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A Bicyclic Ketone as a Solid-State ^{13}C NMR Intensity Reference

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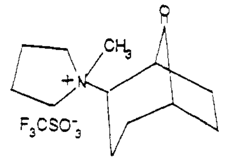
The synthesis and NMR relaxation properties of 4-(*N*-methylpyrrolidino)bicyclo[3.2.1]octan-8-one triflate (321) are described. The use of ^{13}CO-enriched 321 as a ^{13}C intensity reference is justified and demonstrated. The dominant ^{13}C peak in 321-^{13}CO is 217 ppm, far from most other ^{13}C chemical shifts of diamagnetic organic compounds. Hence, this intensity reference peak avoids peak overlap problems for most organic compounds.

INTRODUCTION

The introduction of magic-angle spinning (MAS) techniques has rendered solid-state NMR a highly versatile and powerful technique for the analysis of a wide range of solids, including polymers, resins, humic and fulvic materials, plant materials, and biological macromolecules.[1-6] In most ^{13}C MAS spectra that are reported, the spectral intensities of all the peaks are presented and interpreted in a *relative* sense, i.e., in terms of *ratios* of peak intensities *within* a spectrum. However, in order to maximize the value of any analytical technique, it is necessary to relate intensities to the quantity of analyte in an *absolute* sense. This usually requires the use of an absolute *intensity standard*, providing a peak in the spectrum for which the intensity has an *absolute* meaning, which in NMR represents a known number of nuclei.

The properties required of a suitable intensity standard in NMR are (1) chemical inertness and stability (so that the integrity of intensity interpretations is maintained in time), (2) suitable chemical shift in a vacant region of the spectrum (to avoid peak overlaps), (3) small (but ideally not too small) line width (so that small amounts of the intensity standard yield a substantial integrated intensity, for convenience and accuracy of measurement), (4) relaxation behavior that permits accurate absolute intensity measurements under the conditions of the NMR technique(s) of interest, and (5) the possibility of physical isolation from the analyte or convenient separation from it after the NMR analyses (e.g., a salt that can be extracted readily from a nonionic organic solid). For solid-state ^{13}C NMR the peaks most commonly employed as absolute intensity references have been methyl peaks of $(CH_3)_3Si$– compounds.[7] Such peaks, while often suitable, are frequently extremely sharp, making accurate peak integration somewhat difficult (albeit certainly not impossible), and sometimes overlap with analyte peaks in the region of 0 ppm (relative to tetramethylsilane, TMS) that occur in some kinds of samples, e.g., coals, oil shales, humic acids, and fulvic acids.[5,6] This laboratory has often employed the $-CH_2O-$ peak due to Delrin in a Delrin/zirconia MAS rotor as a "built-in" absolute intensity reference, which was calibrated in terms of an effective number of ^{13}C nuclei represented by the Delrin peak.[8,9] However, this peak, which occurs at 88.8 ppm in the ^{13}C NMR spectrum, also overlaps with spectral intensity for a variety of analytes. Of course, there is no single standard that avoids overlap problems with all peaks in the ^{13}C spectra of all possible analytes. The best one can hope for is to have a series of intensity standards suitable for various regions of the spectral ranges of possible interest, with the combination of these regions covering the entire ^{13}C chemical shift range. The present paper addresses the need for a ^{13}C intensity standard at the low-shielding end of the normal ^{13}C spectral range for diamagnetic materials.

An intensity standard in the 210–220 ppm range would be suitable for this purpose, falling beyond the ^{13}C chemical shift range for most samples, including organic geochemical samples. This chemical shift range has previously been reported for certain keto groups in five-membered rings, e.g., in some bicyclic compounds.[10] In this paper we describe the characteristics of 4-(*N*-methylpyrrolidino)bicyclo[3.2.1]octan-8-one triflate (referred to herein as compound 321) as a solid-state ^{13}C MAS intensity standard, especially for cross-polarization (CP) experiments. The synthesis and relevant relaxation behavior of 321 material that is ^{13}C-enriched at the carbonyl position are described.



