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Communication

Two-dimensional *J*-spectra with absorption-mode lineshapes

Andrew J. Pell, James Keeler *

University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, CB2 1EW, UK

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Abstract

Two-dimensional *J*-spectroscopy offers the possibility of a complete separation of chemical shifts and *J*-couplings. However, the usefulness of the experiment is considerably reduced by the fact that peaks in the spectra have the phase-twist lineshape. We present a simple new spectroscopic method for recording *J*-spectra in which the peaks are both in the absorption mode and retain their natural intensities, albeit at the cost of a considerable reduction in the signal-to-noise ratio. No special data-processing is required. The method is tested on quinine, and the steroid dehydroisoandrosterone.

Keywords: NMR; *J*-spectroscopy; Broadband homonuclear decoupling

1. Introduction

In this communication we describe a new method for recording two-dimensional *J*-spectra with absorption-mode lineshapes. After tilting by 45°, such spectra offer a complete separation of chemical shifts in one dimension and *J*-couplings in the other, thus facilitating the analysis of overlapping spectra [1]. However, when recorded in the conventional way, *J*-spectra show the phase-twist lineshape which is not suitable for high-resolution work on account of the broad dispersive component. There are a number of ways of eliminating this phase-twist lineshape, but these all suffer from various drawbacks. The most popular method, which is to use a pseudo-echo weighting function to eliminate the dispersive component, results in severe intensity distortions [2]. Other methods which rely on special data processing methods have proved to be successful, but have not achieved wide acceptance [3–10]. In contrast, our method is simple to implement, requires no special data processing, and retains the natural intensities of the conventional one-dimensional proton spectrum. A key element of our pulse sequence is the combination of a selective 180° pulse with a simultaneous weak gradient—a pulse sequence element introduced by Zangger and Sterk [11]. This ingenious element has the effect of separately inverting each spin while leaving all its coupled partners unaffected.

We have recently shown that this separation of shifts and couplings, while retaining absorption-mode lineshapes, can also be obtained from a modified anti *z*-COSY experiment [12]. The experiment described in this communication is an alternative to the anti *z*-COSY approach, and has some advantages in terms of simplicity. However, in common with our earlier experiment, the generation of an absorption mode spectrum comes at the cost of a significant reduction in the signal-to-noise ratio.

Our method for recording absorption-mode *J*-spectra is based on the well-known idea that a P-type and an N-type spectrum can be combined to give an absorption-mode spectrum [13,14]. Both the P- and N-type spectra have phase-twist lineshapes, but if the ω_1 axis of one of the spectra is reversed, and the two-spectra added together, it turns out that the dispersive parts of the phase-twist lineshapes cancel. The pulse sequence for conventional *J*-spectroscopy, along with the coherence transfer pathway (CTP) is shown in Fig. 1(a). We cannot really describe this as the P- or N-type spectrum, since the coherence order changes in the middle of t_1. Fig. 1(b) shows the CTP of the complementary *J*-spectrum which will have a phase-twist in the

* Corresponding author. Fax: +1223 336913.
E-mail address: jhk10@cam.ac.uk (J. Keeler).

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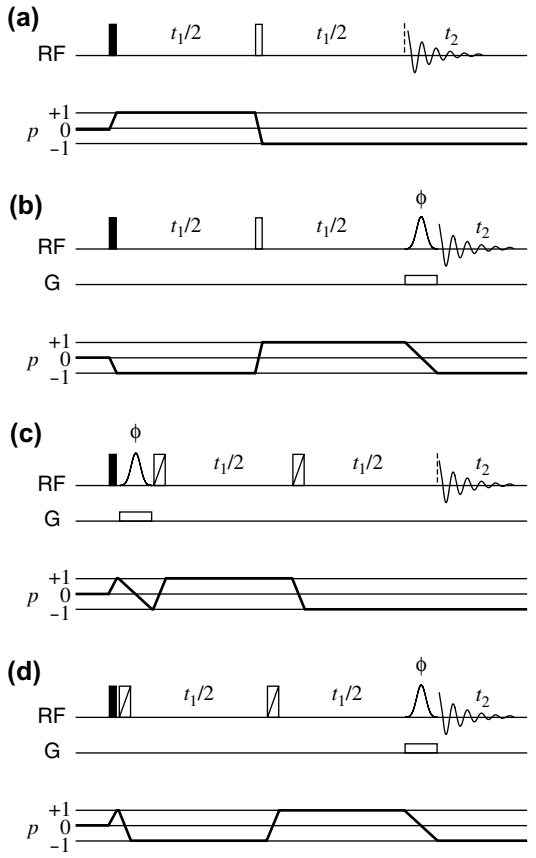

