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

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To understand the heat-induced *cis-trans* isomerization of ethylenic bonds in octadecatrienoic acids, pine seed oil, which contains the unusual nonmethylene-interrupted pinolenic (*cis*-5,*cis*-9,*cis*-12 18:3) acid as a major component, was heated under vacuum at 240°C for 6 h together with linseed and borage oils. As a result, a small percentage of pinolenic acid undergoes *cis-trans* isomerization. The main isomer that accumulates is the *trans*-5,*cis*-9,*trans*-12 18:3 acid. Minor amounts of the three mono-*trans* isomers are also present. Identification of isomers was realized by combining gas-liquid chromatography on a CP Sil 88 capillary column, argentation thin-layer chromatography and comparing the equivalent chainlengths of artifacts to those of isomers present in NO_2-isomerized pine seed oil. Hydrazine reduction was used to demonstrate that there was no positional shift of double bonds. Heat-induced geometrical isomerization of pinolenic acid differs from that of α- and γ-linolenic acids in at least two aspects. The reaction rate is slower (about one-fourth), and mono-*trans* isomers are formed in low amounts.

KEY WORDS: Geometrical isomers, heated oil, α-linolenic acid, γ-linolenic acid, pinolenic acid, reaction mechanism, *trans* fatty acids.

Octadecatrienoic acids such as α-linolenic (*cis*-9,*cis*-12,*cis*-15 18:3) acid and γ-linolenic (*cis*-6,*cis*-9,*cis*-12 18:3) acid undergo *cis-trans* isomerization of their ethylenic bonds when they are heated under vacuum at temperatures higher than *ca.* 200°C (1,2). Heating in the absence of oxygen does not promote any noticeable positional shift of double bonds in octadecatrienoic acids or in linoleic acid. Although the two octadecatrienoic acids differ by the absolute location of their double bonds, they give rise to several geometrical isomers that have *trans* double bonds in the same relative position along the hydrocarbon chain, in approximately the same proportions (2). Moreover, because the rates of isomerization of the two octadecatrienoic acids are similar (2), it is probable that the reaction mechanism of geometrical isomerization is the same for both acids. The main feature of this reaction is the formation of two major isomers with one *trans* double bond located either on the side of the carboxylic group or on the side of the methyl group. The internal double bond is considerably more resistant to geometrical isomerization than the two external double bonds (1,2). At least in the case of α-linolenic acid, it has been demonstrated (1) that the formation of the di-*trans*, *trans*-9,*cis*-12 ,*trans*-15, 18:3 isomer is made at the expense of the two main mono-*trans*, *trans*-9,*cis*-12,*cis*-15 and *cis*-9,*cis*-12,*trans*-15, 18:3 isomers. Its initial probability of formation at the very beginning of the reaction is close to zero, and its level increases as the reaction continues (1).

Pinolenic acid (*cis*-5,*cis*-9,*cis*-12 18:3 acid) is an uncommon C_{18} nonmethylene-interrupted trienoic acid that is relatively abundant in pine seed oil (3). This fatty acid and α- and γ-linolenic acids have the presence of three *cis* ethylenic bonds, two of these being located in positions 9 and 12, in common. Although pine seed oil has no recognized nutritional interest, pinolenic acid can be of some help to understand the mechanism of the *cis-trans* isomerization that occurs upon heating in α- or γ-linolenic acids. With this in mind, we have focused our attention on the separation and identification of pinolenic acid geometrical isomers (PAGI). For this purpose, pine seed oil was isomerized with nitrous acid or heated in ampoules sealed under vacuum, and the fatty acids in the resulting products were analyzed by combining gas-liquid chromatography (GLC) and argentation thin-layer chromatography (Ag-TLC) of their methyl esters.

