Improvement in the Resolution of Individual *trans*-18:1 Isomers by Capillary Gas–Liquid Chromatography: Use of a 100-m CP-Sil 88 Column

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ABSTRACT: Doubling the length of a CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands) from 50 to 100 m remarkably improves the resolution of individual *trans*-18:1 isomers from either ruminant fats or partially hydrogenated oils. Although the use of a 50-m column gives interesting results, it does not allow sufficient resolution of the *trans*-10 and *trans*-11 18:1 isomers. Moreover, the *trans*-6 to *trans*-9 18:1 isomers emerge as a single group of peaks, whereas the *trans*-12 isomer is only partly resolved from the adjacent *trans*-11 and *trans*-13 plus *trans*-14 isomers. With the 100-m column, the *trans*-9, *trans*-10, and *trans*-12 18:1 isomers are almost baseline resolved from other isomers. However, with both columns, it is not possible to separate the critical pair of *trans*-13 and *trans*-14 18:1 acids which co-elute under a single peak. Despite this minor drawback, the 100-m CP-Sil 88 column appears to be of great interest for the separation and the quantitation of most individual *trans*-18:1 acids. Except for the use of argentation thin-layer chromatography, there is no need for complementary techniques, such as ozonolysis. This simple and powerful tool may be applied to ruminant fats, partially hydrogenated oils, and human tissue lipids.

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KEY WORDS: Butterfat, equivalent chainlengths, gas–liquid chromatography, human milk, isopropyl esters, methyl esters, octadecenoic acids, hydrogenated oil, separation, tallow, *trans* isomers.

The study of *trans*-18:1 isomer distribution in partially hydrogenated oils (1–9), ruminant fats (10–14), or human tissue lipids (6) generally requires, as a first step, the isolation of monoenoic acids, usually by argentation thin-layer chromatography (Ag–TLC). The *trans*-18:1 acid mixture is sometimes purified by preparative gas–liquid chromatography (GLC) and then submitted to ozonolysis, followed by reduction of the ozonides. The resulting fragments (generally aldehydes and aldesters) are then analyzed by GLC. This procedure allows the quantitation of individual *trans*-18:1 isomers (1–14). However, in addition to the length and the tediousness of the procedure, another drawback is that the shorter fragments are more or less volatile and may be partly lost during the experiment. Moreover, the accuracy of the method relies on the use of convenient correction factors for the unequal response of the flame-ionization detector. Although several attempts were made to analyze individual *trans*-18:1 acids directly by GLC on capillary columns, only a few authors have obtained promising results (15–17). However, the resolution between individual isomers still remained incomplete.

In recent studies on the *trans*-18:1 acid content and distribution in ruminant fats and human milk lipids (17,18), we could obtain a rather good resolution of several, but not all, isomers using a 50-m CP-Sil 88 capillary column (Chrompack, Middelburg, The Netherlands). Several overlaps occurred, and often only groups of isomers could be quantitated. For example, the *trans*-10 18:1 isomer appeared as a simple shoulder on the leading edge of the main *trans*-11 18:1 acid, and these two isomers could seldom, if ever, be individually quantitated. Moreover, the *trans*-12 18:1 isomer was not particularly well resolved from the adjoining *trans*-11 and *trans*-13 plus *trans*-14 18:1 acids. Nevertheless, the separations obtained with the 50-m CP-Sil 88 column were encouraging, and it seemed possible to improve these separations by, for example, increasing the length of the column. Recently, 100-m CP-Sil 88 capillary columns (actually two 50-m columns joined end-to-end) have been made commercially available, and we report here how such columns can improve the resolution of *trans*-18:1 isomers, following their fractionation by Ag–TLC.

