Journal of Magnetic Resonance **141,** 312–321 (1999)
Article ID jmre.1999.1889, available online at http://www.idealibrary.com on IDEAL®

A Procedure for Obtaining Pure Absorption 2D *J*-Spectra: Application to Quantitative Fully *J*-Decoupled Homonuclear NMR Spectra

P. Mutzenhardt, F. Guenneau, and D. Canet

Laboratoire de Méthodologie RMN, Faculté des Sciences, Université Henri Poincaré, BP 239, 54506 Vandoeuvre-lès-Nancy Cedex, France

Received May 5, 1999; revised July 20, 1999

The phase-twisted lines, resulting from a double complex Fourier transform, can greatly alter the quality of two-dimensional spectra. This problem, which is generally overcome by experimental procedures (requiring twice as much time as a standard measurement), is inevitable in the two-dimensional *J*-resolved experiment. This experiment is revisited theoretically and a postprocessing treatment, leading to pure 2D *J*-spectra, is deduced. Quantitative fully *J*-decoupled homonuclear spectra are accordingly obtained by means of a projection onto the F2 axis after a 45° tilt. © 1999 Academic Press

INTRODUCTION

In the early days of two-dimensional NMR, the obtention of pure absorption spectra was thought to be impossible because of the hypercomplex nature of the data (of the form $\exp(2i\pi\nu_1 t_1)^* \exp(2i\pi\nu_2 t_2)$). This time modulation rests of the necessity of "quadrature detection" in both dimensions, arising from instrumental and practical considerations (i.e., the setting of the carrier frequency somewhere near the middle of the conventional one-dimensional spectrum). A double Fourier transform of the above time-domain data leads invariably to a mixture of absorption and dispersion lines (referred below as phase-twisted lineshapes) which proves impossible to correct by data processing. One method for overcoming this drawback consists of creating a cosine (or sine) modulation in the t_1 dimension instead of the $\exp(2i\pi\nu_1 t_1)$ modulation. This is easily achieved in a heteronuclear experiment by (i) setting the carrier frequency at one extremity of the spectrum and (ii) avoiding the phase cycling which yields the complex exponential modulation. In homonuclear experiments, as one prefers to keep the advantage of quadrature detection in the t_2 dimension (for sensitivity and for limiting the transmitter power), this method would require the carrier frequency to be shifted prior to the t_1 evolution period with the major problem of phase retrieval at the beginning of the t_2 acquisition period. Fortunately elegant strategies have been devised for effectively (and not physically) moving the carrier frequency toward one extremity of the spectral window. They are based either on the incrementation of the initial pulse phase for each t_1 increment, divided by 2 with the respect to the normal increment (TPPI procedure (*1*)), or by repeating the experiment with the initial pulse shifted by 90° in a separate acquisition block (STATES procedure (*2*)). Yet these methods fail for the simple spin echo experiment and numerous studies based on postprocessing of the raw data have been proposed to overcome this difficulty. These studies could be divided into two categories. On the hand, the approach of Freeman and co-workers based on an analysis of the multiplet patterns allows them to extract each individual chemical shift in a coupled crowded proton spectrum and leads to a quantitative "decoupled" proton spectrum (*3, 4*). This clever method is restricted to the *J*-resolved experiment (i.e., arising from a homonuclear spin-echo experiment) and does not solve the general phase-twist problem. On the other hand, one can find methods based on a partial or total analysis of the data (considered a sum of damped sine functions). ALPESTRE (*5*) (a linear predictive estimation of signal time reversal) uses a backward linear prediction to reconstruct full echoes in the t_1 dimension and that way achieves a partial analysis of the signal. Furthermore, an emergent algorithm called filter diagonalization method (FDM) (*6, 7*) has been successfully applied to obtain (i) decoupled proton spectra (*8, 9*) and (ii) pure absorption spectra in electron spin echo experiments and gradient HSQC experiments (*10, 11*). In this paper, we expand our previous work on quantitative time-domain analysis of two dimensional data (*12*) to derive a general algorithm for getting rid of phase-twist problems in 2D spectra. It will be applied here with the aim of obtaining quantitative fully decoupled homonuclear spectra. The next section presents a detailed analysis of the *J*-decoupled experiment and mathematically demonstrates the potentiality of our approach for obtaining (1) pure absorption 2D spectra and (2), as a by-product, fully decoupled homonuclear spectra for which a theoretical expression provides the undisputed evidence of their quantitative nature.

THE *J*-DECOUPLED EXPERIMENT REVISITED

The relevant experiment corresponds to the classical two-dimensional sequence used in the so-called homonuclear *J*-resolved spectroscopy,

1090-7807/99 $30.00

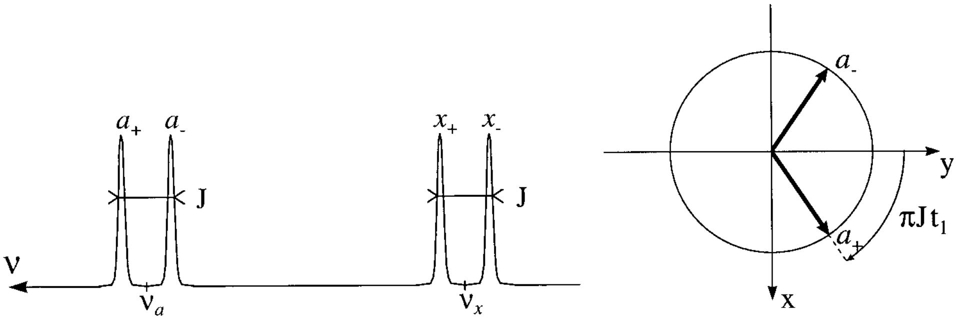

FIG. 1. Left: A schematic representation of the spectrum of a *J*-coupled two-spin-$\frac{1}{2}$ system with the notations used in the text. Right: The state of the two lines a_+ and a_- at the echo maximum.

$$(\pi/2)_x\text{–}t_1/2\text{–}(\pi)_y\text{–}t_1/2\text{–Acq}(t_2). \quad [1]$$

Because the central π pulse removes all chemical shift effect (with the chosen radiofrequency phases, refocusing occurs along the y axis of the rotating frame), evolution arises only from the indirect coupling J, so that, at the beginning of the acquisition period (maximum of the echo), the x and y components of magnetizations associated with a_+ and a_- (Fig. 1) are, respectively,

$$I^+_{x_0} = \sin \pi J t_1 \qquad I^+_{y_0} = \cos \pi J t_1 \quad [2a]$$

$$I^-_{x_0} = -\sin \pi J t_1 \quad I^-_{y_0} = \cos \pi J t_1. \quad [2b]$$

Each of these quantities should be multiplied by $\exp(-t_1/T_2)$, provided that the two lines are affected by the same transverse relaxation processes (i.e., in the absence of CSA-dipolar cross-correlation; otherwise two transverse relaxation times T_2^+ and T_2^- should be introduced). Physical detection during t_2 is assumed to be of the quadrature type with, as a convention, sampling of the quantity $(I_y + iI_x)$.