EXPERIMENTAL PROCEDURES

Samples. Pine seed oil (NOF Corporation, Tokyo, Japan) and linseed oil (technical grade) were kind gifts from Dr. J.-L. Sebedio (INRA, Dijon, France). Cold-pressed borage oil was kindly provided by Dr. M. Le Scao (ISTAB, Bordeaux, France). *Aquilegia vulgaris* (Columbine) seeds (*ca.* 1 g) were purchased in a seed shop. The seeds were crushed in hexane, the suspension was filtered, and the solvent from the filtrate was evaporated. The oil was used as such without further purification. *Taxus baccata* (Yew) arils were collected in local parks and gardens (region of Bordeaux, France) during September. Ground yew seeds (about 150 g) were mixed with anhydrous Na_2SO_4, and the oil was extracted in Soxhlet and Kumagawa apparatuses from 10- to 15-g portions with petroleum ether. The combined extracts were evaporated, and the resulting dark-green oil was dissolved in hexane. This solution was washed once with an equal volume of a mixture of methanol/water (1:1, vol/vol). The hexane phase was withdrawn, then stirred with silica gel and filtered on paper. The filtrate was stirred with activated charcoal powder and filtered a second time. After evaporation of the solvent, about 8 mL of a bright-yellow oil was obtained. Synthetic *cis*-3 to *cis*-17 and *trans*-5 to *trans*-15 18:1 acids were generously donated by Dr. L. Svensson (Kabi Pharmacia, Stockholm, Sweden).

Preparation of PAGIs. Pine seed oil was isomerized with nitrous acid, which was generated by mixing nitric acid and aqueous sodium nitrite, as described previously (2,4). Alternatively, pine seed oil was heated in the dark at 240°C for 6 h in a glass ampoule that was sealed under vacuum (1,2). Aliquots of borage oil and linseed oil were simultaneously heated under the same conditions.

Preparation of fatty acid methyl esters (FAME). FAME were prepared mainly (1,2) according to Morrison and Smith (5) with 10% BF_3 in methanol as a reagent.

Ag-TLC. FAMEs were separated according to the number and geometry of their double bonds, and also according to the distance between ethylenic bonds by TLC on silica-gel plates impregnated with $AgNO_3$, as described previously (2).

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Hydrazine reduction of ethylenic bonds. Mono- and di-*trans* isomers of heated pine seed oil plus the all-*cis* isomer itself were isolated as single fractions by multiple Ag-TLC. The methyl esters were saponified and treated with hydrazine hydrate (2). The resulting mixture of partially reduced fatty acids was methylated, and FAMEs were further fractionated by Ag-TLC to isolate *trans*- and *cis*-octadecenoic acids.

GLC. Analyses of FAMEs 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, 100 kPa). It was operated isothermally at 160°C until 20:0 acid or the last octadecatrienoic acid of interest eluted. The temperature was then increased at a rate of 10°C/min to 195°C and held until completion of the analysis. The injection port and detector temperature were maintained at 250°C. Fatty acids from heated borage oil were analyzed as isopropyl esters on the same column operated under previously described conditions (2). Equivalent chainlengths (ECL) were calculated according to Ackman (6) with 16:0, 18:0 and 20:0 acid methyl esters as standards.

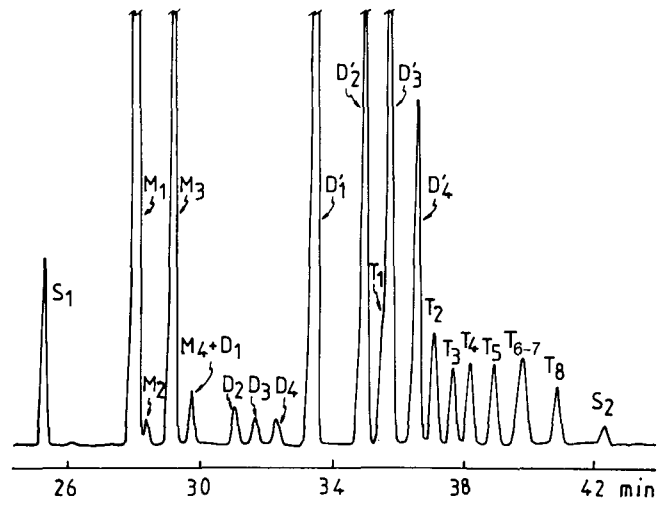

FIG. 1. Partial chromatogram showing the C_{18} region of fatty acid methyl esters prepared from NO_2-isomerized pine seed oil. Analysis on a CP Sil 88 fused-silica capillary column (50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middelburg, The Netherlands) operated at 160°C with an inlet pressure of the carrier gas (helium) of 100 kPa. M, monoenes; D, nonmethylene-interrupted dienes; D', methylene-interrupted dienes; T, nonmethylene-interrupted trienes. Peak identification as in Table 1.

