of the solvent was evaporated on a steam bath, and the remainder was removed *in vacuo* with a rotating evaporator.

Isolation of Capric Acid by Steam Distillation. An 8.79-g. portion of the oil was refluxed with 90 ml. of 0.8*N* ethanolic potassium hydroxide under nitrogen for 3 hrs. Unsaponifiable matter was removed (0.34 g., 3.9%), and free fatty acids were recovered (7.43 g., 84%). The acids were steam-distilled into the following three fractions over a period of 9 hrs.

		g.
Fraction I	(0–1 hrs.)	1.03
Fraction II	(1–5 hrs.)	3.26
Fraction III	(5–9 hrs.)	1.21
Residue		0.75
		6.25 (84% recovery)

Methyl esters from each fraction were prepared by reacting the free acids with diazomethane (2) and were analyzed by gas chromatography (1). The composition of each fraction is listed in Table I.

Identification of Capric Acid. The amide was prepared according to the procedure of Shriner, Fuson, and Curtin (3), using 0.331 g. of acid from Fraction II. The yield of crude product was 0.375 g., m.p. 84–94°C. After three recrystallizations from aqueous ethanol 0.110 g. of white crystals, m.p. 96–97°C., were obtained; lit. value 98.5°C. (4). There was no depression of melting point on admixture with authentic capramide.

Anal. Calcd. for $C_{10}H_{21}ON$: N, 8.2. Found: N, 8.0.

The anilide was prepared essentially by the method of de Jonge *et al.* (5), using a 0.304-g. portion of acid from Fraction II. The crude yield was 0.483 g. of yellow solid. After three recrystallizations from aqueous ethanol 0.256 g. of white crystals, m.p. 62.5–63°C., was obtained; lit. values 69.5–69.9°C. (5) and 62°C. (3). On admixture with authentic capranilide (m.p. 63–64°C.) no depression of melting point was observed.

Anal. Calcd. for $C_{16}H_{25}ON$: N, 5.7. Found: N, 5.6.

TABLE I

Fatty Acid Composition of *Cuphea llavea* Seed Oil and Derived Steam Distillates (Area Percentage of Methyl Ester Peaks)

Acid	Original oil (1)	Steam distillates			
		Fractions			Residue
		I	II	III	
	%	%	%	%	%
Caprylic	0.8	2.4	0.5	.....	0.2
Pelargonic	0.1	0.1	0.1	.....	.....
Capric	82.7	96.8	98.3	93.5	0.9
Unknown	Trace	0.2	0.2	0.1	.....
Lauric	1.2	0.4	0.9	4.7	2.3
Myristic	0.8	.....	.....	1.1	3.8
Palmitic	2.6	.....	.....	0.7	18.8
Stearic	0.5	.....	.....	.....	3.1
Oleic	4.9	.....	.....	.....	71.0
Linoleic	6.3	.....	.....	.....	
Linolenic	0.1	.....	.....	.....	

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Fatty Acids Analysis by High Resolution Nuclear Spin Resonance. A Preliminary Evaluation[1, 2]

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High resolution nuclear spin resonance spectra of four fatty acid methyl esters have been presented and discussed from the standpoint of applying this instrumentation to the analysis of the possible eight-component system resulting from the hydrogenation of linolenic acid. If one of the constituents other than 9- or 12-oleic acid can be neglected or found by another method, the analysis seems possible. Experimental conditions for the accurate reproduction of the relative intensities of the spectral bands must first be found.

RECENT ATTEMPTS in these laboratories to hydrogenate selectively linolenic acid in linseed oil to linoleic acid created a need for improved procedures for analyzing fatty acid mixtures (1). This paper is a preliminary evaluation of the usefulness of high resolution nuclear spin resonance (NSR) spectroscopy in the performance of such analyses. Its presentation at this time, in the absence of sufficient data for an analytical paper, is in response to the request by certain workers in the field of oil chemistry that the results given herein be made more generally available. Spectra from the methyl esters of linolenic, linoleic, oleic, and stearic acids are presented and discussed from an analytical viewpoint. A more general treatment of the application of NSR to oil chemistry has been published elsewhere (2).