It is easy to see that I_{x_0} and I_{y_0} lead, respectively, to the signal

$$iI_{x_0}\exp(2i\pi\nu_0 t_2) \quad [3a]$$

$$I_{y_0}\exp(2i\pi\nu_0 t_2), \quad [3b]$$

where ν_0 is the precession frequency in the rotating frame of the considered line. For a_+ and a_-, we obtain

$$a_+\text{:}\exp(i\pi J t_1)\exp[2i\pi(\nu_a + J/2)t_2] \quad [4a]$$

$$a_-\text{:}\exp(-i\pi J t_1)\exp[2i\pi(\nu_a - J/2)t_2], \quad [4b]$$

where the amplitude of each signal has been set to unity and where relaxation damping factors have been omitted.

A double Fourier transform with respect to t_2 and t_1 yields two signals at ($\nu_1 = J/2$, $\nu_2 = \nu_a + J/2$) and ($\nu_1 = -J/2$, $\nu_2 = \nu_a - J/2$) as schematized in Fig. 2. Tilting the two-dimensional map by 45°, i.e., transforming the frequency variable ν_2 into $\nu_2' = \nu_2 - \nu_1$, produces two peaks at ($\nu_1 = J/2$, $\nu_2' = \nu_a$) and ($\nu_1 = -J/2$, $\nu_2' = \nu_a$); thus their projection upon the ν_2' axis results in a single peak at ν_a, that is a decoupled spectrum. This is of course the objective of this experiment. However, a major drawback arises from the hypercomplex form of expressions [4] for which double Fourier transform leads invariably to phase-twisted peaks, improper to further operations (tilt and projection). One possibility for overcoming these difficulties is to calculate the amplitude spectrum with the inconvenience of broadened lines involving important wings.

It is well known that a phase-sensitive two-dimensional spectrum can be obtained by switching from a t_1 phase modulation to a t_1 amplitude modulation with, in addition, the necessity to shift the frequency origin in the ν_1 domain so that all signals appear (in this frequency domain) at a positive frequency. As indicated in the Introduction, this is usually achieved by a phase incrementation simultaneously with the time incrementation (*1*). Alternatively, the same goal can be reached by devising two experiments, one leading, in t_1, to a sine modulation and the other leading to a cosine modulation (*2*). In the present experiment, the only possibility lies in the relative phases of the two pulses. It turns out that choosing a $(\pi)_x$ pulse (instead of a $(\pi)_y$ pulse in the experiment discussed until now) yields

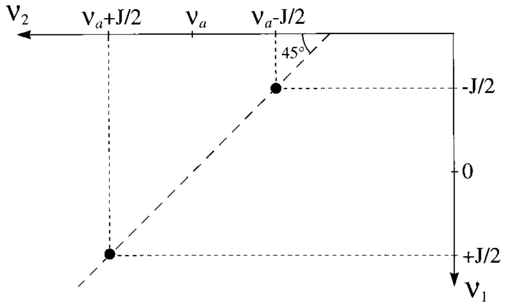

FIG. 2. Location of the two cross-peaks arising from the *a* lines in the two-dimensional diagram obtained after double Fourier transform of data resulting from the spin echo experiment.

$$a_{+}\!: -\exp(i\pi J t_1)\exp[2i\pi(\nu_a + J/2)t_2] \quad [5a]$$

$$a_{-}\!: -\exp(-i\pi J t_1)\exp[2i\pi(\nu_a - J/2)t_2], \quad [5b]$$

which would substitute to [4]. Clearly, except for the trivial sign change, this experiment does not offer any new information. It allows us, however, to compensate for instrumental imperfections through the widely employed EXORCYCLE (*13*) phase scheme.

Since the latter approach fails, one may consider sampling the whole echo or the echo in its reconstruction if it is not available in its totality. If we suppose that it is possible to sample negative t_2 values, the signal for a_+ and a_- can be expressed as

$$\begin{aligned} a_{+}\!:&\exp(i\pi J t_1)\exp[-(t_1 + t_2)/T_2] \\ &\times \exp[2i\pi(\nu_a + J/2)t_2]\exp(-|t_2|/T_2') \quad [6a] \\ a_{-}\!:&\exp(-i\pi J t_1)\exp[-(t_1 + t_2)/T_2] \\ &\times \exp[2i\pi(\nu_a - J/2)t_2]\exp(-|t_2|/T_2'), \quad [6b] \end{aligned}$$

where t_1 is strictly positive and fixed (whereas t_2 is a time variable) and where the contribution from field inhomogeneity to signal damping (T_2') has been explicitly introduced. It can be stressed that the "*J*-echoes" are neither symmetric (for the real part) nor antisymmetric (for the imaginary part). Let us consider the contribution from the a_+ line (ignoring damping effects due to transverse relaxation); the real and imaginary parts can be written, respectively,

$$\cos 2\pi\left[\nu_a t_2 + \frac{J}{2}(t_2 + t_1)\right] \quad [7a]$$

$$\sin 2\pi\left[\nu_a t_2 + \frac{J}{2}(t_2 + t_1)\right]. \quad [7b]$$

Obviously, due to the terms $(J/2)(t_2 + t_1)$, symmetry properties are lost, precluding the direct reconstruction of the whole echo from a single half echo.

Now, let us calculate the Fourier transform with respect to t_2 of the whole echo, again for the a_+ contribution (whose maximum occurs at $t_2 = 0$), that is,

$$\begin{aligned} F(\nu) = \exp(i\pi J t_1)\exp(-t_1/T_2) \int_{-\infty}^{+\infty} \\ \times \exp[2i\pi(\nu_a + J/2t_2)]\exp(-|t_2|/T_2^*)dt_2. \quad [8] \end{aligned}$$

Calculating the Fourier transform first with respect to $t_2 > 0$ and then with respect to $t_2 < 0$ and summing up the two results (as this must be done in practice, owing to the currently available algorithms; *it can be noticed that the Fourier transform calculation for $t_2 < 0$ requires us to reverse t_2 but also to reverse the frequency scale*), we obtain

$$\begin{aligned} F(\nu) &= \exp(i\pi J t_1)\exp(-t_1/T_2)\{A[(\nu_a + J/2) + iD(\nu_a \\ &\qquad + J/2)] + [A(\nu_a + J/2) - iD(\nu_a + J/2)]\} \\ &= \exp(i\pi J t_1)\exp(-t_1/T_2)2A(\nu_a + J/2), \quad [9] \end{aligned}$$

where *A* and *D* represent the absorption and dispersion spectra, respectively. In practice, because of the phase modification introduced at the receiver level, a zero-order phase correction (the same for all t_1 increments) should yield the result given by [9]. Of course $F(\nu)$ is complex but both real and imaginary parts are amplitude modulated by $\pi J t_1$ according to cosine and sine functions, respectively. This is due to the fact that $F(\nu)$ is a pure absorption spectrum as long as the Fourier transform with respect to t_2 is considered; *this property removes immediately the phase-twist problem* since, after the first Fourier transform (with respect to t_2), it suffices to retain the real part of the second Fourier transform (with respect to t_1) so as to obtain pure absorption spectra in both dimensions.

