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About Long-Lived Nuclear Spin States Involved in Para-Hydrogenated Molecules

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Abstract: This study deals with a spin system constituted of three nonequivalent protons, two of them originating from para-hydrogen (p-H_2) after a hydrogenation reaction carried out in the earth magnetic field. It is shown that three singlet states are created provided indirect (*J*) couplings exist between the three spins, implying hyperpolarization transfer toward the third spin. Upon insertion of the sample in the NMR (Nuclear Magnetic Resonance) high field magnet, the following events occur: (i) the longitudinal two-spin orders which are parts of the singlet states survive; (ii) the other two terms (of these singlet states) tend to be destroyed by magnetic field gradients but at the same time are partly converted into differences of longitudinal polarizations. Nuclear spin relaxation is studied by appropriate NMR measurements when evolution takes place in the high field magnet or in the earth field. In the former case, relaxation is classical although complicated by numerous relaxation rates associated with both longitudinal two-spin orders and longitudinal polarizations. In the latter case, an equilibration between the singlet states first occur, their disappearance being thereafter driven by relaxation rates which remain very small because of the absence of any dipolar contribution. Thus, even in the case of a three-spin system, long-lived states exist; this unexpected property could be very useful for many applications.

Introduction

As it is well-known and well documented,[1,2] a dramatic increase of sensitivity in NMR spectroscopy can be achieved by hydrogenation with *para*-enriched hydrogen provided that, in the product, the two hydrogen atoms (*A* and *B*) occupy nonequivalent positions. This phenomenon is known as PHIP (ParaHydrogen Induced Polarization), and very often one uses the term hyperpolarization. The enhanced ^{1}H NMR spectrum is generally supposed to appear in the form of two antiphase doublets (of splitting equal to the *J* coupling between *A* and *B*), which originate from a longitudinal two-spin order which is defined as

$$L_{AB} = I_z^A I_z^B \tag{1}$$

Owing to the fact that a radio frequency pulse "rotates" the spin operators, this longitudinal two-spin order can only be observed by a pulse of flip angle different from 90° (the optimum being a 45° pulse) leading, among other things, to the two observable quantities $I_z^A I_y^B$ and $I_y^A I_z^B$ (the radio frequency field being supposedly able to act along the *x*-axis of the frame in which the above quantities are defined).[3] There exist two experimental procedures: hydrogenation performed inside the magnet (the PASADENA experiment) or outside the magnet (the ALTADENA experiment). In this paper, we shall concentrate on the ALTADENA experiment.

The above considerations are valid as long as the spin system of the hydrogenated molecule is solely constituted by the two protons *A* and *B*. As soon as a third spin (*C*) is added, *J*-coupled to *A* and *B*, complications occur. One generally observes hyperpolarization transfers toward the third spin. This feature has been essentially exploited in cases where the third spin is a heteronucleus.[4−9] Moreover, in some cases, further transfers toward longitudinal polarizations (i.e., I_z^A, I_z^B, I_z^C) can occur. This has been reported quite recently[10] since *in phase* spectra (resulting presumably from a 90° pulse) are invariably shown. It has been stated in this publication that the time at which this

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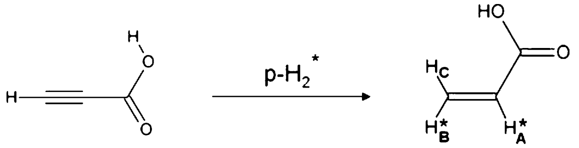

Figure 1. Sketch of the hydrogenation process (by para-enriched H_2) of propiolic acid (Sigma, CAS 471-25-0) homogeneously catalyzed by rhodium complex catalyst (Sigma, CAS 79255-71-3). Because of the absence of *J*-coupling with the labile acidic proton, one ends up with a *J*-coupled three-spin system which permits polarization transfers. *J*-coupling values are: $J_{AB} = 10.5$ Hz, $J_{AC} = 17.2$ Hz, $J_{BC} = 1.8$ Hz.

