Hydrogenation of Soybean Oil: A Thin-Layer Chromatography and Gas Chromatography/Matrix Isolation/Fourier Transform Infrared Study

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By use of gas chromatography/matrix isolation/Fourier transform infrared (GC/MI/FT-IR) spectroscopy, the double-bond configurations of fatty acid methyl esters (FAMEs) derived from hydrogenated soybean oil and margarine were determined from the positions of observed IR bands. However, because of the formation of complex FAME mixtures with overlapping GC peaks, preliminary separation was necessary. Mixtures of FAMEs were separated by silver nitrate thin-layer chromatography and GC using a polar cyanopropylpolysiloxane capillary column. GC eluates were trapped at 12 K under high vacuum in an argon matrix and subsequently analyzed by IR spectroscopy. Because of the high specificity of the MI/FT-IR technique, unique IR spectra were obtained that allowed the characterization of eight C_{18} diene FAME positional isomers with trans,trans or trans/cis configuration. C_{18} FAMEs with identical GC retention times were readily distinguished, and their degree of unsaturation and double-bond configuration were established by IR spectroscopy.

INTRODUCTION

Dietary fats and oils are vital for many metabolic functions. However, epidemiological studies indicate a correlation between cardiovascular disease or atherosclerosis and a high intake of fat; diets high in polyunsaturated oils were reported to lead to lower blood cholesterol levels than do those high in hydrogenated oils, and a relationship between serum cholesterol levels and atherosclerosis was suggested (Emken, 1979). Catalytic hydrogenation is used to modify the physical properties of oils for various purposes. This process partially converts cis fatty acids into trans isomers and causes the migration of double bonds along the fatty acid chain, thus producing a complex mixture of positional isomers with both cis and trans configurations (Allen, 1987). Kummerow (1979) concluded that trans fatty acid isomers can elevate serum cholesterol levels and alter cell membrane properties. Furthermore, trans-9 18:1, which is not endogenously synthesized by the human body, may interfere in the ability of the heart to respond to stress (Kummerow, 1979). Hanis et al. (1989) recently found that hydrogenated fat adversely affected litter size, sperm morphology, and regularity of the oestrous cycle and prolonged the period of gestation in experimental rats. Concern was also expressed about the safety of trans-9, trans-12 18:2, which was reported to impair desaturase activity and prostaglandin synthesis in laboratory animals (Kinsella et al., 1981). The effects on the physiology of the cell and the nutritional value of three other trans,trans 18:2 positional isomers that were identified in the present work remain unknown.

In a recent gas chromatography/matrix isolation/Fourier transform infrared (GC/MI/FT-IR) spectroscopic investigation of margarine and hydrogenated soybean oil (Mossoba et al., 1990), the double-bond configuration of geometric and positional fatty acid methyl ester (FAME) isomers was determined on the basis of distinctive IR spectral characteristics, such as band positions. However, the observed gas chromatograms (Mossoba et al., 1990) indicated the formation of complex FAME mixtures, and a preliminary separation was necessary to reduce the number of overlapping gas chromatographic peaks.

In the present study, we report the separation of FAME isomers, on the basis of the degree of unsaturation and the double-bond configuration, by silver nitrate thin-layer chromatography ($AgNO_3$-TLC) followed by capillary GC with MI/FT-IR spectroscopic identification.

For the first time, MI/FT-IR spectra in the range 4000-600 cm^{-1} were obtained for each of four trans,trans and four cis/trans C_{18} FAME dienes derived from margarines or hydrogenated soybean oil. It was then possible to differentiate these positional isomers by the intensity of the CH_2 asymmetric stretching vibration at 2935 cm^{-1} relative to that of the carbonyl band at 1754 cm^{-1}. These two bands were common to all the FAMEs analyzed.

EXPERIMENTAL PROCEDURES

All materials, standards, and procedures for test sample preparation and for hydrogenation (Mossoba et al., 1990) as well as the official procedures for esterification and determination of iodine value (AOAC, 1984, Sections 28.056-28.059 and 28.023-28.024, respectively) and the GC/MI/FT-IR instrumentation (Bourne et al., 1984; Reedy et al., 1985) were described previously.

