Quantitative Applications of High-Resolution Proton Magnetic Resonance Measurements in the Characterization of Detergent Chemicals

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Abstract

Quantitative hydrogen nuclear magnetic resonance measurements are used for the determination of average structures of detergent chemicals. For alkylbenzenes, alkylphenols, and ethylene oxide (EO) adducts of alkylphenols, the following quantities can be measured: average lengths of alkyl chains, average molecular weights, degree and kind of branching in the alkyl chain, ortho-para distribution of aryl substituents, and average lengths of EO chains. Accuracy is of the the order of ± 2% of the total hydrogen. The method is nondestructive, and sample volumes as small as 0.007 ml have been used.

Introduction

PREVIOUS ACCOUNTS in this journal of the utility of high-resolution nuclear magnetic resonance (NMR) measurements have stressed the qualitative capabilities of the method (1,2). Recent significant advances in commercially available instrumentation have simplified the experimental technique for hydrogen nuclei to the point where reproducible spectra on precalibrated chart paper are now easily obtained. Moreover, accurate electronic integration of peak areas—which under proper operating conditions are directly proportional to the number of hydrogen nuclei giving rise to them (3)—is becoming a routine analytical procedure. This paper describes some quantitative applications of hydrogen NMR in the study and characterization of chemicals which are of interest as raw materials and intermediates in the manufacture of synthetic detergents. Alkylbenzenes, alkylphenols, and ethylene oxide (EO) adducts of alkylphenols have been studied. Average length of alkyl chains, degree and kind of branching in alkyl chains, ortho-para distribution of aryl substituents, and average length of EO chains have been determined.

Experimental

Spectrometer. High resolution NMR spectra were obtained at 60 Mcps on a Varian Associates Model A-60 spectrometer. All measurements were made at the ambient temperature of the probe (ca. 32C) in spinning 5 mm precision bore, glass sample tubes. Prior to runing a series of samples, it was customary to adjust the homogeneity controls of the magnet to obtain a resolution of 0.4 cps or better, as measured by the line width at half height of a degassed sample of acetaldehyde. Peak positions were recorded in cps and ppm of the magnetic field downfield from tetramethylsilane (TMS), which was added at ca. 3% concentration to all samples as an internal reference. The internal calibration of the A-60 spectrometer was periodically checked by an accurate sideband calibration experiment. Spectra were usually recorded at a 500 cps sweep width using a 500-sec sweep time for the absoption spectra and a 50-sec sweep time for the integrals. Spectrum and integral amplitude controls were adjusted to keep all signals on scale. For optimum quantitative results, however, a careful study of the reproducibility of the absorption spectra and integrals revealed that the built-in calibrations of the amplitude controls on the A-60 spectrometer were sufficiently precise for well-separated bands of a spectrum to be integrated separately on an expanded scale, at nearly full pen deflection. Direct comparison of relative peak intensities is then possible by use of the appropriate amplitude factors, with the overall precision of the measurements being improved. It was necessary to record the integrated spectra at a low radio frequency field strength, H_1, to avoid saturation (4) of narrow lines with resulting bias of relative peak areas. Experiments at varying H_1 power indicated that below 0.04 milligauss, relative areas of all peaks were correct within the inherent accuracy of the integrator electronics, or ±2%.

Samples. Sample volume was normally 0.3 ml, although when only small quantities were available for study, microcell techniques (5) yielded useable spectra from as little as 0.025 ml of a CCl_4 solution containing 0.007 ml of alkylbenzene. Low viscosity samples were examined as the undiluted liquids in order to obtain maximum signal to noise ratios. Viscous samples and solids were examined as 25% by volume solutions in CCl_4 for good peak resolution. Tubes were stoppered, but not ordinarily degassed. Samples were obtained from commercial sources as well as from custom synthesis of specific isomers. From mass spectrometric and gas chromatographic analyses, it is known that materials of this type frequently contain a large number of structural isomers and homologs (6). The observed NMR spectra in these cases are a superposition of the spectra of all the sample components, so that average structural information based on the distribution of hydrogen is obtained.