DATA ALGORITHM TO GET RID OF THE TWISTED PHASE PROBLEM IN 2D *J*-SPECTRA

The problem of reconstructing the whole echo in the time domain t_2 remains. It can be pointed out that a similar attempt has been carried out by Nuzillard (*5*), who reconstructed artificial echoes along the t_1 dimension again with the aim of escaping from the phase-twist problem. Fundamentally, his approach, based on linear prediction, is close to the one described here. However, t_1 spectra are likely to be less predictable than t_2 spectra, as the former arise from an unknown *J* modulation while the latter are simply conventional NMR spectra, amplitude, and/or phase modulated. Thus, once all lines in the standard NMR spectrum have been identified, the only issue is to quantify their modulation features according to the t_1 time evolution. It occurred that reconstructing missing parts (actually the missing left part) in t_2 echoes poses severe problems associated with instabilities of linear prediction algorithms. It was then decided to reconstruct the *whole* left part of a t_2 echo, recognizing that the actual information necessary for this operation is actually contained in the echo right part, which is of course available in its totality.

Let us go back to Eq. [6] and change for convenience t_2 in t. Any contribution (labeled by the subscript *m*) to the right part of the t_2 echo signal $\mathcal{S}(t)$ can be written as

$$a_m\exp(-t_1/T_{2m})\exp(i\varphi_m)z_m^t, \quad [10]$$

with $z_m = \exp[-1/T_{2m}^* + 2i\pi\nu_m]$, ν_m standing for the effective precession frequency ($\nu_a \pm J/2$ in (*6*)), whereas φ_m arises from *J* modulation during $t_1(i\pi J t_1$ in [6]) and where the

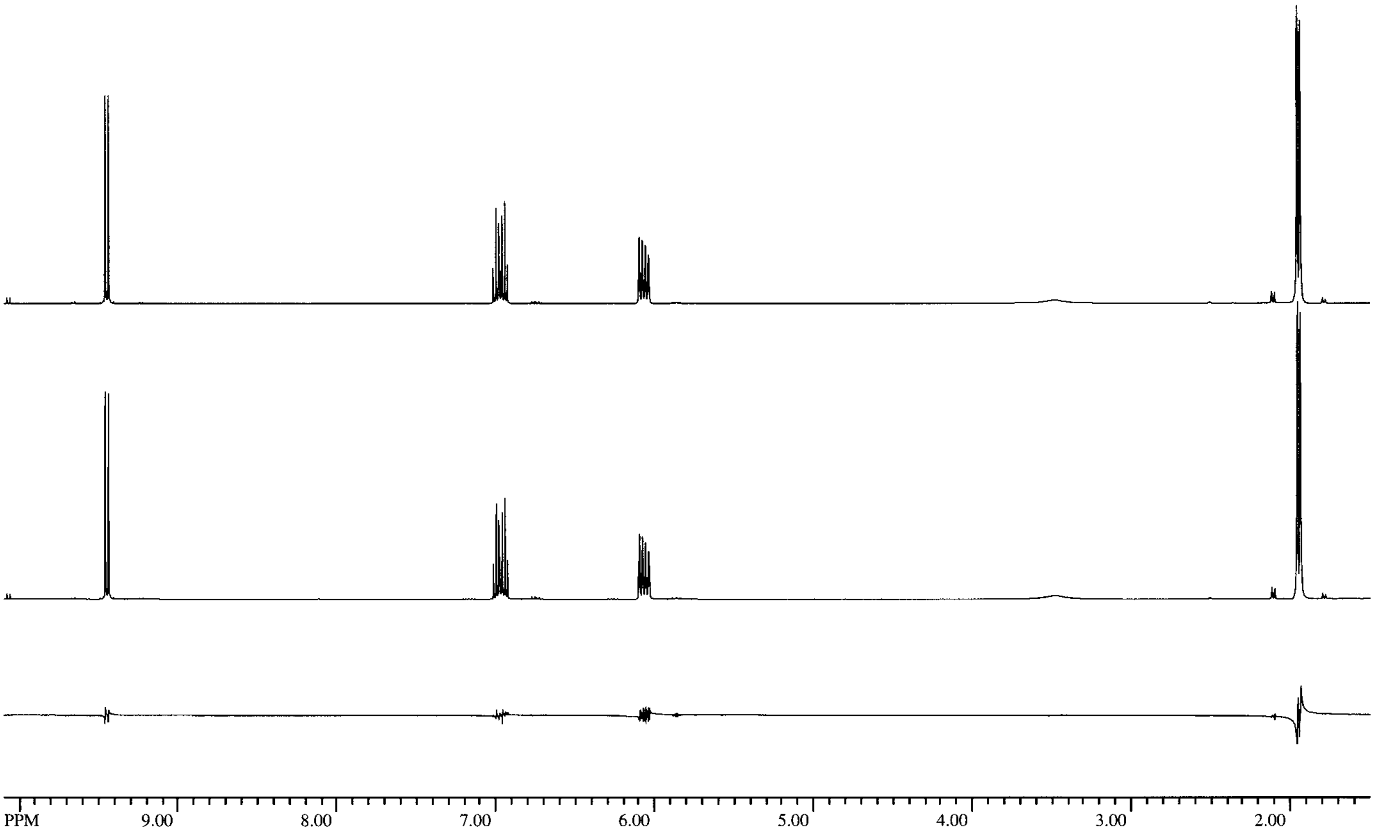

FIG. 3. First stage of the algorithm: Data analysis. Top: Standard proton spectrum of tanscrotonaldehyde (TCA) measured on an Avance Bruker DRX spectrometer operating at 9.4 T. 16 K complex time domain data, spectral width: 5000 Hz, four scans. Middle: Reconstructed spectrum with the spectral parameters provided by an HD analysis of the whole time-domain data. Bottom: Difference of the two spectrum multiplied by a factor of 5, exhibiting some small residual signals.

effective transverse relaxation time T^*_{2m} has been reintroduced ($1/T^*_{2m} = 1/T_{2m} + 1/T'_2$). Altogether assuming that M different signals contribute to $\mathcal{S}$, one has

$$\mathcal{S}(t) = \sum_{m=1}^{M} a_m \exp(-t_1/T_{2m}) \exp(i\varphi_m)\, z^t_m. \qquad [11]$$

Let us assume in the following equations that t is positive (right half of the echo). Equation [11] can be rewritten as

$$\mathcal{S}(t) = \sum_m b_m z^t_m, \qquad [12]$$

with $b_m = a_m \exp(-t_1/T_{2m}) \exp(i\varphi_m) = a'_m \exp(i\varphi_m)$.

The parameters b_m and z_m can in principle be deduced from the right half echo (e.g., by Hankel decomposition algorithm (HD) (*14*) or any other time-domain analysis method) for each t_1 increment. We shall now try to mimic the Fourier transform of a whole echo (see Eqs. [8] and [9]). With this goal in mind let us, for each t_1 increment, construct a signal $\mathcal{S}'(t) = \sum_m b_m (z^t_m)^*$ (where the asterisk stands for the complex conjugate, which amounts to change any resonance frequency into its opposite). Taking the Fourier transform of both $\mathcal{S}(t)$ and $\mathcal{S}'(t)$ yields

$$FT[\mathcal{S}(t)] = \sum_m a'_m[\cos \varphi_m + i \sin \varphi_m][A(\nu_m) + iD(\nu_m)] \qquad [13a]$$

$$FT[\mathcal{S}'(t)] = \sum_m a'_m[\cos \varphi_m + i \sin \varphi_m] \times [A(-\nu_m) + iD(-\nu_m)]0 \qquad [13b]$$

$$FT[\mathcal{S}(t)] + FT[\mathcal{S}'(t)] = 2 \sum_m a'_m \exp(i\varphi_m) A(\nu_m), \quad [13c]$$

where A and D stand for absorption and dispersion spectra, respectively, and where the properties $A(-\nu_m) = A(\nu_m)$, $D(-\nu_m) = -D(\nu_m)$ have been used.