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EXPERIMENTAL SECTION

4-(*N*-Methylpyrrolidino)bicyclo[3.2.1]octan-8-one Triflate. The natural-abundance 321 material was prepared via a three-step sequence, for which the first step is the synthesis of the pyrrolidine enamine of cyclopentanone via the reaction of pyrrolidine (Aldrich) with cyclopentanone (Aldrich), as described by Stork et al.[11] The distilled enamine (bp 71–75 °C at 4 Torr) was converted to bicyclo[3.2.1]octan-8-one by reaction with acrolein (Aldrich, dried over calcium sulfate and distilled under

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Ar, bp 68 °C at 650 Torr) in a 1:1 (molar) mixture in dry dioxane (0.5 mL of dioxane/mmol of enamine) with stirring at 0–25 °C for 3 h on the basis of the procedure of Stork and Landesman.[12] The dioxane was removed using a rotary evaporator (42 °C at 10 Torr) prior to distillation of the product (bp 72–78 °C at 2 Torr, 50% yield). The bicyclo[3.2.1]octan-8-one was converted to 321 by reaction with an equimolar quantity of methyl trifluoromethanesulfonate (Aldrich methyl triflate) in methylene chloride (5 mL/g of ketone) with stirring at 0 °C (during addition) to 25 °C over 3 h. Unreacted methyl triflate was quenched with an amount of methanol equal to the molar amount of methyl triflate added initially. The reaction mixture was then concentrated on a rotary evaporator (35 °C, 10 Torr), ethyl acetate was added to the resulting yellow viscous liquid, and the resulting mixture was again concentrated on a rotary evaporator (45 °C, 10 Torr) until off-white crystals formed. The impure solid was resuspended in cold ethyl acetate and then vacuum filtered. Recrystallization of the residue from a 9:1 (by volume) mixture of ethyl acetate:ethanol and isolation of crystals from the reconcentrated filtrate, followed by washing with ethyl acetate, yielded white crystals in 30% yield (mp 151–152 °C) after drying in a vacuum oven (75 °C, <1 Torr, 12 h).

The 321-^{13}CO compound (^{13}C-enriched at the carbonyl) (C-8) position) was prepared by a six-step sequence, the first three of which are the synthesis of cyclopentanone that is ^{13}C-enriched at the carbonyl position and the last three of which constitute the synthesis of 321-^{13}CO from cyclopentanone-^{13}*C* by the procedure described above for the natural-abundance case. In the first step (the synthesis of [1,6-$^{13}C_2$]adiponitrile), 10.8 g of 1,4-dichlorobutane (Aldrich) was added dropwise to 10.0 g (10% stoichiometric excess) of sodium cyanide (MSD Isotopes, 99 atom % ^{13}C, dried at 120 °C) in a slurry of 50 mL of dry dimethyl sulfoxide (Fischer DMSO, dried over CaH and distilled, bp 68 °C at 8 Torr) in a 300-mL flask according to a procedure adapted from that of Baran.[13] Before addition of $ClCH_2CH_2CH_2CH_2Cl$, the NaCN/DMSO mixture was first heated to 90 °C; then the dihalide was added slowly from an addition funnel at a rate such that the temperature of the reaction mixture, under reflux, did not rise above 160 °C. After all of the dihalide was added, the reaction mixture was stirred at 25 °C for 12 h and then filtered. The residual solids were washed with four 50-mL portions of CH_2Cl_2. The combined filtrate was washed with 100 mL of H_2O (to remove any NaCN) in a separatory funnel, and the aqueous layer was saturated with NaCl and then extracted five times with 20-mL portions of CH_2Cl_2. The combined organic phases were then concentrated on a rotary evaporator (30 °C, 10 Torr). The DMSO was removed from the concentrated organic phase by distillation (24 °C, 5 Torr); the ^{13}C-enriched adiponitrile (yield of this step: 88%) remained in the distillation pot (with a few mole percent DMSO, as determined by liquid-sample ^{13}C NMR on a Bruker AM-270 spectrometer).

