *trans*-9,*cis*-12,*cis*-15 18:3.

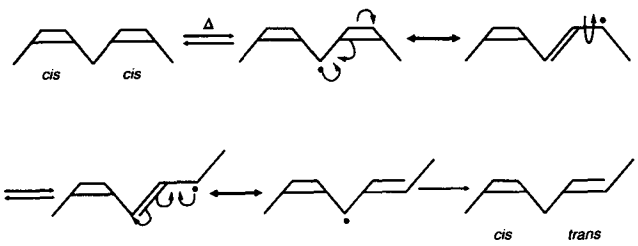
ture is equal to or less than 270°C. Such a temperature appears to be the highest used for industrial oil deodorization, and the *trans*-9,*trans*-12,*cis*-15, *cis*-9,*trans*-12,*trans*-15 and *trans*-9,*trans*-12,*trans*-15 18:3 isomers should not be present in commercial deodorized oils in amounts higher than traces.

If one considers a methylene-interrupted dienoic acid structurally related to α-linolenic acid, the *cis*-9,*cis*-12 18:2 acid for example, the addition of the third *cis*-15 double bond considerably increases the isomerization rate of the *cis*-9 ethylenic bond. Alternately stated, this last bond is weakened vis-à-vis *cis-trans* isomerization when it is associated with a methylene-interrupted diethylenic system in a methylene-interrupted manner. Two double bonds exert a more powerful influence on this isomerization than a single ethylenic bond.

TABLE 3
Quantitative Data Concerning the Heat-Induced Isomerization Products of Octadecadienoic Acids Structurally Related to α-Linolenic Acid

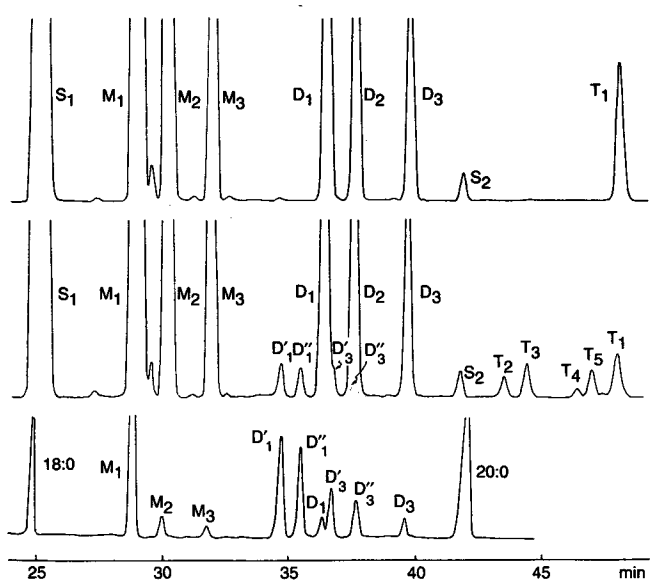
	9,12-18:2	12,15-18:2	9,15-18:2
DI[a]	9.6	9.5	0
c,t[b]	57.4	56.1	—
t,c	42.6	43.9	—
t,t	0	0	—

[a]Degree of isomerization: sum of *trans* isomers divided by total isomers times 100 (mean of two analyses).
[b]*c-cis; t-trans.* Percentages relative to total *trans* isomers (mean of two analyses).



SCHEME 1

To explain the isomerization of adjacent methylene-interrupted ethylenic bonds, it seems appropriate to postulate the existence of a transient free radical on the methylene group as that shown in Scheme 1. However, this working hypothesis deserves further experimental support. It is not clear how this intermediate radical is formed (temperature alone and thermal agitation, traces of oxygen and formation of unstable peroxides), but it should be stabilized by delocalization of the five available electrons. The isomerization may then occur in both directions and give rise to two mono-*trans* isomers in roughly equal amounts. An important step in the isomerization reaction would be the formation of an unstable conjugated *cis-cis* radical, that would give rise, following rotation of the former *cis*-ethylenic bond, to the more stable *cis-trans* methylene-interrupted structure. From experimental observations, this structure is apparently unable to isomerize a second time. Consequently, one must deduce that it cannot generate a new radical that could lead to the di-*trans* isomer. Once formed, the *cis*-9,*trans*-12,*cis*-15 18:3 isomer, because it cannot give a free radical, is an end-product of the reaction. Practically, the *trans*-ethylenic bond behaves like a single bond, and the *cis*-9,*trans*-12,*cis*-15 18:3 isomer is equivalent to the *cis*-9,*cis*-15 18:2 acid regarding heat-induced *cis-trans* isomerization. If one considers, for example, the *trans*-9,*cis*-12,*cis*-15 18:3 isomer, this isomer would be able to generate a free radical on the methylene in position 14 (because it is present between two *cis*-ethylenic bonds), but not on that in position 11 (because it is present between one *cis*- and one *trans*-ethylenic bonds): consequently, it could only generate the *trans*-9,*cis*-12,*trans*-15 18:3 acid, but not the *trans*-9,*trans*-12,*cis*-15 18:3 isomer. For the same reasons, the *cis*-9,*cis*-12,*trans*-15 18:3 isomer could only produce the *trans*-9,*cis*-12,*trans*-15 18:3 acid. The main allowed *cis-trans* reactions in heated α-linolenic acid, based on experimental evidences, are summarized in Scheme 2.



