

^{13}C NMR SPECTROSCOPY: RELAXATION TIMES OF ^{13}C AND METHODS FOR SENSITIVITY ENHANCEMENT

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ABSTRACT

Developments in techniques for studying ^{13}C nmr at natural abundance are reviewed with particular emphasis on Fourier transform (FT) methods and multiple pulse versions of the FT technique, such as driven equilibrium Fourier transform (DEFT).

The mechanisms of spin-lattice relaxation of ^{13}C are discussed in the light of T_1 data for a number of small molecules. Contributions from dipolar interactions, spin-rotation, chemical shift anisotropy and scalar coupling interactions have been found. T_2 for ^{13}C can be appreciably shorter than T_1 when ^{13}C is coupled to a more rapidly relaxing nucleus, such as a proton. The implications of this result on sensitivity enhancement by FT and DEFT methods are discussed.

INTRODUCTION

NMR study of carbon-13 is now an extremely active area; in fact, at the 12th Experimental NMR Conference six months ago exactly half the papers dealt in some way with this nucleus. This popularity has not always been enjoyed by ^{13}C . For many years following the initial reports of ^{13}C nmr in a number of organic molecules ^{13}C remained in the class often described as 'less receptive nuclei,' and its study was undertaken by only a few investigators who foresaw the importance of such investigations and who had the patience to work under very adverse conditions. At this commemoration of the discovery of nmr 25 years ago, it seems appropriate to review briefly the early efforts in the study of ^{13}C nmr and to point out the improvements in technique effected at various stages.

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Table 1. Some properties of carbon-13 nuclei

Property	^{13}C	^1H
Spin	1/2	1/2
Frequency	25.1 MHz	100 MHz
Sensitivity	0.016	1.00
Abundance	1.1%	99.9%
T_1 (typical)	1-40 s	1-20 s
Chemical shift range	220 ppm	12 ppm

First we might summarize with the aid of *Table 1* some of the properties of ^{13}C in comparison with hydrogen in order to indicate both the problems and promise of ^{13}C studies. The widespread desire to study ^{13}C nmr stems primarily from the ubiquitousness of carbon in organic compounds and from the large shift relative to that of the proton, the latter property holding much hope for spectral resolution of carbon atoms that differ only slightly in their chemical properties. Unfortunately the magnetic moment of ^{13}C is only about one-fourth that of the proton, so that at constant field the sensitivity of ^{13}C nmr is reduced by a factor of about 60. To make matters worse, the natural abundance of ^{13}C is only 1.1 per cent, so that studies of ^{13}C in its natural form suffer an overall sensitivity loss relative to ^1H of about 5700. For many years it was thought that ^{13}C relaxation times were very long, and

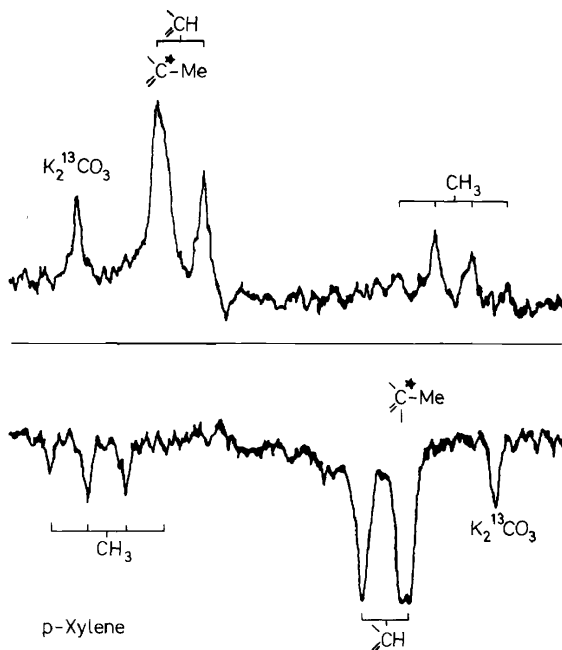


Figure 1. ^{13}C spectrum of *p*-xylene, obtained by rapid passage method. Upper trace, H_0 swept toward higher values; lower trace, H_0 swept toward lower values. Lauterbur.²