Fig. 1. Pulse sequences for recording absorption-mode *J*-spectra. Shown in (a) is the sequence and CTP for the conventional *J*-spectroscopy experiment. The filled rectangle represents a 90° pulse, and the unfilled rectangle represents a 180° pulse. Sequence (b) is for the anti *J*-spectrum which, when combined with the *J*-spectrum, results in an absorption-mode lineshape. The selective 180° pulse is indicated by the Gaussian profile. In practice, the sequences in (c) and (d) are used to record the *J*- and anti *J*-spectra respectively, so that both spectra have the same intensity. Note the use of the BIPs in (c) and (d), which are indicated by the unfilled rectangles with the diagonal stroke. All pulses have phase x apart from the selective pulses, which are phase-cycled as $\phi = [x, y, -x, -y]$, along with the cycle $\phi_{rx} = [x, -x, x, -x]$ for the receiver phase.

opposite sense: we term this the *anti J-spectrum*. Note that in this sequence the coherence order is -1 during the first half of t_1, and $+1$ during the second half, opposite to the CTP in Fig. 1(a).

Normally, we can switch between the P- and N-type CTP simply by changing the phase cycling or gradient pulses. However, it is not possible to switch between the CTPs for the *J*- and anti *J*-spectra in this way on account of the fact that there is no mixing period in the *J*-spectroscopy experiment.

It might be thought that placing a non-selective 180° pulse at the end of t_1 in the anti *J*-spectroscopy sequence would achieve the desired effect, since this will change the coherence order from $+1$ to -1. While this is true, the pulse also has the effect of flipping the spin states of *all* the passive spins; as a result, when the *J* and anti *J*-spectra are combined it turns out that the dispersive parts of the lineshape do not cancel. In fact, the desired result can only be achieved by applying a selective 180° pulse to one spin, such that the spin states of all of the passive spins are not flipped. Such a selective experiment could only be performed on one spin at a time, which would not lead to a very useful two-dimensional spectrum.

The key point of our new method is that we can achieve the effect of this selective 180° pulse by placing the Zangger–Sterk pulse sequence element at the end of t_1, as shown in Fig. 1(b). This element consists of a selective 180° pulse in combination with a weak gradient. The gradient strength is chosen such that the spread of frequencies across the sample matches the width of the whole spectrum. This means that a spin at a particular chemical shift comes into resonance with the selective pulse at a particular *position* in the sample. Thus, when the signal from the whole sample is measured, each spin has experienced precisely the kind of selective 180° pulse we require. It is important that the selective pulse be phase cycled so as to select that part of the sample which experiences a refocusing pulse.

There are other ways in which this apparent selective inversion of all of the passive spins can be achieved, such as the time-reversal method of Sørensen, Griesinger and Ernst [15], and the anti *z*-COSY mixing scheme employed in our earlier work [12]. However, both of these methods lead to the generation of cross peaks which, on account