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Presentation and Discussion of the Spectra

The application of high resolution NSR to the analysis of fatty acid mixtures is most simple conceptually as reference to the spectra in Figures 1–4 will show. The spectra may be separated into bands, each of which arises from the hydrogen nuclei or protons in some particular functional group in the molecule. The area under a band, or its intensity, is proportional to the number of protons and hence to the number of functional groups giving rise to the band. Reference to Table I will show that most of the fatty acid methyl esters in the system contribute an unique balance of those functional groups

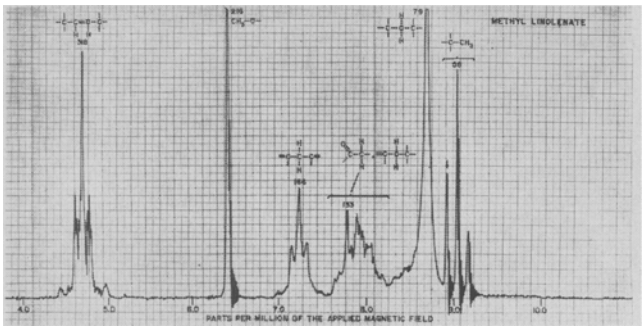

FIG. 1. High resolution NSR spectrum of 9,12,15-linolenic acid methyl ester.

that NSR distinguishes. A number of simultaneous equations can be written, one for each band which bears unique information. The unknowns in each equation are the mole fractions of fatty acid methyl esters present; the coefficients are proportional to the number of functional groups each ester contributes to the band; and the right-hand side is a number proportional to the measured intensity of the band in a spectrum from the mixture of esters. Solution of this system of equations gives the mole fraction of each of the constituents of the mixture. Such is the essence of this paper. The remainder of this section will be concerned with a more detailed discussion of the analytical features of the spectra.

The independent variable against which all the spectra are plotted is the polarizing magnetic field. The magnetic field variation is expressed in parts per million (p.p.m.); the steady state value of the magnetic field is 14,100 gauss. One p.p.m. variation is therefore equivalent to a variation of 14.1 milligauss. In all spectra the magnetic field is scanned linearly in the direction of increasing field. Functional groups in which the protons are less diamagnetically shielded from the applied polarizing field give bands at the lower applied fields. Olefinic protons are poorly shielded while methyl protons are well shielded. Such separations between bands are called chemical shifts.

There are six bands from which analytical information can be drawn. The intensity of the olefinic II band at 4.7 p.p.m. is directly dependent upon the number of double bonds in the mixture. The band at 7.2 p.p.m. from methylene groups adjacent to two doubly-bonded carbons distinguishes the 9, 15-diolefin from the 9, 12- and 12, 15-diolefins, and its presence signifies the presence of methyl linolenate and methyl linoleate. The band at 7.9 p.p.m. arises from methylene groups adjacent to the carbonyl group and to a single doubly-bonded carbon. The band from the methylene group adjacent to the carbonyl appears separately in the methyl stearate spectrum. Nothing would be gained by resolving these two different sorts of methylenes because the single methylene adjacent to carbonyl is present in the spectra from all constituents of the system. Similarly no attempt need be made to separate the band from the saturated chain at 8.7 p.p.m. from the terminal methyl band in order to gain more information. However later discussion will show that the terminal methyl band is a measure of the presence of double bonds at the 15-position.