In step 2, the conversion of [1,6-$^{13}C_2$]adiponitrile to [1,6-$^{13}C_2$]adipic acid, 100 mL of concentrated HCl was added to the [1,6-$^{13}C_2$]adiponitrile from step 1 and the mixture heated at 50 °C for 1 h, after which 50 mL of H_2O was added and the mixture heated to reflux (80–110 °C) for 3 h, according to the procedure of Mauthner.[14] The reaction mixture, including suspended solids, was transferred to a Buchner funnel and washed with 150 mL of saturated brine. The solid collected on the filter paper and the precipitate formed in the filtrate were pooled and recrystallized twice from H_2O. The yield of pure, dry [1,6-$^{13}C_2$]adipic acid [mp 151–152 °C (lit.[14] 153 °C)] was 82%.

In step 3, 7.9 g of the ^{13}C-labeled adipic acid from step 2 was pyrolyzed to cyclopentanone that was ^{13}C-enriched at the carbonyl position, using the procedure of Rand et al.[15] The adipic acid and anhydrous potassium fluoride (Aldrich) catalyst were dried in a vacuum oven (80 °C, <1 Torr) overnight prior to use. The pyrolysis/distillation equipment was based on a 4-in. Vigreux column wrapped with glass tape and a collection flask adapter terminated at a mineral oil bubbler. The reaction mixture, consisting of 7.9 g of [1,6-$^{13}C_2$]adipic acid and 157 mg of KF, was heated to 280–310 °C for ca 3 h until no more CO_2 formation (bubbling) was evident. The reaction mixture (4.5 g) was collected as a distillate. Liquid-sample ^{13}C NMR analysis of a 5-mg portion of this mixture in acetone-d_6 showed a 25:1 molar ratio of cyclopentanone (carbonyl chemical shift: 219 ppm) to adipic acid (carboxyl chemical shift: 174 ppm), with an overall 77% yield in step 3.

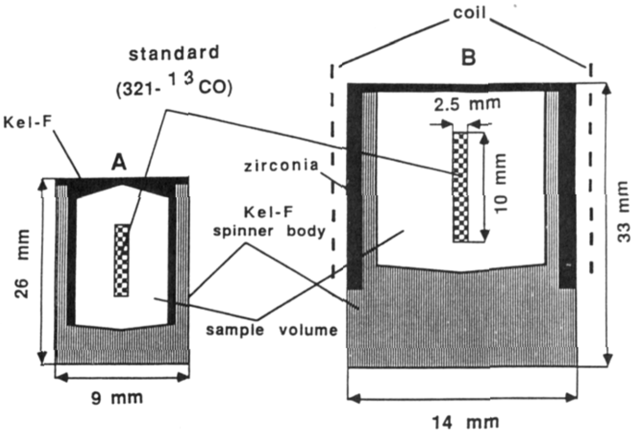

Figure 1. Design of the MAS rotor used in this study, shown as cross sections: (A) rotor designed for 0.33-cm^3 sample; (B) rotor designed for 2.4-cm^3 sample.

Steps 4–6 were carried out on the ^{13}C-enriched material in the same manner described above for the conversion of natural-abundance cyclopentanone to natural-abundance 321. The overall yield of carbonyl-^{13}C-enriched 321-^{13}CO was 1.8 g, starting with 10 g of Na^{13}CN in step 1. Mass spectrometric analysis of 321-^{13}CO, carried out at the Midwest Center for Mass Spectrometry at the University of Nebraska (Lincoln) using fast atom bombardment (FAB) ionization, confirmed the ^{13}C NMR analysis and verified the isotopic enrichment of the carbonyl carbon at 99% ^{13}C.