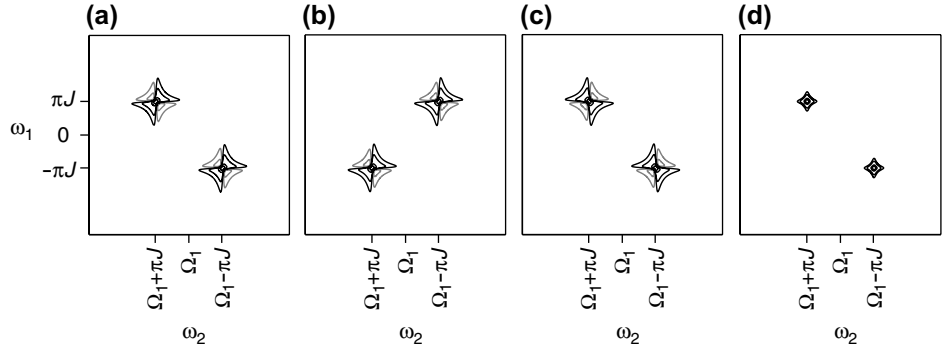

Fig. 2. Simulated spectra from one of the doublets of a two-spin system. The conventional *J*-spectrum is shown in (a), and the anti *J*-spectrum, in which the multiplets tilt in the opposite direction, is shown in (b). The peaks have the characteristic phase-twist lineshape. The ω_1-axis of the spectrum in (b) is reversed to give (c), which is then added to (a) to give the absorption-mode spectrum in (d). Positive contours are coloured black, and negative contours are grey.

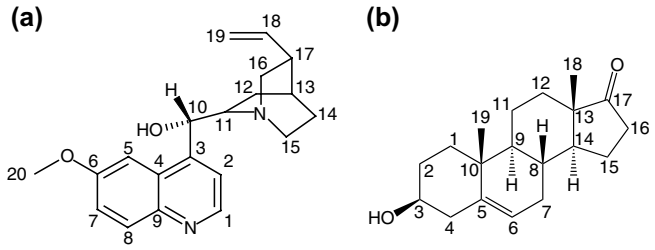

Fig. 3. The structures of (a) quinine, and (b) the steroid dehydroisoandrosterone.

of the refocusing employed in t_1, will fall directly on top of the absorption mode multiplets we are interested in. The beauty of the Zangger–Sterk pulse sequence element employed here is that, since it is a true 180° pulse, no cross peaks are generated.

The way in which an absorption-mode spectrum is generated is illustrated with the simulations, shown in Fig. 2, of a multiplet from a typical two-spin system. The conventional J-spectrum is shown in Fig. 2(a), the anti J-spectrum is shown in (b), and (c) is spectrum (b) with the ω_1-axis reversed. The absorption-mode spectrum resulting from

