Gas–Liquid Chromatographic Analysis of Geometrical and Positional Octadecenoic and Octadecadienoic Acid Isomers Produced by Catalytic Hydrogenation of Linoleic Acid on Ir/Al_2O_3

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ABSTRACT: Catalytic hydrogenation of linoleic acid was studied on Ir/Al_2O_3. A detailed analysis of geometrical and positional isomers of octadecenoic acid (18:1) in the products was performed by capillary gas–liquid chromatography with a new capillary column coated with isocyanopropyl trisilphenylene siloxane (TC-70). Well-resolved peaks of 10 species of 18:1 were observed in the product. In addition to monoenoic acid isomers, four species of *trans*-dienoic isomers and conjugated dienoic isomers were found. From the distribution of 18:1 isomers, it was found that the double bond closer to the methyl end (Δ12) showed higher reactivity than that closer to the carboxyl end (Δ9) for hydrogenation. Because *cis*-8 18:1 and *trans*-8 18:1 were not observed but *cis*-10 18:1 and *trans*-10 18:1 were observed in the products, the double-bond Δ9 did not migrate to the carboxyl end but migrated to the methyl end. On the other hand, the Δ12 bond migrated to both methyl and carboxyl ends. From the distribution of 18:1 isomers in the reaction pathway, the hydrogenation of linoleic acid proceeds *via* half-hydrogenation states. *Cis*-18:1 isomers were produced predominantly in the initial stage of the reaction, while *trans*-18:1 isomers were produced during progress of the reaction. The *cis/trans* and positional isomerization took place by readsorption of 18:1 produced by the partial hydrogenation of linoleic acid.
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KEY WORDS: Capillary GLC analysis, catalytic hydrogenation, *cis/trans* isomerization, *cis-,trans*-18:2 isomers, conjugated 18:2 isomers, double-bond migration, Ir/Al_2O_3, isocyanopropyl trisilphenylene siloxane capillary column, linoleic acid, reaction mechanism.

Margarines and shortenings have been prepared by catalytic hydrogenation of vegetable oils. *Trans* fatty acids contained in them became a focus of concern beginning in the 1950s, and since 1990 they have been a prominent topic in the biomedical literature (1–5). One major problem in many of the reports published on *trans* fatty acids is how to measure their amounts in various foodstuffs and consequently how to estimate consumption levels in the diets. The existing capillary gas–liquid chromatography (GLC) methods on highly polarized stationary phases are limited in their applicability for these samples owing to overlap of certain *cis* and *trans* positional isomers. Two analytical methods are used to determine the *trans* fatty acid composition: infrared spectroscopy (IR) and capillary GLC (6–9). Duchateau *et al.* (10) obtained well-defined peaks of *cis*- and *trans*-18:1 positional isomers with a capillary column coated with cyanopropyl polysiloxane stationary phase (CP™-Sil 88) with a temperature-programmed GLC method. Wolff *et al.* (11) succeeded in the separation of *trans*-18:1 methyl ester positional isomers by using a 100×0.25 mm i.d. CP™-Sil 88 fused-silica capillary column. The resolution of *trans*-18:1 positional isomers was also achieved after converting them to 4,4-dimethyloxazoline derivatives with a 100-m capillary column of SP2560 and CP™-Sil 88(12). However, in both cases, the separation of *cis*-18:1 and *trans*-18:1 fractions had to be carried out by Ag^+-thin-layer chromatography (Ag^+-TLC) and Ag^+-high-performance liquid chromatography (Ag^+-HPLC), respectively, prior to GLC analysis. A 60-m capillary column rather than a 100-m capillary column of CP™-Sil 88 was effective for the isolation of *cis*-18:1 and *trans*-18:1 fractions (10,11).