Preparative Thin-Layer Chromatography. Preparative $AgNO_3$-TLC was conducted on FAME mixtures by using 20 cm × 20 cm plates precoated with a 1-mm layer of silica gel G containing 20% $AgNO_3$. Plates were activated at 100 °C for 1 h. Methyl esters of fatty acids were applied with a streaker (Alltech Associates, Deerfield, IL) and were developed in chloroform (Mallinckrodt). TLC bands were sprayed with a 0.2% solution of 2,7-dichlorofluorescein (Sigma, St. Louis, MO) in ethanol, visualized under UV light (Spectraline), and then scraped off. The FAME analytes were extracted from the silica gel with chloroform, and the extracts were filtered through Whatman 2V fluted paper. A rotary evaporator (Buchi) removed the chloroform, and the residue was dissolved in hexane (Mallinckrodt) or distilled-in-glass isooctane (Burdick & Jackson Laboratories, Inc., Muskegon, MI) for further separation by GC.

RESULTS AND DISCUSSION

Complex FID profiles were obtained when FAMEs derived from margarines and hydrogenated soybean oil

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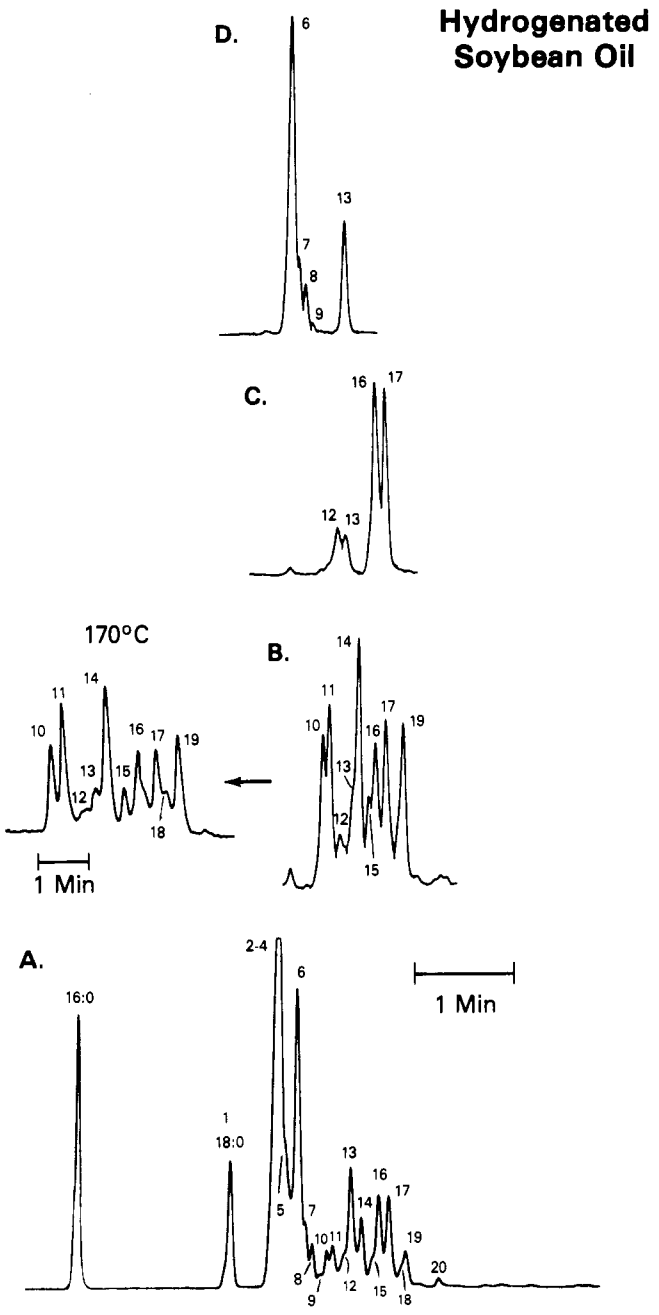

Figure 1. Partial GC/FID chromatograms for FAMEs derived from soybean oil hydrogenated to an iodine value of 96 by using a sulfur-containing nickel catalyst: (A) before TLC; (B–D) after TLC. FAME peaks are numbered sequentially, starting with 18:0 = 1. Temperature was ramped to 200 °C. Insert for TLC fraction (B) shows a partial chromatogram obtained at a final temperature of 170 °C. Peak identities are given in the text and/or Table I.

