Identification of *trans*-Diene Isomers in Hydrogenated Soybean Oil by Gas Chromatography, Silver Nitrate Thin-Layer Chromatography, and ^{13}C NMR Spectroscopy

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Soybean oil was hydrogenated with use of a sulfur-containing nickel catalyst to produce high levels of *trans*-diene isomers. The isomers were derivatized to methyl esters and then fractionated by preparative silver nitrate thin-layer chromatography (TLC). Each TLC band was recovered and analyzed by capillary gas chromatography (GC). The identity of specific *trans*-dienes was confirmed by ^{13}C nuclear magnetic resonance (^{13}C NMR). Packed-column GC using a 6.1 m × 2 mm OV-275 column gave an accurate indication of the total amount of *trans*-dienes present, while a 50 m × 0.22 mm capillary column containing (cyanopropyl)polysiloxane separated the individual *trans*-diene isomers. By ^{13}C NMR, the three major *trans*-diene isomers were identified as *cis*-9,*trans*-12-octadecadienoic acid, *trans*-9,*cis*-12-octadecadienoic acid, and *trans*-9,*trans*-12-octadecadienoic acid.

trans-Monoene and -diene isomers are formed during the partial hydrogenation of vegetable oils. There has been considerable controversy regarding adverse physiological effects of these isomers. A report by the Federation of American Societies for Experimental Biology (FASEB) ad hoc review panel on trans fatty acids (Senti, 1985) concluded that the available scientific literature suggests little reason for concern with the safety of dietary trans fatty acids. The FASEB panel did, however, recommend additional studies to "clarify several unanswered questions".

It has been reported that gas chromatography (GC) using OV-275 packed columns can very effectively quantitate *trans*-monoenes in hydrogenated oils (Gildenberg and Firestone, 1985). Capillary GC has been used for several years to determine specific fatty acid isomers in foods (Slover and Lanza, 1979; Slover et al., 1985). The determination of the bond positions of fatty acid isomers involves chemical reaction at the point of unsaturation followed by additional chromatography (Johnston et al., 1978; Dutton et al., 1988).

trans,trans-18:2 dienes include conjugated, methylene-interrupted (MI), and other positional isomers of linoleic acid. The *trans*-9,*trans*-12-octadecadienoic acid (*trans*-9,*trans*-12-18:2) isomer has been shown to interfere with linoleic acid metabolism (Anderson et al., 1975; Privett et al., 1977; Hwang and Kinsella, 1979). There is some evidence (Holman et al., 1983) that other geometrical and positional isomers of octadecadienoic acids also interfere with essential fatty acid metabolism.

It has been reported that a few margarine oils contain 3% *trans,trans*-18:2 isomers (Sahasrabudhe and Kurian, 1979). This was refuted by Jackson et al. (1980), who analyzed similar margarine oils and found not more than 0.55% of these isomers. More recently Slover et al. (1985) found that some commercial margarines contain more than 1% of the *trans*-9,*trans*-12-18:2 isomer by using a capillary gas chromatographic method in which other isomers do not interfere. Gottenbos (1983) reported that most trans isomers in hydrogenated soybean oil had double bonds at the 9- or 12-position. Dutton (1983), also, claimed that most *trans*-dienes in hydrogenated oils were 9,12-isomers.

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Due to the variable amount of *trans*-diene isomers reported for hydrogenated products in the literature, a method using capillary GC, silver nitrate TLC, and ^{13}C NMR was developed that was used to confirm the presence of specific *trans*-diene isomers in hydrogenated soybean oil. Another purpose of our investigation was to compare packed and capillary GC analysis of hydrogenated soybean oil.

EXPERIMENTAL SECTION

Iodine Value. The Wijs iodine method was used to determine the iodine value of hydrogenated soybean oil according to the official AOAC (1984) method (sections 28.023-28.024).

Methyl Esters. Fatty acid methyl esters (FAMEs) were prepared by transesterification with BF_3/methanol according to the official AOAC (1984) method (sections 28.056-28.059).

Gas Chromatography. Packed-column GC was performed on a Perkin-Elmer Sigma 300 gas chromatograph equipped with a flame ionization detector (FID), using the method described by Gildenberg and Firestone (1985). A 6.1 m × 2 mm stainless steel column packed with 15% OV-275 coated on 100/120-mesh Chromosorb P, AW-DMCS (Supelco, Inc., Bellefonte, PA), was used. Operating conditions: column temperature, 220 °C; injector, 240 °C; detector (FID), 250 °C; carrier gas, He; flow rate, 15 mL/min; injection volume, 1 μL (approximately 10 mg of FAME/mL in hexane).

Capillary GC was performed on a Perkin-Elmer gas chromatograph with an FID detector. A 50 m × 0.22 mm fused silica WCOT CP-Sil 88 column (Chromapack, Inc., Bridgewater, NJ) was used with 80 to 1 split injection. Operating conditions: column temperature, 170 °C; injector and detector temperature, 300 °C; carrier gas, He; column pressure, 152 kPa; injection volume, 1 μL (approximately 10 mg of FAME/mL in hexane).