spectra are recorded (after the hydrogenation reaction) leads to the determination of the relaxation time pertaining to the so-called long-lived (or long-standing) nuclear spin states.[11−13] These long-lived states are singlet states which, in the case of the two *A* and *B* spins, correspond to the wave function $1/\sqrt{2}(\alpha_A\beta_B - \beta_A\alpha_B)$ and can be represented by the following linear combination of product operators

$$S_{AB} = E/4 - (I_x^A I_x^B + I_y^A I_y^B + I_z^A I_z^B) = E/4 - \left[\frac{1}{2}(I_+^A I_-^B + I_-^A I_+^B) + I_z^A I_z^B\right] \tag{2}$$

where *E* is the identity operator (which, anyway, will not affect the forthcoming calculations), while I_+ and I_- represent the raising and lowering operators. (In order to understand this latter property, we can construct the matrix associated with S_{AB} on the following basis: $\alpha_A\alpha_B$, $1/\sqrt{2}(\alpha_A\beta_B + \beta_A\alpha_B)$, $1/\sqrt{2}(\alpha_A\beta_B - \beta_A\alpha_B)$, $\beta_A\beta_B$, and verify that the only nonzero element is the diagonal element corresponding to the third function of this basis.)

In this communication, we would like to present our own experiments dealing with para-hydrogenation of propiolic acid (see Figure 1), a molecule which belongs to the same family as that of ref 10. In this three-spin system, we shall demonstrate that anti-phase doublets (arising from a longitudinal two-spin order as explained above) are indeed observed by means of a 45° pulse, superposed to in-phase multiplets. In the case of a 90° pulse, the longitudinal two-spin orders are evidently filtered out and only in-phase multiplets are visible as in ref 10. A qualitative theory will explain how, starting from singlet states, the spin system is able to switch to longitudinal two-spin orders *and* longitudinal polarizations.

Experimental Section

Sample Preparation. The solutes are commercially available from *Sigma*, and the perdeuterated solvent from *Eurisotop*. All these compounds have been used without further purification. The reactions have been directly performed in o.d. 5 mm NMR tubes equipped with Young valves and connected to a vacuum/hydrogen line. The hydrogenation process is homogeneously catalyzed. It has to be carried out in two steps, as the catalyst has to be activated before being used. Thus, the Rhodium complex (CAS 79255-71-3), dissolved in acetone-d_6, is first activated by hydrogenation using normal hydrogen at ca. 1.4 bar. The solute is then added and hydrogenated using para-enriched hydrogen at ca. 1.4 bar. The sample has to be carefully degassed before the introduction of hydrogen at each step of the reaction. A typical sample is prepared using about 2 mg of precatalyst, 350 μL of solvent, and 10 μL of solute. Para-enriched hydrogen is obtained by storing normal hydrogen at 77 K in the presence of activated charcoal for 2−3 h.

NMR Spectroscopy. The NMR spectra have been recorded at room temperature on a *Bruker* DRX400 high-resolution spectrometer operating at 9.4 T and equipped with a 5 mm TBI-Z probe. A typical measurement consists of two successive $\pi/4$ and $\pi/2$ read pulses with signal acquisition immediately after each pulse.

Theory

We are going to treat the problem with the help of the density operator which represents the spin states of the ensemble of the spin systems. As it is an operator, it can be expanded on an appropriate basis, for example, the basis of product operators. In that way, if we denote by σ_0 the density operator corresponding to the two equivalent protons *A* and *A′* of p-H_2 (which will become *A* and *B* after hydrogenation), one has

$$\sigma_0 = KS_{AA'} \tag{3}$$

K being the p-H_2 enrichment factor.

We have previously shown[8,9] how, at the outcome of the hydrogenation reaction, hyperpolarization can be transferred to other spins belonging to the system (i.e., involved in a *J* coupling network) in which are embedded the two protons originating from p-H_2. As we are dealing with the ALTADENA experiment, the three homonuclear spins are actually magnetically equivalent because, in the earth field, the differences in resonance frequencies are smaller than the line width. In this case, it can be shown that the useful part of the new Hamiltonian reduces to the *J* coupling terms and is able to create the three singlet states S_{AB}, S_{AC}, and S_{BC} in proportions depending in a complicated manner on the various *J* coupling values. *This is the basis of the hyperpolarization transfer process* along with the fact that the density operator must commute with the Hamiltonian. In order to make this statement more explicit and according to what has been done in previous papers,[8,9] it can been noticed that the density operator must reach a steady state when the hydrogenation reaction is completed. The density operator at that moment, denoted by σ_1, must fulfill the following commutation equation.

$$[\sigma_1, H] = 0 \tag{4}$$

H is the Hamiltonian governing the spin system and here

$$H \equiv H_J = J_{AB}(E/4 - S_{AB}) + J_{AC}(E/4 - S_{AC}) + J_{BC}(E/4 - S_{BC}) \tag{5}$$