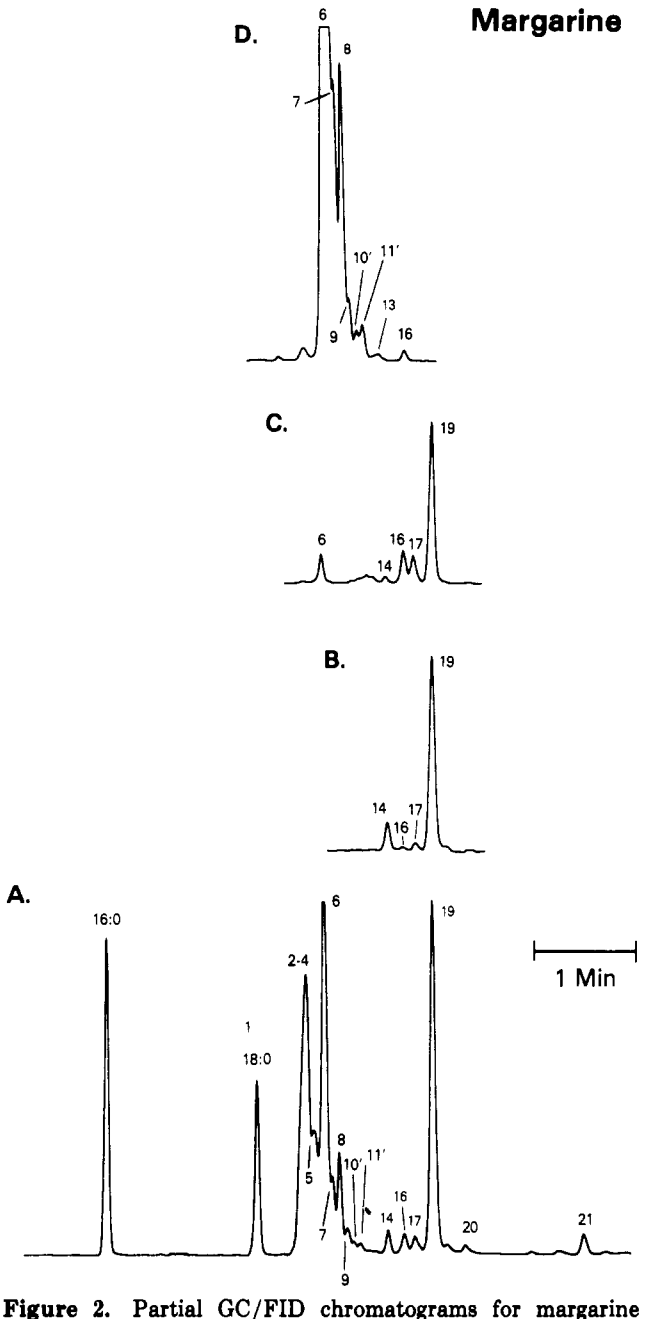

Figure 2. Partial GC/FID chromatograms for margarine FAMEs: (A) before TLC; (B–D) after TLC. FAME peaks are numbered sequentially, starting with 18:0 = 1. Temperature was ramped to 200 °C. Peak identities are given in the text and/or Table I. Peak 21 is *c*9,*c*12,*c*15–18:3.

were examined by GC (Figures 1A and 2A). Some GC peaks overlapped, thus preventing the IR characterization of positional isomers with the same configuration, such as the methylene-interrupted (mi) and non-methylene-interrupted (nmi) trans,trans-diene C_{18} positional isomers. Also, certain FAMEs coeluted, namely, the pair of trans,-trans 18:2 isomers (peaks 10 and 11, Figure 1A) and the two cis 18:1 isomers (peaks 10′ and 11′, Figure 2A); isomers 10 and 10′ eluted at 14.66 min, while isomers 11 and 11′ eluted at 14.72 min. To further separate cis from trans and diene from monoene FAMEs, $AgNO_3$-TLC was used before analysis by GC. The FAMEs in two margarine and three hydrogenated soybean oil test portions, covering the iodine value range 96–85, were separated by this procedure.

Parts B–D of Figure 1 show FID traces for a hydrogenated soybean oil (iodine value 96) fractionated by TLC. The corresponding TLC bands were the first three from the origin (R_f 0.02, 0.04, and 0.16, respectively) scraped off the TLC plate. Similar data are presented in parts B–D of Figure 2 for a commercial margarine. Such TLC and GC separations allowed us to obtain distinguishable MI/FT-IR spectra in the 4000-600-cm^{-1} range and thereby to identify the different positional isomers. Unique IR band positions for FAMEs were recently reported (Mossoba et al., 1990). FAME double-bond configuration and structural characteristics were derived from IR spectral data; information deduced from the order of the TLC and GC elutions complemented the spectroscopic results.