For both packed-column and capillary GC, results were quantitated by area normalization using a Perkin-Elmer Sigma 10 data station. A reference standard was included in each group of test samples. This standard contained the major fatty acids in the hydrogenated soybean oil, and the results were used to calculate a correction factor for each FAME.

Infrared (IR) Spectrophotometry. Methyl esters were dissolved in carbon disulfide, and the total trans content was determined on a Perkin-Elmer 1330 IR spectrophotometer by using the official AOAC method (sections 28.086-28.091). Since methyl elaidate (*trans*-9-18:1) was used as the standard, some test samples that contained *trans*-diene isomers gave percent trans values of over 100%.

Preparative TLC. Silver nitrate preparative TLC was conducted on the FAMEs by using 20 cm × 20 cm plates coated with a 1000-μm layer of silica gel G containing 20% silver nitrate (Wood and Snyder, 1966). Plates were developed with chloroform and examined under long-wavelength UV light after they were sprayed with a 0.2% solution of 2′,7′-dichlorofluorescein in ethanol.

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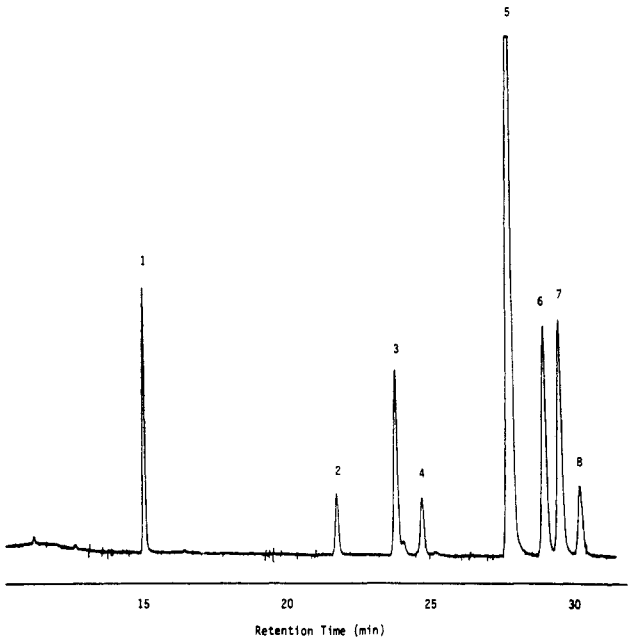

Figure 1. Capillary gas chromatogram of the fatty acid methyl esters of isomerized safflower oil, obtained by using a CP-Sil 88, 50 m × 0.22 mm column. Peaks: 1 = 16:0; 2 = 18:0; 3 = *trans*-9-18:1; 4 = *cis*-9-18:1; 5 = *trans*-9,*trans*-12-18:2; 6 = *cis*-9,*trans*-12-18:2; 7 = *trans*-9,*cis*-12-18:2; 8 = *cis*-9,*cis*-12-18:2.

^{13}C Nuclear Magnetic Resonance (^{13}C NMR). Natural abundance ^{13}C NMR spectra were produced on a Nicolet NMC 300-MHz FT-NMR spectrometer by using a 5-mm carbon-13 probe tuned to 75.457 MHz with an aquisition time of 1–2 h. The standard two-level decoupling experiment was used with a preacquisition delay of 15 s. Although relative peak heights at specific ppm volumes were used to indicate relative concentrations of certain FAMEs, no attempt was made to quantitate FAME composition by ^{13}C NMR. Chemical shifts are reported relative to $CDCl_3$ at 77.0 ppm.

Hydrogenation of Soybean Oil. Soybean oil was hydrogenated for 3 h by using 0.1% of a commercial catalyst that contained 20% nickel and 1% sulfur at 200 °C and 69-kPa hydrogen pressure in a 1-gal pressure vessel reactor manufactured by Autoclave Engineers (Erie, PA). The methyl esters were then prepared and analyzed by GC and preparative TLC. For analysis by ^{13}C NMR, the TLC fractions were scraped from the plate and eluted with chloroform. Each silica gel fraction was washed twice with chloroform, and then the eluate and washings were evaporated to dryness under a stream of nitrogen. Each residue (approximately 50 mg) was dissolved in 0.4 mL of $CDCl_3$ for ^{13}C NMR analysis.

Safflower Oil Isomerization. Safflower oil was isomerized to high levels of trans isomers by using a modification of the method described by Gibson and Strassburger (1976). A 20-g portion of *p*-toluenesulfinic acid (PTSA) was dissolved in 100 mL of 1-propanol, and the solution was added to 100 g of safflower oil in a flask. The contents were flushed with nitrogen and refluxed 2 h. When cool, the mixture was diluted with hexane, extracted with 1 N NaOH, and washed twice with 20% NaCl solution. The hexane solution was dried over Na_2SO_4, filtered, and concentrated on a rotary evaporator to recover the isomerized oil. The isomers were converted to methyl esters and then analyzed by GC and ^{13}C NMR.