Postulating that σ_1 is a linear combination of singlet states (other quantities would not satisfy eq 4), we can write

$$\sigma_1 = x_{AB}S_{AB} + x_{AC}S_{AC} + x_{BC}S_{BC} \tag{6}$$

Inserting $\sigma_1$and *H* in eq 4 and noticing further that $[S_{AB},S_{AC}] = -[S_{AB},S_{BC}] = [S_{AC},S_{BC}]$, we arrive at the following equation

$$(J_{AB} - J_{BC})x_{AB} + (J_{BC} - J_{AC})x_{AC} + (J_{AB} - J_{AC})x_{BC} = 0 \tag{7}$$

Two further conservation equations[9] would lead to the actual calculation of x_{AB}, x_{AC}, and x_{BC} (which are anyway proportional to *K*), but this is not the goal of the present study.

The next step to be envisioned is the insertion of the sample into the NMR probe (and thus in a high magnetic field), possibly

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after a period during which it remains in the low magnetic field (this will be considered below). Anyway, the question is to determine what is the fate of any singlet state upon this insertion. Experimentally, an NMR signal is observed and it is a simple matter to show that it cannot result from a singlet state (say S_{AB}). For that purpose, let us consider a radio frequency pulse of flip angle θ acting along the x direction. From the $I_z^A I_z^B$ quantity, it yields as observable quantities $\sin\theta\cos\theta(I_z^A I_y^B + I_y^A I_z^B)$, whereas from $I_y^A I_y^B$ one obtains $-\sin\theta\cos\theta(I_z^A I_y^B + I_y^A I_z^B)$ that is zero as a net result: S_{AB} cannot lead to a net NMR signal. Thus, what is experimentally observed originates either from $I_z^A I_z^B$ *or* from $I_y^A I_y^B$. It can be noticed that a signal would also be observed from $I_z^A I_z^B + k I_y^A I_y^B$ with $|k| < 1$. The explanation of the experimental observations lies in the fact that the sample, when it reaches its final position at the magnet center, has traveled across magnetic field gradients which tend to destroy all transverse quantities, especially $I_y^A I_y^B$ and $I_x^A I_x^B$ (their fate will be discussed later), without obviously affecting $I_z^A I_z^B$. Thus, if we only consider the longitudinal two-spin order $I_z^A I_z^B$, this means that, when the sample arrives at its final position, the density operator can be written as

$$\sigma_2 = z_{AB}L_{AB} + z_{AC}L_{AC} + z_{BC}L_{BC} \tag{8}$$

The values of the coefficients z_{AB}, z_{AC}, z_{BC} depend on a possible waiting period outside the magnet.

We still have to explain the appearance of enhanced longitudinal polarizations. Although the effect of chemical shift anisotropy (csa)−dipolar cross-correlation rates could be invoked,[14,15] the systematic occurrence of in-phase multiplets (as indicated above) lends itself to another explanation independent of molecular properties. As a matter of fact, the creation of the quantity $\langle I_z^A - I_z^B\rangle$ (where brackets denote "expectation value") has been mentioned before.[1] If, for the sake of simplicity, we limit ourselves to a AB two-spin system, we can relatively easily account for this feature: starting from the Liouville−von Neumann equation and calculating the relevant commutators, we arrive at

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle I_z^A - I_z^B\rangle = -2J_{AB}\langle I_x^A I_y^B - I_y^A I_x^B\rangle \tag{9a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle I_x^A I_y^B - I_y^A I_x^B\rangle = 2(\nu_A - \nu_B)\langle I_x^A I_x^B + I_y^A I_y^B\rangle + \frac{J_{AB}}{2}\langle I_z^A - I_z^B\rangle \tag{9b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}\langle I_x^A I_x^B + I_y^A I_y^B\rangle = -2(\nu_A - \nu_B)\langle I_x^A I_y^B - I_y^A I_x^B\rangle \tag{9c}$$

