^{13}C Nuclear Magnetic Resonance of Homologous Methyl Esters of Saturated Acids and the Effect of Chemical Shift Reagents

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ABSTRACT

A homologous series of methyl esters was studied with ^{13}C Nuclear Magnetic Resonance (CMR) spectroscopy and compared to results which have been obtained with proton nuclear magnetic resonance (PMR) spectroscopy. Chemical shift reagents (CSRs) were used to improve the separation of resonances. The broader chemical shift range for CMR made possible the identification of each carbon as a unique resonance for methyl hexanoate through methyl nonanoate. The use of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octadione) Ytterbium (III) enables the separation of all carbons into unique resonances through methyl dodecanoate at 5KHz bandwidth. Carbon magnetic resonance is a useful tool for distinguishing members of homologous series and certainly more versatile than PMR.

Proton nuclear magnetic resonance (PMR) like other spectroscopic techniques cannot readily distinguish members of a homologous series. Additional methylene groups in a carbon chain do not produce large changes in the spectra. In the case of the series of methyl esters of saturated acids, first order spectra have been obtained for methyl acetate, propionate, and butyrate (1). However, by use of chemical shift reagents (CSR) the spectra of all the methyl esters up to methyl octanoate could be obtained with complete separation of all methylenes by PMR (1). Walters showed that carbon disulfide (CS_2) was a better solvent for the CSR than chloroform and allows additional complexation of ester and CSR (2). Sakamoto and Oki (3) used the CSR Eu(fod)$_3$ [tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octadione) europium III], to determine conformations of cyclic esters by the ratio of chemical shift to mole fraction of CSR, Pfeffer and Rothbart (4) and Almquist et al. (5) examined the effects of CSR on the spectra of triglycerides and attempted to explain the upfield and downfield shifts associated with the addition of CSR and to describe the equilibriums involved. Swern and Wineburg (6) and Wineburg and Swern (7) used CSR to investigate the structure of lipid derivatives. Pfeffer (8) in a recent review of PMR of lipids described the problem of distinguishing methylene groups in lipid materials and the use of CSR to broaden the range of chemical shifts. The use of these europium and praesodynium reagents, however, still constitutes a derivatization from which recovery of lipid materials is difficult.

Batchelor et al. (9-11) used CMR to study the conformations of unsaturated fatty acids to determine the role of electric fields and steric effects on their spectra. The broader chemical shift of ^{13}C-NMR (CMR) vs. PMR, i.e., 5000 vs. 1000 Hz, and the simplicity of CMR spectra because of broadband noise decoupling, suggested that it would be more useful than PMR for these type studies.

We report herein the utility of CMR over PMR for the structural determination of saturated methyl esters and the use of CSR in CMR spectroscopy.

EXPERIMENTAL PROCEDURES

^{13}C spectra were obtained on a JEOL PS/PFT 100 pulsed NMR spectrometer. A Nicolet 1083 (20 bit word) computer system was interfaced to the spectrometer to collect the free induction decay (FID) and perform the FFT and integration of peak areas. The observing, lock, and irradiating frequencies were calibrated with a Dana 8010B frequency counter. Line positions were referenced to tetramethylsilane (TMS), calculated by the computer, and are accurate to the line-width described by one data point (0.81 Hz). The instrument was tuned before each data acquisition and locked on the deuterium resonance of deuterochloroform. Spectra were normally taken with a 5000 Hz bandwidth, although bandwidths between 100-5000 Hz were sometimes used as noted, with 8K data points assigned to the FID. A 90° pulse of 20.5 μsec at a 20.0 sec pulse repetition rate was generally used.

