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EFFECT OF PARAMAGNETIC RELAXATION REAGENT CONCENTRATION
ON THE NUCLEAR OVERHAUSER ENHANCEMENT AND ON THE USE OF
GATED PROTON DECOUPLING IN QUANTITATIVE CARBON-13
NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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BRIEF

The concentration effect of $\text{Cr}(\text{acac})_3$ on the pyrene ^{13}C NMR resonances demonstrates that all NOE's are not completely quenched until 0.25 M $\text{Cr}(\text{acac})_3$ is present. In quantitative analyses using lesser $\text{Cr}(\text{acac})_3$ concentrations, gated proton decoupling will be necessary.

ABSTRACT

To determine the effect of the concentration of the paramagnetic relaxation reagent chromium (tris)acetylacetonate [Cr(acac)₃] when used in quantitative carbon-13 nuclear magnetic resonance spectroscopy (¹³C NMR), the spectrum of pyrene, a compound with extreme values of spin lattice relaxation times (T₁) and nuclear Overhauser enhancements (NOE), was studied. The NOE, line broadening and shifting of the five pyrene resonances were determined for up to 0.3 M Cr(acac)₃. The quaternary and protonated carbons displayed different NOE's until all were completely quenched with the Cr(acac)₃ greater than 0.25 M. Employing this Cr(acac)₃ concentration in quantitative ¹³C NMR analyses eliminated errors due to differing NOE's, but led to considerable line broadening and shifting. The use of lower Cr(acac)₃ concentrations in these analyses, such as the commonly reported 0.05 - 0.15 M, necessitates the joint use of gated proton decoupling to eliminate the NOE differences which still exist at these concentrations.

INTRODUCTION

Over the past decade, natural abundance ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) has undergone a dramatic development. Use of ^{13}C NMR spectral data has become an established and powerful method for analysing organic substances (1,2). For the most part, this information has been non-quantitative, mainly because of the approach used in obtaining these natural abundance spectra — rapid accumulation for reasonable signal-to-noise (S/N) in a minimum of time. The instrumental conditions required in this approach lead to loss of the quantitative condition; in other words, equal numbers of carbons often yield different peak areas because the carbons have differing spin-lattice relaxation times (T_1) and nuclear Overhauser enhancements (NOE). Although there are other instrumental factors which can affect signal intensity — e.g., adequate digitization in the frequency domain spectrum, pulse strength (H_1) in relation to the sweep width, choice of either single side band crystal filter or Butterworth filter, and choice of filter bandwidth (2-5) —, surmounting the T_1 and NOE problems is the subject of this paper.

Overcoming inequalities among carbons due to different T_1 's is well understood. One need only insert a delay between successive pulses which is greater than or equal to five times the longest T_1 of the carbons in the sample. A complication which arises, however, is that some quaternary carbons have a T_1 in excess of 100 seconds. Samples with such slowly relaxing carbons will require delay periods of 8-12 minutes between successive pulses. For 500 accumulations, a

conservative estimate even for a concentrated sample, the total experimental time is measured in days, making the approach impractical for routine analysis of a large number of samples. One way to overcome this limitation is to consider the T_1 's and NOE's of the system and to base the analysis of the compounds of interest on the signals from fast relaxing, NOE-enhanced carbons. This will be applicable to simple mixtures, and necessary in those cases in which addition of a relaxation reagent (vide infra) is not possible due to a need for sample recovery upon completion of the analysis. An example of this approach and the precautions to be observed have been published for the analysis of binary mixtures (6).

Commonly, the method of choice for overcoming long relaxation times is addition of a paramagnetic relaxation reagent (PARR) (7-10) such as chromium (tris)acetylacetonate [$\text{Cr}(\text{acac})_3$]. The introduction of such a species provides a powerful spin-lattice relaxation mechanism for all carbons. Consequently all T_1 's are decreased to the point that rapid pulse repetition rates (the sum of the acquisition time and pulse delay, typically ≤ 6 seconds) are possible. Use of the PARR technique is mandatory when the sample contains quaternary carbons for analysis, or when the sample is a complex mixture on which one must perform a total analysis and account for all carbons. Examples of such mixtures are fossil fuel substances, for which it is practically impossible to know the exact molecular constituents. One cannot assume that there are so few carbons with long T_1 's that it is unnecessary to use long pulse delays in order to accurately account for such carbons.