NMR Equipment and Techniques. All of the solid-state ^{13}C NMR experiments reported in this paper were carried out at 25 MHz on a home-built 100-MHz spectrometer, based on a wide-bore Nalorac superconducting magnet, a Nicolet 1180 data system with a 283B pulse programmer, and a home-built MAS probe. The MAS system is based on an updated version of the Delft design, utilizing a Kel-F rotor with a sample volume of about 0.33 cm^3 and a Kel-F tube (o.d., 2.5 mm; i.d., 1.0 mm) along the rotor axis for containing the intensity reference. Figure 1 shows a diagram of this MAS rotor and a similar one with a sample volume of about 2.4 cm^3.

As in any NMR experiment, the geometrical relationship between the sample and the transmitter/receiver coil is important. Unfavorable geometrical relationships can lead to distortions of both line shapes and intensities. Once a good geometrical arrangement is achieved (e.g., as shown in Figure 1, yielding no apparent line shape distortions), it is critical to reproduce this arrangement as one changes from sample to sample, including especially the calibration sample, hexamethylbenzene (HMB) in the present work. We found that in 10 repetitive independent assemblies of the rotor/sample/capillary-reference system containing fixed masses of HMB and 321-^{13}CO (in the capillary), the ratio of the HMB to 321-^{13}CO intensities showed a standard deviation of ±1.3%.

The MAS rotor speed for these experiments was about 3.5 kHz. All of the ^{13}C MAS pulse sequences employed in this study have been reported previously.

RESULTS AND DISCUSSION

1. ^{13}C NMR Spectrum of 321-^{13}CO. Figure 2A shows the ^{13}C NMR spectrum of a 250-mg natural-abundance sample of compound 321, obtained via $^1H \rightarrow {}^{13}C$ cross polarization (CP) and MAS, i.e., CP-MAS. The main peak of interest is the carbonyl carbon peak at 217 ppm. This is in a chemical shift region that is characteristic of the carbonyl carbons of

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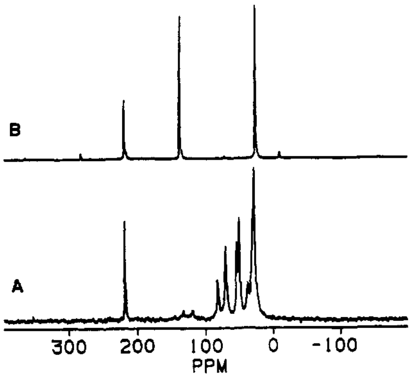

Figure 2. ^{13}C CP-MAS spectra of (A) natural-abundance 321 and (B) 12.1 mg of 321-^{13}CO in a capillary surrounded by 241 mg of HMB.

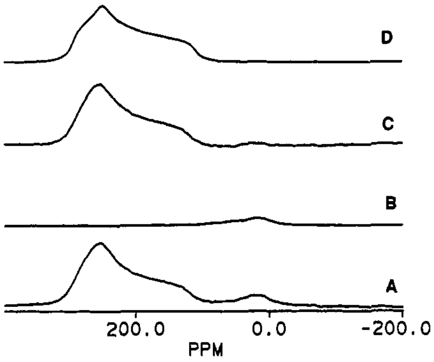

Figure 3. Chemical shift anisotropy pattern of 321 samples, obtained from CP experiments on static samples: (A) 321-^{13}CO standard; (B) natural-abundance 321; (C) difference spectrum, (A) - (B), the 321-^{13}CO CSA pattern due ideally to the carbonyl peak; (D) simulation of spectrum C.