Although the carbomethoxy peak at 6.4 p.p.m. is present in all spectra, the constant ratio of its intensity to the number of molecules in the sample serves to give the intensities of the other bands meaning in terms of the number of protons that they represent. The intensity of the carbomethoxy band can be considered as representing three protons, and the intensities of all of the other bands in terms of protons can be found by measuring the ratios their intensities bear to that of the carbomethoxy band. More directly, these intensity ratios alone can be used for the coefficients (by computation from the molecular structures) and for the right-hand sides of the simultaneous equations. For this reason the use of the methyl ester form of the fatty acids is recommended for use in analysis. If the fatty acids were used, a broad carboxyl band of one-third the intensity at a much lower field would replace the carbomethoxy band. Employment of the carboxyl band would not prove so convenient as that of the carbomethoxy band.

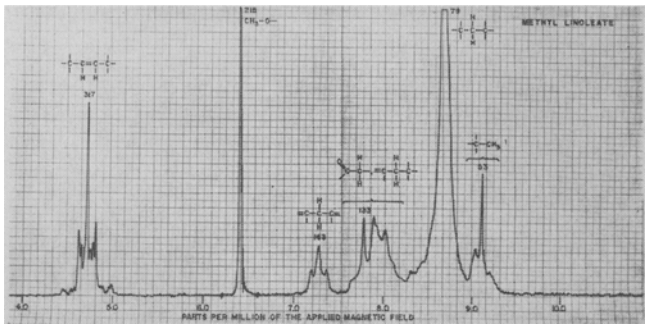

FIG. 2. High resolution NSR spectrum of 9,12- linoleic acid methyl ester.

TABLE I

Number of Protons in Each Analytically Significant Functional Group of Each Fatty Acid Molecule (Methyl Ester Form)

Fatty Acid	Functional Groups					Peak from terminal methyl band at 8.9 p.p.m.?
	$-C=C-H$	$H-C(-C=C-)-H$	$H-C(-C=C-)-H$ and $O=C-C(H)-H$	$H-C(-C-)-H$	$H-C-O-H$	
9, 12, 15-Linolenic [a]	6	4	6	10	3	yes
9, 12-Linoleic [a]	4	2	6	16	3	no
9, 15-Linoleic	4	..	10	14	3	yes
12, 15-Linoleic	4	2	6	16	3	yes
9-Oleic [a]	2	..	6	22	3	no
12-Oleic	2	..	6	22	3	no
15-Oleic	2	..	6	22	3	yes
Stearic [a]	..	..	2	30	3	no

[a] Spectra from these substances are presented in this paper.

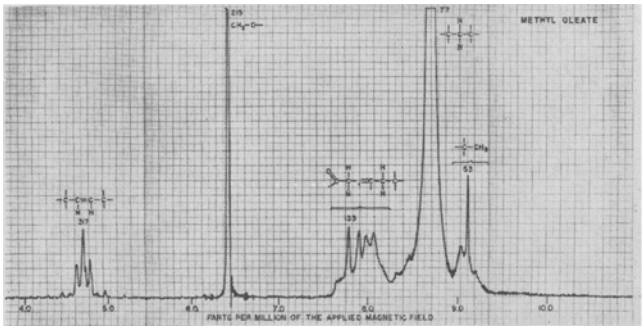

FIG. 3. High resolution NSR spectrum of 9-oleic acid methyl ester.

Thus far, the intensity ratios between the carbomethoxy band and the four other separable bands yield four simultaneous equations. Comparison of the appearance of the terminal methyl bands in the other three spectra will yield a fifth equation. The appearance of the spectral band from protons in a particular functional group is determined both by the magnitude of spin-spin coupling between these and protons in adjacent groups and by the chemical shifts of the groups of protons. The terminal methyl band in the methyl linolenate spectrum may be considered a clear triplet by interaction with the two protons of the adjacent methylene group. Since this methylene group is adjacent to a doubly-bonded carbon, it appears at approximately 8.00 p.p.m. in the spectrum. Removal of the double bond at the 15- position moves the appearance of this methylene group upfield to 8.7 p.p.m., which in turn results in the altered appearance of the terminal methyl band in Figures 2, 3, and 4. In addition, the terminal methyl band has moved upfield slightly, leaving a valley at 8.9 p.p.m. between the methyl and its adjacent methylene band. In this valley falls the low field peak of the terminal methyl bands of fatty acids with double bonds at the 15-position. The intensity of this peak is a measure of the concentration of these acid esters and yields a fifth equation.

