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Determination of Homonuclear Scalar Coupling Constants by Combining Selective Two-Dimensional NMR Spectroscopy with Convolution and Deconvolution: Applications to Paclitaxel (Taxol)

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Homonuclear proton–proton coupling constants can be determined accurately by recording selective two-dimensional correlation spectra and by analyzing the resulting multiplets by two-dimensional convolution and deconvolution methods. In this work, these methods were evaluated by attempting to determine the complete set of homonuclear couplings in paclitaxel (taxol). Convolution methods have been applied successfully to ten pairs of complementary multiplets recorded by selective correlation spectroscopy (soft-COSY) and pure in-phase correlation spectroscopy (PICSY). These pairs of multiplets gave reliable measurements of ten active couplings. In some cases, satisfactory PICSY spectra could not be obtained, so that the complementary information of soft-COSY and PICSY could not be exploited. Furthermore, couplings involving two strongly-coupled diastereotopic protons (14H$^{\alpha}$ and 14H$^{\beta}$) could not be determined in a satisfactory manner. Further problems arose due to partial overlap of the chemical shifts of two other protons (10H and 13H). Deconvolution has been applied successfully to all 14 soft-COSY multiplets, yielding 14 estimates of active couplings and 38 estimates of passive splittings. This work provides between one and seven estimates for 21 coupling constants. The accuracy of these different estimates must be weighed with the complexity of the methods used. This work may help to assess the perspectives of completely automated computer-supported analysis of two-dimensional spectra.

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INTRODUCTION

Homonuclear scalar coupling constants play an essential role in the determination of molecular constitution and conformation. Because of overlapping multiplets and imperfect lineshapes, the traditional method of simply measuring splittings in conventional one-dimensional proton spectra tends to be unreliable in all but the simplest cases. Two-dimensional (2D) double-quantum filtered correlation spectra (DQF-COSY) are not very suitable for the accurate determination of coupling constants because of the complexity of the multiplets and their limited digital resolution. Selective 2D methods, such as selective correlation spectroscopy (soft-COSY)[1,2] and pure in-phase correlation spectroscopy (PICSY),[3,4] have the advantage that they allow one to 'zoom in' on selected cross-peak multiplets and therefore offer much higher digital resolution. Moreover, they provide complementary multiplet patterns which lend themselves to analysis by convolution methods.[5–7] The convolution of complementary multiplets with two-dimensional arrays of delta functions separated by a trial coupling constant J^* leads to pairs of spectra which have a high degree of similarity when J^* is equal to the true active coupling constant J_{act}. An alternative method is provided by deconvolution[8,9] of a single multiplet obtained by soft-COSY. This allows one to simplify multiplet structures by removing splittings due to different coupling partners in successive steps. Both active and passive coupling constants can thus be determined. This paper describes applications of various methods to paclitaxel (taxol) (Fig. 1) in order to evaluate their advantages and drawbacks.

THEORETICAL

In two-dimensional correlation spectra, a scalar coupling between two weakly coupled spins A and X gives rise to a two-dimensional multiplet with four components that have alternating phases $\begin{bmatrix} + & - \\ - & + \end{bmatrix}$ in COSY, DQF-COSY or soft-COSY, and identical phases $\begin{bmatrix} + & + \\ + & + \end{bmatrix}$ in TOCSY or PICSY. The dimensions of these squares