Methyl esters were obtained from the Hormel Institute of the University of Minnesota and certified 99% pure. Gas chromatography, infrared analysis, and thin layer chroma-

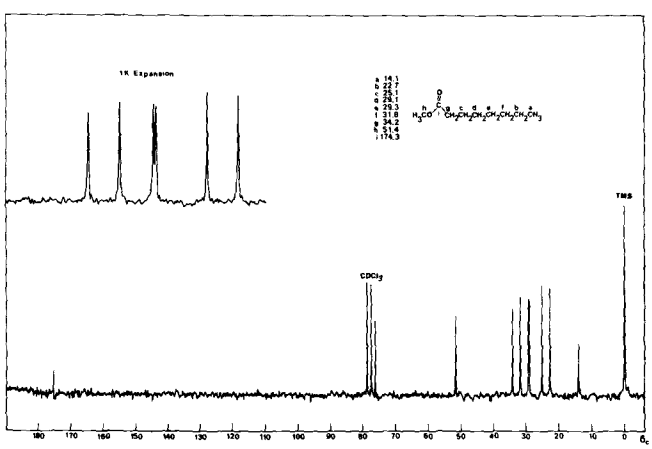

FIG. 1. A 5000 Hz (200 ppm) spectrum of methyl octanoate 20% in $CDCl_3$ referenced to TMS. Inset is a 1K expansion of the 20-40 ppm portion.

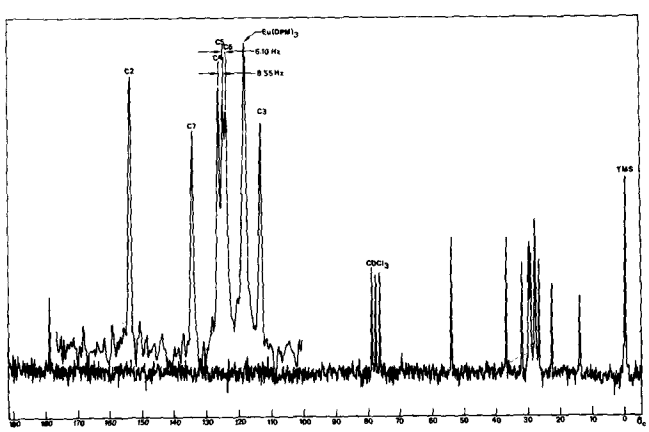

FIG. 2. A 5000 Hz (200 ppm) spectrum of methyl nonanoate saturated 20% in $CSCl_3$ saturated with Eu(DPM)$_3$ referenced to TMS. Inset is a 1K expansion of 20-40 ppm portion.

tography were used to verify the purity of these methyl esters.

Chemical shift reagents were obtained from Ventron and Norell Chemical Companies and kept in a vacuum desiccator over phosphorus pentoxide (P_2O_5) until used. The CSR was added in increments to enable determination of both structure, by the magnitude of chemical shift, and the minimum amount required to completely separate all carbon resonances when possible.

Spectra were taken at various concentrations of methyl ester in deuterochloroform. Usually 15-25% ester gave optimal results. Some samples, as small as 40 mg, were run in a Wilmad ^{13}C microcell. The time required for data acquisition varied from 10-30 min. Spin-lattice relaxation times (T_1's) were measured and calculated with the auto-T_1 program of the Nicolet 1083. Samples were not degassed. Spin-spin relaxation time (T_2*) were measured by the peak width at half height in Hz and converted by use of the equation $T_2^* = 1/\pi\Delta\delta\frac{1}{2}$, where $\Delta\delta\frac{1}{2}$ is the peak width at half height.

RESULTS AND DISCUSSION

Carbon magnetic resonance was more versatile than proton magnetic resonance in the analysis of a homologous series of methyl esters. The CMR chemical shifts (δ in ppm downfield from internal TMS) are shown in Table I, and were from survey type studies. The sweep width was 5000 Hz, limiting resolution to 2.44 Hz. Some shorter sweep widths were used in T_1 experiments. Chemical shifts were assigned on the basis of literature values and incremental additions of CSR. Spectra with each carbon in the ester exhibiting a unique resonance were obtained for methyl octanoate and all the lower homologues. For esters of the series C-9 through C-20 acids all but the central methylenes showed unique resonances. Methyl eicosanoate gave eleven unique resonances and one resonance for the ten central methylenes (C6-C15). Attempts at quantitative analysis of the mixture of saturated methyl esters were not successful, since the resonance for the methoxyl carbon was the same for all samples. Walters used this technique (1) with PMR to quantitate C_4, C_6 and C_7 acids as methyl esters with CSR. However, quantitative analysis using CMR with unsaturated methyl esters has been accomplished by Barton et al. (12).