^{13}C RELAXATION AND SENSITIVITY ENHANCEMENT

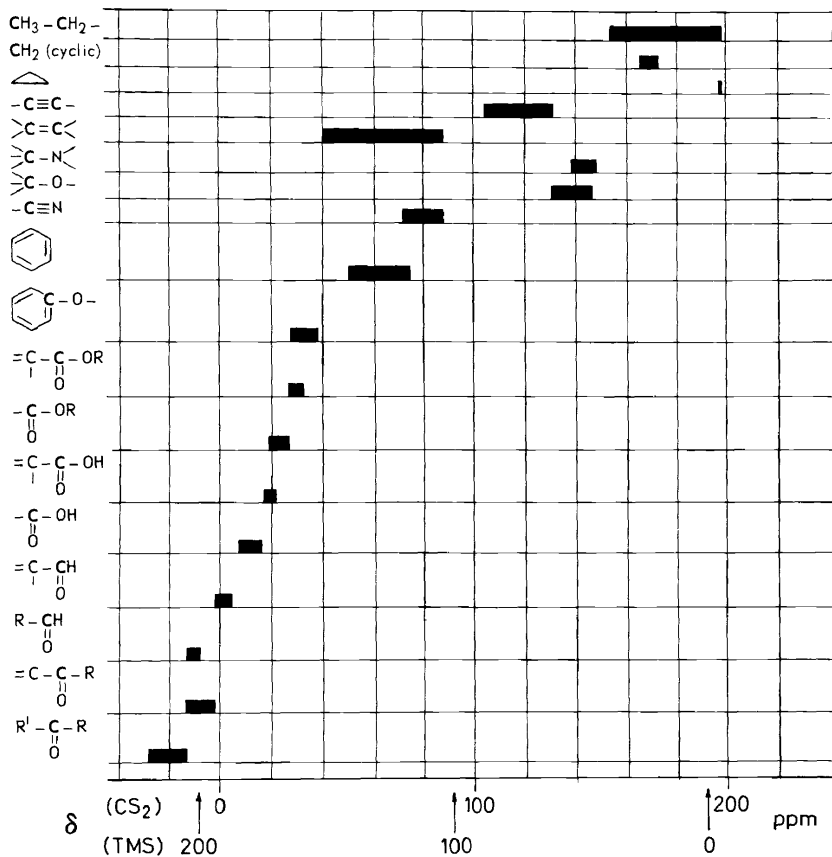


Figure 2. Correlation of ^{13}C chemical shift with functional groups. Scales referenced both to ^{13}C in CS_2 and to ^{13}C in tetramethylsilane are shown.

that saturation problems would be severe, but, as we shall see in much more detail later, that fear was exaggerated.

The first report of ^{13}C resonance at natural abundance was published in 1956 by Lauterbur¹, who used adiabatic rapid passage at 8.5 MHz to study a series of neat organic liquids contained in 15 mm sample tubes. With sweep rates ranging up to 100 Hz s^{-1} and large values of H_1 , small couplings were obscured, but chemical shifts and large, one-bond $^{13}\text{C}-\text{H}$ couplings could be studied. An example of such rapid passage spectra, from one of Lauterbur's early papers², is shown in Figure 1. Because of artifacts associated with the rapid passage, the spectra display pronounced asymmetry and require scans with both increasing and decreasing field to obtain reliable data. With these methods many ^{13}C spectra were obtained, and a large amount of chemical shift data tabulated and interpreted, particularly by Lauterbur², Stothers³, and Spiess and Schneider⁴. The general features of ^{13}C chemical shifts have been summarized by Stothers⁵ and are illustrated in Figure 2.

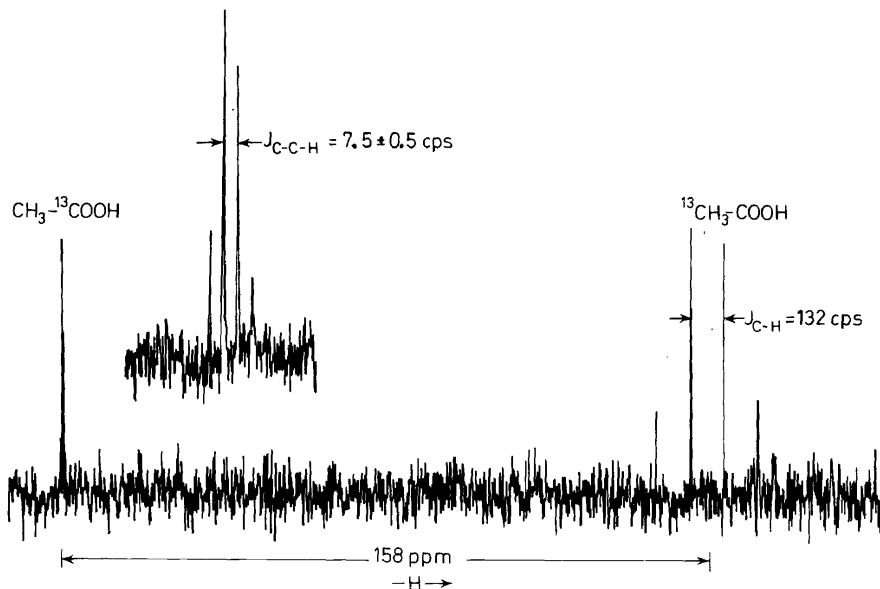


Figure 3. ^{13}C spectrum of acetic acid, showing one-bond and two-bond ^{13}C -H couplings. Shoolery⁶

Two major advances in ^{13}C technique were reported at the 3rd Experimental NMR Conference, in 1962. Shoolery⁶ showed that it was possible to obtain good 15 MHz high resolution *slow passage* spectra of neat liquids in *spinning* 12 mm sample tubes. With the greatly improved resolution (ca. 1 Hz) it was possible to resolve features not previously observed, as indicated in Figure 3, where splitting due to the two-bond ^{13}C -H coupling in acetic acid is clearly shown. The second technical improvement to come to light at this time was the use of selective proton decoupling to enhance the signal not only by collapsing multiplets to a single line but also by increasing the

