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Methyl esters for fatty acids. Model compounds for assignment of signals in high resolution NMR spectra of edible oils

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Abstract

^{1}H- and ^{13}C-NMR spectra for saturated, unsaturated and polyunsaturated methyl esters of fatty acids are presented. Signal assignments and general features of the spectra are discussed.

Introduction

While routine Low Resolution NMR techniques have been established as analytical methods for foods, High Resolution NMR spectroscoy is still regarded as a research tool rather than a routine in method.

One of the fields where analytical protocols based on High Resolution NMR spectroscopy might be widely accepted in the future as an alternative to classical chemical methods is in the analysis of edible oils. Previous work[1-12] has demonstrated both the analytical power of the technique (e.g. the possibility of determination of distribution of fatty acid chains within the triglyceride) and the advantage of eliminating the tedious preparation of the sample necessary for gas chromatographic analysis.

In the context of the increasing interest in this straightforward analytical method[13-15], the assignment of the NMR signals for the fatty acid moieties is a required condition for the optimal choice of signals to be integrated. Assignment of signals from the ^{1}H- and ^{13}C-NMR spectra of methyl esters of some fatty acids chosen as models for fatty acid triglycerides are presented in this paper.

Experimental Method

Compounds 1-17 noted in Table 1 were used in the experiments.

Table 1 Pure compounds used for signal assignment of NMR spectra

	Fatty acid compound name	Sample	CAS registry no.
1	Hexanoic (caproic) (C6:0)	P	106-70-7
2	Heptanoic (oenanthic) (C7:0)	P	106-73-0
3	Octanoic (caprylic) (C8:0)	P	111-11-5
4	Nonanoic (pelargonic) (C9:0)	P	1731-84-6
5	Decanoic (capric) (C10:0)	P	110-42-9
6	Undecanoic (hendecanoic) (C11:0)	P	1731-86-8
7	Dodecanoic (lauric) (C12:0)	P	111-82-0
8	Tetradecanoic (myristic) (C14:0)	P	124-10-7
9	Hexadecanoic (palmitic) (C16:0)	M	112-39-0
10	Eicosanoic (arachidic) (C20:0)	M	1120-28-1
11	Docosanoic (behenic) (C22:0)	M	929-77-1
12	Tetracosanoic (lignoceric) (C24:0)	M	2442-49-1
13	9- trans-octadecanoic (elaidic) (C18:1 *trans*)	M	2462-84-2
14	9-cis-octadecanoic (oleic) (C18:1 *cis*)	M	112-62-9
15	13-cis-docosenoic (erucic) (C22: 1 *cis*)	M	1120-34-9
16	9, 12 *cis, cis*-octadecadienoic (linoleic) (C18: 2 *cis*)	M	112-63-0
17	9,12,15-*cis, cis, cis*-octadecatrienoic (linolenic) (C18: 3 *cis*)	M	301-00-8

Key: P =Polyscience Corporation IL grade sample
M = Merck grade samples

Compounds 1-8 were used in the form in which they were supplied. Compound 9 was supplied as a 10% w/w solution in ethyl benzene. The ethyl benzene was vacuum evaporated before being dissolved in deuterated chloroform. Compounds 10-17 were also used in the form in which they were supplied.

The NMR spectra were recorded on a Varian Gemini 300 Broad Band spectrometer at 300.075 MHz and 75.462 MHz for ^{1}H- and ^{13}C- nuclei respectively. The samples were