RESULTS AND DISCUSSION

To identify PAGIs that may appear upon heat treatment of pine seed oil, we first studied NO_2-isomerized pine seed oil. A partial chromatogram of FAMEs, prepared with the modified oil and analyzed on a CP Sil 88 capillary column, is given in Figure 1. Note the excellent resolution that is obtained inside each family of isomers—monoenes, nonmethylene-interrupted dienes (5,9-18:2 acids), methylene-interrupted dienes (9,12-18:2 acids), nonmethylene-interrupted trienes (5,9,12-18:3 acids) (peaks M, D, D' and T, respectively, in Fig. 1). For the last group, seven peaks are baseline resolved. Enlargement of one of the peaks (peak T_{6-7} in Fig. 1) indicates that it contains two isomers that are on the verge of separating. However, some overlaps occur between the different families. Peak T_1 in Figure 1 is eluted as a shoulder on the leading edge of one mono-*trans* isomer of 18:2n-6 acid (peak D'_3). Components M_4 and D_1 are not separated at all. The fact that PAGIs are easier to resolve than γ-linolenic acid geometrical isomers (G-LAGI) (2) on the CP Sil 88 column is probably linked to the contribution of the double bond nearest the carboxylic group to the ECL of the various isomers. For example, the difference on cyanoalkyl polysiloxane stationary phases between ECL of *cis*-6 and *cis*-9 18:1 acids is only about 0.05 carbon units (7,8). On the other hand, the difference between ECL of *cis*-5 and *cis*-9 18:1 acids is *ca.* 0.18 carbon units (7,8).

Fractionation of the mixture of FAMEs (from NO_2-isomerized pine seed oil) by Ag-TLC allows separation of the tri-*trans*, di-*trans* and mono-*trans* isomers from the all-*cis* pinolenic acid (Fig. 2). No less important is the fact that it is also possible to separate geometrical isomers of the minor C_{18} nonmethylene-interrupted dienoic acid, *cis*-5,*cis*-9 18:2 acid, which is initially present in low amounts (*ca.* 2% of total fatty acids) in pine seed oil. This uncommon fatty acid is easily located on chromatograms by comparison with FAME prepared from *T. baccata* seed

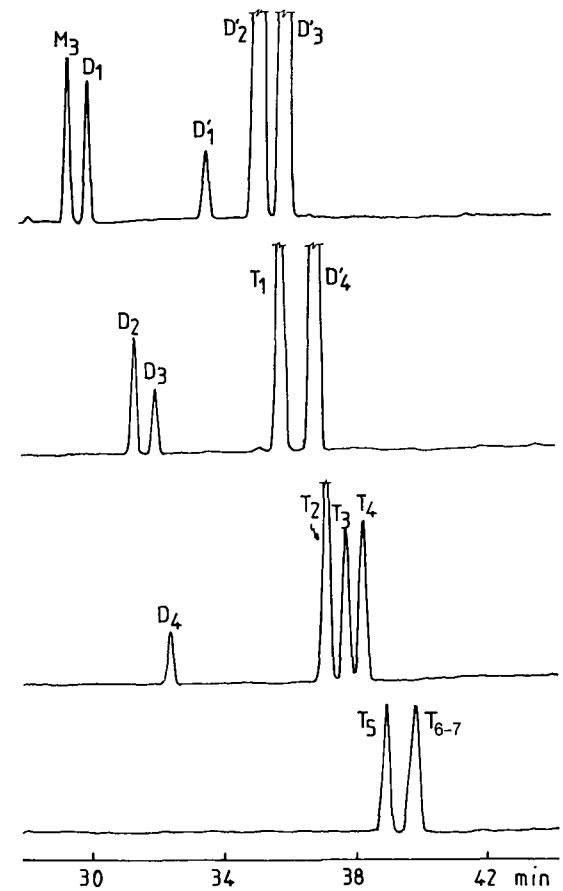

FIG. 2. Partial chromatograms on a CP Sil 88 capillary column of some fractions of fatty acid methyl esters (FAME) separated by argentation thin-layer chromatography, starting with FAME prepared with NO_2-isomerized pine seed oil. Peak lettering as in Figure 1, identification of peaks as in Table 1. See Figure 1 for characteristics, operating conditions and company source of CP Sil 88.