five-membered-ring ketones[10] but is outside the region, on the low-shielding side, of most other ^{13}C chemical shifts of diamagnetic organic compounds. Figure 2B shows a ^{13}C CP-MAS spectrum of a 241-mg sample of powdered hexamethylbenzene (HMB, aromatic carbon peak at 132 ppm, methyl peak at 17.6 ppm) in a Kel-F spinner with 12.1 mg of 321-^{13}CO contained in the capillary tube positioned along the spinner axis (Figure 1). Because of the small amount of 321-^{13}CO employed and the fact that the carbonyl carbon of this compound has 99 times as much ^{13}C as any other position in the 321 molecule, only the 217 ppm peak of 321 shows up in Figure 2B, and its intensity is comparable to that of the HMB peaks. This demonstrates, as expected, that the carbonyl ^{13}C signal of a small sample of 321-^{13}CO is sufficiently intense to serve as a suitable intensity reference, assuming the relaxation behavior of the 321 carbonyl resonance is suitable. Furthermore, the small size of the MAS sidebands of the 321 carbonyl peak are sufficiently small that adequate intensity corrections can be made for them, at least at a low magnetic field like 2.3 T. Figure 3 shows the ^{13}C chemical shift anisotropy (CSA) pattern of 321-^{13}CO. In Figure 3A, obtained from a ^{13}C CP experiment on a static sample of 321-^{13}CO, one sees the CSA pattern of the carbonyl peak, together with a less intense pattern due to natural-abundance resonances of the other carbons. The chemical shift anisotropy pattern of unlabeled 321 compound is presented in Figure 3B. After subtraction of these two spectra, one obtains a spectrum dominated by the CSA pattern of the carbonyl resonance of 321-^{13}CO. This pattern was simulated (Figure 3D) in order to find the δ_{11}, δ_{22}, and δ_{33} values. From the simulation of the CSA pattern the following principal elements of the chemical shift tensor have been derived: $\delta_{11} = 290$ ppm, $\delta_{22} = 253$ ppm, $\delta_{33} = 108$ ppm.

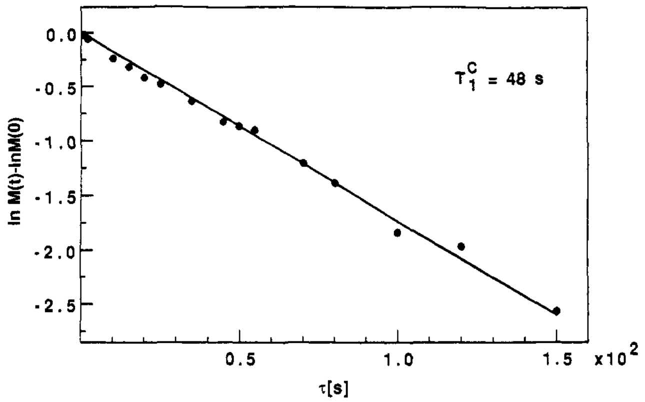

Figure 4. Plot of [ln M(t) - ln M(0)] vs t (the inversion recovery time) in a Freeman-Hill version of a ^{13}C CP-MAS inversion recovery experiment for T_1^C in 321-^{13}CO.

Table I. Relaxation Parameters Measured on a 321-^{13}CO/HMB Mixture[a]

^{13}C Peak	T_1^C (s)	T_1^H (ms)	$T_{1\rho}^H$ (ms)	T_{CH} (ms)
321 (carbonyl)	48 ± 2	620 ± 15	168 ± 5	0.33 ± 0.01
HMB (aromatic)	1.09 ± 0.04	125 ± 5	85.3 ± 3	1.35 ± 0.06
HMB (aliphatic)	0.63 (68%)[b] 0.14 (32%)	125 ± 5	60.0 ± 3	4.1 (55%)[b] 0.12 (45%)

[a] 12.1 mg of 321-^{13}CO and 241 mg of HMB. [b] Two components are seen in the relaxation behavior.