SCHEME 2

REFERENCES

1. Ackman, R.G., S.N. Hooper, and D.L. Hooper, Linolenic Acid Artifacts from the Deodorization of Oils, *J. Am. Oil Chem. Soc. 51*:42–49 (1974).
2. Wolff, R.L., and J.-L. Sebedio, Geometrical Isomers of α-Linolenic Acid in Low-Calorie Spreads Marketed in France, *Ibid. 68*:719–725 (1991).
3. Wolff, R.L., *Trans*-Polyunsaturated Fatty Acids in French Edible Rapeseed and Soybean Oils, *Ibid. 69*:106–110 (1992).
4. Wolff, R.L., Further Studies on Artificial Geometrical Isomers of α-Linolenic Acid in Edible Linolenic Acid-Containing Oils, *Ibid. 70*:219–224 (1993).
5. Wolff, R.L., Occurrence of Artificial *trans*-Polyunsaturated Fatty Acids in Refined (deodorized) Walnut Oils, *Sci. Alim. 13*:155–163 (1993).
6. O'Keefe, S., S. Gaskins-Wright, V. Wiley, and I.C. Chen, Levels of *trans* Geometrical Isomers of Essential Fatty Acids in Some Unhydrogenated U.S. Vegetable Oils, *J. Food Lipids 1*:165–176 (1994).
7. Wolff, R.L., Heat-Induced Geometrical Isomerization of α-Linolenic Acid: Effect of Temperature and Heating Time on the Appearance of Individual Isomers, *J. Am. Oil Chem. Soc. 70*:425–430 (1993).
8. O'Keefe, S.F., V.A. Wiley, and D. Wright, Effect of Temperature on Linolenic Acid Loss and 18:3 Δ9-*cis*,Δ12-*cis*,Δ15-*trans* Formation in Soybean Oil, *Ibid. 70*:915 (1993).
9. Wolff, R.L., and J.-L. Sebedio, Characterization of γ-Linolenic Acid Geometrical Isomers in Borage Oil Subjected to Heat Treatments (deodorization), *Ibid. 71*:117 (1994).
10. Wolff, R.L., *cis-trans* Isomerization of Octadecatrienoic Acids During Heating. Study of Pinolenic (*cis*-5,*cis*-9,*cis*-12 18:3) Acid Geometrical Isomers in Heated Pine Seed Oil, *Ibid. 71*:1129–1134 (1994).
11. Ratnayake, W.M.N., Studies on Fatty Acids from Nova Scotian Seaweeds and on the Specificity of Hydrazine Reduction of Unsaturated Fatty Acids, Ph. D. Thesis, University of Dalhousie, Halifax, Canada, 1981.
12. Conway, J., W.M.N. Ratnayake, and R.G. Ackman, Hydrazine Reduction in the Gas–Liquid Chromatographic Analysis of the Methyl Esters of Cyclopropenoic Fatty Acids, *J. Am. Oil Chem. Soc. 62*:1340 (1985).
13. Wolff, R.L., Resolution of Linolenic Acid Geometrical Isomers by Gas–Liquid Chromatography on a Capillary Column Coated with 100% Cyanopropyl Polysiloxane Stationary Film (CP^{TM}Sil 88), *J. Chromatogr. Sci. 30*:17 (1992).
14. Ackman, R.G., The Analysis of Fatty Acids and Related Materials by Gas–Liquid Chromatography, *Prog. Chem. Fats other Lipids 12*:167–284 (1972).
15. Sebedio, J.-L., and R.G. Ackman, Calculation of GLC Retention Data for Some Accessible C_{20} Isomeric *cis*-Unsaturated Fatty Acids, *J. Chromatogr. Sci. 20*:231–234 (1982).
16. Sebedio J.-L., A. Grandgirard, and J. Prevost, *J. Am. Oil Chem. Soc. 65*:362–366 (1988).

[Received January 16, 1995; accepted November 13, 1995]