Results

Alkylbenzenes. Figure 1 shows the NMR spectrum of a typical dodecylbenzene made from tetrapropylene. Two major resonance bands are apparent. The one at lower field arises from hydrogens on the aromatic ring, while the one at higher field is produced by the hydrogens of the alkyl chain. Since the lower band results from exactly five hydrogens per molecule, assuming only monoalkylation, the average number of hydrogens in the alkyl chain, the average carbon number of the alkyl chain, as well as the average molecular weight of the sample can be readily obtained from the relative integrals of these two bands. The general formula for a monoalkylbenzene is:

$$C_6H_5 - C_nH_{2n+1}$$

If $\ominus$ is defined as the measured fraction of the total hydrogen integral which arises from *non-alkyl* hydrogen, then n, the average number of carbons per alkyl chain, is given by the expression

$$n = (5-6\ominus)/2\ominus \qquad [A]$$

From the value of n, the average molecular weight

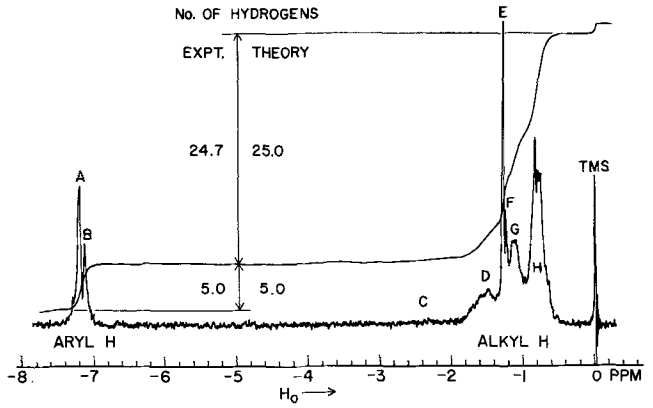

FIG. 1. NMR spectrum of a typical dodecylbenzene made from tetrapropylene.

A = $C_6H_5-CR_3$
B = $C_6\underline{H_5}-CHR_2$
C = $\alpha-\underline{CH}$
D = other $-\underline{CH}$ and $\beta-CH_2-$
E and F = $\beta-CH_3$
G = other $-CH_2-$
H = other $-CH_3$

of the alkylbenzene may be obtained from the formula

$$\text{mol wt} = (14.026)n + 78.108 \qquad [B]$$

Using the same spectrum, additional information about the degree and kind of branching in the alkyl chain can be obtained from the relative intensities of the overlapping peaks within the separate bands. The origin of the individual peaks is indicated on the spectrum in Figure 1. Assignment of peaks in the spectrum to specific structural units was made on the basis of observations on a large number of pure reference compounds of known structure. Although the individual peaks of the alkyl band are not completely resolved, the alkyl integral can be assigned and arbitrarily divided at the inflections corresponding to peak minima to give a consistent measure of the branching in the alkyl chain. Figure 2 shows for comparison the NMR spectrum of a straight chain dodecylbenzene. The quantitative NMR data on chain branching for Figures 1, 2 are compared in Table I.

Alkylphenols. Figure 3 shows the NMR spectrum of a typical commercial nonylphenol consisting of para and ortho isomers in a ratio of 9:1, respectively. The shape of the alkyl resonance qualitatively resembles that from an analogous alkylbenzene, except that the chemical shifts of hydrogens attached to carbon alpha or beta to the aromatic ring are shifted to lower

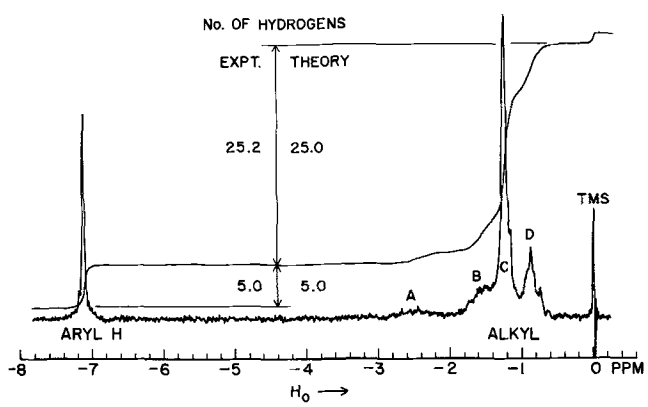

FIG. 2. NMR spectrum of a straight chain dodecylbenzene.