oil, a relatively rich source of *cis*-5,*cis*-9 18:2 acid (9) (11% of total fatty acids in the oil we prepared). Geometrical isomers of this nonmethylene-interrupted dienoic acid are also separated according to their number of *cis* and *trans* double bonds by Ag-TLC. The fast-moving diene is the all-*trans* isomer (peak D_1 in Fig. 2). Following this band is a band containing, among other components, a mixture of the *trans*-5,*cis*-9 and *cis*-5,*trans*-9 18:2 acids (peaks D_2 and D_3 in Fig. 2). Note that the geometrical isomers of 5,9-18:2 acid move at a slightly lower rate during Ag-TLC than do the corresponding isomers of 9,12-18:2 acid. Although the *cis*-5 and *trans*-5 18:1 acids have the least mobilities during Ag-TLC among the whole series of *cis*- and *trans*-octadecenoic acids (10), this retardation is probably explained by the fact that the two double bonds are nonmethylene-interrupted (11–13). However, when isomers of 5,9-18:2 acid are compared to isomers of the all-*cis* 5,9,12 18:3 acid, the observations previously made with 18:2n-6 and 18:3n-3 isomers (4,13) are verified: One *cis* double bond has the same effect on the migration rate of methylene- or nonmethylene-interrupted acids as two *trans* double bonds. Identification of the *trans*-5,*cis*-9 18:2 acid is made possible by comparison with the same compound present in low amount in *A. vulgaris* seed oil (3,14) (2.4% in the oil used in this study). Consequently, all four isomers of 5,9-18:2 acid are identified. Their order of elution on the CP Sil 88 capillary column is *trans*-5,*trans*-9 18:2 < *cis*-5,*trans*-9 18:2 < *trans*-5,*cis*-9 18:2 < *cis*-5,*cis*-9 18:2 (Table 1). The elution order of 18:2n-6 acid geometrical isomers is well established on cyanoalkyl polysiloxane-coated capillary columns: *trans*-9,*trans*-12 18:2 < *cis*-9,*trans*-12 18:2 < *trans*-9,*cis*-12 < *cis*-9,*cis*-12 18:2 (7,15). Note that the elution order of geometrical isomers of the two dienes is the same: *trans*,*trans* < *cis*,*trans* < *trans*,*cis* < *cis*,*cis*.

From the ECL of 5,9-18:2 acid and of 9,12-18:2 acid geometrical isomers together with the ECL of *trans*-9 and *cis*-9 18:1 acids (both present in the NO_2-isomerized pine seed oil; Fig. 1), it is possible to calculate the ECL of all PAGIs. For example, the calculated ECL for *trans*-5,*trans*-9,*trans*-12 18:3 acid will be the sum of the base value 18.00 plus the fractional chainlength (FCL = ECL − 18.00) of the *trans*-5,*trans*-9 18:2 acid, plus the FCL of the *trans*-9,*trans*-12 18:2 acid, minus the FCL of the *trans*-9 18:1 acid (which is counted twice). The result of this calculation is equivalent to summing up the FCL of each of the *trans*-5, *trans*-9 and *trans*-12 18:1 acids and of the two dienoic adjustments (plus the base value 18.00) (16). The calculated figure for the ECL of *trans*-5,*trans*-9,*trans*-12 18:3 acid will be: 18.00 + 0.66 + 1.09 − 0.40 = 19.35 (Table 1). The experimental ECL value for this acid is 19.34. When all combinations are taken into account, calculated values for ECL of all eight PAGIs differ from experimental values by only 0.00 to 0.03 carbon units (Table 1). This agreement between calculated and experimental figures is even better than that for α- and γ-linolenic acids (4). Consequently, identification of all eight PAGIs is achieved. The number of *trans* ethylenic bonds in PAGIs is also supported by the migration rate during Ag-TLC. For at least one isomer, the *trans*-5,*cis*-9,*cis*-12 18:3 acid (columbinic acid), the identification could be confirmed by comparison of its ECL with that of the same acid present in great abundance (more than 50% of total fatty acids) in *A. vulgaris* seed oil (3,14). The elution order

TABLE 1

Experimental and Calculated Chromatographic Retention Data for Fatty Acid Methyl Esters Prepared with NO_2-Isomerized Pine Seed Oil