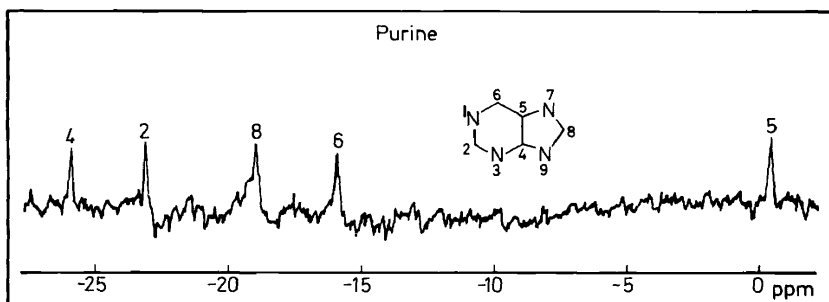


Figure 4. ^{13}C spectrum at 15 MHz of purine with proton decoupling. Pugmire, et al.⁹

area under the line through the nuclear Overhauser effect (NOE). Shoolery⁶, Lauterbur⁷, and Grant⁸ independently and concurrently showed the advantages of proton decoupling; and Grant and coworkers⁹ demonstrated that the decoupling frequency could be changed manually as the spectrum was swept in order to enhance signals from different carbon atoms and to provide an aid to their assignment. An example of this technique is shown in *Figure 4*.

A great leap forward in nmr techniques in general came with the introduction of special-purpose computers to permit signal-to-noise enhancement by time averaging, and ^{13}C nmr shared in the benefits of this method. In addition, the availability of frequency synthesizers permitted the development about 1966 of frequency sweep instruments using a heteronuclear (usually proton) lock. Roberts¹⁰ and Grant¹¹ and their coworkers used 15 MHz frequency sweep instruments of this sort to obtain and interpret a large mass of very valuable spectral data on ^{13}C resonances in complex organic molecules. More recently the development of the present generation of commercial spectrometers equipped with frequency sweep and heteronuclear lock, together with magnets that permit the use of sample tubes of 12 mm diameter or more, has permitted widespread studies of ^{13}C at frequencies of 22–25 MHz.

About the time that the first frequency sweep ^{13}C spectrometers were introduced, Ernst¹² demonstrated that random noise modulation of the decoupling frequency permits effective broad band decoupling. Weigert, Jautelat and Roberts¹³ were the first to report ^{13}C studies in complex molecules with noise-modulation to decouple simultaneously all protons in the molecules. A 'noise decoupler' is now considered an absolutely essential part of any ^{13}C spectrometer. While noise modulated decoupling usually provides the optimum ^{13}C signal, the concomitant loss of all information on spin-spin couplings between carbon and protons is sometimes too large a price to pay in terms of making spectral assignments. Johnson¹⁴ showed that the sensitivity-enhancing advantage of the nuclear Overhauser effect could be retained but information on one-bond C—H coupling acquired as well by the use of 'off-resonance decoupling,' in which the bandwidth of the coherent proton decoupling power is adjusted to leave 'residual splittings' in the ^{13}C spectrum. The number of lines in the residual multiplet indicates the number of directly attached protons, and the magnitude of the residual splitting is related to the difference between the proton resonance frequency and the decoupling frequency¹².

The most recent advance in ^{13}C nmr techniques is the development of Fourier transform methods—truly an order of magnitude improvement over conventional time averaging procedures in enhancing signal-to-noise. Although the use of FT methods in proton nmr had been demonstrated by Ernst and Anderson¹⁵ in 1966, the application to ^{13}C was impeded by spectrometer and computer limitations and by fears that long T_1 s would negate much of the advantage of the FT technique. The first successful applications of the FT method to ^{13}C nmr were reported by Farrar, et al.¹⁶, Keller¹⁷, and Sternlicht¹⁸ less than two years ago. Since then the method has mushroomed in popularity with a number of commercial and home-made instruments now available. An example of the type of study that can

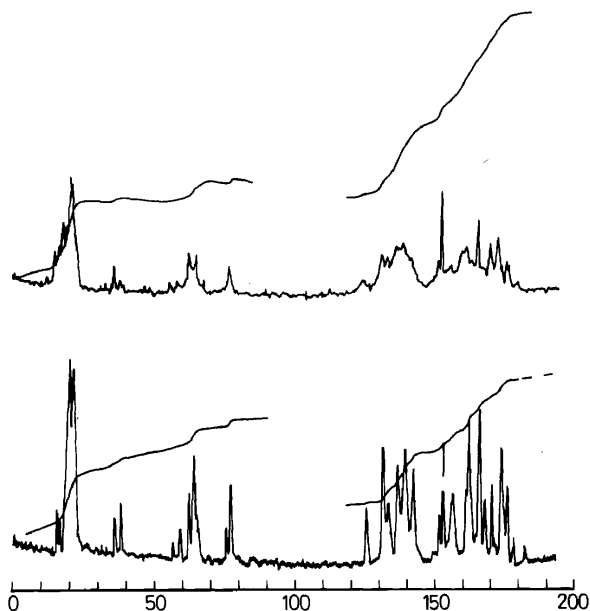


Figure 5. FT ^{13}C spectrum of ribonuclease A at 15 MHz with proton noise-modulated decoupling. (A) Native protein, 0.017 M, 32,768 scans in 6 h. (B) Denatured protein, 0.015 M, 31,284 scans in 12 h. Scale is ppm from $^{13}\text{CS}_2$. Allerhand, et al.¹⁹

now be readily accomplished by ^{13}C FT nmr (with noise decoupling) is the elegant work of Allerhand et al.¹⁹, on ribonuclease, illustrated in Figure 5. Spectra with generally acceptable signal-to-noise (~ 10) can be obtained with concentrations as low as 0.02 M in times of the order of 6–12 hours.