Therefore, further reversing the frequency scale of $FT[\mathcal{S}'(t)]$ (as should be done for the left part of an actual echo) and adding it to $FT[\mathcal{S}(t)]$ leads to a result similar to Eq. [9] (that is, the disappearance of $D(\nu_m)$, which represents the

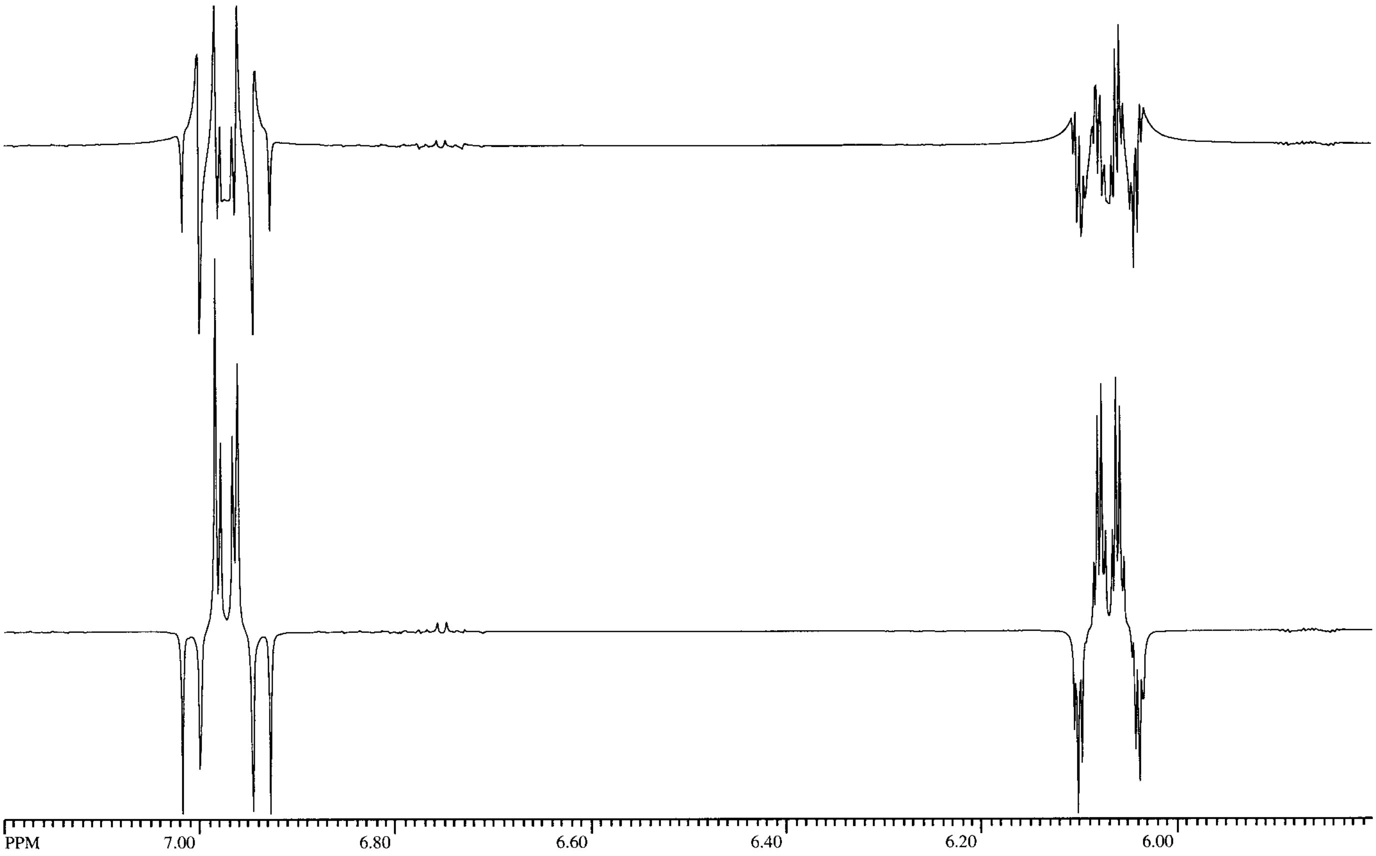

FIG. 4. Second stage of the algorithm: Reconstruction of t_2 echoes and addition to the experimental data. Top: A selected t_1 increment ($t_1 = 30$ ms) extracted from a *J*-resolved experiment on TCA after the Fourier transform in the t_2 dimension. Bottom: Same spectrum after the addition of reconstructed echo (see text). Phase twist is seen to be completely removed, the intensity of each line reflecting a cosine modulation which arises from the sole evolution of scalar couplings during the t_1 period.

condition for avoiding phase-twist problems). It can be noted that this transformation amounts to change t into $-t$ without affecting the phase factor included in b_m. Finally, it can be emphasized that the whole process rests on a successful analysis of the standard NMR spectrum, that is, a search for the resonance frequencies ν_m and their associated effective transverse relaxation time T^*_{2m}.

We now summarize the above-described procedure which can be considered a general method for obtaining phase-sensitive 2D spectra whenever phase modulation (and not amplitude modulation) leads to phase-twist lineshapes:

(1) Extract the z_m's from a reference spectrum.

(2) For each t_1 increment, deduce the relevant complex amplitudes (b_m) from the corresponding experimental data, denoted by $\mathcal{S}(t)$.

(3) Construct $\mathcal{S}'(t)$, compute its Fourier transform, and reverse the frequency scale.

(4) Add this result to the Fourier transform of $\mathcal{S}(t)$. This leads to an absorption spectrum, with a complex modulation factor depending on t_1. Further Fourier transform with respect to t_1 will yield a real part exhibiting absorption features along both ν_1 and ν_2. This removes the phase-twist problem arising from the hypercomplex nature of data along both t_1 and t_2.

(5) Continue the processing in a standard way, that is, to compute the Fourier transform along t_1 without using magnitude calculation.

The outline of this algorithm is close to the one used to extract quantitative information from the two-dimensional heteronuclear Overhauser effect (HOE) some time ago (*12*). The problem of finding the best analysis method which yields reliable z_m's at the first stage may be critical. We chose the HD method derived from the Hankel singular value decomposition (*15*) because it can be used as a blackbox and does not need prior knowledge of the signal. The total number of lines M contained in the FID has only to be overestimated since any extra line yields negligible amplitude. It can be pointed out that any analysis method, such as FDM or nonlinear least squares, which is capable of estimating the four spectral parameters for each line (i.e., frequency, damping factor, amplitude, and phase), could be used instead of the HD algorithm.