RESULTS AND DISCUSSION

GC Analysis of Isomerized Safflower Oil. Safflower oil was first isomerized with a PTSA catalyst, which produces only 9,12 geometrical isomers (Snyder and Scholfield, 1982). Therefore, a simple profile was provided to check the feasibility of using ^{13}C NMR to identify specific FAME isomers in a FAME isomer mixture.

Isomerization of safflower oil with PTSA converted over 90% of the linoleic acid to *trans*-diene isomers. No positional isomerization during PTSA elaidination was found.

Table I. ^{13}C NMR Shifts for Allylic Carbons of Linoleic Acid Geometrical Isomers (Bus et al., 1976)

isomer	chemical shift, ppm C_{11}	C_8	C_{14}
trans-9,*trans*-12-18:2	35.70	32.60	32.60
cis-9,*trans*-12-18:2	30.50	27.15	32.60
trans-9,*cis*-12-18:2	30.55	32.60	27.20
cis-9,*cis*-12-18:2	25.75	27.35	27.35

Figure 1 shows the GC chromatogram obtained for the isomerized safflower oil by using a CP-Sil 88 capillary column. Peak identifications of FAMEs were made on the basis of retention times of reference standards. Initial identifications of *cis*-9,*trans*-12-18:2 and *trans*-9,*cis*-12-18:2 isomers were made on the basis of the relative retention times reported for these isomers by Mutter and Homan (1987), who used a 50-m CP-Sil 88 capillary column to separate FAME isomers in hydrogenated soybean oil. The isomerized oil contained about 45% *trans*-9,*trans*-12-18:2 and 13% each of the *cis*-9,*trans*-12-18:2 and *trans*-9,*cis*-12-18:2 isomers. The small shoulder on peak 3 indicates the presence of a *trans*-18:1 positional isomer. However, there was apparently no positional isomerization of 18:2 dienes during isomerization since there were no minor diene peaks.

When isomerized safflower oil was analyzed with an OV-275 6.1 m × 2 mm packed column, several FAME isomers were separated. However, the *cis*-9,*trans*-12-18:2 and *trans*-9,*cis*-12-18:2 isomers were not separated.

^{13}C NMR Analysis of Isomerized Safflower Oil. The number, position, and geometrical configuration of double bonds in the fatty acid chain determine the chemical shift of the peaks in the carbon-13 spectrum. Table I shows the published (Bus et al., 1976) ^{13}C NMR chemical shifts for allylic carbons of linoleic acid geometrical isomers. These chemical shifts are unique and separate from shifts for other carbons in the fatty acid chain and can, therefore, be used for identification. For the external allylic carbons (C8 and C14) these shifts are at 32.6 ppm if the carbons are allylic to a trans bond; the shifts are at 27.15–27.35 ppm if the carbons are allylic to a cis bond. The internal methylene carbon (C11) has a unique chemical shift for each isomer. These shifts are 35.70 ppm for *trans*-9,*trans*-12-18:2, 30.50 ppm for *cis*-9,*trans*-12-18:2, 30.55 ppm for *trans*-9,*cis*-12-18:2, and 25.75 ppm for *cis*-9,*cis*-12-18:2. The *cis*-9,*trans*-12-18:2 and *trans*-9,*cis*-12-18:2 isomers could not be distinguished because there was only a 0.05 ppm difference in their C11 chemical shifts.

The ^{13}C NMR spectrum of isomerized safflower oil (Figure 2) shows the unique chemical shifts of the external and internal allylic carbons of the linoleic acid isomers. The peaks at 27.2 and 32.6 ppm show the chemical shifts of the external allylic cis and trans carbons, respectively. The C11 chemical shift of each linoleic acid isomer was found. The chemical shift at 35.7 ppm confirms the presence of *trans*-9,*trans*-12-18:2, while the chemical shift at 30.5 ppm confirms the presence of *cis*-9,*trans*-12-18:2 and *trans*-9,*cis*-12-18:2 isomers. The chemical shift at 25.7 ppm confirms the presence of *cis*-9,*cis*-12-18:2. The relative peak heights for the methylene carbons (C11) at 25.7, 30.5, and 35.7 ppm correlate directly with the relative concentrations of each of the linoleic acid isomers in isomerized safflower oil.