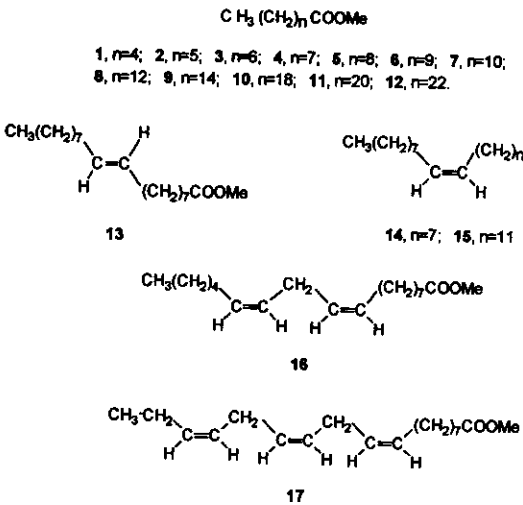
prepared in 5 mm Norell 507 grade sample tubes as 1% v/v and 50% v/v solutions in deuterated chloroform for ^{1}H- and ^{13}C-nuclei respectively. For 2D-NMR experiments the same concentration as for ^{13}C-NMR spectra was used. For the ^{1}H spectra 16 scans were sampled with a 45° pulse width, 2 seconds acquisition time, on 2.6 KHz spectral width. For the fast ^{13}C spectra 1024 scans were sampled with a 30° pulse width, 0.8 seconds acquisition time, on 15 KHz spectral width, using Waltz-16 broad band decoupling for ^{1}H nuclei. The quantitative ^{13}C spectra were recorded in the same conditions described for fast ^{13}C ones but only 512 scans were sampled, an inverse gated Waltz-16 decoupling sequence was employed in order to reduce the NOE effects and a 20 second relaxation delay was also introduced. The H-C COSY pulse sequence used was that supplied by the spectrometer manufacturer and described elsewhere[16-18]. For these experiments an average 140 Hz was used to emulate the first order ^{13}C-^{1}H coupling constants. The Long Range H-C COSY experiments were performed with a COSY sequence which was modified as described elsewhere[19]. The same average 140 Hz for the first order and an average of 7 Hz for the second order ^{13}C-^{1}H coupling constants were used in the latter case.

Results and Discussions

The Model Compounds

The methyl esters of the fatty acids 1-10 noted in Table 1 were studied.

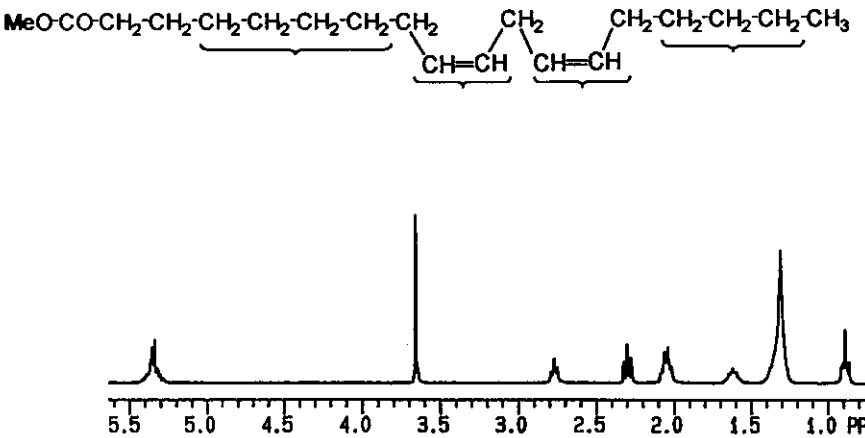
Figure A
Molecular structures of the model compound fatty acids studied



General Features of the NMR Spectra

Typical ^{1}H- and ^{13}C-NMR spectra are presented in Figures 1 and 2 respectively for the case of the ester of compound16. Thus, in the ^{1}H-NMR spectrum (figure 1) it can be seen that the signals for the MeO group (3.66 ppm, s), protons belonging to the two double bonds (5.25-5.45 ppm, m), the CH_2 group in the α position to the COO group (2.30 ppm, t), the CH_2 in the β position to the COO (162 ppm, b quintet), the CH_2 group separating the two double bonds (2.77 ppm, t), the two CH_2 groups flanking the double bonds system (2.00-2.10 ppm, m) and the terminal CH_3 group (0.89 ppm, deformed triplet). All the other CH_2 groups appear as a unique broad singlet at 1.31 ppm. The ^{1}H-NMR spectra of compounds 1-17 are similar. The presence or absence of some of the above described signals, as well as variations in the integrals of these signals indicate the presence or absence of double bonds and the number of CH_2 groups in the chain. Table 2 summarises the ^{1}H-NMR spectra of the compounds studied.