The system in Table I consists of the eight possible constituents of mixtures produced in the hydrogenation of linolenic acid, neglecting *cis-trans* isomerization. But 9-oleic and 12-oleic methyl esters should yield indistinguishable spectra and must be considered as a single substance as a consequence. The resulting seven-component system has one more unknown than the six-component system of which NSR seems capable of providing a complete analysis. If, in practice, only one of these seven remaining constituents can be neglected or determined in another way, then the straight-forward application of NSR should be possible. The following section will discuss experimental aspects of the analysis, beginning with the procedures by which spectra given in this paper were obtained.

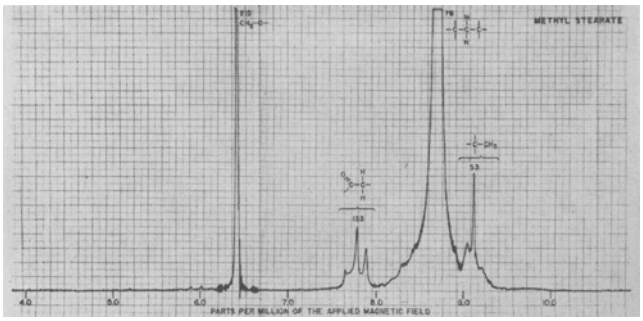

FIG. 4. High resolution NSR spectrum of stearic acid methyl ester.

Experimental Procedures

Samples bearing specified iodine values and other evidences of purity were obtained from the Hormel Foundation. These were stored under refrigeration in sealed vials for approximately two years before the spectra shown in this paper were obtained. However, the latest spectra are not qualitatively different from spectra obtained at the beginning of this period at the applications laboratories of Varian Associates. No quantitative comparison can be made. Spectra were obtained from CCl_4 solutions containing approximately 25% of the fatty acid methyl ester in 4-mm i.d. sample tubes, which were spun. The samples were not degassed. A Varian Associates V-4311 high resolution NSR spectrometer operating at 60 megacycles/second was employed along with the associated electromagnet and power supply. The spectrometer output was fed to a Brown recorder with a full-scale sensitivity of 100 millivolts and a chart speed of 4 in./min. The polarizing magnetic field was scanned at the rate of 18.6 milligauss/min. Spectra were refereneed with respect to less than 5% tetramethylsilane internal to the sample, following the method discussed by Tiers (3). If the signal from tetramethylsilane were to appear on the spectra, it would occur by definition at 10.000 p.p.m. Numbers above the principal peaks are in cycles/sec. from tetramethylsilane; 60 cycles/sec. are equivalent to 1 p.p.m. on the magnetic field scale.

When these display spectra were obtained, there was no intention of reproducing each band with its correct intensity relative to the other bands in the spectrum. The desire was merely to show the separations between bands and the band structures with reasonably high resolution. Accordingly the half-amplitude H_1 of the 60 megacycle/sec. magnetic field was set at 70 db below 140 milligauss, low enough to avoid saturating the proton spin system yet too high apparently for analytical purposes. At this RF level the ratio of olefinic to carbomethoxy band intensity in the methyl linolenate spectrum was found to be 1.83 whereas at 80 db below 140 milligauss it was 1.99. The expected ratio for pure methyl linolenate is 2.00. The lower RF level is expected to favor faithful reproduction of band intensities (4). At this same RF level however the intensity ratios of other bands with the carbomethoxy band are not assuming the expected values. Whether the experimental conditions or the samples are at fault is not presently known. The author merely wishes to point out this experimental barrier which must be overcome before proceeding to test the method on mixtures.

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