Because the sample is traveling through regions of varying magnetic field gradients, both $I_x^A I_x^B$ and $I_y^A I_y^B$ should be almost destroyed at the moment of the NMR measurement. This means a strong time variation of $\langle I_x^A I_x^B + I_y^A I_y^B\rangle$ which has started from a hyperpolarization state. We can see from eq 9c that this variation entails the creation and the time variation of $\langle I_x^A I_y^B - I_y^A I_x^B\rangle$. Conversely from eq 9b this latter time variation leads to the creation of $\langle I_z^A - I_z^B\rangle$. Of course, this is a qualitative account which requires more experimental work in order to be properly assessed. In the present case, we can anticipate that the following quantities could be observed: $\langle I_z^A - I_z^B\rangle$, $\langle I_z^A - I_z^C\rangle$, $\langle I_z^B - I_z^C\rangle$. The estimation of their relative proportion is however beyond the scope of the present study. Anyhow, at the time of the NMR measurement, the density operator should be written as

$$\sigma_3 = z_{AB}L_{AB} + z_{AC}L_{AC} + z_{BC}L_{BC} + z_A I_z^A + z_B I_z^B + z_C I_z^C \tag{10}$$

Of course, the actual values of the coefficients involved in eq 10 depend in a somewhat complicated manner on the various relaxation parameters of the spin system and on the way that hyperpolarization has flown from the singlet states to longitudinal polarizations (their evaluation will be considered elsewhere). The important point is to recognize the presence at that moment of *both longitudinal two-spin orders and longitudinal polarizations*.

Results and Discussion

In order to measure longitudinal two-spin orders and longitudinal polarizations, we have run the following experiment: (i) perform the hydrogenation reaction in a weak magnetic field (e.g., earth field); (ii) possibly, wait to study spin relaxation in this weak field; (iii) move the sample to the high magnetic field with, possibly, a waiting time to study spin relaxation in a high magnetic field. In any situation, we applied first a $(\pi/4)_x$ pulse followed by acquisition yielding the signal $S_{\pi/4}$ and then a $(\pi/2)_x$ yielding the signal $S_{\pi/2}$. Defining the antiphase A spectrum by $A_{AB} = I_y^A I_z^B$, one has

$$S_{\pi/4} = (^1/_2)(z_{AB}A_{AB} + z_{AB}A_{BA} + z_{AC}A_{AC} + z_{AC}A_{CA} + z_{BC}A_{BC} + z_{BC}A_{CB}) + (1/\sqrt{2})(z_A I_y^A + z_B I_y^B + z_C I_y^C) \tag{11}$$

Owing to the fact that part of the polarization has been preserved by the first $(\pi/4)_x$ pulse (the actual factor is $1/\sqrt{2}$), the immediately following $(\pi/2)_x$ pulse leads to

$$S_{\pi/2} = (1/\sqrt{2})(z_A I_y^A + z_B I_y^B + z_C I_y^C) \tag{12}$$

It is a simple matter to recognize that $S_{\pi/2}$ provides a spectrum reflecting *only* longitudinal polarizations, whereas $S_{\pi/4} - S_{\pi/2}$ provides a spectrum reflecting *only* the contribution of longitudinal two-spin orders. This is illustrated by Figure 2 which shows antiphase multiplets on the one hand and in-phase multiplets on the other hand. Antiphase multiplets reflect primarily the hyperpolarization processes (including transfers toward the third spin by means of indirect coupling), and it is especially rewarding to be able to simulate the corresponding spectrum (Figure 2c compared to Figure 2d). This demonstrates unambiguously that these experimental results are artifact-free.

The objective of this work was nevertheless to study the relaxation of the hyperpolarized states resulting from parahydrogenation. There are in fact two ways to deal with the relaxation issue: (i) evolution in the high magnetic field, (ii) evolution in a weak magnetic field (earth field).

We start with the experiment which consists in the parahydrogenation (outside the magnet), immediately followed by the insertion of the sample in the magnet. An evolution delay is then allowed before the sequence ($\pi/4$)-(signal acquisition)-($\pi/2$)-(signal acquisition). As we have seen above, the states of the spin system include both longitudinal two-spin orders and longitudinal polarizations; these classical quantities will then

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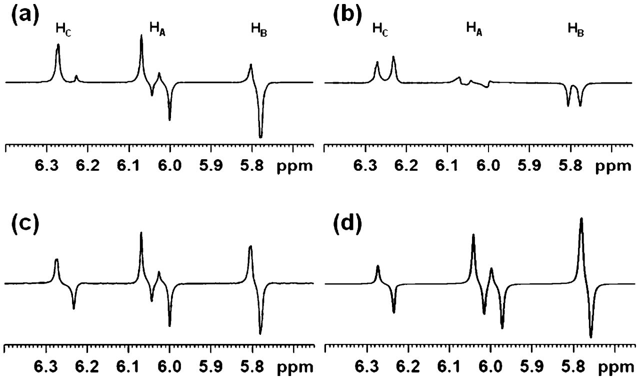

