SHORT COMMUNICATION

^{13}C Nuclear Magnetic Resonance Spectral Confirmation of Δ6- and Δ7-*trans*-18:1 Fatty Acid Methyl Ester Positional Isomers

E.P. Mazzola[a,*], J.B. McMahon[a,1], R.E. McDonald[b], M.P. Yurawecz[c], N. Sehat[c], and M.M. Mossoba[a]

[a]Office of Scientific Analysis and Support, Center for Food Safety and Applied Nutrition (CFSAN), Food and Drug Administration (FDA), Washington, DC 20204, [b]Office of Plant and Dairy Foods and Beverages, National Center for Food Safety and Technology, FDA, Summit-Argo, Illinois 60501, and [c]Office of Food Labeling, CFSAN, FDA, Washington, DC 20204

ABSTRACT: *Trans* octadecenoic acid methyl ester isomers were obtained from a partially hydrognated soybean oil and isolated by silver-ion high-performance liquid chromatography. Recently, the double-bond positions for nine individual *trans* octadecenoic acid positional isomers (Δ8 through Δ16) were confirmed by gas chromatography–electron ionization mass spectrometry after derivatization to 2-alkenyl-4,4-dimethyloxazoline. In this communication, the presence of two additional *trans*-18:1 fatty acid methyl ester positional isomers (Δ6 and Δ7) in the same mixture is confirmed by ^{13}C nuclear magnetic resonance spectroscopy. The identity of the Δ5-*trans*-18:1 fatty acid methyl ester positional isomer is inferred.
JAOCS 74, 1335–1337 (1997).

KEY WORDS: Carbon-13, nuclear magnetic resonance (NMR), partially hydrogenated soybean oil, positional isomers, *trans*-monounsaturated fatty acid.

The nutritional significance of *trans* fatty acids has led to increased interest in chromatographic procedures that can separate and confirm the double-bond configuration and position for individual *trans* fatty acid positional isomers. In 1995, four publications (1–4) detailed the separation of octadecenoic fatty acid positional isomers by silver-ion (Ag^+) chromatography followed by capillary gas chromatography (GC) on polar phases. GC peaks for *trans*-18:1 isomers were correctly identified by comparison with standards (1,3). However, some GC peak assignments were at variance with those of the other two publications (2,4). Therefore, it was decided to confirm the double-bond configuration and position for individual *trans* 18:1 fatty acid positional isomers, obtained from partially hydrogenated soybean oil, by using gas chromatography (GC)–direct deposition–Fourier transform infrared spectroscopy and GC–electron ionization mass spectrometry (EIMS), respectively (5). First, *trans* monounsaturated C_{18} fatty acid methyl esters (FAME) had to be isolated by Ag^+high-performance liquid chromatography (Ag^+–HPLC). A portion of this HPLC fraction was converted to the 2-alkenyl-4,4-dimethyloxazoline derivative and used to confirm the double-bond position for nine individual *trans*-18:1 FAME positional isomers (Δ8 through Δ16) by GC–EIMS. Under the GC experimental conditions used, the minor Δ6 and Δ7 isomers could not be identified because their GC peaks overlapped with those of the relatively more abundant Δ8 and Δ9 isomers, respectively. In a recent publication (6), Δ6- and Δ7-*trans*-18:1 FAME positional isomers were reportedly separated by GC after converting the double bond to an epoxide group. In the present work, the presence of Δ6- and Δ7-*trans*-18:1 FAME positional isomers found in the same mixture of partially hydrogenated soybean oil is confirmed by ^{13}C nuclear magnetic resonance (NMR) spectroscopy, and the identity of Δ5-*trans*-18:1 FAME positional isomer is inferred.

MATERIALS AND METHODS

Hydrogenated soybean oil was donated by Dr. Wayne E. Emmons of SGS Control Services, Inc. (Deer Park, TX). All reagents and solvents were reagent grade and supplied by Nu-Chek-Prep, Inc. (Elysian, MN) and Fisher (Pittsburgh, PA). A previously described, detailed procedure (7) was followed for the preparation of FAME. Standard Δ6-, Δ7-, Δ11-, Δ12-, Δ13-, and Δ15-*trans*-18:1 FAME positional isomers were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC separations were performed utilizing a Waters 600E solvent delivery system (Waters Associates, Milford, MA), a Rheodyne 7125 injector (Rheodyne, Inc., Cotati, CA) with a 20-μL injection loop, and a Waters Model 996 Photodiode Array Detector. A ChromSpher Lipids column (4.6 mm i.d. × 250 mm stainless steel; 5 μ particle size; silver impregnated) was acquired from Chrompack, Inc. (Bridgewater, NJ). Solvent flow was set at 1.0 mL/min. The mobile phase was 0.3% acetonitrile in hexane (isocratic). The test portion size was 100 μg FAME. Semipreparative HPLC fractionations were made using similar conditions with a 10-mm i.d. × 250 mm ChromSpher Lipids column and a flow rate of 5 mL/min.