Peak number[a]	ECL[b]		Calc. − Exp.[c]	Fatty acid structure
	Exp.	Calc.		
S_1	18.00	—	—	18:0
S_2	20.00	—	—	20:0
M_1	18.40	—	—	*trans*-9 18:1
M_2	18.47	—	—	*trans*-11 18:1
M_3	18.57	—	—	*cis*-9 18:1
M_4	18.66	—	—	*cis*-11 18:1
D_1	18.66	—	—	*trans*-5,*trans*-9 18:2
D_2	18.82	—	—	*cis*-5,*trans*-9 18:2
D_3	18.89	—	—	*trans*-5,*cis*-9 18:2
D_4	18.98	—	—	*cis*-5,*cis*-9 18:2
D'_1	19.09	—	—	*trans*-9,*trans*-12 18:2
D'_2	19.27	—	—	*cis*-9*trans*-12 18:2
D'_3	19.36	—	—	*trans*-9*cis*-12 18:2
D'_4	19.45	—	—	*cis*-9,*cis*-12 18:2
T_1	19.34	19.35	0.01	*trans*-5,*trans*-9*trans*-12 18:3
T_2	19.51	19.51	0.00	*cis*-5,*trans*-9,*trans*-12 18:3
T_3	19.57	19.60	0.03	*trans*-5,*cis*-9,*trans*-12 18:3
T_4	19.62	19.62	0.00	*trans*-5,*trans*-9,*cis*-12 18:3
T_5	19.69	19.69	0.00	*cis*-5,*cis*-9,*trans*-12 18:3
T_{6-7}	19.76	19.78	0.02	*cis*-5,*trans*-9,*cis*-12 18:3
		19.77	0.01	*trans*-5,*cis*-9,*cis*-12 18:3
T_8	19.87	19.87	0.00	*cis*-5,*cis*-9,*cis*-12 18:3

[a]Peak numbers refer to Figures 1–5.
[b]Equivalent chainlengths (ECL) determined on a CP Sil 88 capillary column under conditions described in the legend of Figure 1. Exp., experimental values; Calc., values calculated as detailed in the text.
[c]Differences between calculated and experimental ECL values.

of PAGIs on the CP Sil 88 capillary column is: *trans*-5,*trans*-9,*trans*-12 18:3 < *cis*-5,*trans*-9,*trans*-12 18:3 < *trans*-5,*cis*-9,*trans*-12 18:3 < *trans*-5,*trans*-9,*cis*-12 18:3 < *cis*-5,*cis*-9,*trans*-12 18:3 < (*cis*-5,*trans*-9,*cis*-12 18:3 + *trans*-5,*cis*-9,*cis*-12 18:3) < *cis*-5,*cis*-9,*cis*-12 18:3 (Table 1). It should be noted that the same principle as that developed for the elution order of α-linolenic acid geometrical isomers (A-LAGI) (4) can be applied to PAGI. Isomers containing a *trans* ethylenic bond in position 12 are eluted according to the elution order of the 5,9-18:2 isomers. The same holds true for those isomers containing a *cis* double bond in position 12. However, the two series are not fully separated, as it is the case for A-LAGI (4): the *trans*-5,*trans*-9,*cis*-12 18:3 acid (first element of the family with a *cis*-12 double bond; peak T_4 in Figs. 1 and 2) elutes before the *cis*-5,*cis*-9,*trans*-12 18:3 acid (last element of the family with a *trans*-12 double bond; peak T_5).

When a sample of pine seed oil is heated under vacuum in a sealed ampoule at 240°C for 6 h, several artifacts appear in small amounts in the chromatographic zone where PAGI have been shown to elute (Fig. 3). Their level is relatively low when compared to the content of A-LAGI and G-LAGI in samples of linseed and borage oils that were simultaneously heated under the same conditions. The degree of isomerization (DI; percentage of *trans* isomers relative to total isomers, including the all-*cis* one) obtained for pinolenic acid is about one-fourth of the DI obtained for octadecatrienoic acids in linseed and borage oils (8.3 instead of 31.8 and 33.2%, respectively) (Table 2). On the other hand, the DI for linoleic acid are practically the same in the three heated oils (Table 2). That no positional shift of ethylenic bonds takes place during heating was demonstrated as follows. Fractions including the mono-*trans*, di-*trans* and all-*cis* isomers of pinolenic acid were isolated by preparative Ag-TLC. After partial hydrazine reduction, the resulting *cis*- and *trans*-octadecenoic acids were collected after Ag-TLC fractionation and analyzed at high load by GLC. Only three peaks were present in each band. Their ECL were identical to those of authentic *cis*-5,*cis*-9 and *cis*-12 18:1 acids on the one hand, and to *trans*-5, *trans*-9 and *trans*-12 18:1 acids on the other hand. The *trans*-9 18:1 acid was the least abundant monoene in the *trans* fraction, indicating that the 9-double bond isomerizes only to a small extent (results not shown).