Figure 2. 400 MHz proton NMR spectra obtained immediately after the insertion of the sample in the high field magnet subsequently to the hydrogenation reaction shown in Figure 1. (a) Spectrum following the first $(\pi/4)_x$ pulse; (b) spectrum following the subsequent $(\pi/2)_x$ pulse; (c) difference of the two spectra; (d) the corresponding simulated spectrum with the following parameters (in arbitrary units): $z_{AB} = -88.8$, $z_{AC} = 26.4$, $z_{BC} = -37.6$ (second-order effects have been considered in this simulation).

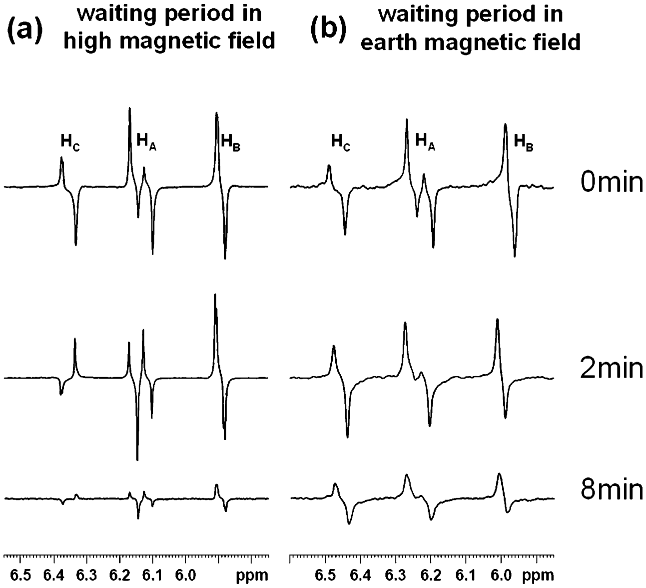

Figure 3. Left (a): Typical spectra showing the evolution of longitudinal two-spin orders (spectrum resulting from the $(\pi/4)_x$ pulse minus the spectrum obtained after the $(\pi/2)_x$ pulse) when the sample is left within the high field magnet. The antiphase multiplets are directly representative of longitudinal two-spin orders. Note that, after 8 min, longitudinal two-spin orders have disappeared. Right (b): Typical spectra reflecting the evolution of singlet states, the sample being left in the earth magnetic field. As in the left part of the figure, the antiphase multiplets are directly representative of longitudinal two-spin orders, themselves being proportional in amplitude to singlet states. As can be seen by the signal-to-noise ratio, for this series of experiment, the p-H_2 enrichment was smaller than those for experiments shown in the left part of the figure. Note that, after 2 min, the three singlet states have equilibrated and that, after 8 min, their amplitudes remain significant.

relax "classically" according to autorelaxation rates, cross-relaxation rates, and possibly cross-correlation rates... This results in a somewhat complicated evolution which will be the subject of a future publication. For the moment, we shall just consider the evolution of longitudinal two-spin orders as measured by subtracting the signal following the $(\pi/2)$ pulse from the signal acquired after the $(\pi/4)$ pulse. The spectrum obtained after a delay of 2 min (Figure 3, left) could give the impression of "long-lived" states. This is in fact deceptive just because we start from an enormously enhanced quantity (10^5-10^4) which arrives at 1% of its initial value after $5T_1$ (T_1 is here the longitudinal two-spin order autorelaxation time, with a typical value of some seconds; it proved impossible to determine an accurate value of this relaxation time just because a new sample is necessary for each evolution period and the reaction yield may be different). At that time, the amplitude of longitudinal two-spin orders is still very high (10^3-10^2), and it must be remembered that, at longer times, the exponential decay is very slow, hence, the apparent long-term persistence of a strong signal.