2. 321-^{13}CO Relaxation Characteristics Relevant to DP-MAS. In order to assess more definitively the suitability of compound 321 as a ^{13}C NMR intensity reference, it is necessary to characterize its relevant relaxation characteristics. For ^{13}C MAS experiments in which ^{13}C magnetization is generated *directly* via ^{13}C spin-lattice relaxation (DP-MAS, for direct polarization-MAS), the relevant relaxation time is T_1^C. DP-MAS experiments are not subject to the often discussed spin-dynamics complexities associated with cross-polarization (CP) techniques; and the advent of large-sample MAS rotors has made it possible in many cases to compensate for the signal-to-noise penalties paid as a result of the long repetition delays required by large ^{13}C spin-lattice relaxation times and by giving up the CP enhancement factor.[16] The solid-state ^{13}C MAS version of the Freeman-Hill version[17] of the inversion recovery experiment was carried out on the sample represented by Figure 2B. The results for the 321 carbonyl peak are shown graphically in Figure 4, and for all three of the dominant peaks they are summarized in Table I. One sees that the measured T_1^C value is 48 s. This value is sufficiently large to place severe restrictions on the use of this material as a ^{13}C intensity reference for DP-MAS experiments. However, this 48-s T_1^C value should not totally disqualify 321-^{13}CO as a DP-MAS intensity reference. Most ^{13}C DP-MAS applications will probably involve relatively long repetition delays because of relaxation constraints imposed by the *analyte*. For example, if a repetition delay (t_d) of 10 s were employed, then the correction factor $(1 - e^{-t_d/T_1})$ for the carbonyl peak of 321-^{13}CO would be 0.188; i.e., the observed ^{13}C signal of 321-^{13}CO will be only 18.8% of what it would be if $t_d > 5T_1^C$. Hence, one must put up with a reduced (by 81.2%) intensity of the 321-^{13}CO signal and a corresponding correction would have to be applied in the quantitative interpretation. Such corrections can be made reliably only if one has accurate values of the relevant T_1 parameters. These numbers are in general field dependent, so one has to determine them for each spectrometer field employed. Furthermore, especially for samples that consist of complex mixtures (even just a distribution of molecular weights in a

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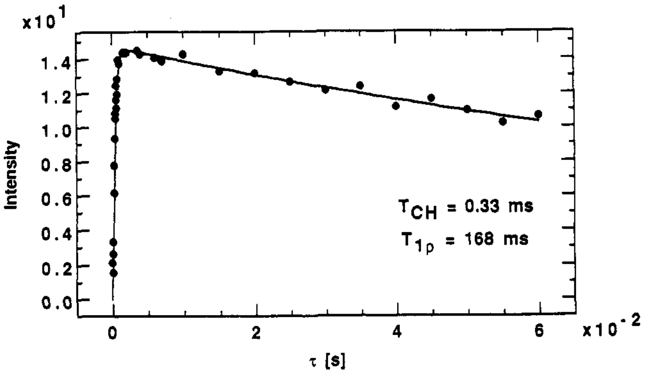

Figure 5. Variable contact-time curve for ^{13}C CP-MAS of 321-^{13}CO. Plot of ^{13}C intensity vs CP contact time, τ.

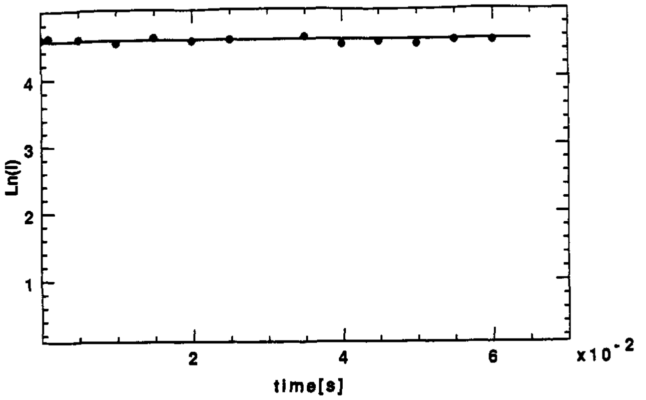

Figure 6. $T_{1\rho}{}^{C}$ relaxation, curve, from the variable ^{13}C spin-lock experiment, of 321-^{13}CO.

polymer), there may be a distribution of T_1 values, in which case a simple $(1 - e^{-t_d/T_1})$ correction is no longer justifiable.