EXPERIMENTAL PROCEDURES

Samples and standards. Ruminant fats and their fatty acid derivatives (isopropyl and methyl esters) were prepared as described previously (17,18). The *trans*-18:1 acid fraction from butterfat used in the present study was a mixture of the *trans*-18:1 acid fractions isolated by Ag–TLC from 14 different samples of butter purchased in July and August 1994. The *trans*-18:1 acid fraction from beef tallow was a composite sample made with the *trans*-18:1 acid fractions isolated by Ag–TLC from nine samples of beef tallow obtained monthly between March and November 1994 and prepared with the fat of cattle

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slaughtered in an abattoir near Paris. The *trans*-18:1 acid fraction from human milk lipids corresponds to the pooled *trans*-18:1 acid fractions isolated by Ag–TLC from lipids of ten samples of human milk (18). Neutralized, bleached soybean oil was partially hydrogenated at 185°C for 16 min under a hydrogen pressure of 200 kPa in the presence of 0.1% nickel catalyst. Standards used to identify *trans*-18:1 acids and determine their equivalent chainlengths (ECL) were synthetic isomers that had ethylenic bonds between positions 5 and 15.

GLC. Analyses by GLC were performed with a Carlo Erba 4130 chromatograph (Carlo Erba, Milano, Italy) equipped with a flame-ionization detector and a split injector that were maintained at 250°C. Two 0.25-mm i.d. CP-Sil 88 fused-silica capillary columns (0.20 μm film) were used, and both were operated at 160°C. Helium was used as a carrier gas with an inlet pressure of 120 and 180 kPa for the 50- and the 100-m columns, respectively. The chromatograph was coupled with an SP 4290 integrator (Spectra Physics, San Jose, CA). ECL of individual *trans*-18:1 acid derivatives (methyl and isopropyl esters) were calculated according to Ackman (19) using 16:0, 18:0, and 20:0 acid derivatives as reference compounds.

RESULTS AND DISCUSSION

The chromatograms of *trans*-18:1 acid isopropyl esters prepared with butterfat and analyzed on 50- and 100-m CP-Sil 88 capillary columns operated under optimum conditions are compared in Figure 1. The improvement in the resolution of individual isomers is immediately observable and deserves only few comments. Isomers that remain unresolved are the *trans*-6 to *trans*-8 18:1, and the *trans*-13 and *trans*-14 18:1. All other isomers, ranging from the *trans*-5 to the *trans*-16 18:1, are fairly well resolved. Other examples of separations on the 100-m column of *trans*-18:1 acid isopropyl esters are given in Figure 2. They include *trans*-18:1 acids from beef tallow, human milk lipids, and partially hydrogenated soybean oil. The fact that the *trans*-6 to *trans*-8 18:1 isomers are not resolved is not a drawback, because the *trans*-6 and *trans*-7 18:1 isomers are generally minor components in either ruminant fats (10–14) or partially hydrogenated oils (1–9). The separation of these last isomers, together with that of the *trans*-13 and *trans*-14 18:1 isomers appears hopeless, at least on this kind of stationary phase (cyanopropyl polysiloxane). This is due to an insufficient difference in the ECLof these two groups of isomers—*ca.* 18.30 for the *trans*-6 to *trans*-8 18:1 acids (as isopropyl esters), and 18.50 for the *trans*-13 and *trans*-14 18:1 acids (Fig. 3). In our conditions, two peaks are well resolved when the difference between their ECLs is 0.04. This holds true for both isopropyl and methyl esters. However, we prefer isopropyl esters for quantitative determinations of *trans*-18:1 acids in ruminant milkfats, as explained previously (17). Isopropyl esters of *trans*-18:1 acids are not better resolved than methyl esters (Fig. 4), except perhaps for the *trans*-10 and *trans*-11 18:1 acids. This is due to a small, though unexplained, difference between the ECL of *trans*-10 and *trans*-11 18:1 acids as isopropyl or methyl esters (Fig. 5). More generally, the mean increase in ECL is 0.041 for each carbon atom shift of the ethylenic bond between the 9- and 13-positions for isopropyl esters, but of only 0.036 for methyl esters. Thus, isopropyl esters appear to have a small advantage over methyl esters, although methyl esters of *trans*-9 18:1 acid are slightly better separated from the mixture of *trans*-6 to *trans*-8 18:1 acids than are the corresponding isopropyl esters. ECL for isopropyl esters and for methyl esters are practically identical on both columns: ECLs of individual *trans*-18:1 acid derivatives do not depend on the column length. The mean difference between ECL of isopropyl esters and methyl esters is 0.10 ± 0.02 on the two columns. The difference between the times of analysis of isopropyl and methyl esters is small, *ca.* 2 min only (Fig. 4).