^{13}C RELAXATION MECHANISMS

One of the major interests of our group at the National Institutes of Health and the National Bureau of Standards has been the investigation of the relaxation of ^{13}C . A knowledge of such relaxation rates and the basic mechanisms that determine those rates is essential for the efficient application of Fourier transform and NOE techniques. We review here briefly the possible mechanisms of spin-lattice relaxation of ^{13}C and summarize our results. A more complete account of this work, together with a review of the pertinent literature, will be published elsewhere²⁰.

Several interactions can contribute to the relaxation rate, R_1 , ($R_1 \equiv 1/T_1$) of a ^{13}C nucleus: (1) dipole-dipole, R_1^d ; (2) chemical shift anisotropy, R_1^{CSA} ; (3) scalar relaxation, R_1^{SC} ; and (4) spin-rotation, R_1^{SR} . The observed rate is the sum of these four terms.

Dipolar relaxation arises primarily from nearby nuclei with large magnetic moments, usually ^1H in the molecules studied here. (We neglect intermolecular dipole-dipole interactions, which should be quite small.) In the

'extreme narrowing condition' that characterizes the motion of small molecules, R_1^d for ¹³C relaxed by ¹H is given by ²¹

$$R_1^d = \frac{\gamma_H^2 \gamma_C^2 \hbar^2}{r^6} \tau_c \quad (1)$$

where γ_H and γ_C are the magnetogyric ratios of ¹H and ¹³C, which are separated by a distance r , and τ_c is the correlation time for the rotational motion of the internuclear vector r . τ_c varies exponentially with temperature as

$$\tau_c = \tau_c^0 \exp E/kT \quad (2)$$

As τ_c decreases with increasing temperature, R_1 also decreases.

R_1^{csa} is proportional to the molecular reorientation time but is also dependent on magnetic field ²¹:

$$R_1^{csa} = \frac{2}{15} \gamma_C^2 H_0^2 (\sigma_{\parallel} - \sigma_{\perp})^2 \tau_c \quad (3)$$

where H_0 is the applied magnetic field, and $(\sigma_{\parallel} - \sigma_{\perp})$ is the anisotropy in the chemical shift.

R_1^{sc} arises from scalar spin coupling between ¹³C and a nucleus that relaxes rapidly, usually by quadrupolar interactions. R_1^{sc} is given by ²¹

$$R_1^{sc} = \frac{8\pi^2 J^2}{3} S(S+1) \frac{T_{1S}}{1 + 4\pi^2 (\nu_C - \nu_S)^2 (T_{1S})^2} \quad (4)$$

where J is the spin-spin coupling constant between ¹³C and a spin S with relaxation time T_{1S} , and Larmor frequency ν_S . ν_C is the Larmor frequency of ¹³C. Of the molecules studied here, only those containing I or Br are possible candidates for scalar relaxation, since these are the only nuclei where $T_{1S} \approx 1/2\pi(\nu_C - \nu_S)$. The behaviour of R_1^{sc} with temperature depends on the relative magnitudes of $(\nu_C - \nu_S)$ and T_{1S} . The contribution of R_1^{sc} from ⁷⁹Br will increase with temperature, opposite to that from ⁸¹Br and ¹²⁷I.

R_1^{sr} depends on the interaction of ¹³C with the overall molecular rotation ²² and is given by

$$R_1^{sr} = (2\pi I k T / \hbar^2) C_{\text{eff}}^2 \tau_J \quad (5)$$

where I is the molecular moment of inertia, C_{eff} is the effective spin-rotation coupling tensor, and the correlation time τ_J is given by

$$\tau_J = I/6kT\tau_c \quad (6)$$

Thus R_1^{sr} varies inversely with τ_c , hence *increases* with increasing temperature. Studies of the temperature dependence of T_1 can, then, provide insight into the contributions from the various relaxation mechanisms.

Figure 6 shows the temperature dependence of R_1 at 15 MHz for seven small molecules, each enriched to 60 per cent in ¹³C. The straight lines with positive slope obtained for CHCl₃ and CH₃¹³COOH indicate that R_1 is mostly dipolar. The curve for CH₃OH shows that R_1^d dominates over most of the temperature range with increasing contributions from R_1^{sr} at higher

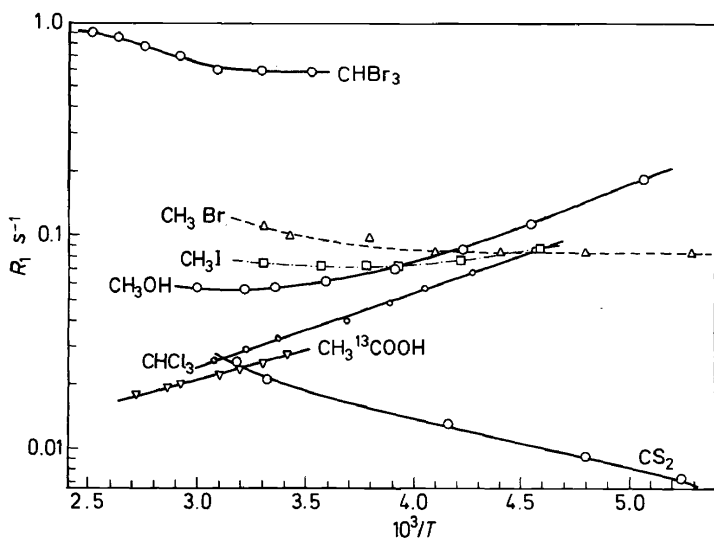


Figure 6. $R_1(^{13}\text{C})$ vs. $1/T$. Data obtained by 180° , τ , 90° pulse method at 15 MHz on 60% enriched neat liquids.