3. 321-^{13}CO Relaxation Characteristics Relevant to CP-MAS. As discussed previously in the literature in several papers, including the detailed analysis of the ^{13}C signal of Delrin as a built-in ^{13}C NMR intensity reference,[8,9] the relaxation parameters relevant to cross polarization are $T_1{}^H$ (the proton spin-lattice relaxation time), $T_{1\rho}{}^H$ (the rotating-frame proton spin-lattice relaxation time), and T_{CH} (the CP relaxation time). In order to determine T_{CH} and $T_{1\rho}{}^H$, a variable contact-time (VCT) ^{13}C CP-MAS experiment was carried out on the 321-^{13}CO/HMB sample represented in Figure 2B. In the usual way, T_{CH} and $T_{1\rho}{}^H$ values were extracted from a computer fit of the data to eq 1,[18] where τ

$$M(\tau) = M^* \frac{1}{1 - T_{CH}/T_{1\rho}{}^{H}} (e^{-\tau/T_{1\rho}{}^{H}})(1 - e^{-\tau/T_{CH}}) \qquad (1)$$

is the CP contact time and M^* is the magnetization that would be obtained if $T_{1\rho}{}^H$ were infinitely large and T_{CH} infinitesimally small. Equation 1 is based on the assumption that $T_{1\rho}{}^C \gg T_{1\rho}{}^H$, a condition that we have verified in this case (vide infra). The VCT curve and computer fit are given in Figure 5; the parameters obtained are $T_{CH} = 0.33$ ms and $T_{1\rho}{}^H = 168$ ms. An independent determination of $T_{1\rho}{}^C$ by the well-known spin-lock technique[19] yielded an estimate of the order of seconds (see Figure 6), which is much larger than $T_{1\rho}{}^H$; the ^{13}C spin-locked magnetization was roughly constant over the entire 60-ms range of spin-lock periods. This combination of parameters is extremely favorable for quantitative measurements via CP-MAS; i.e., it is easy to choose τ such that $T_{CH} \ll \tau \ll T_{1\rho}{}^H$.

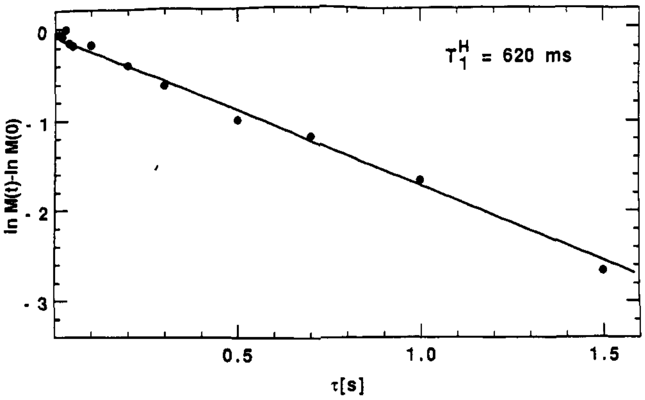

Figure 7. Plot of [ln M(t) – ln M(0)] vs t in a ^{13}C CP-MAS-detected Freeman–Hill version of a proton inversion recovery experiment.

The value of M^* computed from the VCT fit of eq 1 corresponds to 3.92 times the M_0 value one obtains on the same sample by the ^{13}C DP-MAS technique. This compares to the value 3.98 that is derived from the simple thermodynamic view of $^1H \rightarrow {}^{13}C$ cross polarization,[16] assuming that the number of protons is much greater than the number of ^{13}C's ($n^1H/n^{13}C$ is ~22 for 321-^{13}CO). This demonstrates that the CP process in the present case corresponds rather well to the popularly accepted view. The relaxation parameters measured for the 321-^{13}CO/HMB mixture are summarized in Table I.