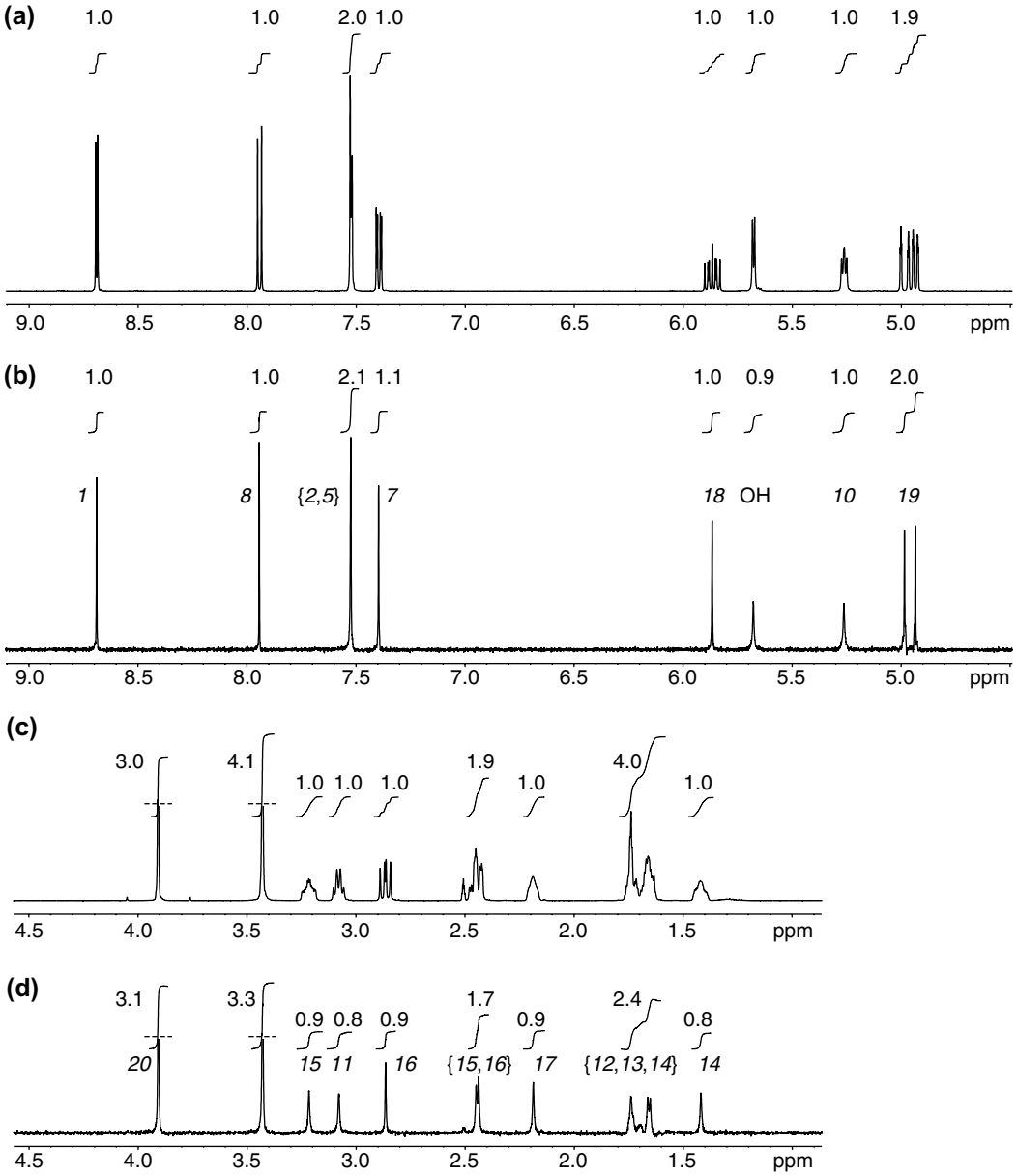

Fig. 4. Conventional spectra and projections of the absorption-mode J-spectra of quinine; the projections show a single line at the shift, i.e. they are decoupled. The spectrum is shown in two parts: (a) and (b) are the aromatic and vinyl region, whereas (c) and (d) are the aliphatic region. The projection shown in (b) is of the region shown in (a); likewise (d) is the projection of the region shown in (c). The assignment of the peaks in (b) and (d) is indicated by the numbers in italics, and corresponds to the labelling in Fig. 3 (a). *Experimental details*: the J-spectrum was recorded at 500 MHz for protons, with 36 scans per t_1 increment. The selective pulse was a Gaussian of length 57.6 ms, truncated at 1% [20]; the accompanying gradient had a strength of 3% of the maximum value of 59.5 G cm^{-1}. The B_1 field strength and length of the BIP were 20 kHz and 100 μs [17]. The spectral widths in ω_1 and ω_2 were 50 and 4496 Hz, respectively. The acquisition time in t_2 was 1.8 s; 85 t_1 increments were recorded, giving a maximum value of t_1 of 1.7 s.