*To whom correspondence should be addressed at the Food and Drug Administration (HFS-717), Center for Food Safety and Applied Nutrition, Washington, DC 20204. E-mail: epm@vm.cfsan.fda.gov.

[1]Summer student researcher.

Proton-decoupled ^{13}C NMR spectra, described by 32,768 data points (real part), were obtained at 100 MHz, with broadband irradiation at 400 MHz, using a Varian NMR Instruments VXR-400S spectrometer (Palo Alto, CA) equipped with a Nalorac Z-SPEC MD-400-3 microprobe (Martinez, CA). Pulse widths of 5 µs were employed at a transmitter power of 53, which corresponds to tip angles of 45° with 3-mm sample tubes. Spectral widths of 20 KHz were used, corresponding to acquisition times of *ca.* 0.82 s. ^{13}C chemical shifts were referenced to $CDCl_3$ and are reported relative to tetramethylsilane.

Long-range, heteronuclear multiple bond correlation NMR spectra were recorded at 400 MHz with spectral widths of 2160 and 20,000 Hz in the proton and carbon dimensions, respectively, and with 2048 data points in the ^1H dimension; 192 incremented ^1H spectra of 64 scans each were acquired by using 10-µs (90°) ^1H pulses at a power of 55 and a 1.8-s repetition rate. Delays based on long-range heteronuclear coupling were optimized for 63 ms (8 Hz); delays based on 1-bond heteronuclear coupling were optimized for an assumed average 1-bond coupling of 140 Hz. The data were acquired as 1024 × (192 × 2) hypercomplex files and were processed by a combination of Gaussian and shifted-Gaussian multiplication prior to the first Fourier transformation and sinebell and shifted-sinebell multiplication prior to the second Fourier transformation. Zero-filling was used in both dimensions to give a final data matrix of 4096 × 1024 points.

RESULTS AND DISCUSSION

^{13}C NMR spectral data observed for the Ag^+–HPLC fraction containing the mixture of *trans*-18:1 FAME positional isomers isolated from a partially hydrogenated soybean oil are presented in Table 1 and Figure 1. The olefinic-carbon chemical shifts observed for the soybean oil mixture components Δ6- (129.43 and 130.87 ppm), Δ7- (129.82 and 130.59 ppm), and Δ15- (129.28 and 131.73 ppm) *trans*-18:1 FAME positional isomers were essentially identical to those obtained for standards. The ^{13}C NMR signals observed for these three isomers exhibited distinctive olefinic-carbon chemical shifts that were characteristically different from those of the majority of the components of this mixture (Δ8–Δ14), whose intense signals fell in a much narrower chemical shift range of *ca.* 0.5 ppm (Fig. 1).

TABLE 1
C_{18} *Trans* Monounsaturated FAME Positional Isomers ^{13}C Nuclear Magnetic Resonance Chemical Shifts (ppm)[a]

	C_n Shift		C_{n+1} Shift		Shift difference	
Δ number	STD	MIX	STD	MIX	STD	MIX
Δ5	128.44[b]	128.71	131.21[b]	131.59	2.77[b]	2.88
Δ6	129.44	129.43	130.88	130.87	1.44	1.44
Δ7	129.78	129.82	130.56	130.59	0.78	0.77
Δ15	129.35	129.28	131.79[c]	131.73	2.44	2.45
Δ16	131.52[d]	131.64	124.75[d]	124.39	6.77[d]	7.25

[a]In $CDCl_3$, ppm from tetramethylsilane; STD, standard; MIX, mixture.
[b]C_{18} *cis* monounsaturated Δ5 fatty acid methyl ester (FAME) positional isomer (Ref. 8).
[c]A three-bond connectivity was observed between this signal and the methyl protons in a heteronuclear multiple bond correlation experiment, thus confirming its assignment.
[d]C_{10} *trans* monounsaturated Δ8 FAME positional isomer (Ref. 8).