A = $\alpha-\underline{CH} + \alpha-CH_2-$
B = other $-\underline{CH} + \beta-CH_2-$
C = other $-CH_2- + \beta-CH_3$
D = other $-CH_3$

TABLE I

Comparison of NMR Data for a Branched [a] and a Straight [b] Chain Dodecylbenzene

Structure [c]	% of Total hydrogen		Average number of hydrogen atoms per molecule	
	Branched	Straight	Branched	Straight
$C_6\underline{H_5}-CR_3$	12.0	0.0	3.6	0.0
$C_6\underline{H_5}-CHR_2$	4.8	16.5	1.4	5.0
$\alpha-\underline{CH}$	1.7	4.0	0.5	1.2
Other $-\underline{CH} + \beta-CH_2-$	13.8	10.6	4.1	3.2
$\beta-CH_3$	14.8	d	4.4	d
Other $-CH_2-$	18.2	52.4	5.4	15.8
Other $-CH_3$	34.7	16.5	10.3	5.0
Total	100.0	100.0	29.7	30.2

[a] See Figure 1.
[b] See Figure 2.
[c] Applicable hydrogen underlined.
[d] $\beta-CH_3$ peak not resolved from other $-CH_2-$.

field in the alkylphenol spectrum. The amount of shift differs for ortho and para substituents. Figure 4 contains a correlation chart of the observed range of chemical shifts for the alkyl portions of thirteen pure monoalkylphenols and thirty-three pure monoalkylbenzene isomers and homologs of known structures. Observed peaks are wider in almost every case than the chemical shift regions indicated in Figure 4. This is due to spin-spin coupling (7) between neighboring nuclei, which splits most peaks into multiplets. Partially overlapping peaks of varying multiplicities from different structural isomers superimpose to give the broad spectral envelopes which are observed with commercial samples.

Methods analogous to those employed for alkylbenzenes can be used to determine average alkyl chain lengths and degree of branching in the alkylphenol chains. Here the general formula is:

$$HO - C_6H_4 - C_nH_{2n+1}$$

the value of n is determined by equation A, and the average molecular weight given by equation B is increased by the weight of one oxygen per molecule.

The low field portion of the alkylphenol spectrum shows a single resonance peak for phenolic hydrogen due to rapid exchange of protons between ortho and para substituted molecules. The chemical shift and line width of this peak are extremely sensitive to the presence of traces of acidic or basic impurities, and under some conditions of slow exchange, separate broad OH peaks for the ortho and para isomers can

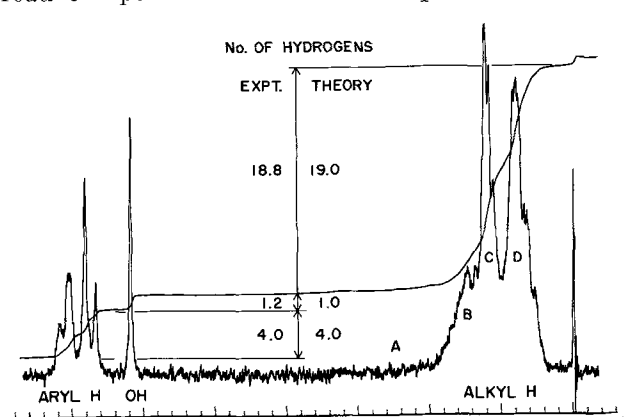

FIG. 3. NMR spectrum of a typical nonylphenol.