GC Analysis of Hydrogenated Soybean Oil. Soybean oil was hydrogenated with a sulfur-containing nickel catalyst to an iodine value of 88. The presence of sulfur during hydrogenation is known to produce high levels of trans isomers (Okonek, 1987). Therefore, the levels of

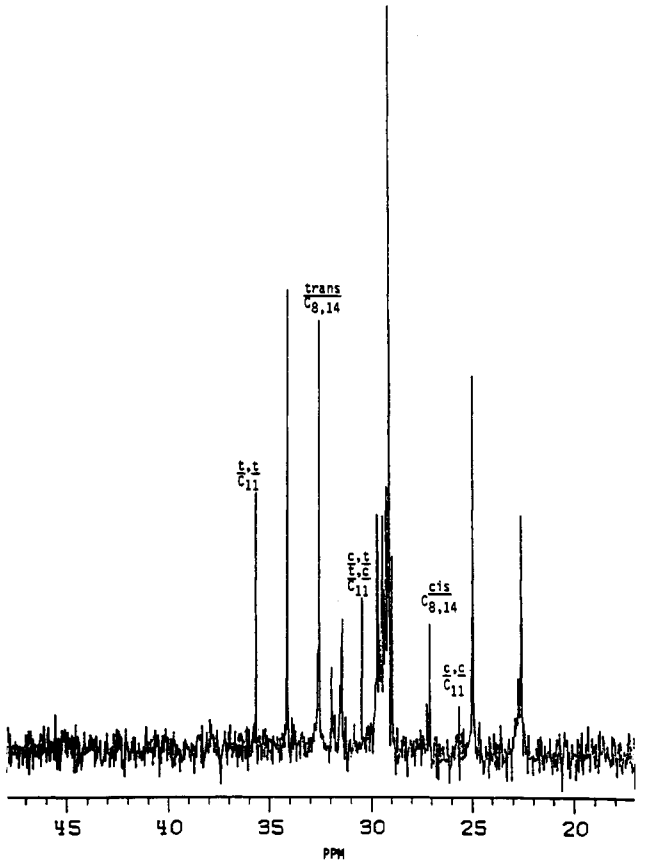

Figure 2. ^{13}C NMR spectrum of isomerized safflower oil: c = cis, t = trans.

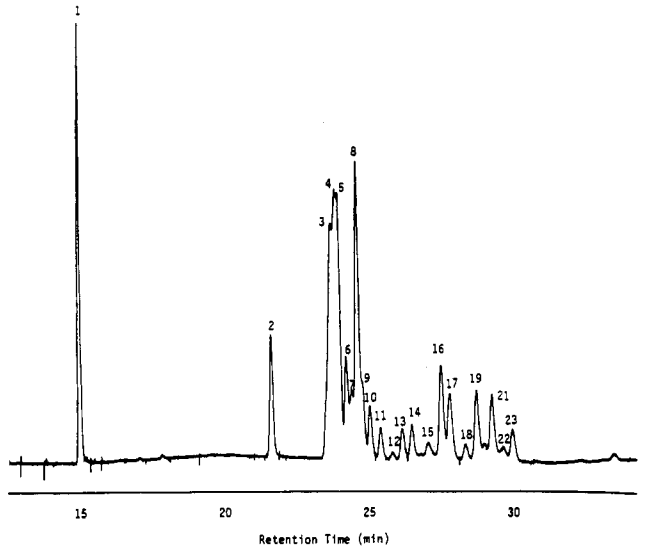

Figure 3. Capillary gas chromatogram of the fatty acid methyl esters of hydrogenated soybean oil, obtained by using a CP-Sil 88 50 m × 0.22 mm column. Operating conditions were the same as those used to obtain Figure 1. Peak identification: see Table II.

trans isomers were enhanced by the processing conditions and are not typical of the trans isomer content of most commercial hydrogenated products. The recommended use of the commercial sulfur-containing catalyst used for this study was for the manufacture of coating fats, vegetable creamers, and margarine blending stock.

As shown in Figure 3, capillary GC separated several isomers present in the hydrogenated soybean oil. Silver nitrate TLC and ^{13}C NMR were used to identify these isomers.

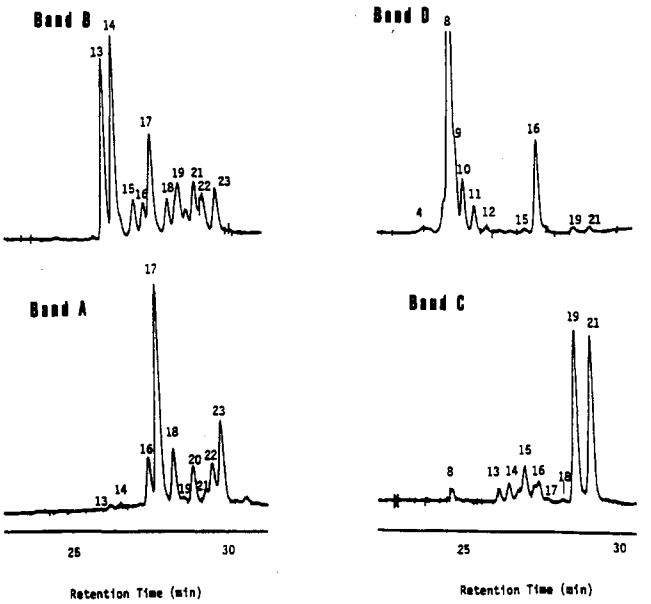

Figure 4. Capillary gas chromatograms showing composition of silver nitrate TLC bands. Peak identification: see Table II.