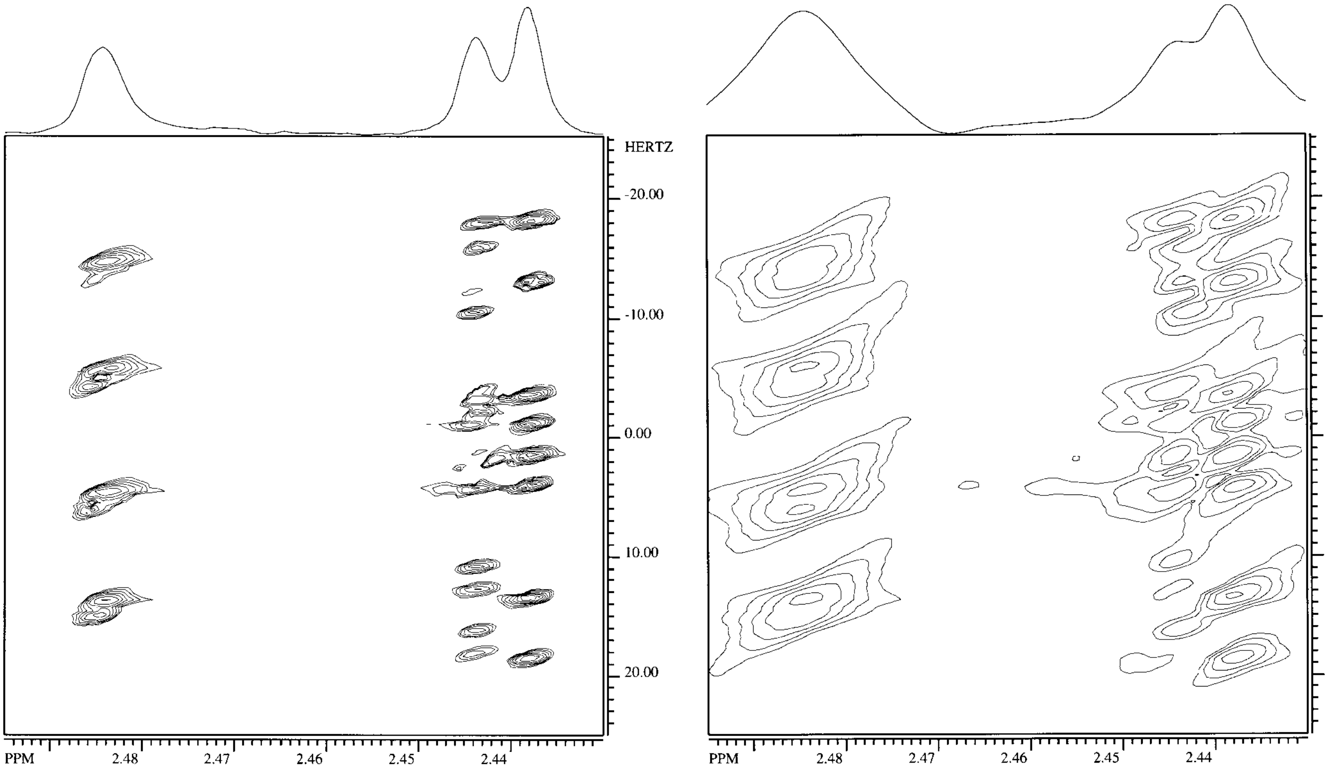

FIG. 5. Partial comparison of final *J*-resolved 2D spectra of TCA. Right: Standard processing (2D FT, 45° tilt, magnitude mode). Left: Data processed with the algorithm described in the text. A better resolution is obtained in both dimensions. Acquisition parameters: 512 t_1 increments of 10 ms and 8 K complex data (t_2) were collected. Spectral width: 5000 Hz, eight scans, EXORCYCLE phase cycling. Final 2D map size 8 K × 512 points.

PROJECTION AND QUANTITATIVE HOMONUCLEAR "DECOUPLED" SPECTRA

Let $S(\nu_1, \nu_2)$ be a pure absorption cross-peak of amplitude a at frequencies ν_{01} and ν_{02} in the frequency domains ν_1 and ν_2 obtained by the method described in the previous section.

$$S(\nu_1, \nu_2) = a\,\frac{T_2}{1 + 4\pi^2 T_2^2(\nu_{01} - \nu_1)^2} \times \frac{T_2^*}{1 + 4\pi^2 T_2^{*2}(\nu_{02} - \nu_2)^2}, \qquad [14]$$

where T_2 and T_2^* are the effective transverse relaxation times in ν_1 and ν_2, respectively. The 45° tilt amounts to change ν_2 into $\nu_2' = \nu_2 - \nu_1$, yielding

$$S(\nu_1, \nu_2') = a\,\frac{T_2}{1 + 4\pi^2 T_2^2(\nu_{01} - \nu_1)^2} \times \frac{T_2^*}{1 + 4\pi^2 T_2^{*2}(\nu_{02} - \nu_2' - \nu_1)^2}. \qquad [15]$$

The projection upon the ν_2' axis corresponds to an integral with respect to ν_1,

$$s(\nu_2') = a \int_{-\infty}^{+\infty} \frac{T_2}{1 + 4\pi^2 T_2^2(\nu_{01} - \nu_1)^2} \times \frac{T_2^*}{1 + 4\pi^2 T_2^{*2}(\nu_{02} - \nu_2' - \nu_1)^2}\, d\nu_1, \qquad [16]$$

which can be recast in terms of inverse Fourier transforms,

$$s(\nu_2') = a \int_{-\infty}^{+\infty} \{\mathrm{Re}[FT(\exp[2i\pi\nu_{01}t_1]\exp(-t_1/T_2))] \times \mathrm{Re}[FT(\exp[2i\pi(\nu_{02} - \nu_1)t_2] \times \exp(-t_2/T_2^*))]\}\, d\nu_1. \qquad [17]$$

Making the Fourier transforms explicit, we can write [15] as

$$s(\nu_2') = a \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} \exp\left(-\frac{|t_1|}{T_2} - \frac{|t_2|}{T_2^*}\right) \times \cos[2\pi(\nu_{01} - \nu_1)t_1] \times \cos[2\pi(\nu_{02} - \nu_2' - \nu_1)t_2]\,dt_1\,dt_2\,d\nu_1. \qquad [18]$$