In a previous report (13), the detailed distribution of monoenoic (18:1) and dienoic acid (18:2) isomers in the hydrogenation product of linoleic acid (*cis*-9,*cis*-12 18:2) with metal catalyst has been described. Two analytical methods (IR absorption and capillary GLC) are used to determine the composition of *cis*-9 18:1 and *trans*-12 18:1. Since *trans*-12 18:1 and *cis*-9 18:1 could not be separated by capillary GLC columns coated with cyanopropyl methylsilicone, their contents were determined by IR method. Because the intensity of IR peak depends on the change in polarization of the molecules during vibration, the peaks due to *trans* isomers are more intense than those due to *cis* isomers. Therefore, quan-

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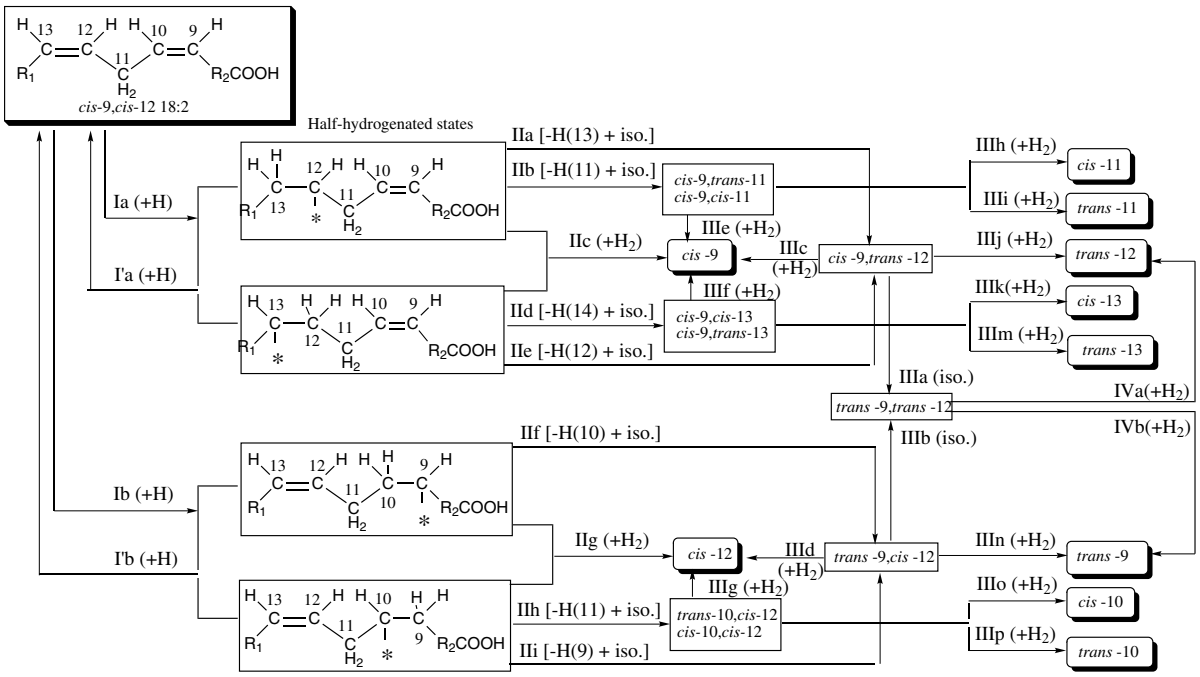
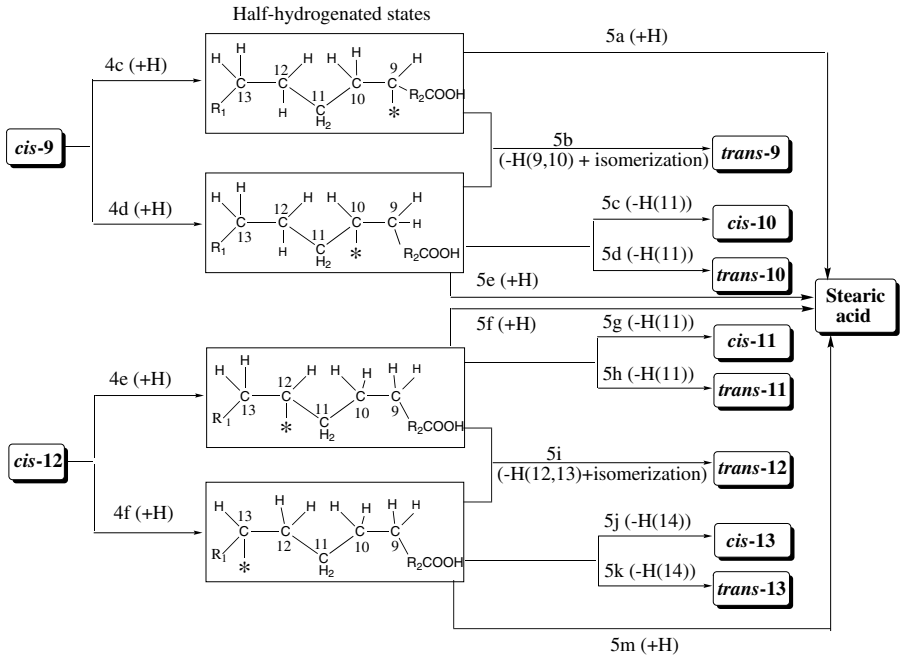