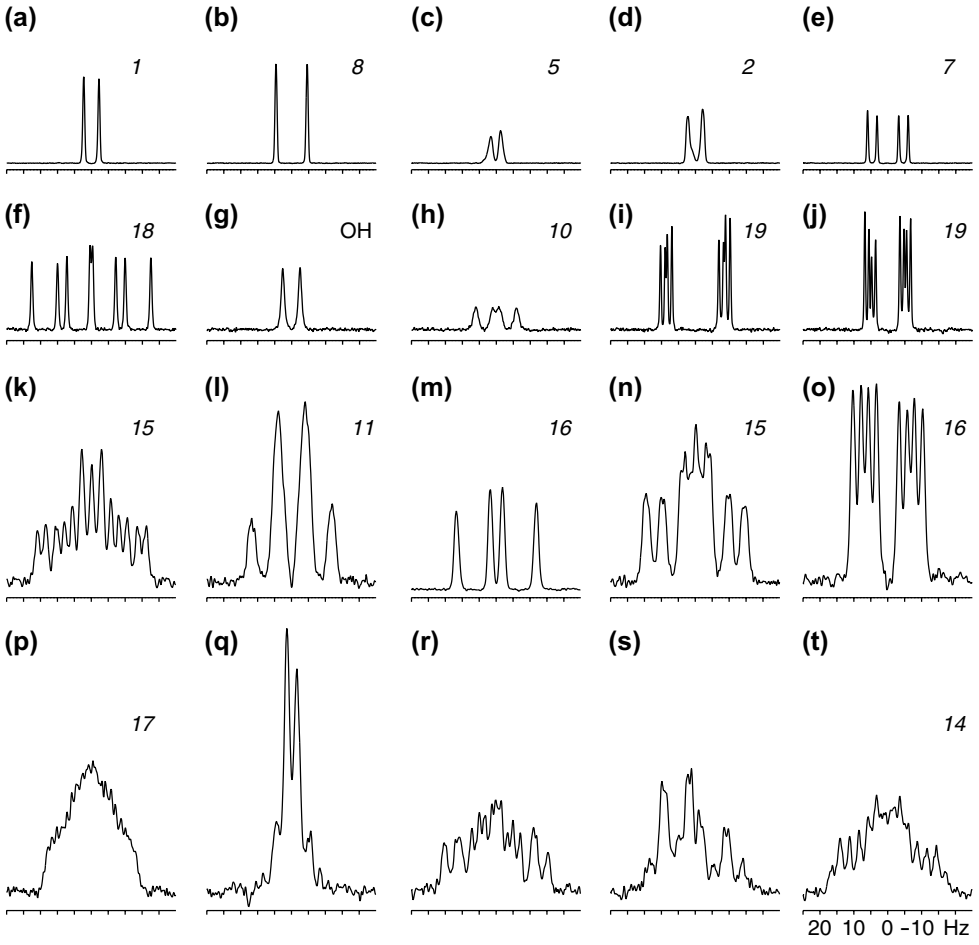

Fig. 5. Cross-sections taken parallel to ω_1 from the tilted absorption-mode *J*-spectrum of quinine. Multiplets (a)–(e) are plotted on the same vertical scale, whereas (f)–(j) are 5-fold expansions. The multiplets (k)–(t) of the aliphatic protons were taken from a separate experiment with a smaller number of t_1 increments. Multiplets (k), (l), and (n)–(t) are plotted on a scale that is expanded by five times relative to (m). The assignment is given by italicized numbers; multiplets (q)–(s) are associated with H_{12}, H_{13}, and H_{14}, but the assignment of which is which is unclear. *Experimental details*: the experimental parameters for (a)–(j) are as given in the caption to Fig. 4. Multiplets (k)–(t) were taken from a spectrum in which the acquisition time in t_2 was 0.9 s, and in which 45 t_1 increments were recorded, giving a maximum value of t_1 of 0.9 s. The number of scans per increment was 160.

the combination of (a) and (c) is shown in (d). Rather than combining the spectra after the double Fourier transformation, it is computationally more efficient to make the appropriate combinations of the time-domain data after the first Fourier transformation [13,14]. The facility provided on many spectrometers for processing P- and N-type data into absorption-mode spectra is exactly what is needed to process the *J*- and anti *J*-spectra.

The drawback with the Zangger–Sterk element is that, for a given spin, only that part of the sample which is on-resonance with the selective 180° pulse contributes to the signal. This means that there is a considerable reduction in the size of the detected signal, of the order $\Omega_B/(\gamma G\, l)$, where Ω_B is the bandwidth of the selective pulse, γ is the gyromagnetic ratio, G is the gradient strength, and l is the length of the sample that lies within the receiver coil. Thus, the more selective the pulse is made, the lower the sensitivity.