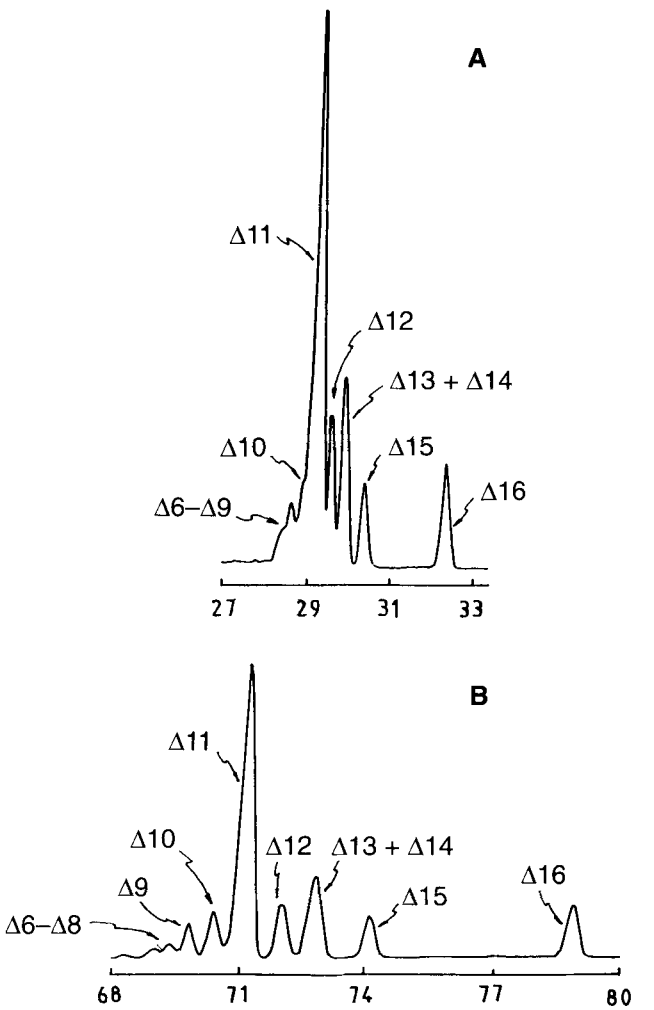

FIG. 1. Chromatograms of *trans*-18:1 acids that were isolated from butterfat and analyzed as isopropyl esters. A: analysis on a 50 m × 0.25 mm i.d. CP-Sil 88 fused-silica capillary column (Chrompack, Middelburg, The Netherlands) operated at 160°C with helium as a carrier gas (inlet pressure, 120 kPa) at 160°C. B: analysis on a 100 m × 0.25 mm i.d. CP-Sil 88 fused-silica capillary column with helium as a carrier gas (inlet pressure, 180 kPa) at 160°C. Identification of individual isomers was by comparison with synthetic compounds, except for the *trans*-16 18:1 isomer (Ref. 15).