SCHEME 1

titative analysis by IR is prone to underestimating the amount of the *cis* isomer. It is important to isolate *cis*-18:1 and *trans*-18:1 isomers. The choice of the stationary phase in capillary GLC analyses strongly influences resolution.

Recently, a new column for the analysis of fatty acids has become available commercially. This paper reports on separations of octadecenoic acid isomers in the partially hydrogenated product of linoleic acid by a capillary GLC equipped with an isocyanopropyl trisilphenylene siloxane-coated column (Ir/Al_2O_3) (trademarked as TC-70). The octadecenoic isomers in the product of the catalytic hydrogenation of linoleic acid could be separated into 10 components, and the isolation of *cis*-18:1 and *trans*-18:1 positional isomers was achieved with this TC-70 capillary column (GL Science Co., Ltd., Tokyo, Japan). The reaction mechanism of hydrogenation of linoleic acid on Ir catalysts is discussed.



SCHEME 2

EXPERIMENTAL PROCEDURES

Materials. Linoleic acid (Wako Pure Chemical Co. Ltd., Osaka, Japan) contained 96% octadecadienoic acid with both double bonds in the *cis* configuration (*cis*-9,*cis*-12 18:2), 0.3% octadecadienoic fatty acid with two double bonds in the *trans,cis* configuration (*trans*-9,*cis*-12 18:2), and 3.5% oleic acid (*cis*-9 18:1). The reactant contained no octadecadienoic fatty acid with both double bonds in the *trans* configuration (*trans*-9,*trans*-12 18:2). Oleic acid (*cis*-9 18:1) was purchased from Kanto Chemicals Co. Ltd. (Tokyo, Japan). Vaccenic acid (a mixture of *trans*-11 and *trans*-12 18:1) and elaidic acid (*trans*-9 18:1) were purchased from Tokyo Kasei Co. Ltd. (Tokyo, Japan). *Cis*-vaccenic acid (*cis*-12 18:1), *trans*-13, and *cis*-13-octadecenoic acid methyl esters (*trans*-13 18:1 and *cis*-13 18:1) were purchased from Sigma Chemical Company (St. Louis, MO). The mixture of *trans*-9,*trans*-12 18:2 (50 wt%); *cis*-9,*trans*-12 18:2 (20 wt%); *trans*-9,*cis*-12 18:2 (20 wt%); *cis*-9,*cis*-12 18:1 (10 wt%) methyl esters was also purchased from Sigma Chemical Co.

Catalysts. The precursor of the Ir catalyst was prepared by impregnating alumina with an aqueous solution of iridium chloride and drying *in vacuo* at room temperature. It was oxidized at 873 K. The iridium content in the precursor was adjusted to be 5 wt% of iridium metal. The catalyst was reduced twice by 600 mm Hg of H_2 at 573 K prior to use.