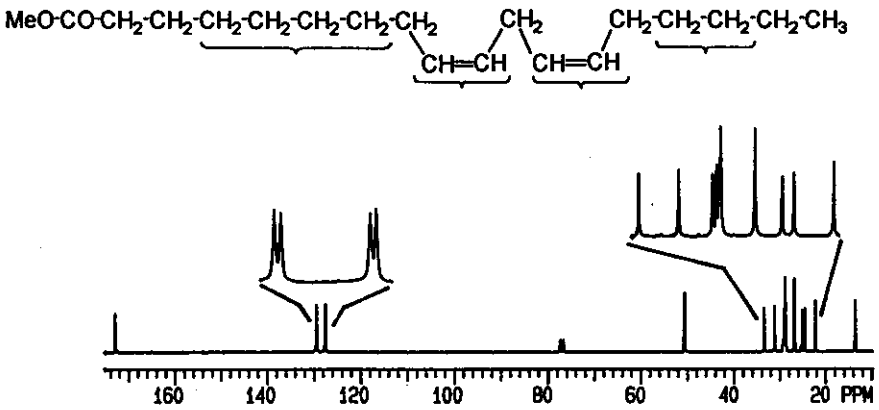
Figure 1 ^{1}H-NMR spectrum of methyl ester of linoleic acid



Although the determination of the type of fatty acid chain from the ^{1}H spectrum is straightforward, the utility of this spectrum for mixtures of fatty acids is limited. The superposition of the signals of similar groups belonging to different types of fatty acids reduces the use of these spectra to a primary qualitative screening of the sample. A typical ^{13}C-NMR spectrum is presented in Figure 2 for the same ester (16). Five principal zones of the spectrum can be distinguished. The CO signal at 174 ppm, the double bonds zone 125-132 ppm, the MeO group at 51 ppm, the CH_2 groups between 19-35ppm, and the terminal CH_3 group at 14ppm.

The CO (esteric) and CH (double bond) zones are by far the easiest signals to be analysed. Thus, most of the NMR studies of edible oils previously published concentrate on these signals. In spite of the relatively small number of these signals and the ease of their assignments of these signals in the mixture the long relaxation times for CO signals and the need of enhanced resolution for both CO and CH signals make their integration less accurate than that of the CH_2 signals.

Figure 2
^{13}C-NMR spectrum of methyl ester of linoleic acid



Among the signals belonging to CH_2 groups, some are easily and unambiguously assignable. Thus, the four signals flanking the group of CH_2 signals are always assigned as follows: 22.00-22.60 ppm the last CH_2 group in the chain (α referred to CH_3), 24.45-25.00 ppm the second CH_2 group in the chain (C^3, β referred to CO), 31.10-31.95 ppm the CH_2 group before the last one (β referred to CH_3) and 33.80-34.15 ppm the first CH_2 group in the chain (C^2, α referred to CO). All the other CH_2 groups resonate between 28.50-30.00 ppm. If double bonds are present, one or two additional typical positions can be emphasised. The two CH_2 groups flanking a *cis* double bond or a system of several *cis* double bonds appear between 27.05-27.25ppm. If the double bond is a *trans* one these signals are shifted to about 32.5 ppm. The CH_2 groups between two double bonds appear between 25.40-25.65 ppm. If a double bond is in the β position to the terminal CH_3 group, the last CH_2 in the chain (α referred to the CH_3) appears around 20 ppm instead of 22 ppm in normal chains.

Table ^{1}H-NMR spectra of compounds 1 - 17 in deuterated chloroform. Chemical shifts expressed in ppm as δ values referred to TMS as internal standard. Chemical shifts of protons belonging to double bonds are underlined.

	MeO	H^2	H^3	H^4	H^5	H^6	H^7	H^8	H^9	H^{10}	H^{11}	H^{12}	H^{13}	H^{14}	H^{15}	H^{16}	H^{17}	H^{18}	H^{19}	H^{20}	H^{21}	H^{22}	H^{23}	H^{24}
1 (C6:0)	3.66	2.31	1.63	1.20–1.40		0.90																		
2 (C7:0)	3.66	2.31	1.62	1.20–1.40			0.89																	
3 (C8:0)	3.66	2.30	1.62	1.20–1.40				0.88																
4 (C9:0)	3.66	2.30	1.62	1.20–1.40					0.88															
5 (C10:0)	3.66	2.30	1.62	1.20–1.40						0.88														
6 (C11:0)	3.65	2.30	1.62	1.20–1.40							0.88													
7 (C12:0)	3.65	2.29	1.62	1.20–1.40								0.88												
8 (C14:0)	3.67	2.30	1.62	1.20–1.40										0.88										
9 (C16:0)	3.66	2.30	1.62	1.20–1.40												0.88								
10 (C20:0)	3.66	2.30	1.62	1.20–1.40																0.88				
11 (C22:0)	3.66	2.30	1.62	1.20–1.40																	0.88			
12 (C24:0)	3.66	2.30	1.62	1.20–1.40																				0.88
13 (C18:1-t)	3.66	2.30	1.62	1.20–1.40				1.96	5.38		1.96	1.20–1.40						0.88						
14 (C18:1-c)	3.67	2.30	1.62	1.20-1.40				2.01	5.35		2.01	1.20-1.40						0.88						
15 (C22:1-c)	3.65	2.29	1.62	1.20-1.40								2.01	5.34	2.01	1.20-1.40							0.88		
16 (C18:2-c)	3.66	2.30	1.62	1.20-1.40				2.05	5.36		2.77	5.34		2.05	1.20-1.40			0.89						
17 (C18:3-c)	3.66	2.30	1.62	1.20-1.40				2.05	5.35-5.37		2.80	5.35-5.37		2.80	5.35-5.37		2.08	0.97						