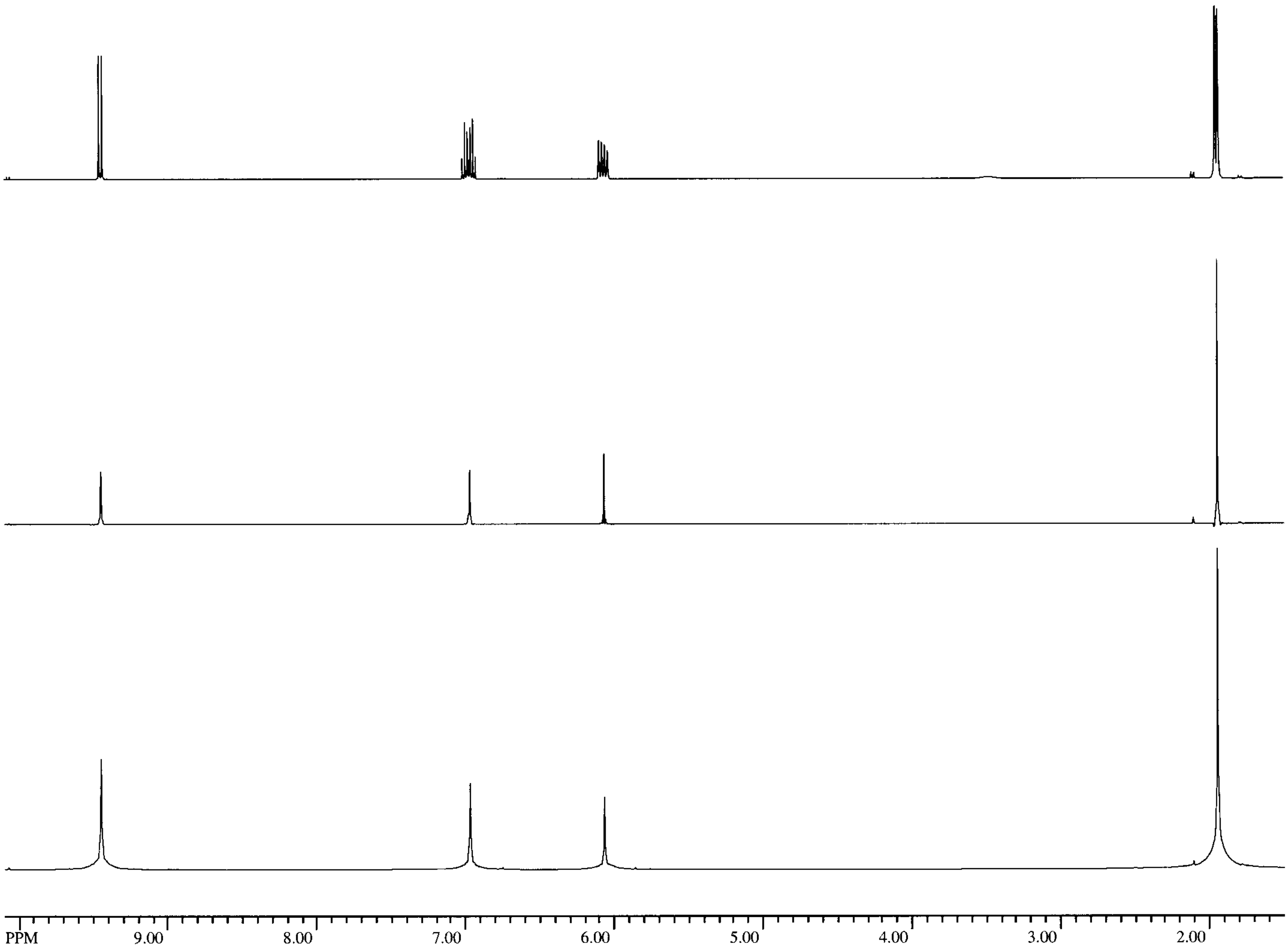

FIG. 6. ν_2 projections of the 2D spectra shown in Fig. 5. Top: Conventional proton spectrum displayed at the same scale. Middle: *Quantitative* projection obtained by the procedures described in this paper. Line areas are within 5% identical to those of the conventional spectrum. Bottom: Normal projection of the standard *J*-resolved experiment exhibiting a large broadening due to the magnitude mode.

Rearranging the trigonometric functions, we obtain

$$\begin{aligned} s(\nu_2') = \frac{a}{2}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty}\exp\left(-\frac{|t_1|}{T_2}-\frac{|t_2|}{T_2^*}\right)\Bigg\{ &\cos 2\pi[\nu_{01}t_1 \\ &+ (\nu_{02}-\nu_2')t_2]\int_{-\infty}^{+\infty}\cos[2\pi\nu_1(t_1+t_2)]d\nu_1 \\ &+ \sin 2\pi[\nu_{01}t_1 + (\nu_{02}-\nu_2')t_2] \\ &\times\int_{-\infty}^{+\infty}\sin[2\pi\nu_1(t_1+t_2)]d\nu_1 + \cos 2\pi[\nu_{01}t_1 \\ &- (\nu_{02}-\nu_2')t_2]\int_{-\infty}^{+\infty}\cos[2\pi\nu_1(t_1-t_2)]d\nu_1 \\ &+ \sin 2\pi[\nu_{01}t_1 - (\nu_{02}-\nu_2')t_2] \\ &\times\int_{-\infty}^{+\infty}\sin[2\pi\nu_1(t_1-t_2)]d\nu_1\Bigg\}dt_1dt_2. \qquad [19] \end{aligned}$$

The ν_1 integrals involving a sine function are zero whereas those involving a cosine function lead to a dirac function δ. This yields

$$\begin{aligned} s(\nu_2') = \frac{a}{2}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty}\exp\left(-\frac{|t_1|}{T_2}-\frac{|t_2|}{T_2^*}\right)&\cos[2\pi(\nu_{01}t_1) \\ &+ 2\pi(\nu_{02}-\nu_2')t_2]\delta(t_1+t_2)dt_1dt_2 \end{aligned}$$

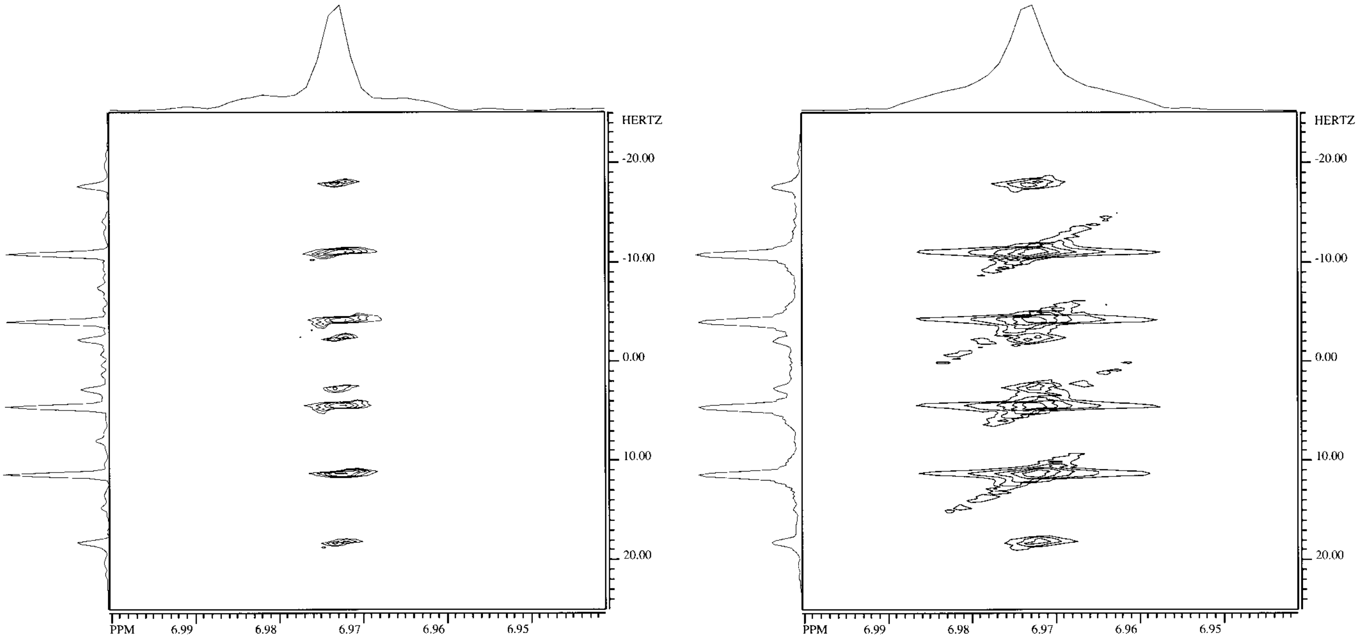

FIG. 7. Partial comparison of the final *J*-resolved spectra for 4-androstene-3,17-dione. Right: Standard processing (2D FT, 45° tilt, magnitude mode). Left: Data processed with the algorithm described in the text. Note the better separation of the two peaks at 2.44 ppm. Acquisition parameters: 512 t_1 increments of 12.5 ms and 2 K complex data (t_2) were collected. Spectral width: 1500 Hz, eight scans, EXORCYCLE phase cycling, repetition time 10 s. Final 2D map size 4 K × 512 points.