Procedure. The catalyst (0.1 g) was placed into a reaction vessel (100 mL) and was reduced by hydrogen gas at 573 K. After reduction of the catalyst, the reaction vessel was evacuated at 573 K for 1 h and cooled to the reaction temperature (413 K). Linoleic acid (0.5 g) was immediately introduced into the reaction vessel *in vacuo*, and 300 torr of hydrogen gas was added to the system. Hydrogenation was carried out in the closed system at 413 K.

Analysis of the products. The reaction products were converted into methyl esters and were analyzed by GLC (GC-14B; Shimadzu Co. Ltd., Kyoto, Japan) with a flame-ionization detector and a flexible fused-silica capillary column coated with TC-70 (60 m × 0.25 mm i.d.). The column is operated isothermally at 453 K, with helium carrier gas pressure at 175 kPa. Injection ports and detector were held at 523 K. Stearic acid (18:0) and eicosanoic acid (20:0) methyl esters were used as reference materials to determine the equivalent chainlength (ECL) values on the TC-70 (6).

RESULTS AND DISCUSSION

Separation of the hydrogenation product by GLC. The catalytic hydrogenation of linoleic acid was carried out on Ir/Al_2O_3. Typical GLC profiles of the reaction product by using cyanopropyl methylsilicone (CPS-1, Tokyo Kasei Co. Ltd., Tokyo, Japan) and TC-70 columns are shown in Figure 1.

Separation of 18:1. Figure 1A shows the profile obtained with the CPS-1 column. Seven resolved peaks due to octadecenoic acid isomers (18:1) were observed. Figure 1B shows a

