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Analytical ^{13}C NMR Spectroscopy of Fatty Quaternary Amines

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ABSTRACT

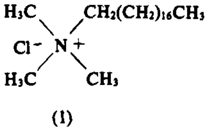
Natural abundance ^{13}C nuclear magnetic resonance spectroscopy (CMR) has been used for the rapid, nondestructive analysis of fatty quaternary ammonium compounds. Quantitative analysis of mixtures of mono-, di- and tri-fatty ammonium chlorides can be accomplished under conditions that do not involve heat or extremes of pH and that are independent of solvent present. In order to determine optimal conditions for quantitative studies, carbon spin lattice relaxation times (T_1) and nuclear Overhauser effects (NOE) were determined for each of the components. The method of internal standard addition was used to determine reproducibility and accuracy of the method for the measurement of mono- and tri-fatty quaternary components in material consisting predominantly (i.e., greater than 90%) of di-fatty-dimethyl ammonium chloride.

INTRODUCTION

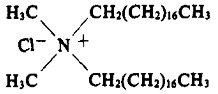
Comprehensive studies of carbon-13 nuclear magnetic resonance spectroscopy (CMR) of aliphatic amines have been reported (1-3). None of these has adequately addressed the specific topic of quaternary amines. Fatty quaternary ammonium compounds have been of significant economic interest for many years. Their utility as fabric softeners, dispersants, emulsifiers and sanitizers is widely appreciated (4).

The properties of fatty quats vary widely and are dependent both on the distribution of the substituent fatty chains and on the nature of the substitution at the quaternary nitrogen. The substituent fatty chain distributions can be easily ascertained by examination of the precursors, but it is of significant importance that something be known about the quaternary substitution.

The primary focus of this paper is on compounds like those shown in Scheme I. Model compounds prepared from pure stearyl precursors were used, as were those having a hard tallow distribution. Only the signals from those carbon atoms directly bonded to nitrogen were used for quantitation. The method is, therefore, independent of chain length or distribution. One of the primary goals of this study was to obtain a method of general applicability.



(1)



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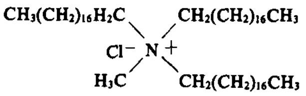


(3)

SCHEME I

Attempts to determine mono-, di- and tri-fatty substitution by gas liquid chromatographic (GLC) methods are complicated by the involatility of the materials, their chemical inability to form volatile derivatives, and their susceptibility to thermal decomposition or rearrangement (i.e., Hofmann degradation, dealkylation/realkylation). Pyrolytic methods yield information about the composition of the chains but are inconclusive with respect to the nature of the nitrogen substitution (5).

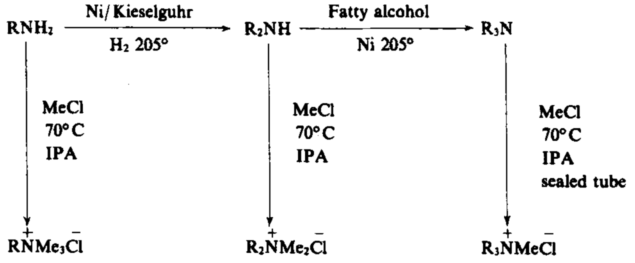
The use of liquid chromatographic methods, particularly ion-pairing, has shown some promise (6). Separation, however, is hindered by the surface-active nature of the compounds and there are considerable difficulties in detecting them at low concentration. Thin layer chromatography may ultimately prove to be a viable method but may present problems for the quantitative assessment of small amounts of contaminant quat in a matrix of other quaternary species.

CMR spectroscopy, on the other hand, does not suffer from any of the previously mentioned difficulties. The sample need only be dissolved in a suitable deuterated solvent (e.g., $CDCl_3$) for spectra acquisition. There is no chance for thermal alteration of the sample and all components of the mixture are measured simultaneously and with equal sensitivity. This makes CMR spectroscopy ideal as a method for the evaluation of other methods. This method cannot, however, be considered routine. It requires a highly sophisticated and expensive NMR spectrometer and frequently needs hours of accumulation time for trace contaminant measurement.