Table II. Identification of Fatty Acid Methyl Esters in TLC Bands by Capillary GC and ^{13}C NMR

TLC band	R_f	% trans by IR	peak	fatty acid methyl ester
A	0.12	54.2	17	*cis,trans-* or *trans,cis*-18:2 (NMI)[a]
			18, 20, 22	unknown
			23	*cis*-9,*cis*-12-18:2
B	0.22	115.3	13, 14	*cis,trans-* or *trans,cis*-18:2 (NMI)
			15	unknown
C	0.34	101.7	19	*cis*-9,*trans*-12-18:2
			21	*trans*-9,*cis*-12-18:2
D	0.50	29.0	8-12	*cis*-18:1 isomers
			16	*trans*-9,*trans*-12-18:2
E	0.60	79.2	3-7	*trans*-18:1 isomers
F	0.68		1	16:0
			2	18:0

[a] NMI = non-methylene interrupted.

Silver Nitrate TLC and ^{13}C NMR Analysis of Hydrogenated Soybean Oil. Methyl esters of the isomers in the hydrogenated soybean oil were prepared and separated into six bands by preparative silver nitrate TLC. These bands were labeled A–F, with band A closest to the origin.

Figure 4 shows the capillary gas chromatogram of the extracted FAMEs in each TLC band that contained *trans*-diene isomers (bands A–D). These GC chromatograms indicate that fatty acid methyl ester (FAME) diene isomers are concentrated in bands A–C. The GC retention times of the FAME isomers in these bands were similar to those of the diene isomers found in the isomerized safflower oil (Figure 1). Band D contained *trans*-9,*trans*-12-18:2 as well as the *cis*-monoene isomers. It has previously been shown by silver nitrate TLC (Carpenter et al., 1976) that, starting from the origin, the elution order of FAMEs containing 18 carbons is (1) *cis,cis*-18:2, (2) *cis,trans-* and *trans,cis*-18:2, (3) *trans,trans*-18:2 and *cis*-18:1, (4) *trans*-18:1, and (5) saturated.

Table II summarizes the identification of the capillary GC peaks shown in Figures 3 and 4 based on the analysis of each TLC band. Single analyses were performed on each isolated FAME because of limited quantities (about 50 mg each).

Band A. Peak 17, the major component of band A, was identified as a *trans,cis-* or *cis,trans*-18:2 non-methylene-interrupted (NMI) diene isomer on the basis of its TLC migration, GC retention time, and ^{13}C NMR chemical

shifts. If peak 17 were a *trans,trans*-diene isomer, it would have migrated closer to band D. The ^{13}C NMR spectrum of band A also showed no chemical shifts at 30.5 and 35.7 ppm, which are specific for C11 of cis,trans (or trans,cis) and trans,trans isomers, respectively, thereby confirming that peak 17 is not an MI *trans*-diene isomer.

Peak 23 was identified as *cis*-9,*cis*-12-18:2 (linoleic acid) on the basis of its GC retention time compared to that of a standard and its TLC migration rate. The presence of this isomer was also confirmed by ^{13}C NMR analysis of band A, which revealed a small chemical shift at 25.7 ppm that is specific for the C11 carbon of *cis*-9,*cis*-12-18:2.

The unidentified peaks that were minor components of band A (peaks 18, 20, and 22) were most likely *cis,cis*-18:2 NMI isomers on the basis of their GC retention times and migration with *cis*-9,*cis*-12-18:2 on the TLC plate. The fact that band A was found to contain 54.2% trans by IR spectrophotometry also indicates that about half the isomers in band A did not contain trans double bonds.

Band B. The major components of band B were peaks 13 and 14. The trans content of 115% found by IR spectrophotometry is a good indication that this band contained *trans*-diene isomers. Using a *trans*-monoene standard and the small test portion size could have caused the deviation from the theoretical 100% trans content expected for *cis,trans*- and *trans,cis*-dienes. *trans*-Monoenes would have migrated closer to band E on the TLC plate and would have had shorter GC retention times. *trans,trans*-Diene isomers would have migrated closer to band D on the TLC plate and would have produced a much higher trans fatty acid content by IR spectrophotometry for band B. On the basis of this evidence, peaks 13 and 14 were tentatively identified as *cis,trans*- or *trans,cis*-18:2 isomers. These peaks were identified as NMI isomers since the chemical shift at 30.5 ppm, which is specific for MI cis,trans, and trans,cis isomers, was not found in the ^{13}C NMR spectrum of band B.

Peak 15, a minor component of band B, is also most likely a *cis,trans*- or *trans,cis*-18:2 isomer on the basis of its TLC migration and the similarity of its GC retention time to those of peaks 13 and 14.