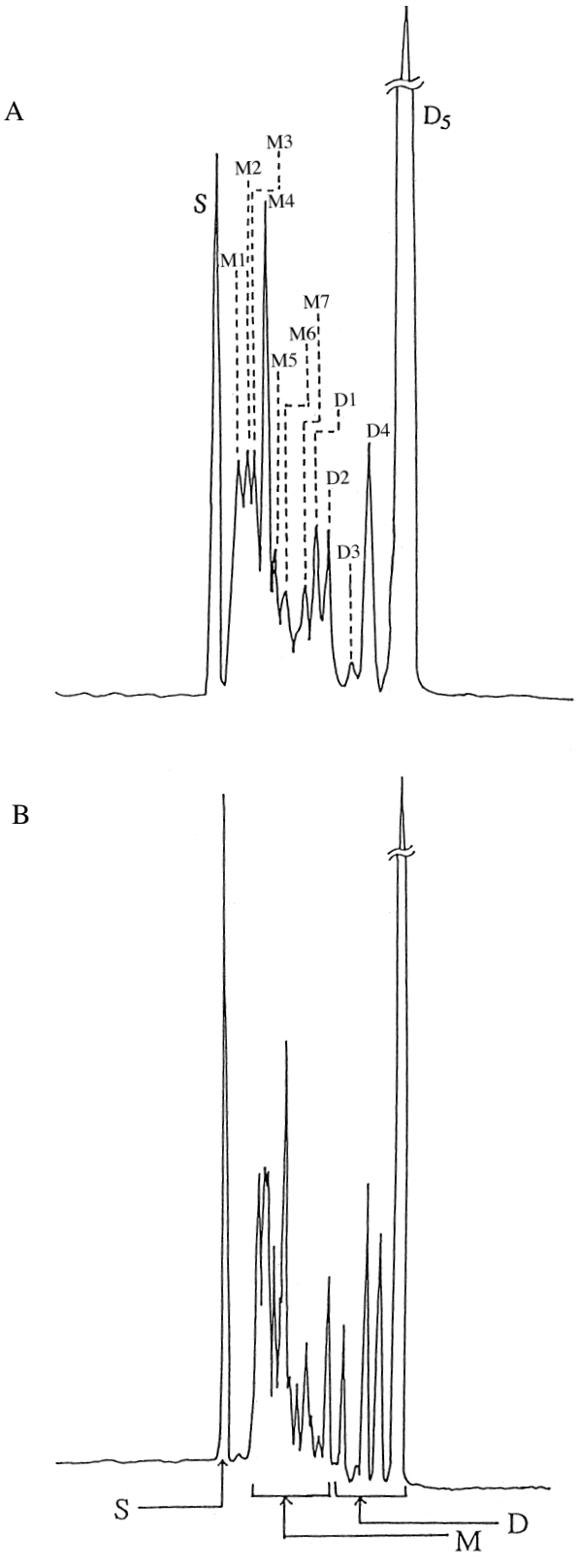


FIG. 1. Comparison of chromatograms of the hydrogenation product of linoleic acid analyzed on CPS-1 (Tokyo Kasei Co. Ltd., Tokyo, Japan) (A) and TC-70 (GL Science Co. Ltd., Tokyo, Japan) (B) flexible fused-silica capillary columns.

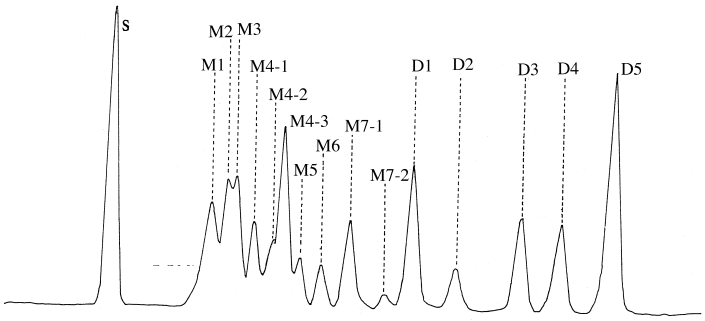


FIG. 2. The magnified GLC profile of 18:1 isomers analyzed on a TC-70 column, derived from Figure 1B. See Figure 1 for company source.

profile analyzed with a TC-70 column. Octadecenoic acid isomers (18:1) were separated into 10 components. The GLC profile due to 18:1 isomers is presented under magnification in Figure 2. The assignments of the peaks have been obtained by using standard samples.

The M4 and M7 peaks in Figure 1A separated into three and two components, respectively, in Figure 1B and Figure 2. Wolff and Bayard (11) reported that *trans*-13 18:1 methyl ester always coelutes with *trans*-14 18:1 methyl ester, even though they used a 100-m capillary column of CP™-Sil 88 for the separation. In Figure 2, the former two peaks (M4-1 and -2) are therefore assigned to *trans*-12 18:1 and unresolved *trans*-13 and *trans*-14 18:1, respectively. The M4-3 peak is assigned to *cis*-9 18:1 by comparison with a standard sample. Even if *trans*-15 18:1 coelutes with *cis*-9 18:1 in the M4-3 peak, the amount of *trans*-15 18:1 would be negligibly small because the amounts of positional isomers of *trans* 18:1 in the reaction product decreased in the order of *trans*-9 > *trans*-10 ≥ *trans*-11> *trans*-12 > *trans*-13 + *trans*-14, and many isomerization steps are required for the formation of *trans*-15 18:1 during the hydrogenation of *cis*-9,*cis*-12 18:2. The M7-1 peak is assigned to *cis*-12 18:1, and peak M7-2 should be assigned to overlapping peaks of *cis*-13 18:1 and *cis*-14 18:1, referring to the results of Wolff and Bayard (11). The peak due to *cis*-9 18:1 (M4-1) was isolated from the peak due to *trans* 18:1 isomers (*trans*-12, *trans*-13 + *trans*-14 18:1) by the TC-70 capillary column, as shown in Figures 1B and 2. Therefore, the TC-70 capillary column is a suitable column to separate *trans* isomers (*trans*-9 to *trans*-14 18:1) from *cis* isomers (*cis*-9 to *cis*-14 18:1).