temperatures. The data can readily be fit to two straight lines. The magnitude of R_1 for CH_3Br and CH_3I indicates that R_1^d dominates also in these cases, but the shape of the curves demonstrates either the presence of R_1^{sr} or R_1^{sc} or both. Scalar relaxation is expected to be important for ^{13}C bound to Br. The importance of this mechanism very probably accounts for R_1 being an order of magnitude larger in the case of CHBr_3 than in the other molecules. The almost linear inverse temperature dependence of R_1 for CS_2 shows that the relaxation at 15 MHz is almost entirely due to spin-rotation.

Further insight into the contribution of dipolar relaxation may be obtained from the results of nuclear Overhauser enhancement studies. Kuhlmann, et al.^{2,3}, showed that η , the fractional increase in the intensity of the ^{13}C signal on irradiation at the proton frequency is given by

$$\eta = 1.988 R_1^d / R_1 \quad (7)$$

Table 2. T_1 and NOE results at 30°C

Molecule	η	T_1 s	$R_{s^{-1}}$	R_s^d s^{-1}	$R_{s^{-1}}^{\text{other}}$
CHCl_3	1.75	32.4 ± 0.5	0.0309	0.027	0.004
$\text{CH}_3^{13}\text{COOH}$	1.44	41.1 ± 0.6	0.0243	0.018	0.006
CH_3OH	0.92	17.5 ± 0.3	0.057	0.027	0.031
CH_3I	0.52	13.4 ± 0.2	0.075	0.019	0.056
CH_3Br	0.30	8.8 ± 0.2	0.114	0.017	0.097
CHBr_3	0.30	1.65 ± 0.05	0.61	0.10	0.51

Thus from a measurement of R_1 and of η , R_1^d can be calculated. Our NOE results, together with values of R_1 and R_1^d , are given in *Table 2* for the six hydrogen-containing molecules. As suggested by the temperature dependence in *Figure 6*, ^{13}C in CHCl_3 is relaxed almost entirely by the dipolar mechanism, with dipolar relaxation the dominant contribution also in CH_3COOH . In CH_3OH at 30° both NOE results and graphical analysis of the temperature-dependent R_1 data indicate that the dipolar contribution is about half the total, with the other half attributable to spin-rotation relaxation. This distribution is, of course, strongly dependent on temperature.

The three bromine and iodine-containing molecules have very low NOEs and are thus relaxed primarily by mechanisms other than dipolar interactions. Gillen, et al.²⁴, found that spin-rotation is an important cause of relaxation in CH_3I . Scalar coupling to I might also be expected to contribute to the ^{13}C relaxation in CH_3I . However, we find no variation of T_1 (^{13}C) over the frequency range 9–19 MHz²⁰; hence we conclude that scalar coupling is at most of very minor importance in the relaxation of $^{13}\text{CH}_3\text{I}$.

In the two bromine-containing molecules, however, scalar coupling is likely to be especially important because of the near coincidence of the Larmor frequencies of ^{79}Br and ^{13}C . The very short T_1 in CHBr_3 certainly seems to be due to scalar coupling relaxation, since the NOE results show a small dipolar contribution, and spin-rotation is not likely to be larger than in CHCl_3 , where it was shown to be negligible.

From our results CS_2 is seen to be relaxed almost entirely by spin-rotation interactions. Spiess, et al.²⁵, in a careful temperature and field dependence study, concluded also that R_1^{sr} is dominant, but that R_1^{csa} contributes at low temperatures and high fields.

We have carried out only limited frequency dependence studies to test for contributions from R_1^{csa} . We found no difference in R_1 for CH_3I and CH_3OH at 9, 15 and 19 MHz; for $\text{CH}_3^{13}\text{COOH}$ we observed a small apparent variation of R_1 with frequency, but recent work by Haeberlen²⁶ indicates that R_1 for this molecule is the same at 62 MHz as our value at 15 MHz.

It is known that the spin-rotation contribution to relaxation in the vapour phase can be orders of magnitude larger than in the liquid²⁷. If care is not taken to impede liquid–vapour interchange, the apparent R_1 for a volatile liquid may be much larger than the true value, since a large portion of the relaxation actually occurs in the vapour. Our results were obtained with tubes constricted between the measured portion of the liquid and the liquid surface²⁸. This type of sample tube effectively precludes rapid relaxation in the vapour from obscuring the liquid phase relaxation. With ordinary sample tubes, however, spurious results may be obtained, as indicated in *Figure 7* for $\text{CH}_3^{13}\text{COOH}$.

All data reported here are for samples that were carefully degassed. We have not studied in detail the effect of dissolved oxygen, but we did make one observation for $\text{CH}_3^{13}\text{COOH}$, which has a rather long T_1 , hence should be sensitive to small changes. Opening the sample tube and shaking it increased R_1 from 0.025 s^{-1} to approximately 0.05 s^{-1} . This increment of about 0.025 s^{-1} is much smaller than that commonly found for dissolved oxygen affecting ^1H relaxation, typically of the order of 0.3 s^{-1} .