Figure 1 was typical of the methyl ester for the lower homologues for which unique resonances were obtained for each carbon. The spectrum of methyl nonanoate was the first in the series to show a multiple carbon resonance. The signals for carbons 4, 5, and 6 (Table I) were coalesced at 29.4 ppm. In 1000 Hz spectra the signals for carbons 5 and 6 were coalesced at 29.4 ppm with carbon 4 at 29.5 ppm.

Figure 2 is the spectrum of methyl nonanoate with the CSR tris-(2,2,6,6-tetramethylheptane-3,5-dianato) europium (III) [Eu(DPM)$_3$]. A saturated solution of Eu(DPM)$_3$ was required to shift the carbon resonances so that each one was a single carbon resonance. Other more effective CSRs were the partially fluorinated β-diketone, 1,1,1,2,2,3,3-heptafluoro-7,7 dimethyl-4,6-octadione (fod), of europium (III) and Ytterbium (III). Single carbon resonances in methyl nonanoate could be obtained using 100 mg of Eu(fod)$_3$ and that the separation of the downfield shifted signals was greater than obtained with a saturated solution of Eu(DPM)$_3$. Table II shows the effect of incremental additions of Eu(fod)$_3$. One hundred mg of CSR was needed for complete separations of the resonances for each carbon in methyl nonanoate. Ytterbium (fod)$_3$ [Yb(fod)$_3$] was the best of the three CSRs tried. The separations achieved by the addition of 100 mg of Yb(fod)$_3$ were greater than that for 100 mg of Eu(fod)$_3$, (C4-C5 12.21 Hz vs. 3.66 Hz and C5-C6 8.55 Hz vs. 4.88 Hz, respectively; see Table II). The chemical shift for carbons 4,

TABLE I

CMR Chemical Shift (δ in ppm from TMS) for a Homologous Series of Methyl Esters[a,b,c]

Methyl ester	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}
Hexanoate	37.9	26.9	31.5	23.0	14.1														
Heptanoate	34.2	25.1	29.0	31.6	22.6	14.1													
Octanoate	34.2	25.1	29.1	29.3	31.8	22.7	14.1												
Nonanoate	34.2	25.1	29.5	(29.4	29.4)	31.9	22.8	14.1											
Decanoate	34.2	25.1	29.6	(29.4	29.4)	29.3	32.0	22.8	14.1										
Dodecanoate	34.2	25.0	29.5	29.6	(29.7	29.7	29.4	29.3	32.0	22.8	14.1								
Tetradecanoate	34.1	25.0	29.5	29.6	(29.8	29.8	29.8	29.8)	29.4	29.3	32.0	22.8	14.1						
Hexadecanoate	34.2	25.1	29.5	29.6	(29.8	29.8	29.8	29.8	29.8	29.8)	29.4	29.3	32.1	22.8	14.1				
Octadecanoate	34.2	25.1	29.5	29.6	(29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8)	20.3	29.3	32.1	22.8	14.1		
Eicosanoate	34.1	25.0	(29.6	29.6)	(29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8)	29.4	29.3	32.0	22.8	14.1

[a] δ For carbonyls = 174.2, δ for methoxyl = 51.3.
[b] δ Values within parenthesis are coalesced resonances.
[c] All spectra were 500Hz, 20% in $CDCl_3$.

TABLE II

The Effect of Solvent and Incremental Addition of $Eu(fod)_3$ on the Chemical Shifts of Methylnonanoate

Carbon atom	δ In ppm for mg $Eu(fod)_3$ added						
	Neat	0	10	30	50	100	150
2	33.95	34.17	34.32	34.61	34.95	35.68	36.50
3	25.29	25.09	25.19	25.34	25.53	25.97	26.45
4	29.80	29.27	29.32	29.36	29.46	29.66	29.90
5	29.68	29.27	29.32	29.36	29.46	29.51	29.66
6	29.62	29.27	29.32	29.32	29.32	29.32	29.41
7	32.34	31.94	31.94	31.94	31.94	31.99	32.04
8	23.07	22.76	22.76	22.76	22.76	22.76	22.81