Separation of 18:2. Five peaks due to dienoic acid isomers (18:2) were observed. The profile of the peaks due to 18:2 isomers obtained on the TC-70 capillary column was similar to that obtained on the CPS-1 capillary column. Ratnayake and Pelletier (14) reported that the peak of *trans*-8,*cis*-12 18:2 overlapped with that of *cis*-9,*trans*-13 18:2 in peak D2 in Figure 1. The Δ9 double bond did not migrate to the carboxyl end, although Δ12 migrated to the methyl end during hydrogenation, as described later. Therefore, peak D2 in Figure 1B should be assigned to *cis*-9,*trans*-13 18:2. The ECL values,

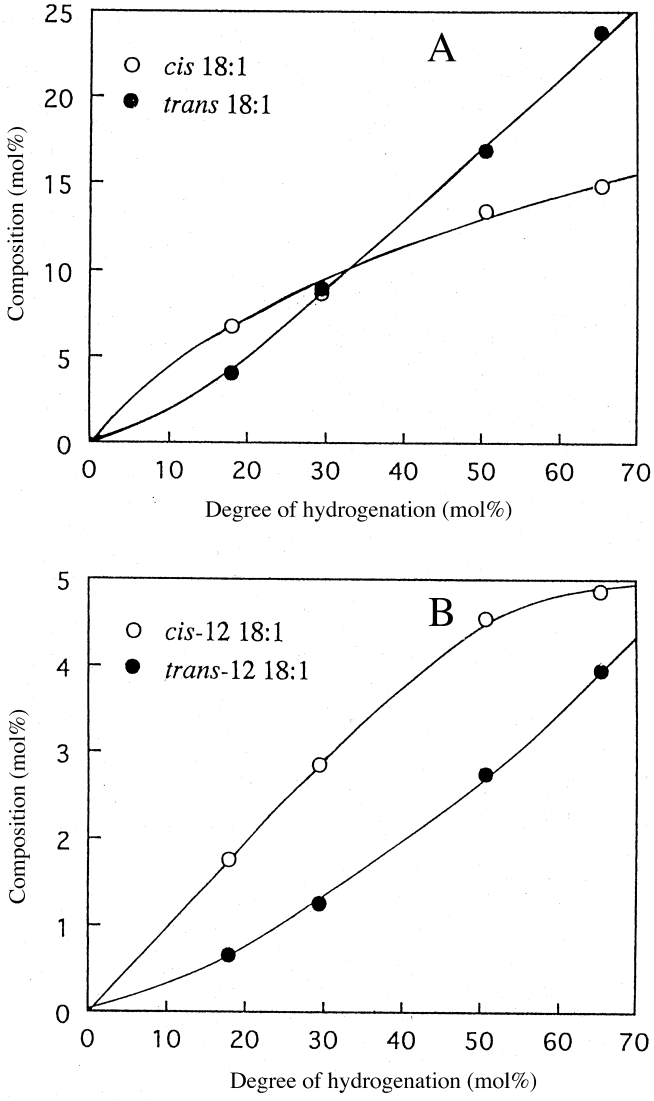


FIG. 3. (A) *Trans*-18:1 and *cis*-18:1 as a function of the degree of hydrogenation. (B) *Trans*-12 18:1 and *cis*-12 18:1 as a function of degree of hydrogenation.

determined experimentally on the TC-70 capillary column, are listed in Table 1. The experimental ECL values for 18:1 isomers were smaller than those obtained by Wolff *et al.* (15) on a CP™-Sil 88 capillary column. Several peaks that appeared after the peak of linoleic acid (*cis*-9,*cis*-12 18:2) in Figure 1B were assigned to conjugated octadecadienoic acid isomers as shown in a previous paper (16).