A = $\alpha-\underline{CH} + \alpha-CH_2-$
B = other $-\underline{CH} + \beta-CH_2-$
C = other $-CH_2- + \beta-CH_3$
D = other $-CH_3$

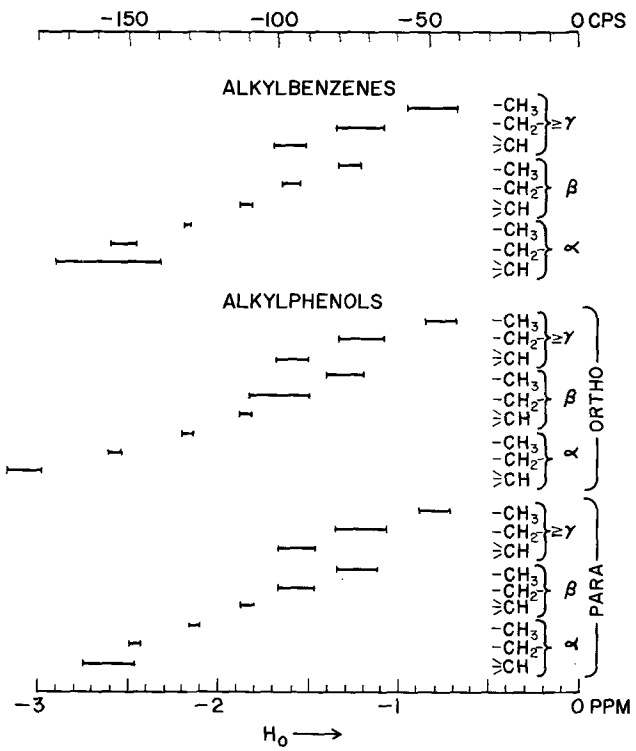

FIG. 4. Range of chemical shifts of alkyl hydrogens. Bands are based on observations of 13 pure monoalkylphenols and 33 pure monoalkylbenzene isomers and homologs of known structure.

be seen. The aromatic band of alkylphenols shows considerable fine structure due to the spin-coupling of ring hydrogens made nonequivalent by the hydroxyl substituent. Superposition of different overlapping spectra for ortho and para mixtures adds to the complexity of the aryl resonance, although in suitable cases this band can be used to advantage to determine the ortho-para distribution of isomers in a mixture. Figure 5 shows, e.g., a calibration curve obtained from prepared mixtures of pure ortho and para sec. butyl phenol. Enlarged spectra of the region between 360 and 460 cps below TMS were run on 25% by weight solutions in CCl_4. Since the aryl hydrogen bands for ortho and para isomers overlap, there are no peaks in that region arising entirely from one isomer. Two major peaks, due largely to ortho and para isomers, respectively, were chosen at −400.5 and −403.0 cps. The ratio of these peak heights plotted versus mole % para isomer in the region 65–100% gives a curve which can be used for

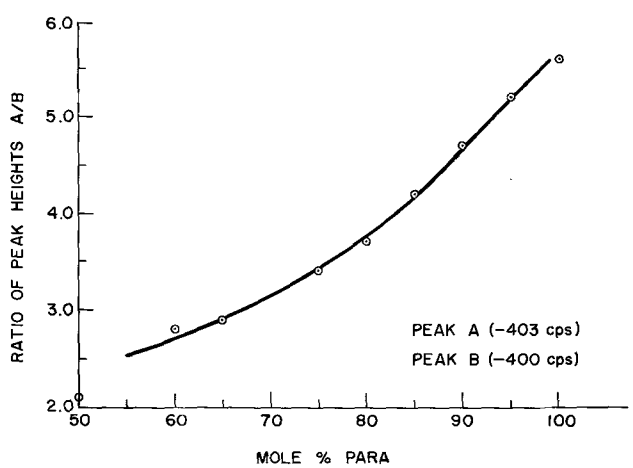

FIG. 5. Calibration curve for mixture of ortho and para sec.-butylphenol.