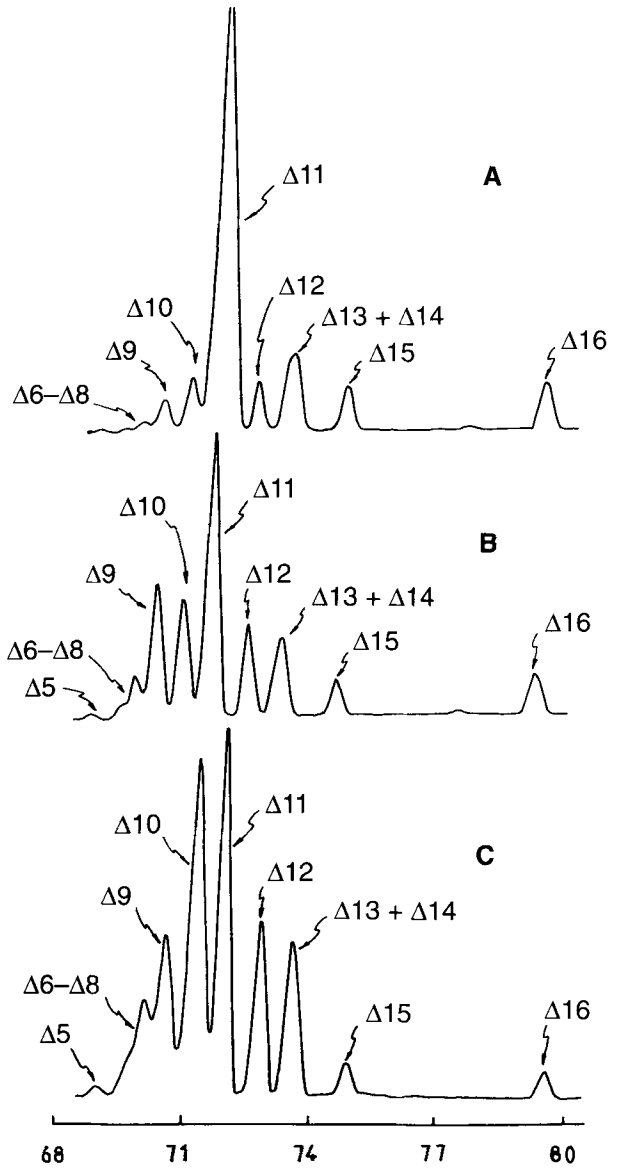

FIG. 2. Chromatograms of the *trans*-18:1 acid isopropyl esters isolated from: A, beef tallow; B, human milk lipids; and C, partially hydrogenated soybean oil. Analyses were done on a 100 m × 0.25 mm i.d. CP-Sil 88 fused-silica capillary column. Operating conditions and company source as in Figure 1.