Distribution of isomers in the hydrogenation products. Stearic acid (18:0), 10 monoenoic acid isomers (18:1), five dienoic acid isomers (18:2), and conjugated dienoic isomers (18:2) were observed in the products of the catalytic hydrogenation of linoleic acid on Ir/Al_2O_3. Because *trans*-8 18:1 was not observed in the products, it was concluded that *cis*-8 18:1 was also not contained in the monoenoic acid isomers. Both *trans*-10: 18:1 and *cis*-10 18:1 were observed. These results indicate that the Δ9 double bond did not migrate to the carboxyl end but migrated to the methyl end during hydrogenation.

All of the *trans*-13 18:1, *cis*-13 18:1, *trans*-11 18:1, and *cis*-11 18:1 isomers were contained in the products. The Δ12 double bond therefore migrates to both methyl and carboxyl ends. If *trans*-14 18:1 and *cis*-14 18:1 coelute with *trans*-13 18:1 and *cis*-13 18:1, *trans*-14 18:1 and *cis*-14 18:1 should be negligibly small because they are formed *via* isomerization of *trans*-13 18:1 and *cis*-13 18:1, and several reaction steps are required for double-bond migration from Δ12 to Δ14 *via* Δ13 during the hydrogenation of linoleic acid.

Reaction path. To clarify the reaction pathway of the hydrogenation of linoleic acid, the time course of the reaction was investigated and summarized in Table 2. The reaction schemes of the catalytic hydrogenation and isomerization of linoleic acid are shown in Schemes 1 and 2. As mentioned in the previous paper (1), it is assumed that linoleic acid is adsorbed on the surface of the catalyst with one double bond, either Δ9 or Δ12. Hydrogenation and isomerization occur *via* the half-hydrogenated state. The formation of geometrical and positional isomers of dienoic and monoenoic acid occurs *via* the half-hydrogenated state during the hydrogenation.

As shown in Table 2, the reactivity for hydrogenation of the two double bonds was different. The reactivity of Δ12 for hydrogenation was higher than that of Δ9 because the ratios of Δ9/Δ12 were 1.3–1.7 and *cis*-9 18:1 was produced predominantly at a lower degree of hydrogenation. This shows that step Ia in Scheme 1 occurred predominantly. This is supported also by the fact that the sum of *cis*-9,*trans*-13 18:2 and *cis*-9,*trans*-12 18:2 (4.4%) was larger than *trans*-9,*cis*-12 18:2 (2.8%) at the lower degree of hydrogenation (the initial stage of the reaction). The amounts of *trans*- and *cis*-octadecenoic acid (18:1) isomers are shown as a function of the degree of hydrogenation of linoleic acid in Figure 3A. At the lower degree of hydrogenation, the total amount of *trans*-18:1 isomers was smaller than that of *cis*-18:1 isomers, while the total amount of *trans*-18:1 isomers exceeded that of *cis*-18:1 isomers upon progress of the reaction (Fig. 3A). Only the amount of *cis*-12 18:1 was larger than that of *trans*-12 18:1 over all the reaction range (Fig. 3B). The positional isomers of *trans*-18:1 except *trans*-9 and *trans*-12 18:1 exceeded the corresponding *cis*-18:1 positional isomers all over the reaction range. From these results, *trans*-18:1 positional isomers have the possibility of being produced by two pathways: (i) readsorption of *cis*-9 18:1 and *cis*-12 18:1 (steps 5c, d, g, h, j, and k in Scheme 2); and (ii) hydrogenation of *trans*- and

TABLE 1
Fatty Acid Composition in the Hydrogenation Products of Linoleic Acid on Ir (5 wt%)/Al_2O_3 and Their ECL Values on TC-70 at 180°C