A number of precautions must be taken in the quantitative interpretation of CMR data (7). Paramagnetic relaxation reagents are quite frequently used in this situation to minimize errors due to variation in nuclear Overhauser enhancement (NOE) and spin-lattice relaxation (T_1) (8). We, however, obtained relaxation time and NOE data for the 3 compounds of interest and selected parameters that precluded the use of added relaxation reagent, thus retaining NOE and increasing sensitivity. The comparative nature of this particular experiment minimizes the effect of these factors and allows rapid data acquisition.

MATERIALS AND METHODS

Commercial stearyl amine and hard tallow amine were used as the starting material for the synthesis of the 3 quaternary amines shown in Scheme I. The compounds were synthesized and purified according to the general format of Scheme II (IPA = isopropyl alcohol).



SCHEME II

The instrument used was a Bruker WM-200 NMR spectrometer equipped with an Aspect 2000 data system. All spectra were obtained in pulse mode at a frequency of 50.391 MHz with simultaneous broadband decoupling of protons at a power of 2 W. The pulse width used was typically 17.5 μsec (90°) and the pulses were repeated at 2.75-sec intervals. The 16K data points were used at a sweep width of 11 KHz (1.36 Hz/pt). Exponential multiplication of the resultant free induction decays was performed with a line broadening constant of 0.5 Hz. Most spectra required about 2000 scans (1.5 hr) for determination of peaks as low as 1%.

Samples were run in 10-mm tubes using $CDCl_3$ (Merck 99.8% D) as solvent and for deuterium lock. Samples varied between 280 and 450 mg in 2 mL of solvent, depending on solubility. Samples used for relaxation and NOE measurements were degassed using the freeze-thaw technique, with a minimum of 4 freeze-thaw cycles. Integration and peak height measurements were done with the Aspect 2000 data system.

RESULTS AND DISCUSSION

The physical behavior of many quaternary ammonium products is adversely affected by very small changes in composition. The primary thrust of this study was the quantitation of small amounts of mono-fatty and tri-fatty quaternary amine contamination in a matrix of dimethyl distearyl ammonium chloride and solvent. The appearance of unquaternized amines is also significant, but better means already exist for this determination. The goal was development of a method capable of accurate quantitation in the range of 0.5-20% for the mono- and tri-fatty contaminants.

The first step was obviously the interpretation of the spectra for each of the pure components. The spectrum of compound 2, a typical di-fatty quaternary amine prepared from a stearoyl precursor, is shown in Figure 1. Spectral interpretation was straightforward and was verified by off-resonance decoupling, which will not be detailed in this paper. Interest centers on the signals at 51.5 and 63.7 ppm in the CMR spectrum. The coupled spectrum shows the signal at 51.5 ppm to be a quartet and the signal at 63.7 ppm to be a triplet. These are the signals due to carbons bonded directly to nitrogen; the triplet is due to the fatty methylenes and the quartet is due to the quaternary methyls. The deshielding of carbons attached to nitrogens bearing more methyls can also be seen by comparison with values obtained from the spectra of compounds 1 and 3, as shown in Table I.

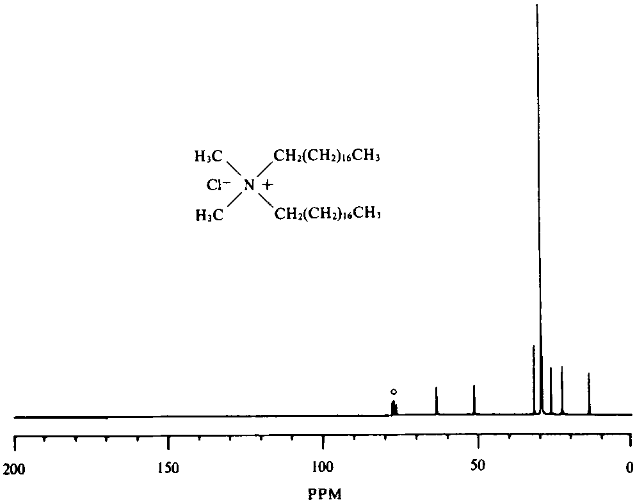


FIG. 1. Broadband decoupled ^{13}C NMR spectrum of compound 2. ○—Solvent (deuterochloroform).