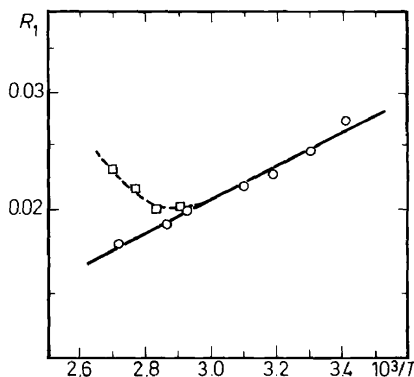


Figure 7. $R_1(^{13}\text{C})$ for 60% enriched $\text{CH}_3^{13}\text{COOH}$. Solid line, sample in constricted tube (see text); broken line, sample in ordinary cylindrical nmr tube

On the basis of the new data presented here, together with other recently reported results, we can make the following observations about ^{13}C relaxation in small molecules.

1. The relaxation is primarily due to ^1H - ^{13}C dipole-dipole interaction if ^{13}C is bonded directly to one or more protons.

2. Spin-rotation can be expected to contribute in small molecules, especially at temperatures near the boiling point, and to be a dominant relaxation mechanism for CH_3 groups due to the free internal rotation about the $\text{CH}_3\text{—R}$ bond.

3. Chemical shift anisotropy is, in general, not a significant relaxation mechanism.

4. Scalar relaxation only rarely contributes to R_1 and then only when ^{13}C is bonded to an appropriate quadrupolar nucleus, such as ^{79}Br and ^{81}Br , but probably never ^{127}I .

DRIVEN EQUILIBRIUM FOURIER TRANSFORM METHODS

One of our major objectives in studying ^{13}C relaxation times was to obtain the necessary information to optimize conditions for Fourier transform experiments. For signal enhancement purposes an FT experiment consists of coherently adding the data from the free induction decays following repetitive pulses of 90° or smaller angles. The optimum time for data acquisition after each pulse depends on the resolution desired, the range of resonance frequencies expected and the computer memory available²⁹. For most ^{13}C studies acquisition times τ usually do not exceed 0.5–1 s. If $T_1 \gg \tau$, little restoration of the magnetization along the z axis occurs during the period τ ; hence the response to subsequent pulses is reduced. For large molecules (e.g., steroids) Allerhand and coworkers³⁰ have shown that ^{13}C relaxation times rarely exceed 1 s, so that the normal FT method is quite efficient. For smaller molecules, however, T_1 is longer, as we have seen. For such cases, Becker et al.³¹, proposed a method—driven

equilibrium Fourier transform (DEFT) nmr—to force the magnetization back to its equilibrium position along the z axis in a time shorter than that required for spin–lattice relaxation. The method consists of the pulse sequence $90^\circ, \tau, 180^\circ, \tau, 90^\circ$ as indicated in *Figure 8*. The first two pulses constitute a spin–echo sequence, and at time 2τ the dephasing of magnetization by field inhomogeneity has been reversed. The final 90° pulse restores the refocused magnetization to the z axis. The entire sequence can be repeated

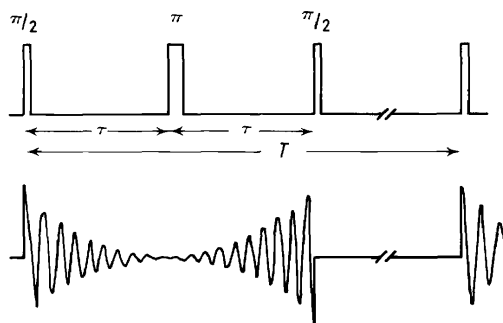


Figure 8. Pulse sequence used in DEFT nmr

after a waiting period $(T - 2\tau)$, and data acquired during the free induction decay following the initial 90° pulse of each sequence and during the rising portion of the echo. Waugh³² and Wallace³³ have examined theoretically the behaviour of the magnetization in the DEFT method and in other related multiple sequences^{32,34} and have calculated that very substantial savings in signal accumulation time (*ca.* a factor of 20) are possible provided $T_2^* \ll T_2 \approx T_1$. We summarize here our recent experimental results that confirm the theoretical calculations, but we point out some instrumental limitations that often prevent the achievement of the predicted improvement in signal-to-noise concurrent with good resolution. In addition we examine the frequently-occurring case in which $T_2 \ll T_1$; as expected the advantage of DEFT and related methods in this situation is considerably reduced.

Expressions for the behaviour of the magnetization and for signal-to-noise have been derived^{32,33} for both ordinary FT experiments (a succession of 90° pulses) and for DEFT. When a steady state has been established by application of a sufficiently large number of pulse sequences we may write

$$(S/N)_{\text{DEFT}} = T^{-\frac{1}{2}} \bar{M}_z = T^{-\frac{1}{2}} M_0 \left\{ 1 - \exp \left(-\frac{T-2\tau}{T_1} \right) \right\} / \left\{ 1 - \exp \left(-\frac{T-2\tau}{T_1} + \frac{2\tau}{T_2} \right) \right\} \quad (8)$$

$$(S/N)_{\text{FT}} = T^{-\frac{1}{2}} \bar{M}_z = T^{-\frac{1}{2}} M_0 \left\{ 1 - \exp \left(-\frac{T}{T_1} \right) \right\} \quad (9)$$