For this 180° pulse to lead to the removal of the splitting due to a particular coupling it must be sufficiently selective such that it affects only one of the coupled partners. The selectivity of the pulse, and hence the sensitivity of the experiment, is thus set by the closest separation between two spins which it is desired to decouple from one another. In their original experiments Zangger and Sterk estimated that they retained about 2% of the original signal, whereas Nilsson and Morris [16] use a 9 ms pulse which we estimate retains around 8% of the signal. In our experiments we have tended to use somewhat longer pulses, trading off the improvement in decoupling between closely-spaced multiplets with a further reduction in the signal-to-noise ratio. For the experiments reported here, we estimate that around 0.3% of the signal is retained.

It is also necessary to make sure that the *J*- and anti *J*-spectra have the same intensity, otherwise the dispersive component of the lineshape will not be eliminated. In prac-

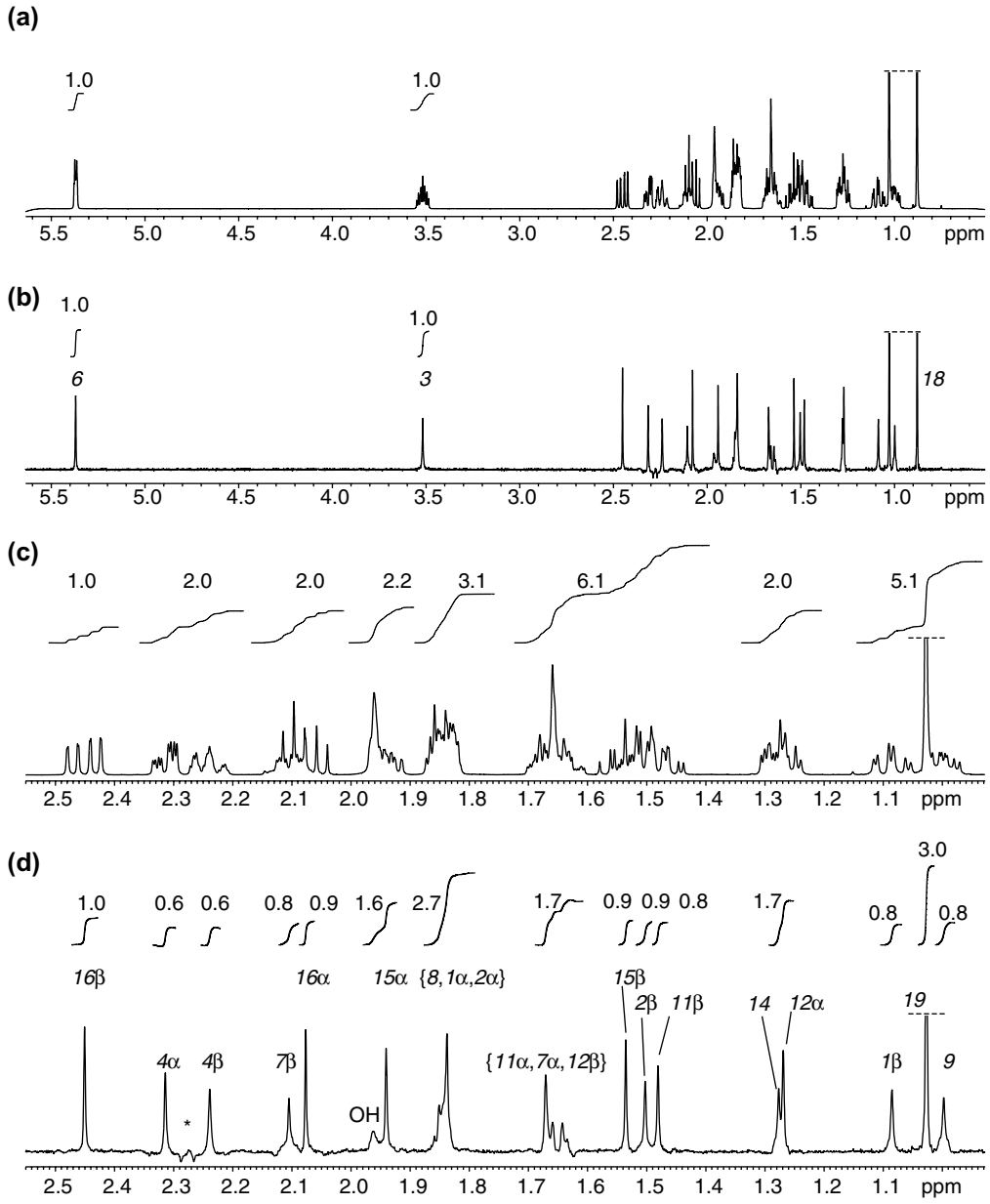

Fig. 6. The conventional spectrum and projection of the absorption-mode *J*-spectrum of dehydroisoandrosterone. In (a) is shown the conventional spectrum of the full chemical shift range. The projection of the *J*-spectrum of the same region is shown in (b). Expansions of the crowded region of the conventional and decoupled spectra are shown in (c) and (d). In the projection (d), the * shows the position of the residual intensity of the strong coupling