TABLE I

^{13}C Chemical Shifts for Fatty Quaternary Ammonium Chlorides[a]

Compound no.	Chemical shift (ppm) -N-CH$_2$-[b]	 -N-CH$_3$[c]
1	66.8	53.5
2	63.7	51.5
3	61.7	49.2

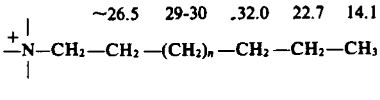
[a] Shifts relative to internal tetramethylsilane for samples dissolved in deuterochloroform.

[b] Signal appears as triplet in coupled spectrum.

[c] Signal appears as quartet in coupled spectrum.

Another spectral feature that simplified interpretation was the relative intensity of the 2 nitrogen-bound carbon signals in both mono-fatty and tri-fatty quat. In the mono-fatty quat, the methylene carbon at 66.8 ppm is only one-third of the intensity of the methyl carbon at 53.5 ppm. The reverse is true for the tri-fatty quat: the methyl carbon signal at 49.2 ppm is one-third the intensity of the methylene at 61.7 ppm.

As mentioned previously, the feature that makes CMR an appealing choice for this study is the fact that the nature of fatty substituents has no effect on the groups attached to the nitrogen. Typical chemical shift values (in ppm) for the carbons of a fully saturated chain are shown in Scheme III. Only unsaturated or highly branched chains differ markedly from these values.



$$\overset{+}{-N}-\overset{\sim 26.5}{CH_2}-CH_2-\overset{29\text{-}30}{(CH_2)_n}-\overset{32.0}{CH_2}-\overset{22.7}{CH_2}-\overset{14.1}{CH_3}$$

SCHEME III

The remainder of this paper will deal with the signals

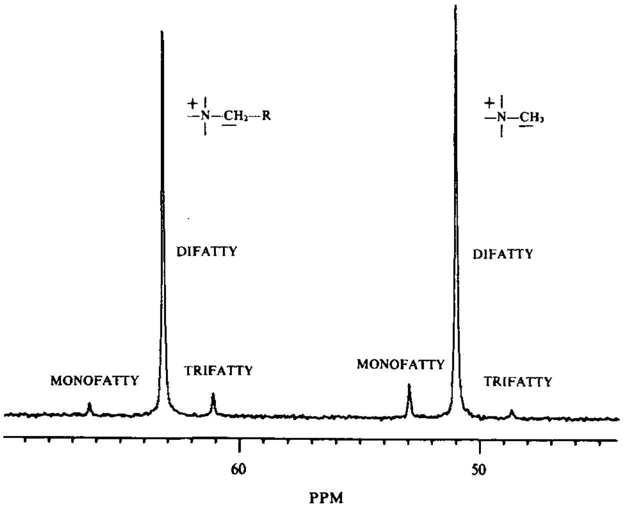


FIG. 2. Expansion of broadband decoupled ^{13}C NMR spectrum (Fig. 1) showing minor components (compounds 1 and 3).