Figure 3 Expansion of the H-C COSY spectrum of methyl ester of linoleic acid

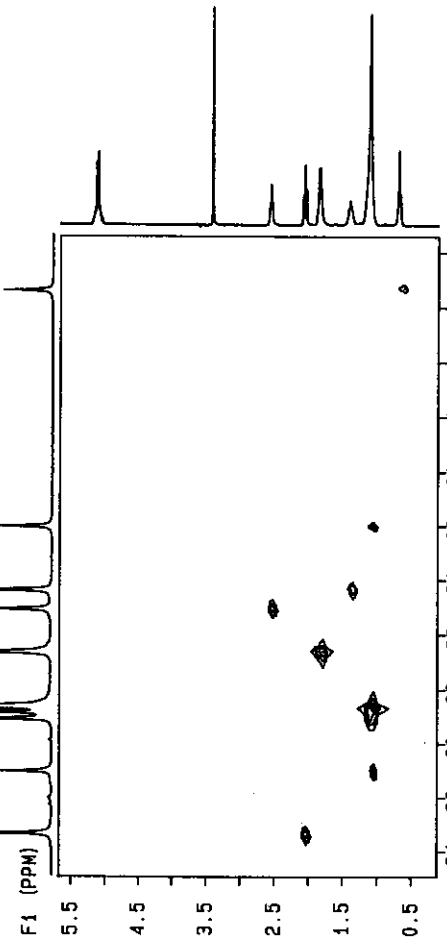


Figure 3. Expansion of the H-C COSY spectrum of methyl ester of linoleic acid (16).

Figure 4 Expansion of the long range H-C COSY spectrum of methyl ester of linoleic acid

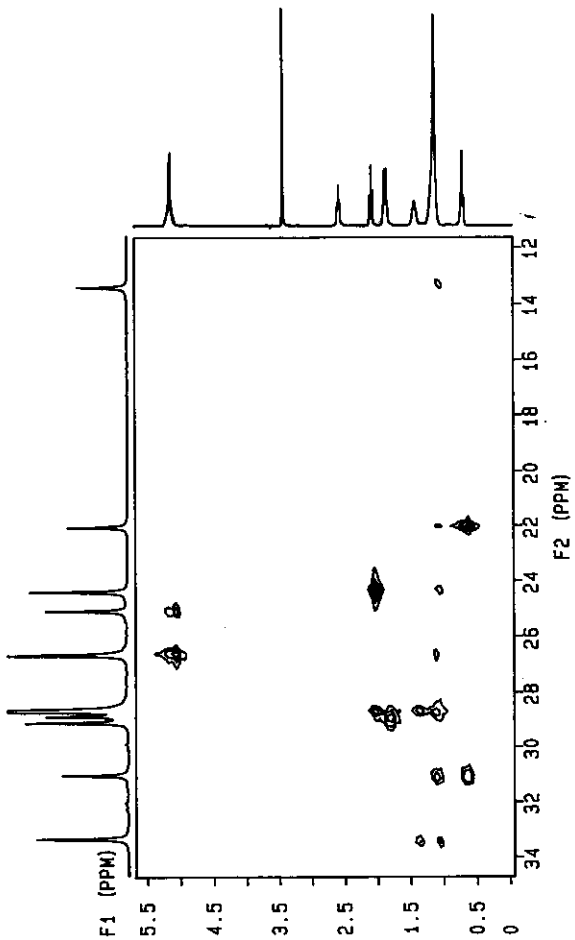


Table ^{13}C-NMR spectra of compounds 1 - 17 in deuterated chloroform. Chemical shifts expressed in ppm as δ values referred to $CDCl_3$ signal (77.00 ppm) as internal standard. Chemical shifts of carbon atoms belonging to double bonds are underlined.