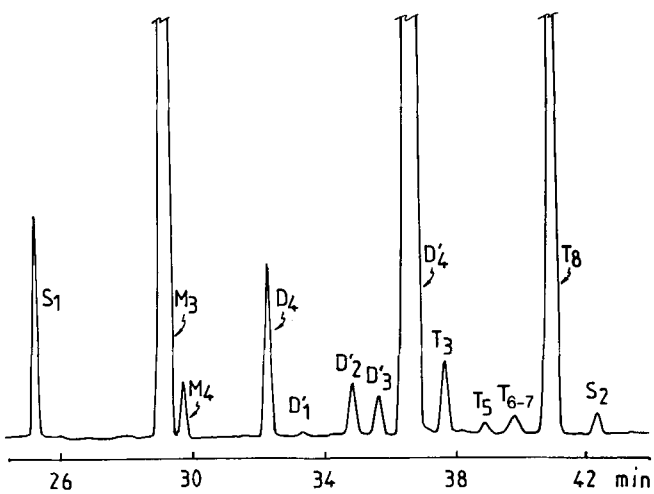

FIG. 3. Partial chromatogram on a CP Sil 88 capillary column of fatty acid methyl esters prepared from pine seed oil heated under vacuum at 260°C for 6 h. Peak lettering as in Figure 1, identification of peaks as in Table 1. See Figure 1 for characteristics, operating conditions and company source of CP Sil 88.

TABLE 2

Quantitative Data for Polyunsaturated Fatty Acids in Pine Seed, Borage and Linseed Oils After Heating at 240°C for 6 h in Ampoules Sealed Under Vacuum

	Pine seed oil	Borage oil	Linseed oil
Initial 18:2n-6 content[a]	44.9	38.4	23.1
DI of 18:2n-6[b]	2.3	2.4	2.4
Initial 18:3 content[c]	14.2	25.4	45.7
DI of 18:3	8.3	33.2	31.8

[a]Percentage of the all-*cis* acid relative to total fatty acids prior to heating.
[b]Degree of isomerization (DI) (percentage of *trans* isomers relative to total isomers including the all-*cis* one) after heating the oils.
[c]*cis*-5,*cis*-9,*cis*-12 18:3 Acid in pine seed oil, *cis*-6,*cis*-9,*cis*-12 18:3 acid in borage oil and *cis*-9,*cis*-12,*cis*-15 18:3 acid in linseed oil.

The main artifact that accumulates in heated pine seed oil is the *trans*-5,*cis*-9,*trans*-12 18:3 acid (*ca.* 64% of total *trans* isomers). This identification is based on the ECL of the peak and on its characteristic migration during Ag-TLC (peak T_3 in Fig. 4). Three other minor isomers, identified by their ECL and by their migration rate during Ag-TLC, are also present. These compounds are the three mono-*trans* isomers of pinolenic acid (peaks T_5 and T_{6-7} in Fig. 4). These observations are at variance with those made with α- and γ-linolenic acids. Upon heating, each of these two acids gives rise mainly to two mono-*trans* isomers (1,2). The formation of the two mono-*trans* isomers does not depend, at least for α-linolenic acid-containing oils, on the initial content of α-linolenic acid in the oils (from *ca.* 6% in soybean oil up to 45% in linseed oil) (1,17–19). No traces of the all-*trans* isomer of pinolenic acid could be detected. This isomer should have migrated along with the all-*cis* linoleic acid during Ag-TLC (Fig. 2). If present, mono-*trans* isomers of the 5,9-18:2 acid should also have been observed in this fraction. However, these isomers were not found (Fig. 4). On the other hand, it would seem that a small amount of the all-*trans* isomer of 5,9-18:2 acid is present. It migrates during Ag-TLC with the mono-*trans* isomers of linoleic acid, just between the *cis* monoenes and the all-*cis* linoleic acid (peak D_1 in Figs. 2 and 4). The *trans*-5,*trans*-9 18:2 acid has the same ECL as the *cis*-11 18:1 acid (Table 1). However, it cannot be confused with this last fatty acid after fractionation by Ag-TLC—it migrates below the *cis*-9 plus *cis*-11 18:1 acid fraction, whereas the *cis*-11 18:1 acid is known to have a slightly higher R_f than the *cis*-9 18:1 acid (10,20). In fact, the upper third of the *cis*-monoenoic acid band is enriched with the *cis*-11 18:1 acid, whereas the lower third of the band is exclusively made up of the *cis*-9 18:1 acid (results not shown). To confirm that the *cis*-5,*cis*-9 18:2 acid gives rise to the *trans*-5,*trans*-9 isomer upon heating and not to the mono-*trans* isomers like *cis*-9,*cis*-12 18:2 acid, we have heated an aliquot of *T. baccata* seed oil at 260°C under vacuum for 5 h. This treatment produces an apparent increase in the height of peak M_4 (*cis*-11 18:1 acid; chromatogram b in Fig. 5), due to the appearance of component D_1 (*trans*-5,*trans*-9 18:2 acid) having the same ECL. Mono-*trans* isomers could not be detected (Fig. 5).