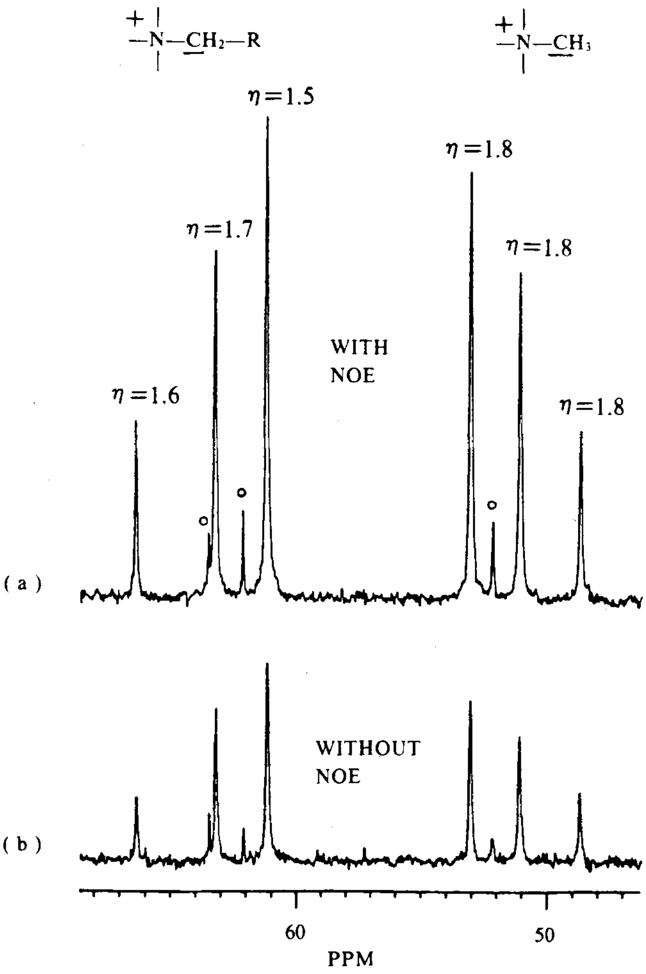


FIG. 3. Broadband decoupled ^{13}C NMR spectra of equimolar mixture of compounds 1, 2 and 3: (a) with nuclear Overhauser enhancement, and (b) without nuclear Overhauser enhancement. o—Impurities.

shown expanded in Figure 2. This particular range of relative intensities is quite typical of a commerical di-fatty dimethyl quat.

In order to compare the signals from different compounds using CMR spectroscopy, one must be assured that the compounds exhibit similar NOE and relaxation behavior. We compared these effects with single-component samples and, as shown in this paper, with equimolar mixtures of the 3 components. The results were identical, within experimental error.

Shown in Figure 3a are spectra obtained without decoupler gating and in Figure 3b those with decoupler gating. By gating off the decoupler, except during scan acquisition, and allowing a long relaxation time between scans, one obtains spectra that exhibit no nuclear Overhauser enhancement.

Table II shows that the quat methyl groups all show identical NOE values of 1.8 (maximum possible is 1.98), whereas there is slight variation in the NOE of the nitrogen-bound methylenes.

Spin-lattice relaxation differences only become significant for multipulse experiments. As long as the interpulse delay is greater than ca. 6.5 × T_1 and the pulse width is 90°, equilibrium magnetization will be achieved. The values in Table III thus indicate a necessary interpulse delay of >2.5 sec. Spin-lattice relaxation data were obtained on the sample used for NOE measurement. The inversion-recovery method (IRFT) was used for T_1 measurement (9). As with NOE, the major variations in relaxation time are seen in the fatty methylenes.

All of the previously mentioned studies were directed at ensuring the accurate measurement of small quantities of mono-fatty and tri-fatty material in commercial di-fatty quat. To determine the accuracy and reproducibility of measurements made by CMR, 2% (by wt) aliquots of the quats to be quantified were added successively to a typical commercial di-fatty quaternary ammonium chloride. The enlargements of the spectra obtained are shown in Figure 4.

The integration and peak height data for added mono-

TABLE II

Nuclear Overhauser Effects (NOE) for Compounds 1, 2 and 3[a,b]

Compound no.	NOE	
	$-N-CH_2-$	$-N-CH_3$
1	1.6	1.8
2	1.7	1.8
3	1.5	1.8

[a] Data were obtained on a single freeze-thaw degassed sample consisting of an equimolar mixture of the 3 compounds.

[b] Sample temperature, 20 C.

TABLE III

Spin-lattice Relaxation Times (T_1) for Compounds 1, 2 and 3[a]

Compound no.	T_1 (sec)	
	$-N-CH_2-$	$-N-CH_3$
1	0.34	0.39
2	0.22	0.20
3	0.11	0.32

[a] Determined by 3-parameter exponential fit of data obtained by inversion-recovery Fourier transform (IRFT) technique (9).