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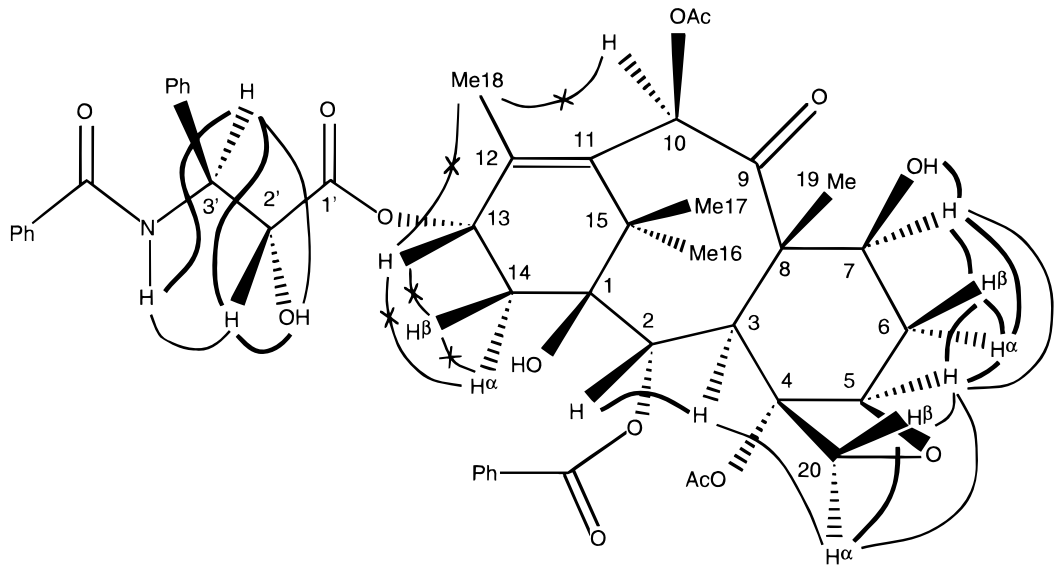

Figure 1. Molecular structure of paclitaxel (taxol), with thick curves representing J-couplings that could be determined by both convolution and deconvolution methods and thin curves representing couplings that could only be measured by deconvolution. Curves with crosses represent couplings that could not be reliably measured because of strong coupling or overlap.

are determined by the active coupling constant J_{AX} in both ω_1 and ω_2 dimensions. Such multiplets can be reconstructed by convolution of a two-dimensional square array of delta functions with a basic peak shape, which is determined by transverse relaxation and by the homogeneity of the static field. In a system with more than two spins, each passive coupling partner K gives rise to additional splittings. In DQF-COSY and TOCSY these splittings can be represented by a (generally rectangular) matrix containing four delta functions with identical signs $[^{+\ +}_{+\ +}]$, while in soft-COSY and PICSY these splittings can be represented by a rectangular matrix containing only two delta functions $[_{+}\ ^{+}]$, again with the same signs. In both cases, the displacements of these delta functions are given by J_{AK} in the (vertical) ω_1 dimension and J_{XK} in the (horizontal) ω_2 dimension.

These principles can be expressed by a few simple relationships:

$$\text{soft-COSY} = \text{peak shape} \otimes \begin{bmatrix} + & \leftarrow J_{AX} \rightarrow & - \\ \uparrow & & \uparrow \\ J_{AX} & & J_{AX} \\ \downarrow & & \downarrow \\ - & \leftarrow J_{AX} \rightarrow & + \end{bmatrix} \otimes \begin{bmatrix} & & + \\ & & \vert \\ & & J_{AK} \\ & & \downarrow \\ + & \leftarrow J_{XK} - & \end{bmatrix} \otimes \ldots \quad (1)$$

$$\text{PICSY} = \text{peak shape} \otimes \begin{bmatrix} + & \leftarrow J_{AX} \rightarrow & + \\ \uparrow & & \uparrow \\ J_{AX} & & J_{AX} \\ \downarrow & & \downarrow \\ + & \leftarrow J_{AX} \rightarrow & + \end{bmatrix} \otimes \begin{bmatrix} & & + \\ & & \vert \\ & & J_{AK} \\ & & \downarrow \\ + & \leftarrow J_{XK} - & \end{bmatrix} \otimes \ldots \quad (2)$$

Coupling constants can be determined very accurately if one uses a pair of multiplets with complementary structures.[5] Soft-COSY and PICSY multiplets have identical structures as far as the passive couplings are concerned, but the pattern of the signs associated with the active couplings is different. The latter can be retrieved[6,7] by convoluting the soft-COSY multiplet with a square array of in-phase delta functions $[^{+\ +}_{+\ +}]$ separated by a trial splitting J^*, while the PICSY multiplet is convoluted with a square array of antiphase delta functions $[^{+\ -}_{+\ +}]$ with the same trial splitting:

$$\mathbf{S} = \text{soft-COSY} \otimes \begin{bmatrix} + & \leftarrow J^* \rightarrow & + \\ \uparrow & & \uparrow \\ J^* & & J^* \\ \downarrow & & \downarrow \\ + & \leftarrow J^* \rightarrow & + \end{bmatrix} \quad (3)$$

$$\mathbf{S}' = \text{PICSY} \otimes \begin{bmatrix} + & \leftarrow J^* \rightarrow & - \\ \uparrow & & \uparrow \\ J^* & & J^* \\ \downarrow & & \downarrow \\ - & \leftarrow J^* \rightarrow & + \end{bmatrix} \quad (4)$$