TABLE III

Spin Lattice Relaxation Times (T_1's) 25% Methyl Nonanoate in $CDCl_3$ and NEAT at 23 C

Carbon no.	δ ppm	T_1 (sec) solution	δ ppm	T_1 (sec) NEAT
4	29.5	3.3	29.8	2.4
5	29.4	3.0	29.7	2.4
6	29.4	3.0	29.6	2.5

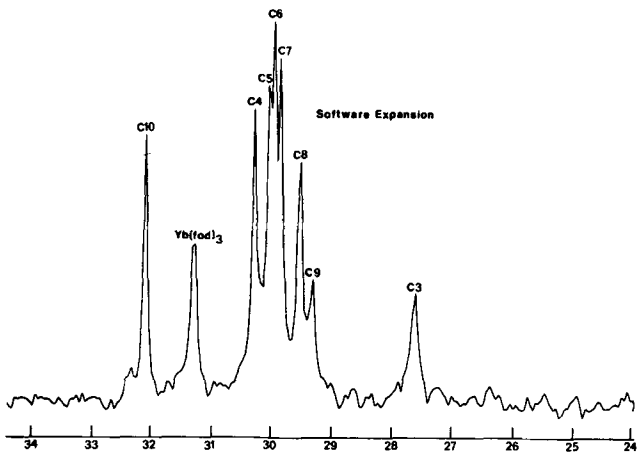

FIG. 3. A software expansion of Figure 9 showing the 28-34 ppm portion of methyl dodecanoate 20% in $CDCl_3$ with 300 mg of $Yb(fod)_3$ added referenced to TMS.

5, and 6 in methyl nonanoate with 100 mg $Yb(fod)_3$ added were 32.0, 30.2, and 29.8 ppm, respectively.

The chemical shift data from the spectrum of methyl nonanoate taken (neat) using tetramethylsilane in a coaxial cell are given in Table II. Carbons 4, 5, and 6 were all unique resonances. This separation was probably due to a shortening of the T_1 values for the neat sample. The T_1's of carbons 4, 5, and 6 were determined and are shown in Table III. A difference noted in the neat sample of methyl nonanoate and in the T_1 experiments was a change in chemical shift due to removal of solvent. Not only were the T_1's shorter but line widths were narrower for the neat sample as compared with the solution sample. The spin-spin relaxation time (T_2*) for solution was less than half the value of the neat sample for carbon 4 (0.13 vs. 0.30 sec). With an increase in chemical shift and a decrease in line width, the coalesced peaks can easily be separated. Separation of the signals for the three carbons (4, 5, and 6) was about 0.1 ppm or close to 2.5 Hz.

The spectrum of methyl dodecanoate showed one peak for carbons 6 and 7. These two carbons were separated into unique resonances by the addition of 300 mg $Yb(fod)_3$ (Fig. 3). None of the other CSRs could separate these two signals. The resonances for these carbons were not separable in a spectrum of a neat sample. The methyl esters of the C-14 through C-20 acids also gave coalesced signals which could not be separated by any of the CSR tested.

Bus et al. (13) were able to assign separate resonances for almost all the carbons in the C-11 and C-18 unsaturated methyl esters. The presence of a double or triple bond and its location in the molecule could be determined. However, some resonances in the C-11 methyl undecenoates and methyl undecynoates were coalesced. Tulloch and Mazurek (14) examined the saturated methyl octadecanoates and the unsaturated methyl oleate and petroselinate. From spectra of specifically synthesized deuterated analogs, these workers were able to assign resonances to each carbon in the unsaturated esters. Seven carbons remained coalesced in the spectrum of methyl octadecanoate even with 100 Hz sweep width. At a 100 Hz sweep width acquisition time would be longer, but the theoretical resolution would be less than 0.1 Hz, thus, if the lines were resolvable it would be possible to obtain the unique resonances. It is doubtful that these lines can be resolved.

^{13}C magnetic resonance can be successfully used as a tool for the structural analysis of lipid materials. Its utility can exceed that of PMR. Good quantitative analysis can be obtained as shown by Barton et al. (12). The use of CMR in studies of biologically and agriculturally important materials can be of tremendous assistance to researchers in these areas.

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[Received January 11, 1978]