where \bar{M}_z refers to the magnetization once a steady state has been established, T is the period of one complete sequence, and τ was defined above. It has been shown³² that the optimum period for a standard FT experiment is

$$T_{\text{opt}} = 1.269 T_1 \quad (10)$$

Using this value of T in equation 9, we obtain an expression for the optimum S/N in an FT experiment for a given T_1 . We can define an enhancement factor, E for DEFT as $(S/N)_{\text{DEFT}}$ from equation 8 divided by $(S/N)_{\text{FT, opt}}$ from equation 9 with $T = T_{\text{opt}}$. E as defined in this way accounts for the signal during the free induction decay following the initial 90° pulse in each DEFT sequence but does not take account of the additional signal that can be acquired during the rising portion of the echo. If the latter signal is essentially a mirror image of the free induction decay (very long T_2 and no homonuclear couplings—see below), then the enhancement would actually be greater than E by a factor of $2^{\frac{1}{2}}$. On the other hand, E refers to the FID, not to the Fourier transformed spectrum, the intensity of which depends not only on the S/N in the FID but also on the resolution. Hence truncation of the FID (i.e., a short value of τ) may adversely affect S/N in the transformed spectrum.

In principle values of E of 5 or more (i.e., a saving in time of 25 or more) can be obtained when T_1 is long. Experimental verification of this prediction for ^{13}C resonance is difficult, since most ^{13}C systems observed in practice do not conform to the condition, assumed by all previous theoretical treatments of DEFT^{32,33}, that $T_1 = T_2$. Shoup and VanderHart³⁵ showed that scalar coupling between ^{13}C and ^1H or other more rapidly relaxing nuclei furnishes a mechanism for shortening T_2 (^{13}C). An example of the effect of proton scalar relaxation contributions to T_2 for ^{13}C is shown in Figure 9,

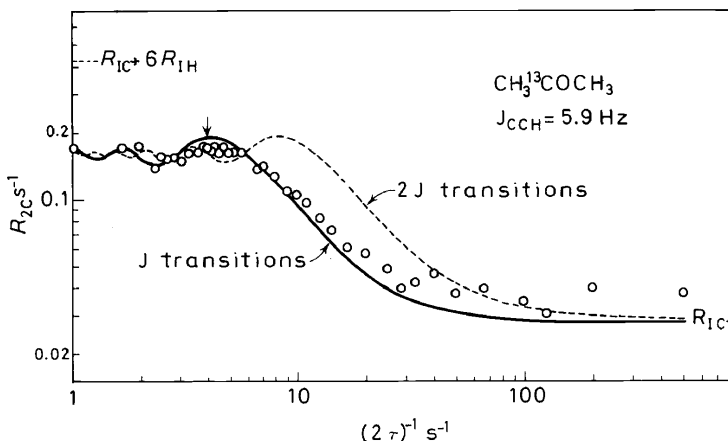


Figure 9. T_2 (^{13}C) in acetone-2- ^{13}C as a function of pulse repetition rate in a Carr–Purcell sequence. Experimental points are indicated; curves are based on the theory of Gutowsky et al.,³⁶ as discussed by Shoup and VanderHart.³⁵ J and $2J$ transitions refer to $\Delta I_z = \pm 1$ and ± 2 transitions between proton spin states

^{13}C RELAXATION AND SENSITIVITY ENHANCEMENTTable 3. T_1 and T_2 for ^{13}C

	T_1, s	T_2, s
$\text{CH}_3^{13}\text{COOH}$	41	3
CH_3I	13	4
$^{13}\text{CH}_3\text{COOCD}_3$	19	6
CHCl_3	32	0.3
CS_2	48	38 ^a

^aMay be near 48; see text.

where the transverse decay time for $\text{CH}_3^{13}\text{COCH}_3$ measured by the Carr Purcell technique is plotted vs. pulse repetition rate. The decay time measured in the slow pulsing limit represents the real $T_2 = 6.0 \text{ s}$ which is considerably shorter than $T_1 = 19.2 \text{ s}$. Values of T_1 and T_2 for some other ^{13}C -enriched molecules are given in Table 3. For the first three molecules, in which ^{13}C is coupled to ^1H , either through one or two bonds, T_2 is appreciably shorter than T_1 . In chloroform T_2 is especially short because of scalar coupling to the chlorine atoms.

We have made tests of the signal enhancements for all the molecules listed in Table 3. Details of this work will be published elsewhere³⁷, but the results for methyl acetate, which are typical for the hydrogen-containing molecules, are given in Figure 10. In this plot of enhancement factor against period, T , the experimental points are indicated; the solid curves are the theoretical plots from equations 8 and 9, while the dashed curves are the