If $J^* = J_{AX}$, the matrices S and S′ should be similar. Two methods have been used to assess the similarity of these matrices. In the first method, each matrix is represented by a normalized N-dimensional vector, and a scalar product of these two vectors is calculated. This scalar product approaches unity if the two normalized vectors are nearly parallel, which occurs when J^* is a good estimate of the active coupling constant.[7,10,11]

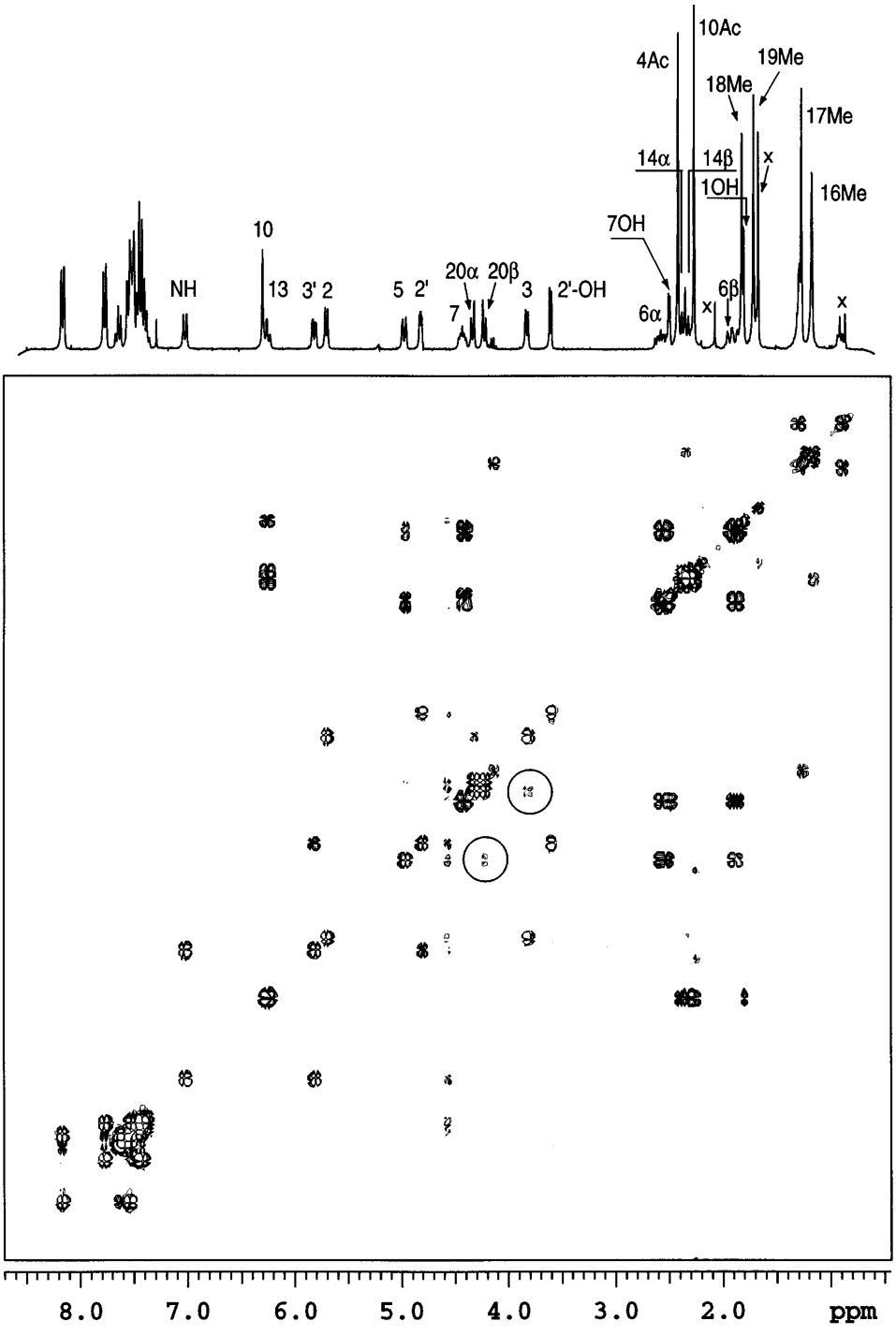

Figure 2. One-dimensional proton spectrum and double-quantum filtered correlation spectrum (DQF-COSY) of paclitaxel (taxol) recorded at 300 MHz (7 T) in $CDCl_3$ at 300 K. The spectrum was recorded with a spectral width of 2480 Hz in both dimensions, using 512 × 2048 points before and 1024 × 2048 points after zero-filling prior to Fourier transformation. Two cross peaks due to long-range $^4J_{HH}$ couplings are emphasized by circles (see text).

The second method consists in plotting the sum of the squares of the differences between the N points of the two convoluted spectra as a function of the trial coupling constant J^*. The best coupling constant is obtained when the χ^2 function reaches a minimum.

Deconvolution is based on a different principle. In favorable cases, it allows one to extract all splittings that appear within a given multiplet, and identify both the squares due to active couplings and the displacement vectors due to passive spins. In soft-COSY, the active coupling constant is determined by deconvolution with an antiphase square array of delta functions $[^{+}_{-}\ ^{-}_{+}]$ with a splitting J^*_{AX}. Passive coupling constants can be identified by deconvolution of the multiplet with a (usually rectangular) array of delta functions $[_{+}\ ^{+}]$ separated by two splittings J^*_{AK} and J^*_{XK} in the ω_1 and ω_2 dimensions, respectively. A measure of the degree of simplification[8] achieved by deconvolution can be obtained by calculating either a 'global' or a 'marginal' integrated absolute value, obtained by summation over

the entire surface or suitably chosen strips of the absolute value of the deconvoluted two-dimensional spectrum. The minima of these functions correspond to the true coupling constants.