$$+\frac{a}{2}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty}\exp\left(-\frac{|t_1|}{T_2}-\frac{|t_2|}{T_2^*}\right)\cos[2\pi(\nu_{01}t_1) - 2\pi(\nu_{02}-\nu_2')t_2]\delta(t_1-t_2)dt_1dt_2. \qquad [20]$$

From the properties of dirac distributions, the first integral becomes

$$\int_{-\infty}^{+\infty}\exp\left(-\frac{|t_2|}{T_2}-\frac{|t_2|}{T_2^*}\right)\cos[-2\pi\nu_{01}t_2 + 2\pi(\nu_{01}2-\nu_2')t_2]dt_2$$
$$=\int_{-\infty}^{+\infty}\exp\left[-\left|t_2\right|\left(\frac{1}{T_2}+\frac{1}{T_2^*}\right)\right] \times\cos[2\pi(\nu_{02}-\nu_{01}-\nu_2')t_2]dt_2.$$

This is just a Lorentzian function representing a signal of resonance frequency $\nu_2' = \nu_{02} - \nu_{01}$ and of transverse relaxation time $T_2T_2^*/T_2 + T_2^*$. It can be recalled that for the *m*th component of a multiplet centered on frequency ν_0, one has $\nu_{02} = \nu_0 + mJ$ (or $\nu_0 + mJ/2$) and $\nu_{01} = mJ$ (or $mJ/2$), m being an integer or a half-integer, positive or negative, so that $\nu_2' = \nu_0$.

The same result is obtained for the second integral and, finally,

$$s(\nu_2') = a\frac{\dfrac{T_2T_2^*}{T_2+T_2^*}}{1+4\pi^2\left(\dfrac{T_2T_2^*}{T_2+T_2^*}\right)^2(\nu_0-\nu_2')^2}. \qquad [21]$$

Equation [21] provides the analytical function for the lineshape in the decoupled spectrum. It is a Lorentzian function, whose maximum occurs of course at $\nu_2' = \nu_0$, and whose linewidth corresponds to an effective transverse relaxation time T_2^{eff} such that $1/T_2^{\mathrm{eff}} = 1/T_2 + 1/T_2^*$. This line broadening in the decoupled spectra is quite negligible compared with conventional amplitude spectra.

RESULTS AND DISCUSSION

The described algorithm has been applied to a sample of transcrotonaldehyde (TCA) as a pedagogical example. A standard homonuclear *J*-resolved experiment was run with an Avance DRX Bruker spectrometer operating at 9.4 T. It must be pointed out that, due to the mathematical expression of the projection, which involves both T_2 and T_2^*, quantitative results cannot be reached without collecting a complete data set. Figure 3 shows the conventional 1D proton spectrum of this compound, compared with a spectrum reconstructed by means

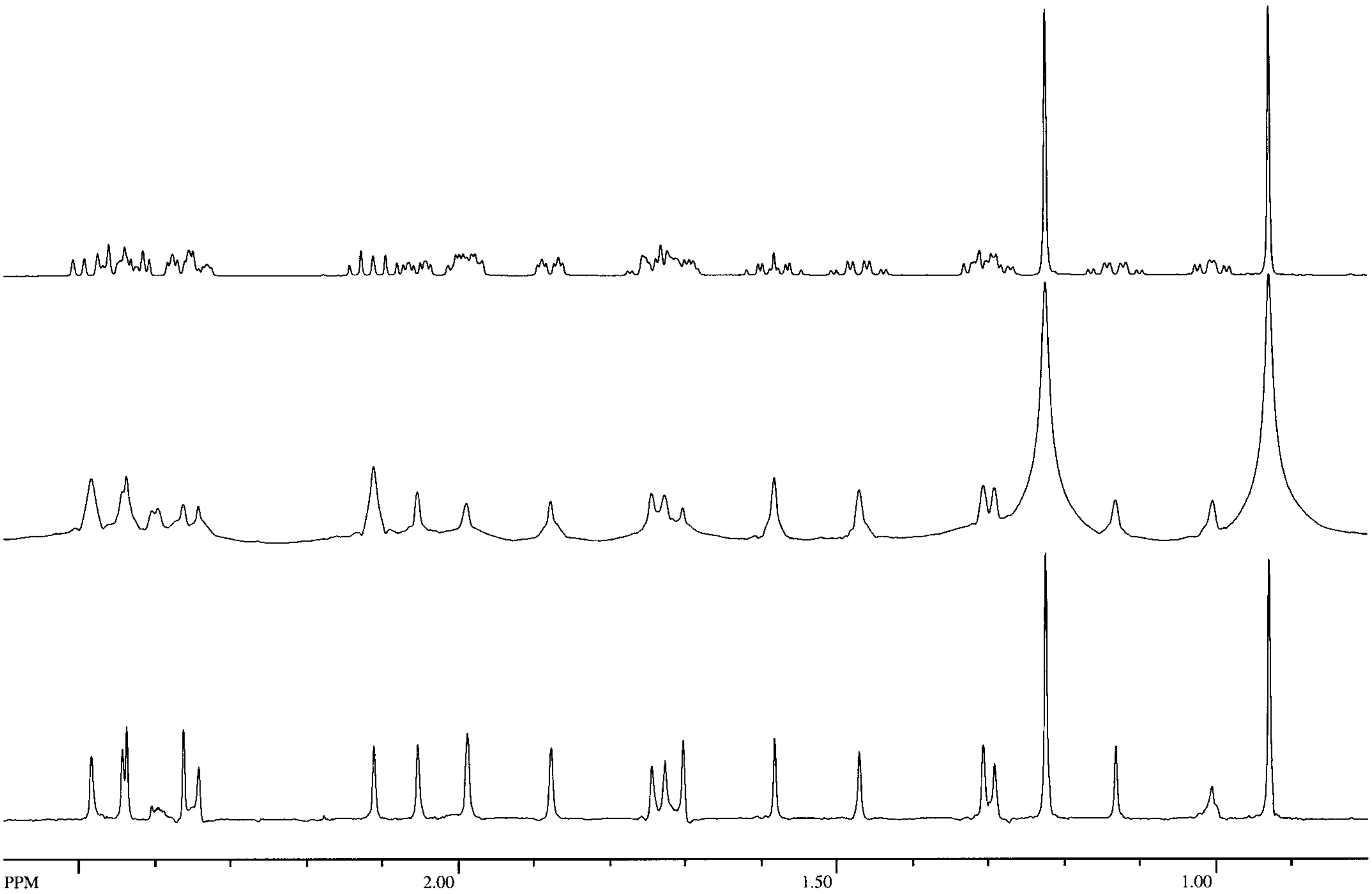

FIG. 8. ν_2 projections of the 2D J-resolved experiment of 4-androstene-3,17-dione at 14.09 T. Top: Conventional proton spectrum scaled in order to be compared with the projections. Middle: Normal projection of the J-resolved experiment exhibiting a large broadening and a poor resolution due to the magnitude mode. Bottom: *Quantitative* projection obtained by the procedure described in this paper. Note the increased resolution and the quantitativity (each line corresponds to one proton, except the two methyl groups which are easily identifiable). A remarkable simplification occurs with respect to the conventional overcrowded spectra.