	MeO	CO[1]	C[2]	C[3]	C[4]	C[5]	C[6]	C[7]	C[8]	C[9]	C[10]	C[11]	C[12]	C[13]	C[14]	C[15]	C[16]	C[17]	C[18]	C[19]	C[20]	C[21]	C[22]	C[23]	C[24]
1	51.11	173.98	33.81	24.46	31.14	22.14	13.64																		
2	51.42	174.31	34.15	25.01	28.93	31.56	22.57	14.05																	
3	51.19	174.07	33.92	24.83	28.81	28.98	31.53	22.46	13.87																
4	51.09	173.95	33.87	24.79	28.99	28.99	29.08	31.66	22.47	13.84															
5	51.04	173.87	33.84	24.77	28.99	29.10	29.26	29.10	31.70	22.48	13.82														
6	51.01	173.83	33.83	24.77	28.99	29.14	29.30	29.39	29.14	31.73	22.49	13.82													
7	50.93	173.70	33.78	24.74	28.97	29.09	29.28	29.42	29.42	29.15	31.73	22.47	13.78												
8	51.35	174.26	34.11	24.96	29.15	29.24	29.43	29.57	29.62	29.63	29.62	29.32	31.90	22.66	14.06										
9	51.37	174.31	34.12	24.96	29.15	29.24	29.44	29.65	29.65	29.65	29.65	29.65	29.65	29.34	31.91	22.67	14.07								
10	51.42	174.34	34.11	24.96	29.15	29.26	29.45	29.60	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.37	31.93	22.69	14.11				
11	51.34	174.25	34.12	24.97	29.17	29.25	29.45	29.59	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.69	29.35	31.93	22.67	14.07		
12	51.38	174.31	34.13	24.98	29.17	29.26	29.45	29.59	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.70	29.36	31.93	22.69	14.08
13	51.33	174.21	34.08	24.94	28.91	29.10	29.16	29.29	32.52	130.45	130.18	32.57	29.64	29.54	29.47	29.10	31.88	22.65	14.05						
14	51.37	174.24	34.10	24.96	29.09	29.13	29.31	29.31	27.16	129.74	125.99	27.22	29.77	29.68	29.52	29.13	31.90	22.67	14.07						
15	51.17	173.39	33.94	24.85	29.07	29.18	29.22	29.22	29.37	29.46	29.53	29.53	27.10	129.70	129.70	27.10	29.68	29.68	29.46	29.22	31.83	22.59	13.97		
16	51.34	174.19	34.08	24.94	29.11	29.11	29.32	29.57	27.18	130.00	127.87	25.63	128.01	130.17	27.18	29.11	31.51	22.54	14.00						
17	51.20	174.01	33.93	24.80	28.98	28.98	28.99	29.44	27.07	130.06	127.61	25.49	128.11	128.11	25.40	126.99	131.75	20.42	14.11						

For the middle group of CH_2 signals where (dependent on the chain length and the field strength of the spectrometer) superposition of signals appear, several orderings were proposed for related saturated long chain compounds [20, 21].

For the compounds 1-17 analysed as samples in CDCl3 we experimentally assigned the first four positions in the chain (C^1 and C4) and the last three (C^n-C^{n-2}). An example of an assignment experiment is presented for compound 16. Figures 3 and 4 present the H-C COSY and Long Range H-C COSY experiments respectively for this case. The complete ^{13}C-NMR spectra for compounds 1-17 are presented in Table 3. The middle chain positions were assigned according to Bengsch's rule[21].

Conclusion

The unambiguous assignment of several CH_2 groups in the ^{13}C-NMR spectra of the saturated, unsaturated and polyunsaturated fatty acid esters should allow the quantitative analyses of the mixtures of these esters or edible oils to be performed by integrating only the CH_2 signals. The integration of the signals of CH_2 groups is generally more accurate than for CO or CH groups as problems arising from both relaxation times and need of resolution enhancement are less critical.

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