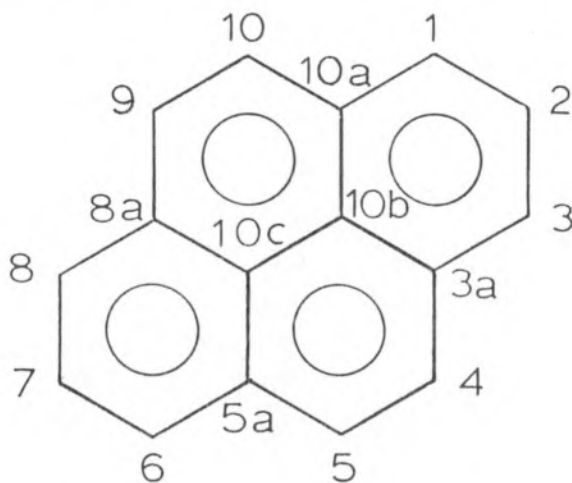
In fact it is reasonable to assume that in these fossil fuel substances there are many structures, such as large polynuclear aromatic hydrocarbons, with quaternary carbons that are well removed from protons and have very long T_1 's. The routine analysis of such samples requires that each ^{13}C spectrum be obtained with reasonable S/N in a relatively short time (1-4 hrs). Such considerations constitute the case for use of the PARR technique in overcoming the difficulties long T_1 's present.

The addition of a paramagnetic species also has a marked effect on a carbon's NOE (7,11,12). There is a quenching and suppression of the nuclear Overhauser effects, but the degree of suppression varies from carbon to carbon, depending on the mechanisms of relaxation of each carbon and on the concentration of relaxation reagent. Equilibration of the different NOE's may not be achieved unless one fully considers these two factors. It was originally suggested (10) that 0.1 M $\text{Cr}(\text{acac})_3$ would efficiently suppress all NOE's. A more recent study of the NOE's of a steroid in a solution with 0.1 M $\text{Cr}(\text{acac})_3$ has demonstrated a substantial and different NOE for each carbon (13). This residual inequality of NOE values introduces errors into quantitative ^{13}C analyses.

Elimination of this residual NOE problem, while still retaining ^{13}C - ^1H decoupling, can be achieved by two means: increasing the concentration of paramagnetic species; or use of a gated ^1H decoupling sequence (14). One report (5) has indirectly demonstrated the relationship between $\text{Cr}(\text{acac})_3$ concentration and NOE. By studying various ratios of acenaphthene's peak areas, the author concluded that the NOE's were not fully equalized until 0.1 M $\text{Cr}(\text{acac})_3$ had been

exceeded. In another study, Thiault and Mersseman (15) recommended a $\text{Cr}(\text{acac})_3$ concentration of 0.4 M to completely quench NOE's. However, the 0.1 M concentration has been suggested as the maximum that can be used without a solubility problem in some solvents or without introducing unacceptable line broadening (10,12). The shifting of the line positions of various organic molecules brought about by 0.1 M $\text{Cr}(\text{acac})_3$ has also been reported (16). However, there are no reports relating the variation of NOE's of a variety of carbon types over a wide range of relaxation reagent concentrations.

In order to carry out accurate quantitative ^{13}C NMR analysis in a routine manner on a large number of fossil fuel samples, with a minimum of machine time per sample, it was necessary to determine the optimum method for removing NOE differences while employing a relaxation reagent for eliminating T_1 problems. To examine the relationship between the $\text{Cr}(\text{acac})_3$ concentration and a carbon's NOE, line width and resonance position, a study was carried out on the model compound pyrene. This compound was chosen because it is a typical fossil fuel



constituent and because it contains carbons with a variety of relaxation times and nuclear Overhauser enhancements. Carbons C-10b,10c are expected to have a T_1 in excess of 100 seconds and a greatly reduced NOE; the protonated peripheral carbons are expected to have very short T_1 's (1-5 seconds) and full NOE's. The peripheral quaternary carbons, C-3a,5a,8a,10a, are expected to have a T_1 and NOE intermediate between these two extremes. The effects of $\text{Cr}(\text{acac})_3$ concentration on the NOE, line width and position of the five pyrene resonances are presented here, as are the implications for the instrumental conditions and sample composition for quantitative ^{13}C NMR analyses using relaxation reagents.

EXPERIMENTAL

SAMPLES. Pyrene was purchased from Aldrich Chemical Company and chromium (tris)acetylacetonate, $\text{Cr}(\text{acac})_3$, from Alfa Products. Both were used without further purification. The pyrene (0.2220 g) and the appropriate amount of $\text{Cr}(\text{acac})_3$ were weighed directly into an NMR tube and 1.30 ml of deuteriochloroform added as a solvent. This high concentration of pyrene - 0.845 M - and its fourfold symmetry greatly decreased the experimental time. All samples were sealed with a cork and Parafilm to prevent changes in concentration due to solvent evaporation. It is the author's experience that the red plastic tube tops supplied with commercial NMR tubes provide a poor seal for volatile compounds. It is important that evaporation of sample components (and a reference compound in the case of absolute analysis) be minimized in a quantitative analysis. A cork and Parafilm do this efficiently. As well, the lips of the NMR tube were flared to increase their strength and prevent breakage while inserting the cork tightly.