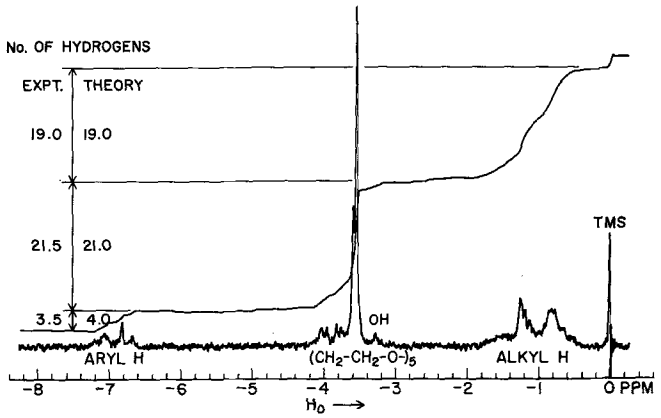

FIG. 6. NMR spectrum of short chain EO adduct of alkylphenol.

analytical purposes to within ± 1 mole %. Similar curves can be prepared for other alkylphenols if pure isomers are available. In the absence of pure isomers, an estimate of ortho-para distribution can still be made from the relative sizes of the integrals of hydrogens on the alpha-carbon, although this method is somewhat less precise due to the broad peaks.

EO Adducts. Figures 6 and 7 show spectra of long chain and short chain EO adducts of alkylphenol. Adducts with this and other hydrophobes have also been studied by Flanagan et al. (8). NMR provides an excellent method of measuring average EO chain lengths. One simply ratios the integral of the $(-CH_2CH_2O-)_x$ peak to the integral of another portion of the molecule, say the four aryl hydrogens or, alternatively, the alkyl band. If the average length of the alkyl chain is not known it can also be determined in the same experiment. The precision with which the value of x can be determined varies from ± 0.2 for short chains (less than five moles of EO per mole of alkylphenol) to ± 2 for long chains (ca. 30 moles of EO). For long chains, it is desirable to integrate the EO band and the aryl band on an expanded scale under different degrees of amplification. The determination is not dependent on the alkyl chain length, ring position, or degree of alkyl branching. Empirical tests, such as cloud point measurements, which have been correlated with EO chain lengths do not have this advantage.

Discussion

Precision and Accuracy. For concentrated solutions which give strong well-resolved signals, the precision of the total quantitative NMR measurements is limited primarily by the inherent stability of the electronics,

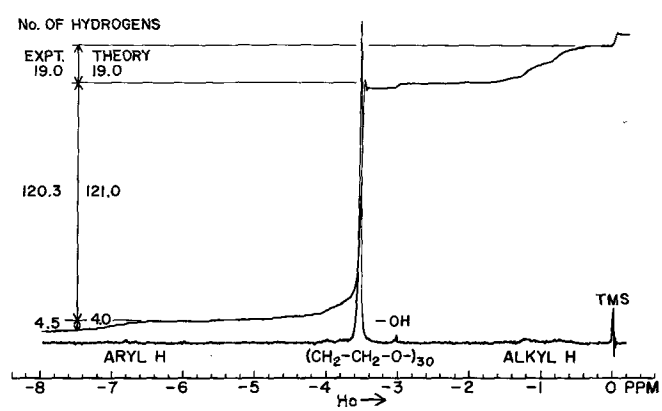

FIG. 7. NMR spectrum of long chain EO adduct of alkylphenol.