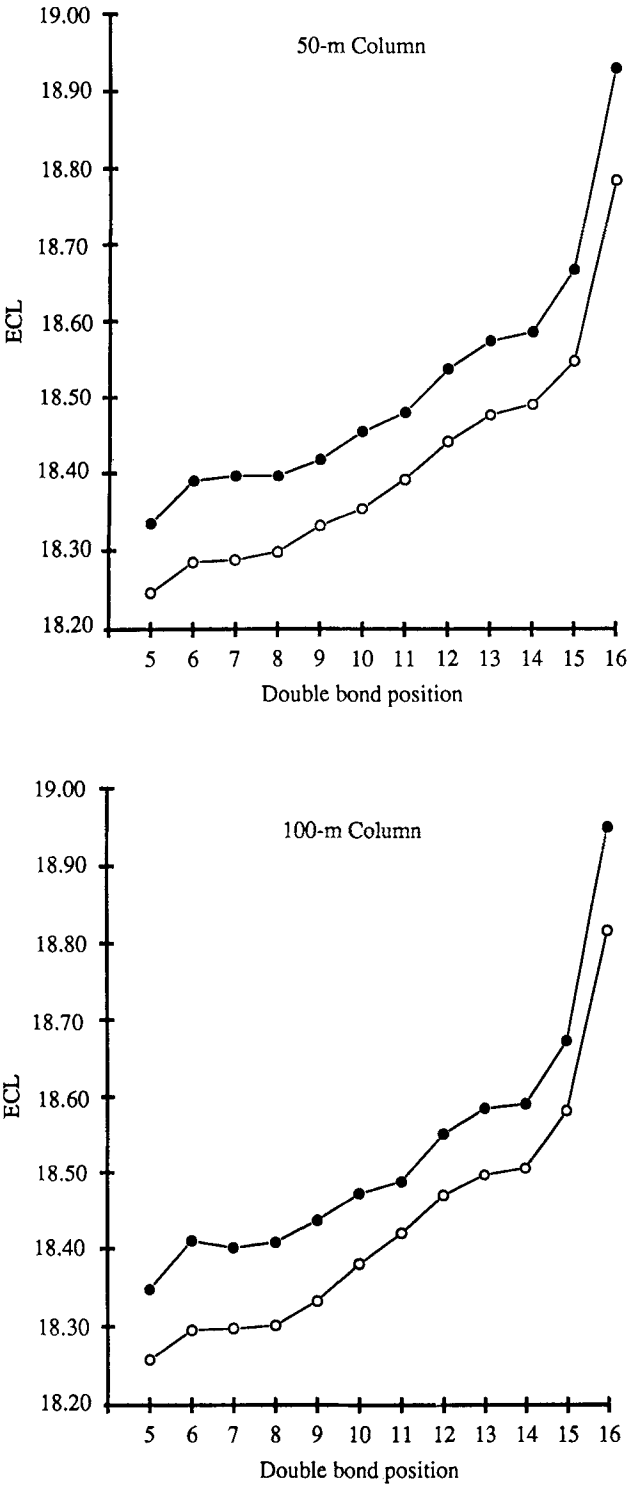

FIG. 3. Equivalent chainlengths (ECL) of *trans*-18:1 acids as methyl esters (●) and isopropyl esters (○) on 50-m and 100-m CP-Sil 88 fused-silica capillary columns (0.25 mm i.d.). Operating conditions and company source as in Figure 1. Isomers with ethylenic bond between positions 5 and 15 were synthetic compounds, whereas the *trans*-16 18:1 isomer was located using butterfat (Ref. 15).

Clearly, the 100-m CP-Sil 88 column appears to be a simple and powerful tool to analyze most individual *trans*-18:1 isomers. The time of analysis is reasonable (1.3 h for the elution of the *trans*-16 18:1 isomer), especially when compared to the ozonolysis procedure. Although there is a small loss of information for some isomers as compared to ozonolysis-based procedures, the direct analysis of *trans*-18:1 acids by GLC eliminates the problems of response factors and volatility of the shortest cleavage fragments. The 100-m column also gives good results when applied to *cis*-18:1 isomers (Fig. 5). However, a prerequisite to such analyses is the isolation of the *trans* or *cis* monoenoic acid fractions by Ag–TLC prior to GLC. Otherwise, some intermingling exists between these two sets of isomers, even with the high resolution achieved by the 100-m CP-Sil 88 column (Fig. 5). It should be noted that when using isopropyl esters, the *trans*-12 18:1

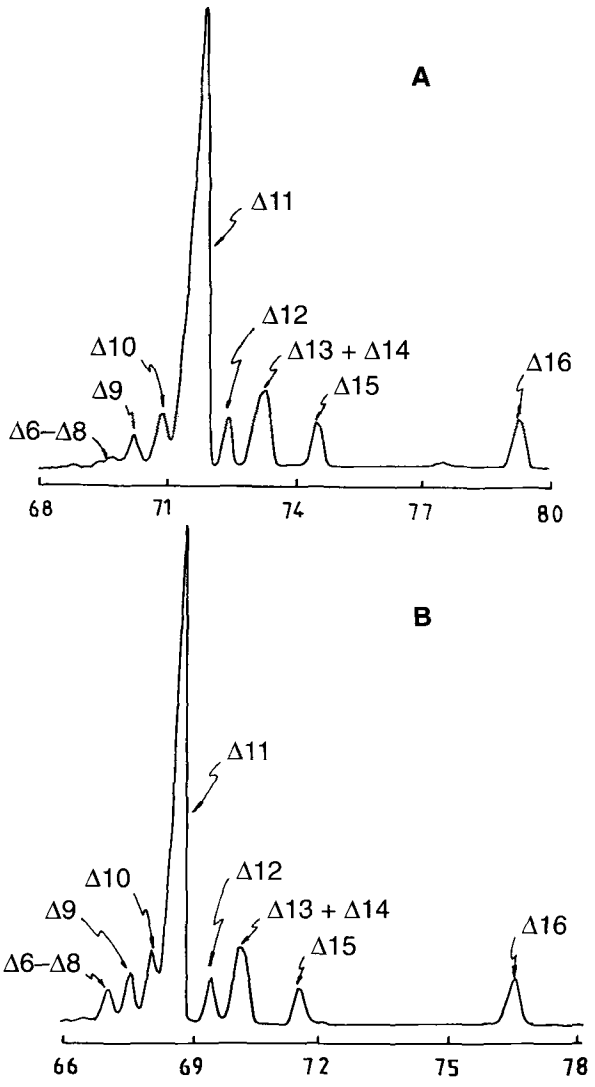

FIG. 4. Comparison of chromatograms of isopropyl and methyl esters of *trans*-18:1 acids isolated from beef tallow (A and B, respectively). Analyses were done on a CP-Sil 88 fused-silica capillary column (100 m × 0.25 mm i.d.). Operating conditions and company source as in Figure 1.

acid elutes under the main *cis*-9 18:1 acid, but that using methyl esters leads to a good separation between these two isomers, the former eluting from the column before the latter (Fig. 5). In these conditions, overlaps between *cis*- and *trans*-18:1 isomers are minimized. Analysts should be warned against the practice of measuring out the *trans*-18:1 acid content in lipids directly through a single chromatographic run of total fatty acids. Results obtained in such a way are basically erroneous and should be considered with reservation.