INSTRUMENTATION. All ^{13}C NMR spectra were obtained at 20 MHz on a Varian CFT-20 pulse Fourier transform spectrometer which utilized a Varian 620L data system. The transmitter was positioned near $\delta = 116$ (relative to internal tetramethylsilane, TMS), and a sweep width of 1000 Hz and a data acquisition time of 2.048 s used (4 K data points). For all spectra the flip angle used was a 90° rf pulse of 17 μs duration. Prior to sampling each transient, the signal was passed through a single side band crystal filter with a bandwidth of 1000 Hz.

To avoid a frequency-dependent attenuation across the pyrene frequency range, the transmitter carrier frequency was placed near the pyrene frequency range, and a large sweep width and filter bandwidth and a short 90° pulse width were used. The line positions of each resonance were computer determined, and the line width at half maximum intensity was measured from an expanded plot of each spectrum.

NOE MEASUREMENT. The NOE of each of the five resonances was measured at each $\text{Cr}(\text{acac})_3$ concentration by obtaining an Overhauser-enhanced spectrum (continuous proton noise decoupling) with peak areas M_i^{CD} , and a suppressed Overhauser spectrum (gated proton decoupling) with peak areas M_i^{GD} . The apparent NOE, η , was then calculated using the formula $\eta = (M_i^{\text{CD}}/M_i^{\text{GD}}) - 1$. The gating of the proton decoupler (on during data acquisition and off for a delay time between end of acquisition and the next pulse) was accomplished using the existing software. With this spectrometer control program it was not possible to obtain one transient in the NOE-enhanced mode and then one in the NOE-suppressed mode, store them separately on a peripheral storage device and then repeat the sequence until the necessary number of transients had been taken for each. With such a sequence, spectral differences brought about by a difference in sample temperature of the two experiments (36°C and 34°C for continuous and gated decoupling respectively) would have been minimized (2). Rather, the transients for the NOE-enhanced spectrum were obtained first, followed by the transients for the NOE-suppressed spectrum (200 transients for each spectrum of the sample with no $\text{Cr}(\text{acac})_3$, and 400 transients for each spectrum of samples with

the various $\text{Cr}(\text{acac})_3$ concentrations). To eliminate differences in peak areas arising from differences in T_1 's, long pulse delays were inserted between pulses: 1000 s for the sample with no $\text{Cr}(\text{acac})_3$, 200 s for the sample with 0.025 M $\text{Cr}(\text{acac})_3$, and 75 s for the remaining samples with $\text{Cr}(\text{acac})_3$. These delays are believed to be greater than 5 times the longest T_1 in each sample and thus approach the recommended time delays ($\geq 6T_1$ for continuous decoupling and $\geq 9T_1$ for gated decoupling) for experiments measuring NOE (17-19). The NOE's reported here were reproducible to ± 0.05 to ± 0.1 .

RESULTS AND DISCUSSION

The ^{13}C NMR chemical shifts, δ , for 0.845 M pyrene in CDCl_3 (for C-3a,5a,8a,10a $\delta = 131.28$, for C-4,5,9,10 $\delta = 127.47$, for C-2,7 $\delta = 125.91$, for C-1,3,6,8 $\delta = 125.03$, and for C-10b,10c $\delta = 124.81$) agree well with previously published spectra (20,21). The importance of knowing the range of T_1 's in a sample in which no PARR is used is underlined by the fact that the C-10b,10c resonance is not observed despite good S/N in a continuously decoupled spectrum obtained with a 32° pulse width, no pulse delay and a 2 s acquisition time. To observe and account for such carbons in a reasonable time, a paramagnetic relaxation reagent such as $\text{Cr}(\text{acac})_3$ is a necessity.