Figure 1 shows that the relative amounts of FAMEs are proportionately higher in the three TLC fractions (Figure 1, parts B–D) than in the original mixture (Figure 1A). For example, isomers 10 and 11 in TLC fraction B produced intense GC peaks and hence were identified by IR analysis

Table I. Distribution of C_{18} FAMEs in TLC Fractions of Transesterified Margarine and Hydrogenated Transesterified Soybean Oil As Determined by GC

		retention time,[b] min		TLC B[c]					TLC C[c]					TLC D[c]				
GC peak	FAME[a]	200 °C	170 °C	M1	M2	6A	7H	7M	M1	M2	6A	7H	7M	M1	M2	6A	7H	7M
6	*c*-18:1	14.38	21.30											6	6	6	6	6
7	*c*-18:1	14.45	21.57											7	7	7	7	7
8	*c*-18:1	14.52	21.81											8	8	8	8	8
9	*c*-18:1	14.60	22.08											9	9	9	9	9
10′	*c*-18:1	14.66	22.36											10′	10′	10′		
11′	*c*-18:1	14.72	22.60											11′	11′	11′		
10	nmi-*t*,*t*-18:2	14.66	22.30				10	(10)					10					
11	nmi-*t*,*t*-18:2	14.72	22.52				11	(11)					11					
12	nmi-*t*,*t*-18:2	14.85	22.90				12	12	–[d]	–[d]		(12)	(12)					
13	mi-*t*9,*t*12-18:2	14.90	23.23					13				13	13	13	13	13	(13)	(13)
14	nmi-*c*/*t*-18:2	15.01	23.38	(14)	(14)	(14)	14	14	14	14	14							
15	nmi-*c*/*t*-18:2	–[e]	23.72				15	15										
16	mi-*c*9,*t*12-18:2	15.18	23.99	16	16	16	16		(16)	(16)	(16)	(16)	(16)	16	16			
17	mi-*t*9,*c*12-18:2	15.27	24.34	17	17	17	17		(17)	(17)	(17)	(17)	(17)					
19	mi-*c*9,*c*12-18:2	15.44	24.77	(19)	(19)	(19)	(19)		19	19	19							

[a] mi, methylene-interrupted; nmi, non-methylene-interrupted. [b] ±0.01 min. [c] M1 and M2 refer to margarine samples; 6A, 7H, and 7M refer to hydrogenated soybean oil samples with iodine values of 95 (Ni, 15-min hydrogenation), 96 (S–Ni, 4-h hydrogenation), and 85 (S–Ni, 7-h hydrogenation), respectively. When an isomer was found in more than one TLC fraction, the corresponding GC peak number for the most intense GC peak is shown in parentheses. [d] A small amount of FAME 12 was found by GC/MI/FTIR before TLC fractionation (Mossoba et al., 1990). [e] Shoulder.

Table II. Absorbance at 2935 cm^{-1}/Absorbance at 1754 cm^{-1} for Two Sets of FAME Positional Isomers

GC peak[a]	isomer[b]	A_{2935}/A_{1754}[c]			% RSD[d]
	trans,trans				
10	nmi-*t*,*t*-18:2	0.75	0.77	0.79	2.6
11	nmi-*t*,*t*-18:2	0.78	0.79	0.82	2.6
12	nmi-*t*,*t*-18:2	0.85	0.84	0.84	0.7
13	mi-*t*9,*t*12-18:2	0.87	0.86	0.85	1.2
	cis/trans				
14	nmi-*c*/*t*-18:2	0.74	0.76	0.78	2.6
15	nmi-*c*/*t*-18:2	0.69	0.71	0.73	2.8
16	mi-*c*9,*t*12-18:2	0.89	0.89	0.90	0.6
17	mi-*t*9,*c*12-18:2	0.93	0.92	0.91	1.1

[a] From Table I and Figures 1 and 2. [b] mi, methylene-interrupted; nmi, non-methylene-interrupted. [c] Absorbances of (CH_2 asymmetric/ester carbonyl) stretching vibrations; replicate determinations. [d] RSD, relative standard deviation.

of that fraction. Isomers 12 and 13 were readily identified by IR analysis of fractions C and D, respectively. The TLC and GC separation data for the test materials analyzed show the various distributions of FAME isomers found in the TLC fractions (Table I). When a compound was found in more than one TLC fraction, the corresponding FAME number for the most intense GC peak is shown in parentheses [e.g., (13) in fraction D].