especially the integrating circuit. The accuracy of the integrator is ± 2% according to manufacturer's specifications. We have observed ± 1%. This means that in a molecule containing 50 hydrogen atoms, these can be accounted for to within ± 0.5–1.0 hydrogen atom. For dilute solutions, which give weaker signals, the signal to noise ratio becomes the limiting factor. This error can be reduced by averaging repetitive observations. In ratioing the area of an unknown resonance peak to one which arises from a known number of hydrogens, any uncertainty in the measurement of the known peak area is multiplied by the ratio. This accounts for the greater uncertainty in the measurement of long EO chains. Another possible source of error arises because the process of obtaining the NMR spectrum disturbs the normal distribution of nuclear spin states. This saturation effect (4) must be kept negligibly small if all peaks are to exhibit their theoretical areas. In practice, this means operating at low radio frequency power levels which give less than optimum signal to noise ratios. Thus, in order to avoid biased peak areas, one must sacrifice some precision of measurement. In practice, a suitable compromise is reached by using the instrumental conditions described in the experimental section. A final source of error arises due to incomplete resolution of overlapping peaks, as in the alkyl band. Choice of the integral inflection at the point of minimum intensity between two partially resolved peaks leads to biased answers if the peaks are of different sizes. Data on average alkyl branching should be considered semi-quantitative for this reason.

The ability of NMR to give average structural information about mixtures of isomers and homologs is fortunate, but frequently it is desirable to know something about the distribution of individual isomeric species in a mixture. Fractionation of a sample using high resolution preparative chromatographic techniques yields small samples of more nearly pure isomers which can then be identified by quantitative NMR using microcell techniques (5). Quantitative spectra have been taken of 0.007 ml alkylbenzene in CCl_4, using spherical nylon sample cells of the type described by Shoolery (5).

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Lipolysis of Trivernolin by Pancreatic Lipase

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Abstract

Vernolic acid (*cis*-12,13-epoxy-*cis*-9-octadecenoic acid) occurs as the triglyceride in the seed of *Vernonia anthelmintica.* Incubation of the seed produces a 1,3-divernolin. To determine whether the structure of trivernolin is responsible for the apparent secondary ester position specificity of the natural enzyme, trivernolin and triolein, were incubated with pancreatic lipase and the free fatty acids and monoglycerides were determined after 5 and 15 min digestion periods. The preponderance of 2-monoglyceride produced by the action of pancreatic lipase was interpreted to indicate that the structure of trivernolin was not solely responsible for the secondary position specificity of the *V. anthelmintica* lipase toward trivernolin.

Introduction

TRIVERNOLIN, a simple triglyceride of vernolic acid was found by Krewson et al. (6) as a major constituent of the oil from the seed of *Vernonia anthelmintica.* There was present a hydrolytic enzyme system in the seed which converted trivernolin to 1,3-divernolin and vernolic acid with no apparent formation of monoglyceride. (6) This was of interest in that most lipases are either specific for the primary esters of glycerides, or do not exhibit any positional specificity (5,8,10,12).

If the lipase system of *V. anthelmintica* is specific for the secondary esters of other triglycerides, it would be most useful in determining triglyceride structure. But before pursuing such investigations, it is necessary to know whether the specificity is due to the action of the lipase, or to the epoxy structure of trivernolin. This investigation was undertaken to partially test this hypothesis by incubation of purified trivernolin and pancreatic lipase of known specificity (10,12) and comparing free fatty acid and monoglyceride contents of this system with one of pancreatic lipase and triolein.

Materials and Methods

Purified trivernolin was prepared at the Eastern Regional Research Laboratory from *V. anthelmintica* seed oil by a process of crystallization and column chromatography (7). Commercial triolein (Hormel) was used as purchased. These triglycerides were checked for purity by thin layer chromatography (TLC) using 75:25 ethyl ether and petroleum ether (30–60C) as the developing solvent and a brom-thymol-blue spray (9) to visualize the spots. In the solvent system used, triglycerides travel just below the solvent front. Both substrates gave only one spot and that was in the area of triglycerides.

A crude preparation of pancreatic lipase (Steapsin, Fisher Scientific) was treated four times to remove ca. 10% lipid materials as follows: 2 g of the crude powder was slurried in 200 ml ethyl ether, stirred for 15 min magnetically and the mixture centrifuged; the ether layer was decanted and after the final treatment, the wet powder was dried at room temp over calcium sulfate in a desiccator. Prior to each diges-

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