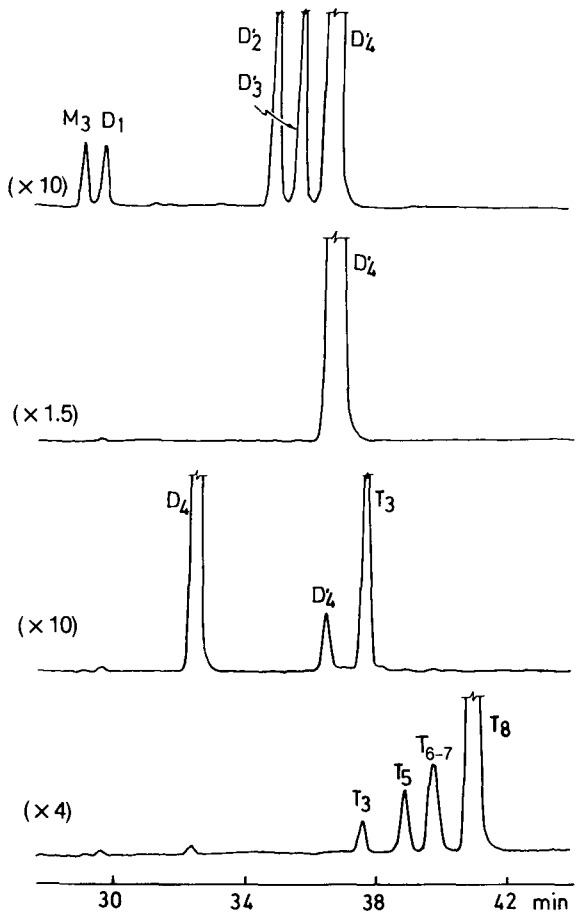

FIG. 4. Partial chromatograms on a CP Sil 88 capillary column of some fractions of fatty acid methyl esters (FAME) separated by argentation thin-layer chromatography, starting with FAME prepared with pine seed oil heated under vacuum at 240°C for 6 h. Values between parentheses are the concentrations relative to the chromatogram in Figure 3. Peak lettering as in Figure 1, identification of peaks as in Table 1. See Figure 1 for characteristics, operating conditions and company source of CP Sil 88.