The information presented in Table I is restricted to TLC fractions B–D. Fractions E (R_f 0.36) and F (R_f 0.68) from all test portions analyzed contained trans 18:1 positional isomers (peaks 2–5, and two minor ones that eluted in the cis-18:1 retention time range) and saturated species (16:0 and 18:0), respectively.

FAME *t*9,*t*12-18:2 (peak 13) was identified from its GC retention time and its IR spectrum by comparison with a standard. However, three other trans,trans 18:2 positional isomers (peaks 10–12) were found in the various test materials. The characteristic IR spectral features from a C_{18} trans,trans diene, specifically, the =C—H stretch vibrations at 3035 and 3005 cm^{-1} and the out-of-plane deformation at 972 cm^{-1}, were found in the spectra of all four of the positional isomers 10–13 (Figure 3). However, the ratio of the intensity of the CH_2 asymmetric stretch vibration (2935 cm^{-1}) relative to that of the ester carbonyl stretching (1754 cm^{-1}) was not the same for the different isomers (Table II) and was used to differentiate them. Absorbance values were obtained by measuring IR band heights on the plotted spectra. When fringes in the baseline occurred, the analyst judged the position of the baseline. In an earlier investigation in which the isomer mixtures were separated by GC only (Mossoba et al., 1990), the overlap between adjacent GC peaks prevented the measurement of such absorbance ratios from the IR spectra, which contained the 2935- and 1754-cm^{-1} bands common to all FAMEs.

Marchand and Beare-Rogers (1982) have shown that mi 18:2 FAME positional isomers migrate further from the origin on $AgNO_3$-TLC plates than do 18:2 FAMEs in which the double bonds are separated by more than a single methylene group (nmi). The trans,trans isomers 10 and 11 were found in TLC band B (R_f 0.02), and isomer 12 was found in TLC band C (R_f 0.04), while isomer 13, which is known to contain mi double bonds at carbons 9 and 12, migrated to TLC band D (R_f 0.16). Therefore, the low R_f values found for isomers 10 and 11 are consistent with positional isomers having nmi trans,trans double bonds. Furthermore, since the R_f values of bands B and C were much closer to each other than to that of band D, isomer 12 is probably another nmi trans,trans positional isomer.

Isomer pair 10 and 11 and isomer pair 10′ and 11′ found in the various test materials had similar GC retention times at 200 °C (14.66 and 14.72 min, for the respective isomers in each pair). However, their configuration and degree of unsaturation were established by IR analysis: Isomers 10 and 11 are nmi trans,trans C_{18} dienes, whereas 10′ and 11′ are cis C_{18} monoenes. This characterization is also consistent with their TLC elution order; the trans,trans positional isomers were found in TLC band B, while the cis positional isomers were found in band D.

The spectra for the four different cis/trans 18:2 positional isomers 14–17 are shown in Figure 4. The deformation vibrations for both cis (730 cm^{-1}) and trans (971 cm^{-1}) groups appear in all four spectra. However, the observed band positions of the relatively weak =C—H stretching vibrations (above 3000 cm^{-1}) allowed us to differentiate between structures having mi or nmi double bonds. The cis/trans isomers 14 and 15 gave rise to trans bands at 3035 and 3005 cm^{-1} and to a cis band at 3010 cm^{-1} (Figure 5); for isomers 16 and 17, which were identified as the mi cis-9, trans-12 and trans-9, cis-12 18:2 isomers, respectively, from their relative retention times (Koba-