The mixture spectrum (Fig. 1) also exhibited two additional pairs of ^{13}C NMR signals at 128.71 and 131.59 ppm, and 124.39 and 131.64 ppm. The former are probably due to the Δ5-*trans*-18:1 FAME positional isomer because (i) the chemical shift separation of its putative olefinic carbons (2.88 ppm) closely agrees with that of the standard Δ5-*cis*-18:1 FAME positional isomer (2.77 ppm) previously reported by Gunstone *et al.* (8), and (ii) separations in chemical shifts for other less abundant *trans*-18:1 FAME positional isomers, such as Δ4, were considerably greater than 2.88 ppm. The lat-

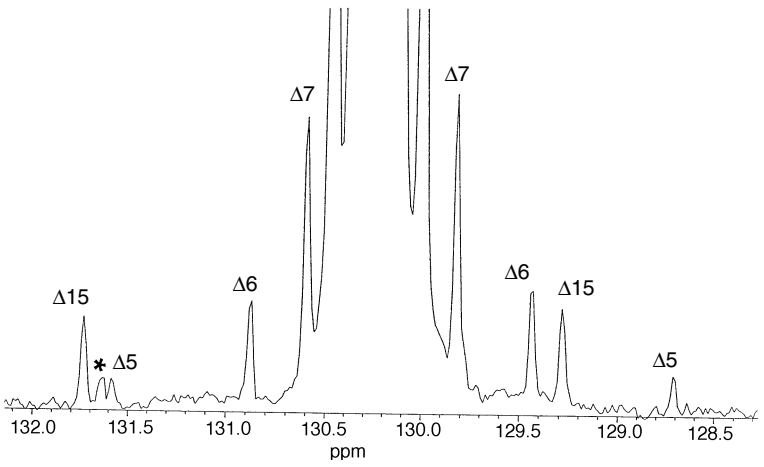

FIG. 1. ^{13}C nuclear magnetic resonance spectrum of the olefinic carbon region for a mixture of *trans*-18:1 fatty acid methyl ester positional isomers. The asterisk denotes the signal at 131.64 ppm.

ter pair of ^{13}C NMR resonances can likely be ascribed to the Δ16-*trans*-18:1 FAME positional isomer. The considerably shielded signal at 124.39 ppm would be assigned to C-17, which possesses one less deshielding β-substituent (9) than other olefinic carbons of the mixture of *trans*-18:1 FAME positional isomers. This resonance is also similar to the 124.75-ppm value reported for the corresponding olefinic carbon (C-8) for the Δ8-*trans*-10:1 FAME positional isomer (8). The signal at 131.64 ppm (denoted by the asterisk in Fig. 1) would be due to C-16.

As expected for partially hydrogenated vegetable oils (10), the isomeric abundance of monounsaturated positional isomers decreased in the sequence Δ5 < Δ6 < Δ7 (see Fig. 1) as the double bond moved away from the center of the fatty acid chain. By using ^{13}C NMR spectroscopy, the identities of the Δ6- and Δ7-*trans*-18:1 FAME positional isomers from a partially soybean oil were confirmed.

REFERENCES

1. Molkentin, J., and D. Precht, Optimized Analysis of *Trans*-Octadecenoic Acids in Edible Fats, *Chromatographia 41*:267–272 (1995).
2. Precht, D., Variation of *trans* Fatty Acids in Milk Fats, *Z. Ernahrungswiss. 34*:27–29 (1995).
3. Wolff, R.L., and C.C. Bayard, Improvement in the Resolution of Individual *trans*-18:1 Isomers by Capillary Gas–Liquid Chromatography: Use of a 100-m CP-Sil 88 Column, *J. Am. Oil Chem. Soc. 72*:1197–1201 (1995).
4. Adlof, R.O., L.C. Copes, and E.A. Emken, Analysis of the Monoenoic Fatty Acid Distribution in Hydrogenated Vegetable Oils by Silver-Ion HPLC, *Ibid. 72*:571–574 (1995).
5. Mossoba, M.M., R.E. McDonald, J.A.G. Roach, D.D. Fingerhut, M.P. Yurawecz, and N. Sehat, Spectral Confirmation of *trans* Monounsaturated C_{18} Fatty Acid Positional Isomers, *Ibid. 74*:125–130 (1997).
6. Pfalzgraf, A., and H. Steinhart, Origin of *trans* Fatty Acids and Their Analysis in Food, Tissue, and Blood Plasma, *Fett/Lipid 98*:81–82 (1996).
7. *Official Methods of Analysis of the Association of Official Analytical Chemists,* edited by K. Helrich, Arlington, 1994, Method 969.33.
8. Gunstone, F.D., M.R. Pollard, C.M. Scrimgeour, and H.S. Vedanayagam, Fatty Acids. Part 50. ^{13}C Nuclear Magnetic Resonance Studies of Olefinic Fatty Acids and Esters, *Chem. Phys. Lipids 18*:115 (1977).
9. Wehrli, F.W., A.P. Marchand, and S. Wehrli, *Interpretation of Carbon-13 NMR Spectra*, John Wiley, New York, 1983, pp. 51–52.
10. Marchand, C.M., Positional Isomers of *trans*-Octadecenoic Acids in Margarines, *Can. Inst. Food Sci. Technol. J. 15*:196–199 (1982).

[Received October 3, 1996; accepted June 22, 1997]