artefact. The assignment in (b) and (d) corresponds to the atom numbering in Fig. 3(b). *Experimental details*: the *J*-spectrum was acquired with 44 scans per t_1 increment. The spectral widths in ω_1 and ω_2 are 100 and 2561 Hz, respectively. The acquisition time in t_2 was 1.6 s; 200 t_1 increments were recorded, giving a maximum value of t_1 of 2.0 s. All other parameters are as given in the caption to Fig. 4.

tice, we have found it convenient to use the pulse sequences shown in Fig. 1(c) and (d) to record the required pair of complementary spectra.[1]

In the *J*-spectroscopy experiment in (c), the selective pulse and gradient combination is inserted before t_1, so that the same part of the sample is selected as for the anti *J*-spectroscopy experiment shown in (d); the two spectra therefore have the same intensities. Both sequences also use the broadband inversion pulses (BIPs) of Smith et al. in place of the conventional 180° pulses [17]; the specific pulse that was used is designated BIP-720-25-40. We found that the inclusion of these BIP pulses reduced the level of artefacts associated with imperfect refocusing to the level where phase cycling of these pulses was not required. It is necessary to use *two* such pulses in the sequence so that the phase errors introduced by the first are refocused by the second.

In strongly-coupled spin systems, it is found that extra peaks appear in the *J*-spectrum, which complicate their

[1] The pulse sequences are available on the WWW at http://www-keeler.ch.cam.ac.uk.