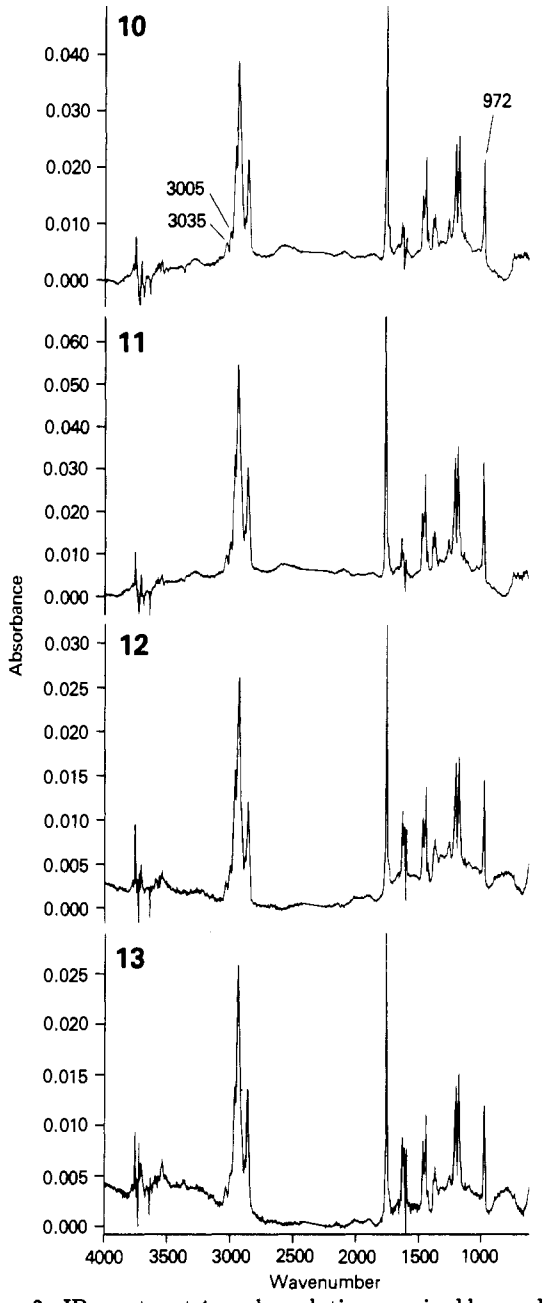

Figure 3. IR spectra at 4-cm^{-1} resolution acquired by coadding 300 scans (2 min, 43 s) for four trans,trans 18:2 FAME positional isomers (peaks 10–13). Isomer identities are given in Table I.

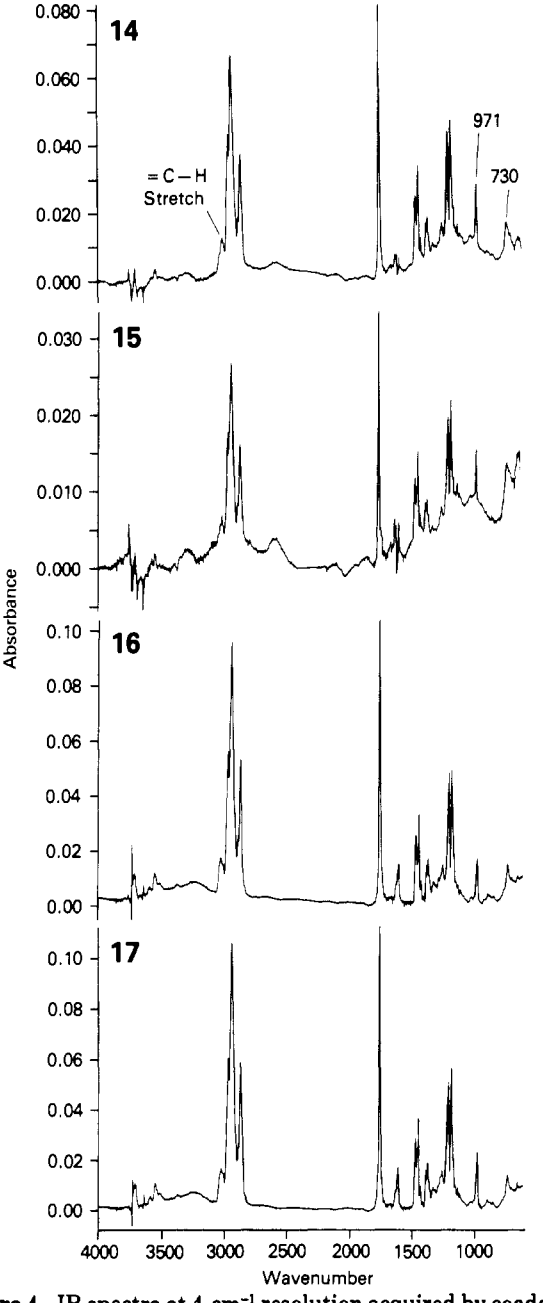

Figure 4. IR spectra at 4-cm^{-1} resolution acquired by coadding 300 scans (2 min, 43 s) for four cis/trans 18:2 FAME positional isomers (peaks 14–17). Isomer identities are given in Table I.