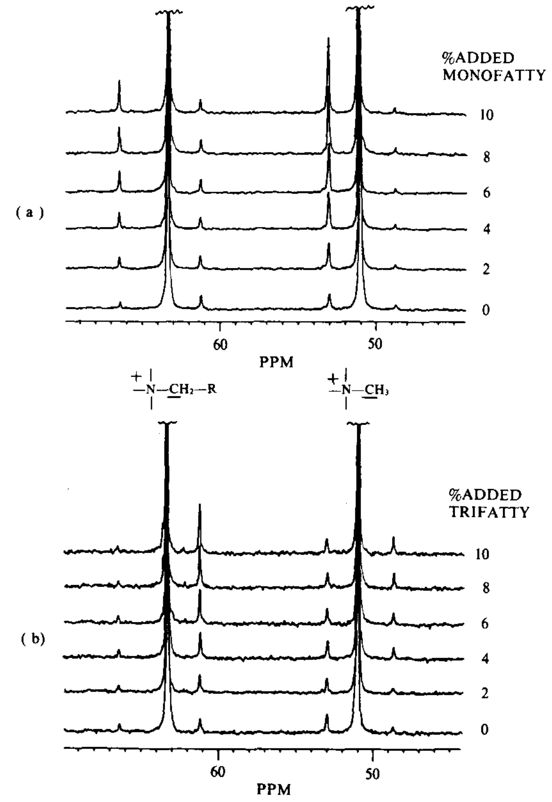


FIG. 4. Effects of additions of compound 1 (a) and compound 3 (b) on the CMR spectrum of compound 2.

TABLE IV

Addition of Compound 1 to Compound 2

% Wt added monofatty	-N-CH$_2$-, 66.9 ppm		-N-CH$_3$, 53.5 ppm	
	Area	Height	Area	Height
0	1.91	2.74	5.25	6.52
2	4.63	4.48	10.07	11.89
4	5.85	6.51	14.24	15.89
6	7.96	8.55	20.30	21.23
8	8.45	10.41	25.74	27.22
10	11.13	12.46	30.11	31.50
Slope	0.85	0.98	2.53	2.52
Intercept	2.39	2.64	4.95	6.45
R^2	0.978	0.999	0.997	0.998

fatty quat are summarized in Table IV. Lines were fit to the data using the method of least-squares and results are also presented in Table IV. As can be seen from the correlation data (R^2), the fit was excellent. It is also noted that the slope of the line fitted for the areas of the methyl peaks is 3 times that for the areas of the corresponding methylenes in the mono-fatty quat. The data here show good agreement for both area and peak height measurement, but peak areas were selected as most appropriate for general use.

The spectra obtained by addition of known weights of material can be used for direct comparison in semiquantitative fashion, or the calibration curves can be used for a more accurate interpolation. Probably most important, however, is the fact that the measure of molar quantities of the constituents may be made directly by comparison of the relative peak areas. It must be remembered that these areas must be normalized in order to apply this approach. In other words, when comparing methyl groups, one must remember that the mono-fatty quat has 3 methyls, the di-fatty quat has 2, and the tri-fatty quat has only one. Therefore, when comparing the 3 methyl group peak areas, one should multiply the area of the tri-fatty methyl by 2 and that of the mono-fatty methyl by 2/3 before comparing data to the area of the di-fatty methyl. In a similar manner, when comparing the peak areas of the methylenes, one multiplies the methylene area for the mono-fatty methylene by 2 and that of the tri-fatty by 2/3 for comparison to the di-fatty. Total agreement is seen between methods when precautions are taken to ensure proper conditions for spectral acquisition.

No mention has been made, so far, of the use of paramagnetic relaxation agents. The previous studies have been done to eliminate the necessity for using them. The primary reason for avoiding their use was to retain as much sensitivity as possible. Comparison of procedures both with and without added Cr(acac)$_3$, showed good agreement in quantitation and the expected loss of sensitivity in the case of added Cr(acac)$_3$. Once again, it must be mentioned that the main feature contributing to the excellent accuracy of the experiment described here is the comparative nature of the experiment.

It is hoped that the procedure described in this paper will assist in the development of other accurate methods for the evaluation of quaternary ammonium compounds. It also provides an ideal nondestructive method for looking at quats in proprietary formulations without the need for sample alteration. It has certainly provided a useful and informative technique, one that can provide quantitative information on compounds that are, at best, troublesome to the analyst.

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