Band C. The major components of band C were peaks 19 and 21, which were identified as *cis*-9,*trans*-12- and *trans*-9,*cis*-12-18:2, respectively. These identifications were based on TLC migration, percent trans by IR spectrophotometry, relative GC retention time, and ^{13}C NMR analysis.

The ^{13}C NMR spectrum of band C is shown in Figure 5. The resonance peak at 30.5 ppm is specific for C11 of *cis*-9,*trans*-12- and *trans*-9,*cis*-12-18:2 isomers and confirmed the presence of these isomers in band C. The external trans allylic carbons (32.6 ppm) and the external cis allylic carbons (27.2 ppm) at C8 and C14 have about the same chemical shift peak heights as the C11 peak height. Each of these isomers has one external allylic cis carbon, one external allylic trans carbon, and one methylene carbon for each isomer molecule. This observation provided additional evidence that the major components of band C were cis,trans and trans,cis MI dienes.

Band D. Band D contained the *cis*-18:1 isomers (peaks 8–12) as indicated by TLC migration and GC retention time. GC analysis also indicated that the concentration of peak 16 in band D was 16.9%. Peak 16 appeared to be *trans*-9,*trans*-12-18:2 on the basis of its GC retention time. This accounted for the 29% trans fatty acids in this band measured by IR spectrophotometry since the theoretical percent trans by IR spectrophotometry for band D was 33.8% (16.9% × 2).

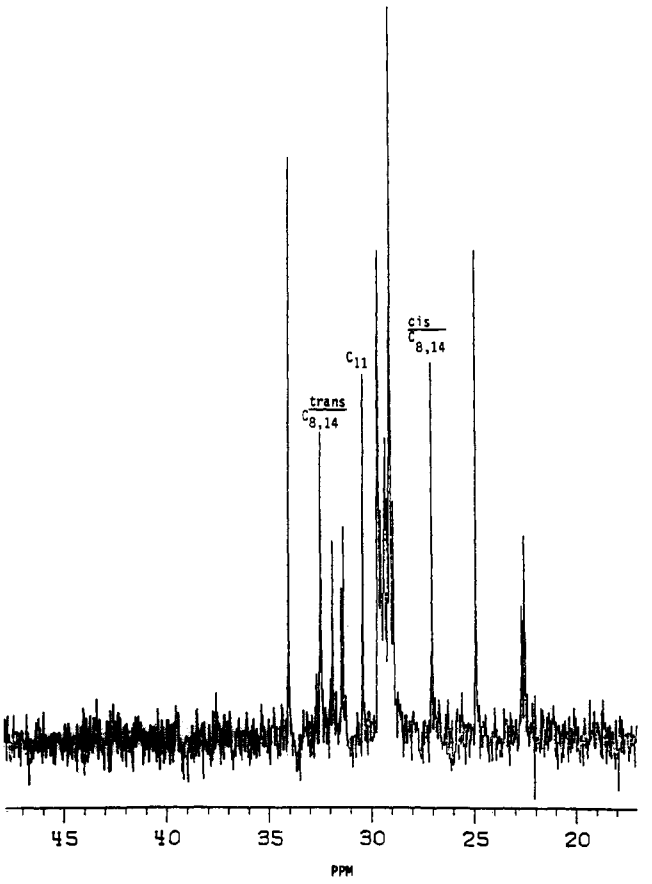

Figure 5. ^{13}C NMR spectrum of band C from preparative silver nitrate TLC.

The presence of *trans*-9,*trans*-12-18:2 in band D was confirmed by the ^{13}C NMR spectrum shown in Figure 6. The chemical shift at 35.7 ppm, which is specific for C11 of *trans*-9,*trans*-12-18:2, is far removed from all other chemical shifts in hydrogenated oil and confirms the presence of this isomer in band D. The expected peak height of the external allylic carbons (C8 and C14) of *trans*-9,*trans*-12-18:2 at 32.6 ppm should be approximately 2 times as high as the peak height for the C11 because there are two external allylic carbons for each methylene (C11) carbon in *trans*-9,*trans*-12-18:2. Since this ratio of relative peak heights was observed, the major trans isomer present in band D was confirmed to be *trans*-9,*trans*-12-18:2.

Band E. The presence of *trans*-monoene isomers (peaks 3–7) in band E was indicated by their TLC migration and GC retention times. The ^{13}C NMR spectrum of this band showed a chemical shift for the trans allylic carbons at 32.6 ppm but no chemical shift at 27.3 ppm, confirming that no cis isomers were present in this band.

Band F. Band F contained saturated FAMEs; their identities were confirmed by comparing their GC retention times with those for palmitic and stearic acid methyl ester standards.

Packed-Column GC. Figure 7 shows the gas chromatogram obtained for hydrogenated soybean oil by using a 6.1 m × 2 mm OV-275 packed column. This chromatographic method has been widely used to determine trans isomers in hydrogenated vegetable oil (Gildenberg and Firestone, 1985). However, most positional and geometrical isomers are not separated by this method.