Evolution outside the magnet is less classical since we are concerned with relaxation of singlet states (see eq 6). As demonstrated experimentally and theoretically by Levitt and cowokers,[11-13] a singlet state alone (contrary to the case of two singlet states, see below) is of a "long-lived" nature because its relaxation does not stem from the most common spin relaxation mechanism, namely the dipolar interaction which is predominant for longitudinal polarizations or longitudinal two-spin orders. We have ourselves tackled this problem by the magnetization modes method[16] and arrived at the following expression for the autorelaxation of the S_{AB} singlet state (which must be multiplied by $\sqrt{4/3}$ for normalization purposes; for these calculations, extreme narrowing has been assumed and possible contributions from the csa mechanism have been neglected)

$$R_1^{S_{AB}} = -2J_{rf(A)} - 2J_{rf(B)} + 4J^{cross}_{rf(A),rf(B)} \tag{13}$$

Here J_{rf} is the spectral density function corresponding to the so-called random field mechanism which arises from randomly fluctuating magnetic fields due to spins other than A or B (e.g., spins outside the considered spin system, for instance, electron spins of paramagnetic species). In order to assess the importance of this mechanism, it can be mentioned that, in the case of polarization of a single spin $^1/_2$, its contribution is given by $-2J_{rf}$. On the other hand, $J^{cross}_{rf(A),rf(B)}$ is defined by the correlation of random fields at spin A and B, meaning that if the source of random fields is a symmetrical position with respect to A and B, then $J_{rf(A)} = J_{rf(B)} = J_{rf}$ and $J^{cross}_{rf(A),rf(B)} = J_{rf}$. In such a (unlikely) situation, $R_1^{S_{AB}}$ would simply cancel. Nevertheless, these considerations lead to the conclusion that $R_1^{S_{AB}}$ is probably very small, hence, the slowly relaxing nature of the singlet state which has thus been dubbed as "long-lived" or "long-standing". In the case of the three-spin system we are dealing with, further complications occur due to cross-terms among the three singlet states S_{AB}, S_{AC}, and S_{BC}. Using again the concept of magnetization modes and the computer program of ref 16, we arrive at the expression given below for the *dipolar* cross-relaxation rate between S_{AB} and S_{AC} (both quantities must be multiplied by $\sqrt{2/3}$ for normalization purposes; extreme narrowing is again assumed)

$$\sigma_{AB,AC} = -\frac{5}{12}\left(J_{BC,BC} - J^{cross}_{AB,BC} - J^{cross}_{AC,BC} + J^{cross}_{AB,AC}\right) \tag{14}$$

In order to establish the meaning of the spectral densities involved in eq 14, it can be indicated that, with these notations, the cross-relaxation term between the longitudinal polarizations I_z^A and I_z^B is expressed as $\sigma_{AB} = (-^5/_{12})J_{AB,AB}$. $J_{AB,AB}$ is deduced

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from the correlation characterizing the reorientation of the vector *AB*. $J^{cross}_{AB,BC}$ is a spectral density of identical nature but related to the reorientation correlation of the two (different) vectors *AB* and *BC*, hence, the terminology "cross-correlation spectral density". Anyway, what is important is the existence of efficient coupling terms (since they arise from dipolar interactions) between the three singlet states. However, these terms will *not* contribute to the decay of the singlet states but rather to their rapid *equilibration* so that z_{AB}, z_{AC}, z_{BC} in eq 8 or 10 become identical. Conversely, the singlet states, on a whole, still decay slowly according to autorelaxation rates such as the one defined in eq 13. In a figurative way, we can compare the three singlet states to three sinks at the same position but initially filled differently; since they are connected by pipes of more or less important diameters (the cross-relaxation terms), they will soon equilibrate. If we assume now small leaks which stand for the autorelaxation terms, we can conceive that the three sinks empty slowly while their contents remain at the same level. This is nicely confirmed by the experimental spectra on Figure 3, right. Spectra recorded 2 and 8 min after the hydrogenation reaction (the sample remaining outside the high field magnet) are characteristic of longitudinal two-spin orders of identical amplitude (according to simulations similar to the one of Figure 2d). Nevertheless, the persistence of long-lived states, much more pronounced than in the case of evolution in the presence of a high magnetic field, can be noted. This is indeed illustrative of the slow relaxation of singlet states with respect to that of longitudinal two-spin orders when the spin system is allowed to evolve in the presence of a low magnetic field.

Conclusion

The present study has demonstrated the slow evolution of singlet states even in a three-spin system involving transfers to a third spin, *J*-coupled to the two spins originating from enriched p-H_2. Such a feature was rather unexpected because dipolar interactions are seen to contribute. They serve merely to bring the singlet states into equilibrium but do not contribute to their decay. Owing to the difficulty of finding a molecule in which the two protons would be nonequivalent and isolated, this should open the way to applications of long-term NMR enhancement by enriched p-H_2, noticeably in the field of molecular imaging.

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