yashi, 1980), the 3010-cm^{-1} band had shifted to 3018 cm^{-1}. Since the 3010-cm^{-1} band is characteristic of an isolated cis monoene group (Mossoba et al., 1990), the band at 3010 cm^{-1} for isomers 14 and 15 suggests that the two double bonds in this positional isomer pair are separated by more than one CH_2 group (nmi). This assignment is also consistent with the fact that the nmi isomers 14 and 15 were found in TLC fraction B, whereas the mi isomers 16 and 17 migrated to fraction C. The intensity of the aliphatic CH stretch band (2935 cm^{-1}) relative to the C=O band at 1754 cm^{-1} in these cis/trans positional isomers (Table II) was found to be lower for the two nmi isomers than for the mi isomer pair.

Recently, McDonald et al. (1989) separated complex mixtures of FAMEs derived from hydrogenated soybean oil by $AgNO_3$-TLC and analyzed each fraction by capillary GC and ^{13}C nuclear magnetic resonance spectroscopy. Each TLC fraction was shown by GC to consist of a mixture of three major and several (six to eight) minor components. The identities of the FAME species in each TLC fraction were determined from the observed chemical shifts for allylic carbons. Their assignments of the FAME configurations are consistent with the IR spectral evidence found in the present work, except for two nmi trans,trans 18:2 positional isomers (10 and 11, Table I), which they tentatively identified as nmi cis,trans or trans,cis 18:2. IR evidence (e.g., 972-cm^{-1} band) supports the trans,trans identity of isomers 10 and 11.

CONCLUSIONS

TLC R_f values and GC retention times complemented IR spectral data (4000–600 cm^{-1}), which were used to determine the degree of unsaturation and the configuration of FAMEs. Four trans,trans and four cis/trans C_{18} FAME positional isomers were characterized by MI/FT-IR spectroscopy.

Of the four trans,trans positional isomers (10–13) found in the present study, two nmi isomers (10 and 11) were

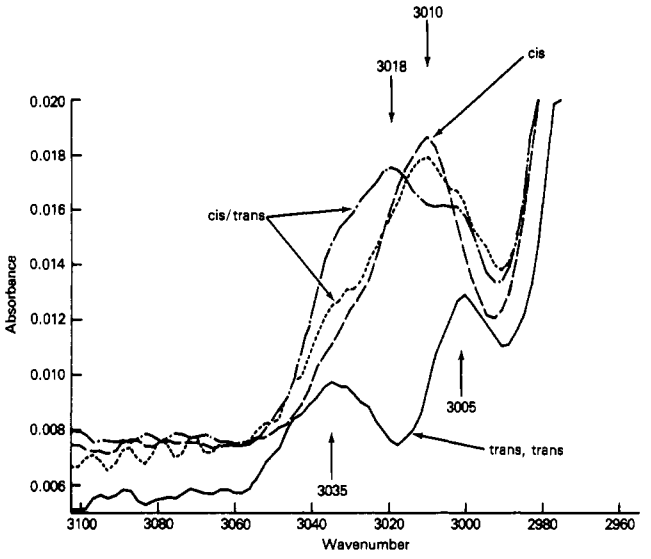

Figure 5. Expanded IR spectra showing the weak =C—H stretching vibrations above 3000 cm^{-1} for FAME compounds. Cis/trans FAMEs exhibited features characteristic of both trans (3035 and 3005 cm^{-1}) and cis (3010 or 3018 cm^{-1}) groups. The band at 3010 cm^{-1}, which is characteristic of a cis 18:1 FAME, suggests that the two double bonds in cis/trans FAMEs are separated by more than a single methylene group.

generated only for soybean oil processed with a sulfur-containing nickel catalyst (test samples 7H and 7M). Because sulfur-containing nickel catalysts are sometimes used to improve the functionality of hydrogenated oils, and nickel catalysts may become contaminated with sulfur, certain hydrogenation conditions can significantly alter the nature and the concentration of trans FAME products in hydrogenated oils. The effect of variations in hydrogenation conditions on reaction rates and trans FAME yields is the subject of further investigation.

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Registry No. *t*9,*t*12-C18:2, 506-21-8; *c*9,*t*12-C18:2, 2420-55-5; *t*9,*c*12-C18:2, 2420-42-0.