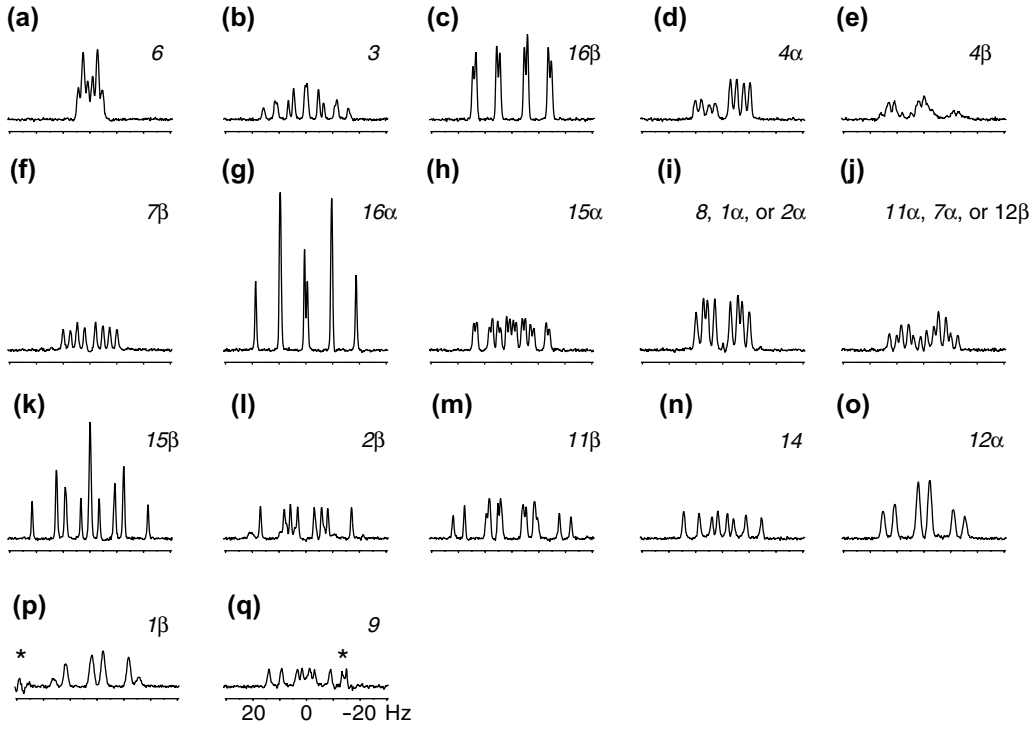

Fig. 7. Cross-sections taken parallel to ω_1 from the tilted absorption-mode J-spectrum of dehydroisoandrosterone. The assignment of each multiplet is given. The positions indicated by the * in (p) and (q) show disturbances due to the t_1 noise from the neighbouring methyl group.

interpretation [18,19]. These so-called strong coupling artefacts arise because the 180° pulse causes a mixing effect which can loosely be described as a transfer of magnetization from the active spin to its strongly-coupled partner. Within each multiplet, these peaks due to strong coupling occur in pairs: one is positive, and the other is negative. For example, in the spectrum of an AB system, there are two pairs of extra peaks, one centred on $\omega_1 = \frac{1}{2}C$, the second centred on $\omega_1 = -\frac{1}{2}C$, where $C = \sqrt{(\Omega_1 - \Omega_2)^2 + (2\pi J)^2}$. When the spectrum is tilted, the peaks in each pair line up on the same ω_2 coordinate, and so are added when the projection is computed. As the peaks have opposite signs they partly cancel in the projection, and it can be shown that the residual intensity in the projection is $2\sin^2 2\theta$, where $\tan 2\theta = (2\pi J)/(\Omega_1 - \Omega_2)$. For modest strong coupling, i.e. first order in θ, the cancellation is complete. This cancellation will only occur if a phase-sensitive spectrum is projected. Thus, we can expect the intensity of the strong coupling artefacts to be much weaker in the absorption-mode spectrum than in the conventionally processed magnitude spectrum.

2. Results

The method is illustrated with the spectra of quinine and the steroid dehydroisoandrosterone, the structures of which are shown in Fig. 3(a) and (b). In Fig. 4 are shown the conventional proton spectra and the projection of the absorption-mode J-spectrum of quinine. The aromatic and vinyl region is shown in (a) and (b), while the aliphatic region is shown in (c) and (d). The integrals of the peaks in the projection are in good agreement with those in the conventional spectrum, except in the case of the overlapping multiplets around 1.7 ppm. This retention of the natural intensities is a particular feature of our method.

Fig. 5 shows the cross-sections taken parallel to ω_1 from the tilted two-dimensional spectrum; the multiplet structures exhibit the narrow lineshapes that are expected from J-spectroscopy in which inhomogeneous broadening is refocused in this dimension.

The second test is the more complex spectrum of dehydroisoandrosterone, shown in Fig. 6(a). There are several overlapping multiplets in the region 1.0–2.5 ppm (expansion shown in (c)), making this spectrum more difficult to assign. The projection of the absorption-mode J-spectrum is shown in (b) and (d). The integrals in the projection are again in good agreement with those in the normal spectrum. There is also much improved separation in the crowded region, as is particularly evident from comparing (c) and (d).

The multiplets taken from the cross-sections are shown in Fig. 7. It is clearly seen that, even in the crowded region, it is possible to obtain well-resolved multiplets.

3. Conclusion

The presence of the phase-twist lineshape is arguably the factor that has limited the applicability of conventional J-spectroscopy, and so over the years a number of

approaches to eliminating this undesirable lineshape have been developed. We have shown that the use of the Zangger–Sterk pulse sequence element in the standard pulse sequence makes it possible to record absorption-mode spectra with natural intensities. The price that must be paid is a reduction in the sensitivity. However, the resulting spectra are of such quality that the reduction in sensitivity may well be tolerable.

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