We observed that the temperature greatly affected the resolution of *trans*-18:1 isomers, the best separations being obtained at the lowest operating temperatures. The inlet gas pressure had little effect. So, it may still be possible to improve the resolution of *trans*-18:1 acids using a temperature lower than 160°C. However, this will exaggeratedly increase the time of analysis. The film thickness and the nature of the carrier gas (hydrogen instead of helium, for example) also

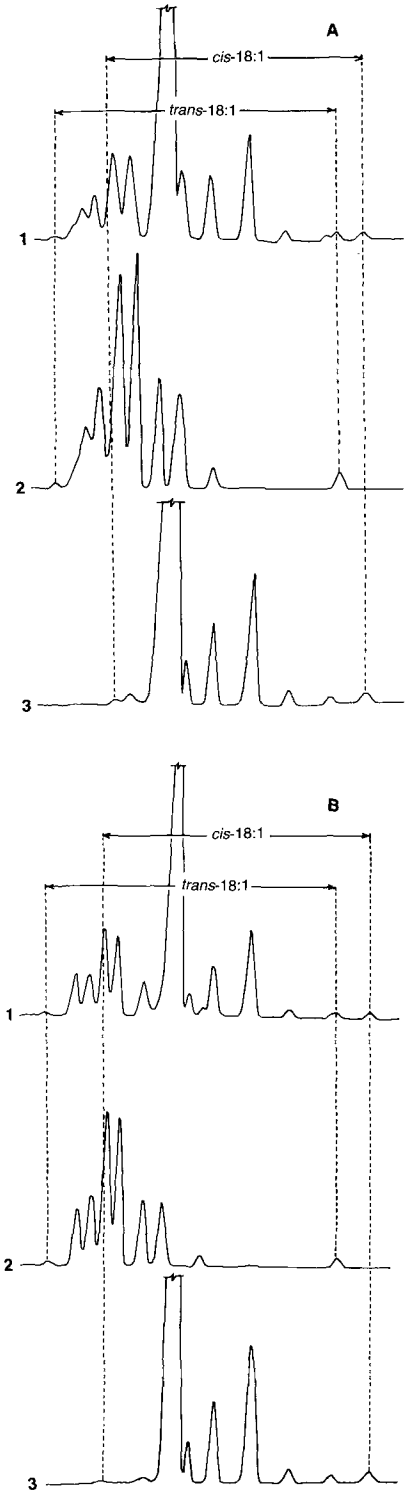

FIG. 5. Chromatograms of the 18:1 acid region of partially hydrogenated soybean oil and of *trans*- and *cis*-18:1 acids isolated by argentation thin-layer chromatography (Ag–TLC) of their isopropyl esters (chromatograms A) and of their methyl esters (chromatograms B). Analyses were done on a 100 m × 0.25 mm i.d. CP-Sil 88 fused-silica capillary column. Operating conditions and company source as in Figure 1. Chromatograms 1, unfractionated fatty acid derivatives. Chromatograms 2, *trans*-18:1 acids isolated by Ag–TLC. Chromatograms 3, *cis*-18:1 acids isolated by Ag–TLC.

should be considered in improving the resolution of *trans*-18:1 acids. Whether equivalent columns from other manufacturers may have properties similar to the CP-Sil 88 column is not known; a comparative study is needed. Chromatograms obtained with 100-m SP-2560 capillary columns (Supelco Inc., Bellefonte, PA) have been published (20,21), and the resolution was not as good as with the 100-m CP-Sil 88 column, or even as good as with the 50-m CP-Sil 88 column. But it is possible that these columns were not operated under optimum conditions. Individual standards of *trans*-18:1 acids are not commercially available. A simple and convenient mean to test the resolutive power of a column is to use *trans*-18:1 acids from butterfat or beef tallow (actually from any ruminant fat) because their distribution profile is almost constant (17,18,22). The *trans*-11 18:1 acid (vaccenic acid), the major isomer in these fats, may be used as a landmark to identify other isomers.

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