Peak number[a]	Fatty acid	ECL value	Product distribution (mol%)
S	18:0	18.00	12.68
M1	*Trans*-9 18:1	18.18	7.09
M2	*Trans*-10 18:1	18.21	4.93
M3	*Trans*-11 18:1	18.23	5.15
M4-1	*Trans*-12 18:1	18.26	3.83
M4-2	*Trans*-13 18:1	18.29	2.15
M4-3	*Cis*-9 18:1	18.31	8.28
M5	*Cis*-10 18:1	18.34	1.96
M6	*Cis*-11 18:1	18.38	1.98
M7-1	*Cis*-12 18:1	18.43	4.74
M7-2	*Cis*-13 18:1	18.49	0.84
D1	*Trans*-9, *trans*-12 18:2	18.55	7.52
D2	*Cis*-9, *trans*-13 18:2	18.62	2.54
D3	*Cis*-9, *trans*-12 18:2	18.74	4.95
D4	*Trans*-9, *cis*-12 18:2	18.81	5.00
D5	*Cis*-9, *cis*-12 18:2	18.91	12.91
K	Conjugated 18:2	20.00–20.72	13.01

[a]See Figure 2 for peak numbers. ECL, equivalent chainlength.

TABLE 2
Distribution of Hydrogenation Products of Linoleic Acid on Ir/Al_2O_3 at Various Degrees of Hydrogenation

	Reaction time (min)			
	38	128	206	516
18:0	3.4	5.7	10.1	13.2
Trans-9 18:1	1.3	2.3	5.1	7.2
Trans-10 18:1	1.2	2.4	3.6	5.2
Trans-11 18:1	0.7	2.1	3.6	5.1
Trans-12 18:1	0.7	1.3	2.8	4.0
Trans-13 18:1	0.4	1.1	2.0	2.5
Cis-9 18:1	2.9	3.6	4.7	5.0
Cis-10 18:1	0.4	1.0	1.8	2.3
Cis-11 18:1	0.6	1.0	1.7	2.0
Cis-12 18:1	1.8	2.9	4.6	4.9
Cis-13 18:1	1.3	0.4	0.8	0.9
Trans-9,*trans*-12 18:2	trace	1.8	4.4	7.9
Cis-9,*trans*-13 18:2	1.4	2.3	3.0	2.7
Cis-9,*trans*-12 18:2	3.0	4.6	5.4	5.1
Trans-9,*cis*-12 18:2	2.8	4.0	4.9	4.9
Cis-9,*cis*-12 18:2	70.6	53.6	31.2	13.3
Conjugated 18:2	3.8	6.3	6.5	9.6
Sum of products	21.9	36.5	58.5	72.9
Degree of hydrogenation	18.1	29.5	50.9	65.5
Σ(18:1)	11.3	18.1	30.7	39.1
Σ(18:2)	7.2	12.7	17.7	20.6
(*cis* 18:1)/(*trans* 18:1)	1.63	0.97	0.80	0.63
(Δ9)/(Δ12)[a]	1.68	1.40	1.32	1.37

[a](Δ9)/(Δ12) = [(*cis*-9 18:1) + (*trans*-9 18:1)]/[(*cis*-12 18:1) + (*trans*-12 18:1)].

dienoic acid (18:2) produced by isomerization of the reactant (steps IIIi, m and p in Scheme 1).

Increases of *cis*-10 18:1, *cis*-11 18:1, and *cis*-13 18:1 at the higher hydrogenation degree indicate that the isomerization proceeds with readsorption of the monoenoic acid in the product on the catalyst (steps 5c, g, and j in Scheme 2). The *cis*- and *trans*-18:1 isomers in higher positions, e.g., 14 and 15, possibly formed during the hydrogenation process, will be negligibly small because the migration of double bonds is required twice or more. A significant amount of conjugated dienoic isomers was found in the hydrogenation products. If the monoenoic acid formation occurs *via* the hydrogenation of conjugated dienoic acid, the 10- and 11-fractions would predominate in the *trans*-fraction, whereas in the *cis*-fraction, the original locations 9- and 12-fractions would predominate (17). At the initial stage of the reaction, *cis*-12 18:1 and *cis*-9 18:1 were produced predominantly as shown in Table 2. This suggests that the conjugated dienoic acid may play a role in the formation of geometrical and positional isomers of 18:1.

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