In applying the results on CP dynamics, one must keep in mind the fact that $T_{1\rho}$ depends on the intensity of the spin-locking rf field, ω_1,[18,20] and that T_{CH} depends on both ω_1 and the MAS speed.[21-23] Furthermore, all of the relevant relaxation parameters depend on motion and, hence, on temperature. Therefore, the application of the CP approach via eq 1 is valid only for a very specific set of conditions. These conditions cannot be expected to carry over from one spectrometer to another; they carry over from day to day only to the extent that these parameters are constant and only if one is careful to maintain a stable Hartmann–Hahn match condition.[16,22-24] Certainly, each spectrometer and each MAS rotor-capillary system must be calibrated individually.

The other relaxation behavior directly relevant to ^{13}C CP-MAS quantitation is determined by $T_1{}^H$. Hence, the well-known ^{13}C CP-MAS-detected ^1H inversion recovery experiment was carried out on the 321-^{13}CO/HMB sample. The results are summarized in Table I and, for the 321-^{13}CO signal, are shown graphically in Figure 7. The measured $T_1{}^H$ value of 321-^{13}CO is 0.62 s, which is suitable for ^{13}C CP-MAS experiments with repetition delays of, e.g., at least 1 s, which is "typical" of ^{13}C CP-MAS experiments on a wide range of analytes. Of course, a suitable correction, based on the factor $(1 - e^{-t_d/T_1{}^H})$ must be applied to the 321-^{13}CO intensities, unless $t_d > 5T_1{}^H = 3.1$ s; this is not a significant limitation of 321-^{13}CO as a ^{13}C CP-MAS intensity reference.

4. Spin Counting with 321-^{13}CO as an Intensity Reference. As a check on the utility of 321-^{13}CO as a ^{13}C intensity standard, we have carried out quantitative ^{13}C CP-MAS and DP-MAS experiments on the sample consisting of 241 mg of HMB in a rotor with 12.1 mg of 321-^{13}CO in a

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capillary. The ^{13}C DP-MAS calibration spectra were obtained with 500 scans and a 300-s repetition delay, so that no $(1 - e^{-t_d/T_1})$ correction was necessary. From the relative integrated intensities of the 321-^{13}CO peak and the HMB peaks and from the known number of ^{13}C spins represented in the 321-^{13}CO resonance, one can calculate the number of spins represented in each HMB resonance: $(5.92 \pm 0.11) \times 10^{19}$ spins for the aromatic peak and $(5.90 \pm 0.10) \times 10^{19}$ spins for the CH_3 peak. Direct calculation for 241 mg of HMB and 1.02% ^{13}C abundance yields the value 5.94×10^{19} spins.

^{13}C CP-MAS calibration spectra were obtained with 400 scans, a 5-ms CP contact time (τ), and a repetition delay (t_d) of 3.5 s. From the integrated intensities obtained in those spectra, using corrections based on eq 1, one obtains the following results for the number of ^{13}C spins represented in each HMB peak: $(5.97 \pm 0.12) \times 10^{19}$ spins for the aromatic peak and $(5.93 \pm 0.12) \times 10^{19}$ spins for the CH_3 peak.

SUMMARY AND CONCLUSIONS

The ^{13}C NMR signal of the ^{13}C-enriched carbonyl position of 4-(*N*-methylpyrrolidino)bicyclo[3.2.1]octan-8-one triflate (321-^{13}CO), contained in a small amount within a capillary tube, is suitable for use as an absolute intensity reference in ^{13}C MAS experiments. This reference is especially suitable in CP-MAS experiments, because the relaxation properties of 321-^{13}CO relevant to CP-MAS experiments are favorable for convenient quantitation. For CP-MAS spin counting of hexamethylbenzene, the precision achieved is ±6%. For DP-MAS spin counting of HMB, the precision achieved directly is ±5% if very long repetition delays are employed.

Overall, 321-^{13}CO is a convenient and successful ^{13}C NMR intensity standard for samples with ^{13}C chemical shifts less positive (more shielded) than about 210 ppm. Hence, it is complementary to other intensity references, which have overlap problems in different regions of the spectrum.

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