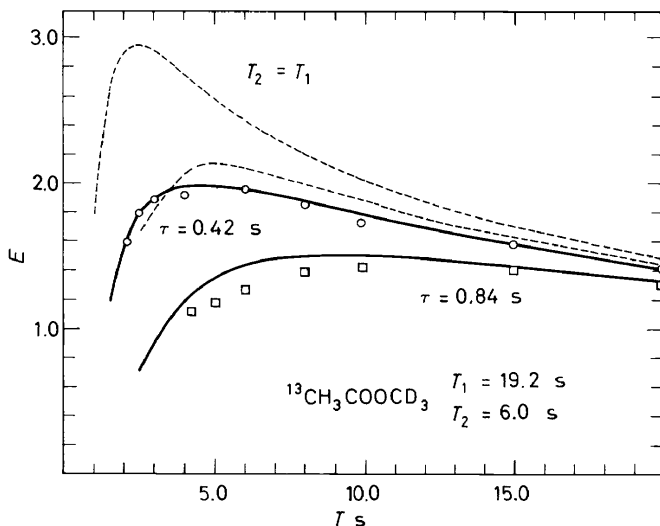


Figure 10. DEFT enhancement factor, E vs. period T for methyl acetate- d_3 . Solid line, theoretical curve for measured values of T_1 (19s) and T_2 (6 s). Broken curve, theoretical curve for hypothetical values $T_1 = T_2 = 19 \text{ s}$. Experimental points are shown.

corresponding theoretical curves for the hypothetical case where $T_1 = T_2 = 19$ s. It is apparent that the theoretical enhancements are readily achieved in practice but that the enhancements are smaller than those predicted for the case $T_1 = T_2$. Note also from *Figure 10* that the optimum value of E , which comes at $T = 6\tau$ for the case $T_1 = T_2$, is displaced to larger values of T when this equality is not obeyed.

From *Figure 10*, the optimum value of E for methyl acetate is about 2.0 for $\tau = 0.42$ s. Thus even in this situation where T_2 is only about one-third as large as T_1 , DEFT provides a saving in time of a factor of four or if the rising portion of the echo is included, a factor of nearly eight. However,

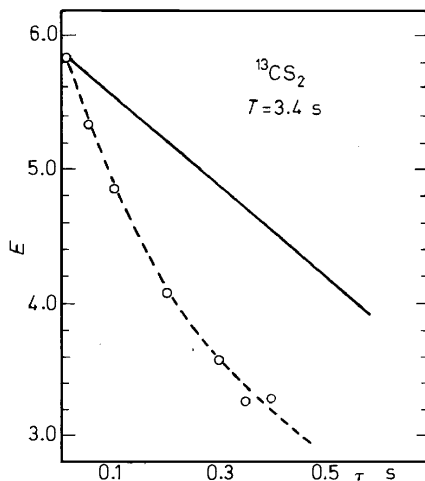


Figure 11. DEFT enhancement factor E vs. pulse separation time τ for $^{13}\text{CS}_2$

with $\tau = 0.42$ s, the resolution attainable is only about 2.5 Hz. If the inherent natural and instrumental linewidths are appreciably less than this value, spectral detail will be sacrificed, and signal-to-noise in the Fourier transformed spectrum will be reduced. With a value of $\tau = 0.84$ s, resolution is improved, but the optimum value of E is appreciably lower. Of course, if limited computer memory and large data rates make it impossible to use a long acquisition time even in the standard FT experiment, then the full enhancement of DEFT is achieved without resolution loss relative to the FT method.

In order to examine one ^{13}C system in which $T_1 \approx T_2$, we turned to CS_2 , where no scalar coupling exists.† *Figure 11* shows the enhancement factor

† The measured value of T_1 is 48 s and of T_2 is 38 s. While the T_1 value should be quite reliable, there can be large systematic errors in the measurement of long T_2 s. We believe that $T_2 \approx T_1 = 48$ s and use that value in the theoretical calculations.

as a function of τ for a fixed value of T . At very small values of τ (0.05 s) the measured value of E agrees well with the theoretical value and is quite large. With increasing τ , however, there is increasing disparity between calculated and observed enhancements. We believe that this effect is an instrument artifact, analogous to the error in long T_2 s alluded to in the footnote, and probably caused by very small magnetic field 'ripple' in our externally-locked magnet system.

In conclusion we can make the following generalizations about DEFT (and *mutatis mutandis* about other multiple pulse refocussing schemes as well):

1. DEFT is designed explicitly for studying nuclei with long T_1 s and is of no value when $T_1 \approx T_2^*$. It is now known that in most large molecules $T_1(^{13}\text{C}) \lesssim 1$ s, so that the possible range of application of DEFT in ^{13}C studies is to rather small molecules. For some other nuclei, such as ^{15}N , the method may have wider applicability.

2. For small molecules, where $T_1(^{13}\text{C})$ may be long, T_2 is usually less than T_1 because of scalar coupling to protons. The enhancement to be expected from DEFT is reduced in these cases. It is possible that noise-modulated proton decoupling with a very large H_1 may lengthen T_2 in some cases, but no evidence has yet been reported to support this proposal.

3. Because of J -modulation of spin echoes DEFT is not applicable to molecules with homonuclear coupling of the observed nucleus³⁸. For ^{13}C at natural abundance this limitation is unimportant.

4. With careful attention to instrument parameters, such as pulse widths and timing, it is possible with the unmodified DEFT method to achieve experimentally the theoretically calculated enhancements for moderate values of τ (~ 0.5 s) provided T_2 is not too long ($\lesssim 6$ s). (Modifications have been proposed which should make some of the instrumental adjustments less critical³², but we have not as yet incorporated these changes in our apparatus.) For long T_2 only very short τ can be used with optimum results unless very good magnetic field stability is achieved.

5. When adequate computer memory is available, the standard FT method is usually capable of providing better resolution than DEFT with approximately equivalent signal-to-noise. However, when computer memory is a limiting factor DEFT may furnish signal enhancement at no loss of resolution.

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