Retention times of standards and other published chromatograms (Gildenberg and Firestone, 1985; Perkins et al., 1977) were used to tentatively identify the *trans*-

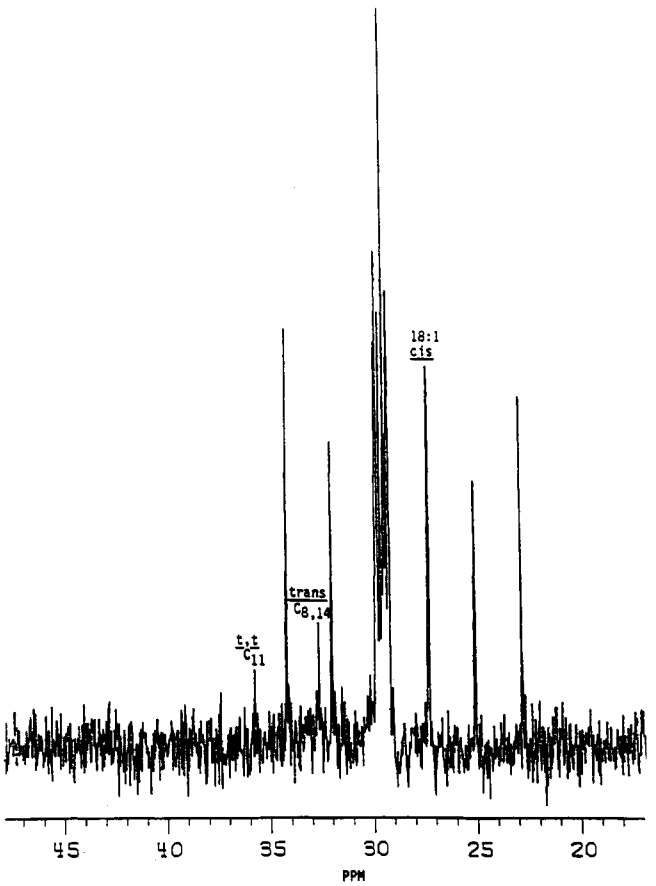

Figure 6. ^{13}C NMR spectrum of band D from preparative silver nitrate TLC.

Table III. Identification of *trans*-Diene Isomers in TLC Bands by OV-275 Packed-Column GC

packed-column GC peak	TLC band	*trans*-diene isomer	capillary GC peak
5	B	*cis,trans*- or *trans,cis*-18:2 (NMI)[a]	13, 14
5	D	*trans*-9,*trans*-12-18:2	16
6	A	*cis,trans*- or *trans,cis*-18:2 (NMI)	17
7	C	*cis*-9,*trans*-12-18:2	19
7	C	*trans*-9,*cis*-12-18:2	21

[a] NMI = non-methylene interrupted.

dienes in peaks 5–7. The TLC bands analyzed by capillary GC (Figure 4) were also analyzed by packed-column GC to identify the diene isomers. The results obtained by packed column GC are summarized in Table III.

Peak 5. Peak 5 had the same retention time as a *trans*-9,*trans*-12-18:2 standard. However, FAME isomers with the same retention time as peak 5 were found in two TLC bands, bands B and D. This indicated that peak 5 contained more than one isomer. The major FAME isomers present in TLC band B were shown to be *cis,trans*- or *trans,cis*-18:2 NMI dienes on the basis of capillary GC, IR, and ^{13}C NMR analyses (Table II).

It was confirmed by ^{13}C NMR analysis (Figure 6) that band D contained *trans*-9,*trans*-12-18:2. According to packed-column GC analysis, TLC band D contained 15.6% of this isomer, which is about the same percent obtained by capillary GC analysis (16.9%).

It can, therefore, be concluded that peak 5 of the packed-column GC chromatogram in Figure 7 contained *trans*-9,*trans*-12-18:2 as well as cis,trans and trans,cis NMI dienes. In the past, some researchers (Sahasrabudhe and Kurian, 1979) have implied that, in the analysis of hydrogenated soybean oil, the peak with the same retention time as the *trans*-9,*trans*-12-18:2 standard contained this

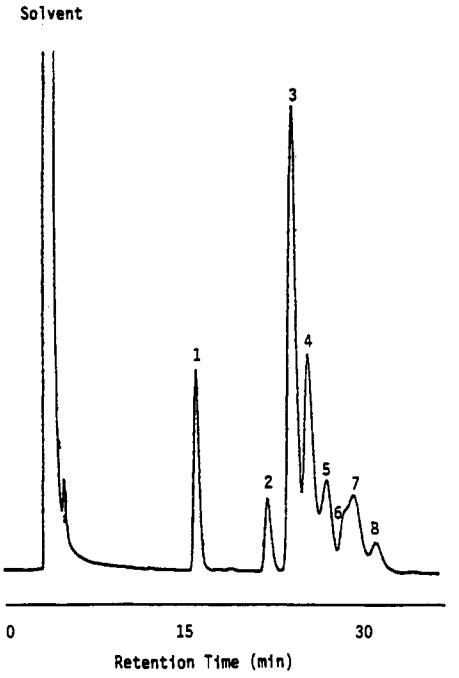

Figure 7. Gas chromatogram of hydrogenated soybean oil, obtained by using an OV-275 packed column. Peaks: 1 = 16:0; 2 = 18:0; 3 = *trans*-18:1; 4 = *cis*-18:1; 5 = *trans*-9,*trans*-12-18:2 and other *trans*-dienes; 6 = *cis,trans*- and *trans,cis*-18:2 geometrical and positional isomers; 7 = cis,trans and trans,cis geometrical and positional isomers; 8 = *cis,cis*-18:2.