of the spectral parameters provided by a HD analysis of the whole time-domain data. Knowing the list of frequencies and damping factors (i.e., the z_m's of previous paragraph), a linear least squares fit was applied for each t_1 increment, thus yielding the complex amplitude of the spectral components. It is then possible to reconstruct, for each increment, the left and missing part of the echo $\mathcal{S}'(t)$. After (i) Fourier transforming of these reconstructed data, (ii) reversing the frequency scale, and (iii) adding them to the Fourier transform of the genuine experimental data, the phase modulation is completely removed as demonstrated in Fig. 4 for a given t_1 increment. Multiplets exhibit a pure cosine modulation in the t_1 dimension and the resulting 2D data set is processed in a standard way by applying Fourier transform to the t_1 dimension, prior to a 45° tilt. It can be compared (Fig. 5) with the resulting 2D spectra obtained through the conventional processing (2D FT, magnitude calculation, and a 45° tilt). As expected, by removing the phase-twist feature this procedure can considerably improve resolution of the J-resolved spectra; this point will be better illustrated by the forthcoming example. The ν_2 projections of both 2D spectra are shown in Fig. 6. Homonuclear decoupling is undoubtedly achieved and a quasi-perfect Lorentzian lineshape is obtained as predicted by Eq. [21]. The integral of each line demonstrates the quantitative feature of this spectrum, the deviation with the conventional spectrum lying within a range of 5%. We turn now to a more demonstrative example constituted by a medium size molecule, namely 4-androstene-3,17-dione. This compound was chosen because it allows the reader to directly compare our results with those obtained by Freeman (*3, 4*) and co-workers. The J-resolved experiment was performed with a Bruker AMX operating at 14.09 T and data were processed as described previously for the TCA molecule. A part of the corresponding 2D spectra, displayed in Fig. 7, exhibits an enhanced resolution with respect to the conventional procedure and therefore leads to an easier analysis of coupling patterns in the ν_1 dimension. The projection along ν_2 (Fig. 8) shows a fully decoupled proton spectrum. Some lines at 2.4 and 1.0 ppm are a little bit distorted because of the limitations inherent to the determination of frequencies and damping factors which are involved in the first step of the time-domain analysis. The set of spectral parameters obtained by HD certainly leads to the best fit with experimental data at the expense of their physical meaning. This point has been developed in a previous paper (*12*) in the case of a lineshape altered by field inhomogeneities. Moreover, harmonic inversion of frequencies (reconstruction of the missing half echo) can be rather tricky and may introduce some distortions in the final spectrum. Nevertheless, we obtained a promising result with some potentiality regarding the quantitative analysis of homonuclear crowded spectra. Finally, it must be mentioned that if only highly resolved qualitative spectra are needed, a valuable solution, which requires only a few t_1 increments, has been proposed recently (*16*).

CONCLUSION

We have proposed an algorithm to get rid of the phase-twist problem which can be useful when this drawback cannot be circumvented by experimental procedures. Application of this algorithm to the *J*-resolved experiment leads to pure absorption 2D spectra but, most importantly, to quantitative fully decoupled spectra. Of course, its performance could be improved by more robust processing at the step of the initial analysis of the conventional one-dimensional spectrum.

REFERENCES

1. D. Marion and K. Wüthrich, Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of ^{1}H–^{1}H spin–spin coupling constants in proteins, *Biochem. Biophys. Res. Commun.* **113,** 967–974 (1983).

2. D. J. States, R. A. Haberkorn, and D. J. Ruben, A two-dimensional nuclear Overhauser experiment with pure absorption phase in the four quadrants, *J. Magn. Reson.* **48,** 286–292 (1982).

3. M. Woodley and R. Freeman, Decoupled proton NMR spectra, *J. Magn. Reson. A* **109,** 103–112 (1994).

4. S. Simova, H. Sengstschmid, and R. Freeman, Proton chemical-shift spectra, *J. Magn. Reson.* **124,** 104–121 (1997).

5. J. M. Nuzillard, Time-reversal of NMR signals by linear prediction. Application to phase sensitive homonuclear *J*-resolved spectroscopy, *J. Magn. Reson. A* **118,** 132–135 (1996).

6. M. R. Wall and D. Neuhauser, Extraction, through filter-diagonalization, of general quantum eigenvalues or classical normal mode frequencies from a small number of residues or short time segment of a signal. I. Theory and application to a quantum-dynamics model, *J. Chem. Phys.* **102,** 8011–8022 (1995).

7. V. A. Mandelshtam and H. S. Taylor, Harmonic inversion of time signals and its application, *J. Chem. Phys.* **107,** 6756–6769 (1997).

8. V. A. Mandelshtam, H. S. Taylor, and A. J. Shaka, Application of the filter diagonalization method to one- and two-dimensional NMR spectra, *J. Magn. Reson.* **133,** 304–312 (1998).

9. V. A. Mandelshtam, Q. N. Van, and A. J. Shaka, Obtaining proton chemical shifts and multiplets from several 1D NMR signals, *J. Am. Chem. Soc.* **120,** 12161–12162 (1998).

10. V. A. Mandelshtam, H. Hu, and A. J. Shaka, Two-dimensional HSQC NMR spectra obtained using a self-compensating double pulsed field gradient and processed using the filter diagonalization method, *Magn. Res. Chem.* **36,** S17–S28 (1998).

11. G. Jeschke, V. A. Mandelshtam, and A. J. Shaka, Pure absorption electron spin echo envelope modulation spectra by using filter-diagonalization method for harmonic inversion, *J. Magn. Reson.* **137,** 221–230 (1999).

12. P. Mutzenhardt, P. Palmas, J. Brondeau, and D. Canet, Quantitative time-domain analysis of two-dimensional heteronuclear Overhauser effect data by the HD method, *J. Magn. Reson. A* **104,** 180–189 (1993).

13. G. Bodenhausen, R. Freeman, and D. L. Turner, Suppression of artifacts in two-dimensional J spectroscopy, *J. Magn. Reson.* **27,** 511–514 (1977).

14. P. Mutzenhardt, J. Brondeau, F. Humbert, and D. Canet, Algorithm based on a new proof of HSVD equations derived without invoking singular value decomposition, *J. Magn. Reson.* **94,** 543–548 (1991).

15. H. Barkhuijsen, R. de Beer, and D. Van Ormondt, Improved algorithm for noniterative time-domain model fitting to exponentially damped magnetic resonance signals, *J. Magn. Reson.* **73,** 553–557 (1987).

16. V. A. Mandelshtam, N. D. Taylor, H. Hu, M. Smith, and A. J. Shaka, Highly resolved double absorption 2D NMR spectra from complex severely truncated 2D phase-modulated signals by filter-diagonalization-averaging method, *Chem. Phys. Lett.* **305,** 209–216 (1999).