Figure 1 shows the relationship between the concentration of $\text{Cr}(\text{acac})_3$ and the NOE of each pyrene resonance. With no relaxation reagent the NOE's are spread over a wide range and agree with those previously reported (22). The three protonated carbons exhibit complete NOE's, while the quaternary carbon atoms located on the periphery of the molecule (C-3a,5a,8a,10a) display an NOE of 0.94. The quaternary carbons in the centre of the molecule, well removed from the protons, have a greatly reduced NOE of 0.31. This trend is consistent with what one would predict based on the expected mechanisms for spin-lattice relaxation and their relative importance for each carbon. The ^{13}C - ^1H dipole-dipole mechanism is expected to be dominant for the protonated carbons and lead to a full NOE, while the NOE's for quaternary carbons will be reduced. This is due to the increasing importance of other mechanisms and the mounting inefficiency of the

dipole-dipole one (22,23).

Upon addition of $\text{Cr}(\text{acac})_3$ the NOE's of the protonated carbons and those of the quaternary carbons display different quenching characteristics, as seen in Figure 1. For a $\text{Cr}(\text{acac})_3$ concentration of less than 0.050 M the NOE's of the two quaternary resonances were reduced to zero, while the mean of the NOE's of the three protonated carbons was 0.27 (± 0.06). At 0.100 M $\text{Cr}(\text{acac})_3$, the mean NOE for the protonated carbons was only reduced to 0.13 (± 0.04). It was only after the $\text{Cr}(\text{acac})_3$ concentration was greater than 0.250 M that all carbons' NOE's were reduced to zero.

In addition, the $\text{Cr}(\text{acac})_3$ also causes some broadening and shifting of the various resonances. Figure 2 shows the relationship between $\text{Cr}(\text{acac})_3$ concentration and corresponding line width for each of the five pyrene lines. The line width reported is the average of the two values observed while measuring the carbon's NOE; one from the spectrum obtained with continuous proton decoupling and the other from that obtained with gated proton decoupling. For all carbons and $\text{Cr}(\text{acac})_3$ concentrations, the two line widths used for an average were within 0.3 Hz of each other. Figure 3 shows the effect of increasing $\text{Cr}(\text{acac})_3$ concentration on the position (relative to the position of the transmitter) of each of the five pyrene resonances.

As Figure 2 shows, the line widths of the quaternary carbons were always less than those of the protonated carbons. The addition of 0.100 M $\text{Cr}(\text{acac})_3$ nearly doubled the width of all five lines. At the highest concentration (0.300 M) the line widths were 3-4 times greater than with no relaxation reagent present. At the same time the positions

of the lines underwent a minor change. With 0.300 M $\text{Cr}(\text{acac})_3$, no line in any spectrum was shifted more than 0.2 ppm, or 4.0 Hz on the CFT-20.

However, the lines were not all shifted in the same direction. The protonated carbon resonances shifted downfield while those of the quaternary carbons shifted upfield - an observation in agreement with that noted for similar molecules in the presence of $\text{Cr}(\text{acac})_3$ (16). The relative positions of two such peaks change twice as fast as the absolute position of either one. Thus any two peaks may be as much as 0.33 ppm closer together or further apart in the presence of 0.300 M $\text{Cr}(\text{acac})_3$. At concentrations of 0.100 and 0.200 M $\text{Cr}(\text{acac})_3$, the separation of two resonances in similar hydrocarbons may experience a maximum change of ca. 0.11 and 0.22 ppm respectively. The actual value will vary depending on the molecule being considered (16).

These relationships between $\text{Cr}(\text{acac})_3$ concentration and NOE, line width and resonance position lead one to conclude that there are two approaches which will lead to elimination of NOE differences. The first method of accomplishing this is to add sufficient $\text{Cr}(\text{acac})_3$ for a concentration of 0.25 M in the sample solution. At this concentration all carbons have quenched NOE's and continuous proton decoupling is appropriate. However, this approach has other consequences. The line widths are two to three times greater and the separation between lines has changed by about 0.27 ppm. For samples with many closely spaced resonances this magnitude of line broadening and shifting can lead to coincidental peaks or to lines crossing and thus a loss of

information. For example in fossil fuel samples, where there are many closely spaced resonances with line widths already broadened due to the complex molecular composition (see for example (24)), such line broadening and shifting must be kept to a minimum to preserve and assign as many resonances as possible and obtain the maximum information. Use of such high $\text{Cr}(\text{acac})_3$ concentrations in the quantitative analysis of this sort of complex mixture would seem to be an unacceptable way of tackling the T_1 and NOE problems.

An alternative to using high concentrations of relaxation reagent while obtaining quantitative proton decoupled ^{13}C spectra is to use gated proton decoupling in conjunction with a lower relaxation reagent concentration. Use of ca. 0.1 M $\text{Cr}(\text{acac})_3$ reduces all T_1 's to a sufficient level to allow a short pulse repetition time (2-5 seconds). Gated proton decoupling is then needed to eliminate the remaining differences in NOE and assure accurate quantitative results.