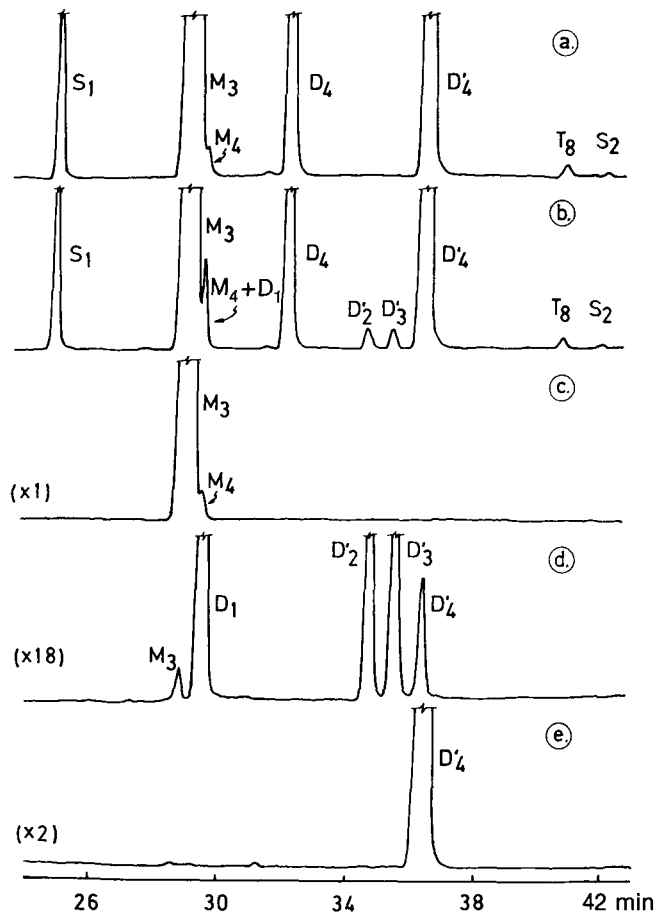

FIG. 5. Partial chromatograms in the C_{18} region on a CP Sil 88 capillary column of fatty acid methyl esters prepared from *Taxus baccata* seed oil. a, Native oil extracted and purified in the laboratory; b, same oil after heating under vacuum at 260°C for 5 h; c, d and e, contiguous fractions isolated by argentation thin-layer chromatography from b. Values between parentheses are the concentrations relative to b. Peak lettering as in Figure 1, identification of peaks as in Table 1. See Figure 1 for characteristics, operating conditions and company source of CP Sil 88.

We could estimate that the DI of *cis*-5,*cis*-9 18:2 acid (*ca.* 8%) was about twice that of *cis*-9,*cis*-12 18:2 acid (4%).

Before trying to explain the differences in the *cis–trans* isomerization pattern of double bonds in methylene- and nonmethylene-interrupted octadecatrienoic acids, the following facts should be considered. First, isolated ethylenic bonds, such as those found in monoenoic acids (mainly *cis*-9 18:1 acid), do not isomerize to any detectable level. Second, two double bonds in the same molecule are required to induce geometrical isomerization. If the dienoic system is methylene-interrupted, heating will lead to the formation of two mono-*trans* isomers (1,2). If it is ethylene-interrupted, the resulting isomer will have the all-*trans* configuration (as shown in this study). Finally, adding a third ethylenic bond increases the rate of isomerization, at least in methylene-interrupted octadecatrienoic acids. This suggests that some interaction between the external double bonds should occur.

One of the main differences between pinolenic acid and α- and γ-linolenic acids is the outside distance between the two external ethylenic bonds spanning seven carbon atoms in pinolenic acid instead of six in the two other octadecatrienoic acids. Apparently, this is sufficient to modify the reaction mechanism of *cis–trans* isomerization—lower rate of overall isomerization and production of a higher relative yield of the di-*trans* isomer. It should also be emphasized that in all three cases, the internal double bond (C_{12}–C_{13} in α-linolenic acid, C_9–C_{10} in γ-linolenic and pinolenic acids) is the most resistant to geometrical isomerization. One can thus speculate that the internal double bond does not participate to a great extent to the geometrical isomerization of the external ethylenic bonds. It is not immediately clear why a one-carbon shift of a double bond has such an effect. However, one may observe that rotations around single bonds allow a methylene-interrupted octadecatrienoic acid molecule, α-linolenic acid for example, to adopt an open hexagonal-ring configuration that brings the two external double bonds in close neighborhood, with C_9 and C_{10} facing C_{15} and C_{16}, respectively. This is not possible if the internal double bond is in the *trans* configuration. In fact, *trans*-9,*trans*-12,*cis*-15 and *cis*-9,*trans*-12,*trans*-15 isomers are formed in trace amounts only, even under the harsher conditions of heating (1). A similar hexagonal configuration may occur

in γ-linolenic acid—in this case, C_6 will be near C_{12} and C_7 near C_{13}. In both cases, the two external ethylenic bonds will be facing each other. So, one can hypothesize that the *cis-trans* isomerizations of the two extreme double bonds are not independent, and that some interaction between these double bonds occurs. In the nonmethylene-interrupted pinolenic acid, these interactions will be modified because the hexagonal-ring configuration only allows C_6 and C_{12} to come into contact. The external ethylenic bonds lie on each side of a C_6-C_{12} axis and are not facing each other.

Because it is highly probable that the reactions are intramolecular, the relative yields of geometrical isomers should not depend on the initial concentration of octadecatrienoic acids in the oils. This was observed with commercial samples of deodorized soybean, rapeseed and walnut oils (17-19). The only parameters that will affect these yields are temperature and time of heating. Consequently, if geometrical isomers of octadecatrienoic acids are to be completely avoided during the deodorization process of octadecatrienoic acid-containing oils, the temperature in the deodorizer should be sufficiently low and the heating time sufficiently short to limit isomerization through heat activation. This should be kept in mind in the light of recent reports that show one of the A-LAGI (*cis*-9,*cis*-12,*trans*-15 18:3 acid) to have peculiar biochemical properties (21-24).

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