isomer only. Marchand and Beare-Rogers (1982) subsequently analyzed commercial margarine and showed that the *trans*-9,*trans*-12-18:2 isomer coeluted with NMI *trans*-dienes and *cis*-monoene positional isomers. Although we found *trans*-18:2 NMI dienes in peak 5, significant quantities of *cis*-monoene positional isomers were not found. For example, about the same results for *trans*-9,*trans*-12-18:2 were obtained when band D was analyzed by packed-column and capillary GC (15.6% vs 16.9%). If any significant amount of *cis*-monoenes had coeluted with the *trans*-9,*trans*-12-18:2 isomer in peak 5 in analyses by OV-275 packed-column GC, then the amount of this isomer would have been higher by packed-column GC.

Peak 6. FAME isomers with the same retention time as peak 6 of the packed-column gas chromatogram (Figure 7) were found in TLC band A. The major isomers in band A were identified as cis,trans or trans,cis NMI isomers by capillary GC, IR spectrophotometry, and ^{13}C NMR (Table II).

Peak 7. The FAME isomers in peak 7 had the same retention time as the *cis*-9,*trans*-12- and *trans*-9,*cis*-12-18:2 isomers that were present in TLC band C. Capillary GC and ^{13}C NMR analysis of band C confirmed that these isomers were *cis*-9,*trans*-12- and *trans*-9,*cis*-12-18:2 (Table II).

Comparison of Capillary and Packed-Column GC Results. Table IV compares the results obtained when hydrogenated soybean oil was analyzed by either capillary or packed-column GC. The trans fatty acids present in the hydrogenated soybean oil totaled 68.7% by IR spectrophotometry. Capillary GC indicated that the hydrogenated soybean oil contained 6.2% *trans*-9,*trans*-12-18:2. Since this isomer has two trans bonds per fatty acid molecule, the total trans isomers by IR spectrophotometry should theoretically be 6.2% higher than the total determined by GC methods. Therefore, the total trans isomers determined by packed-column GC (62.0%), capillary GC

Table IV. Trans Isomer Content of Hydrogenated Soybean Oil

isomer	capillary GC		packed-column GC	
	% compn	CV, %	% compn	CV, %
trans-monoenes	38.8	1.7	38.9	0.5
cis-9,*trans*-12-18:2	4.6	4.5		
trans-9,*cis*-12-18:2	4.7	1.3		
other *cis*,*trans*-18:2	7.0	2.9	13.6[a]	0.5
trans-9,*trans*-12-18:2	6.2	1.6	9.5[b]	0.4
total trans	61.3		62.0	
total *trans*-dienes	22.5		23.1	

[a] Includes *cis*-9,*trans*-12- and *trans*-9,*cis*-12-18:2 methylene-interrupted isomers as well as cis,trans and trans,cis non-methylene-interrupted (NMI) isomers. [b] Also includes cis,trans and trans,cis NMI isomers.

(61.3%), and IR spectrophotometry (68.7% - 6.2% = 62.5%) were in close agreement.

The 23.1% total *trans*-dienes determined by packed-column GC closely approximated the 22.5% *trans*-dienes determined by capillary GC. By comparing the results for *trans*-9,*trans*-12-18:2 obtained with packed and capillary GC columns. it appears that peak 5 of the packed-column gas chromatogram in Figure 7 contained about 65% *trans*-9,*trans*-12-18:2 and 35% *cis*,*trans*- and *trans*,*cis*-18:2 isomers.

CONCLUSIONS

Silver nitrate TLC, capillary GC, and ^{13}C NMR were used to identify specific *trans*-dienes in isomerized hydrogenated soybean oil, including *trans*-9,*trans*-12-18:2, *cis*-9,*trans*-12-18:2, and *trans*-9,*cis*-12-18:2 fatty acid isomers. Over 20% *trans*-dienes, including 6.2% *trans*-9,*trans*-12-18:2, were produced during hydrogenation of soybean oil, using a sulfur-containing catalyst.

Although packed-column GC did not separate individual *trans*-dienes as well as the capillary GC technique did, it was still possible to get an accurate indication of the total *trans*-dienes present with this method. Packed-column GC is a convenient method to use in routine screening of hydrogenated oils for their content of *trans*-monoene and -diene isomers.

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