This accuracy can be demonstrated with the spectra of pyrene obtained at each $\text{Cr}(\text{acac})_3$ concentration. A ratio defined as

$$R = \frac{(\text{sum of the areas of quaternary carbons, } \delta = 131.28 \text{ and } 124.81)}{(\text{sum of the areas of protonated carbons, } \delta = 127.47, 125.91 \text{ and } 125.03)}$$

may be calculated for the gated and continuous proton decoupling experiments. Since long pulse delays were used in these experiments, eliminating the effect on R of residual T_1 differences, R will relate only to the NOE's. Theoretically this ratio should be 0.600 for quantitative conditions. The results for the two types of decoupling are shown in Fig. 4. While the mean of the ratios obtained for the gated decoupling experiments is 0.589 (std. dev. \pm 0.017), the ratio from the continuous

proton decoupling does not approach the theoretical value until more than 0.2 M $\text{Cr}(\text{acac})_3$ is present. These data indirectly indicate the existence of NOE differences up to this $\text{Cr}(\text{acac})_3$ concentration.

Thus at the concentrations of $\text{Cr}(\text{acac})_3$ which have typically been employed (0.05 to 0.10 M) in the reported works, one must also use gated proton decoupling in order to achieve accurate, rapidly accumulated quantitative ^{13}C spectra. The $\text{Cr}(\text{acac})_3$ concentration becomes less critical in these experiments where gated decoupling is used to equalize NOE's since a wide concentration range is effective in achieving the desired T_1 reduction. The pulse repetition time need only be chosen so that at the $\text{Cr}(\text{acac})_3$ concentration used the magnetization of each carbon returns to its equilibrium value prior to the next pulse. For 0.05 and 0.10 M $\text{Cr}(\text{acac})_3$, pulse repetition times of 4-8 s and 2-5 s respectively, are sufficient to eliminate T_1 differences even in samples which have exceptionally long T_1 's when no relaxation reagent is present.

In this way the prime sources of error in quantitative pulse Fourier transform ^{13}C NMR, T_1 and NOE differences, can be eliminated with minimal line broadening and shifting. A precaution one needs to observe with this approach (besides the previously mentioned instrumental and digitization factors) is to be aware of large-scale complex formation between the relaxation reagent and the sample (e.g., via carbonyl, OH, NH_2 functional groups)(10,25). Such preferential association can reduce the effective local concentration of relaxation reagent available to all carbons and lead to a disruption of quantitative

conditions. Other instrumental methods, such as IR spectroscopy, may be used prior to the NMR run to indicate the presence of such functional groups and warn of any impending problems which may invalidate the quantitative ^{13}C results obtained with the PARR technique.

CREDIT

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LIST OF FIGURES

Figure 1. NOE of the five pyrene resonances as a function of $\text{Cr}(\text{acac})_3$ concentration. ($\diamond \cdots \diamond$) C-3a,5a,8a,10; ($\times \text{---} \times$) C-4,5,9,10; ($\square \text{---} \square$) C-2,7; ($\odot \cdots \odot$) C-1,3,6,8; ($\triangle \bullet \text{---} \triangle$) C-10b,10c.

Figure 2. Average line width of the five pyrene resonances as a function of $\text{Cr}(\text{acac})_3$ concentration. ($\diamond \cdots \diamond$) C-3a,5a,8a,10a; ($\times \text{---} \times$) C-4,5,9,10; ($\square \text{---} \square$) C-2,7; ($\odot \cdots \odot$) C-1,3,6,8; ($\triangle \bullet \text{---} \triangle$) C-10b,10c. The correlation coefficients vary between 0.980 and 0.990.

Figure 3. Line position of the five pyrene resonances as a function of $\text{Cr}(\text{acac})_3$ concentration. ($\diamond \cdots \diamond$) C-3a,5a,8a,10a; ($\times \text{---} \times$) C-4,5,9,10; ($\square \text{---} \square$) C-2,7; ($\odot \cdots \odot$) C-1,3,6,8; ($\triangle \bullet \text{---} \triangle$) C-10b,10c. The correlation coefficients vary between 0.951 and 0.985.

Figure 4. Experimental and theoretical ratio (R) of the sum of the quaternary carbons' integrals to the sum of the protonated carbons' integrals of pyrene as a function of $\text{Cr}(\text{acac})_3$ concentration; (---) theoretical ratio = 0.600; ($\square \text{---} \square$) continuous proton decoupling, NOE-enhanced; ($\odot \cdots \odot$) gated proton decoupling, NOE-suppressed.