EXPERIMENTAL

Paclitaxel (Fig. 1) is a natural product that shows significant biological activity against ovarian and breast cancer,[12] which has recently been obtained by total synthesis by the groups of Holton in Tallahassee[13,14] and Nicolaou in La Jolla,[15] while the group of Potier in Gif-sur-Yvette has described a partial synthesis.[16,17]

A sample of 6 mg of paclitaxel, synthesized by Holton and co-workers, was dissolved in *ca* 0.7 ml of $CDCl_3$ (99.96%), obtained from Aldrich, and filtered through a *ca* 1 cm layer of Al_2O_3 powder to neutralize traces of HCl. The solution was subjected to four freeze–pump–thaw cycles before being sealed. The concentration was *ca* 10 mM. All spectra were acquired with a Bruker DMX-300 spectrometer at 300 MHz and 300 K. It is worth noting that even at a much higher field of 720 MHz, the spins $14H^{\alpha}$ and $14H^{\beta}$ are still too strongly coupled to allow extraction of accurate coupling constants by the methods discussed above. The DQF-COSY spectrum was acquired with a spectral width of 2480 Hz in both dimensions, $512 \times 2K$ data points before and $1K \times 2K$ after zero-filling, resulting in a digital resolution of 2.42 and 1.21 Hz per point in the ω_1 and ω_2 dimensions, respectively. All soft-COSY[2] and PICSY[3,4] experiments were acquired with spectral windows of 60×60 Hz centered on the chemical shifts of the relevant spins. For all selective experiments, 128×256 data points were acquired and Fourier transformed without zero-filling, resulting in a digital resolution of 0.469 and 0.234 Hz per point in the ω_1 and ω_2 domains, respectively. The digital resolution was enhanced by trigonometric interpolation (see below). In all selective experiments, the duration of the 270° Gaussian excitation and purging pulses was 30 ms, truncated at 2.5%, with peak RF amplitudes of about 55 Hz. For PICSY, the doubly selective irradiation period τ_{DSI} was set to be close to $1/J$, rounded off to an integer multiple of the inverse of the frequency separation between the relevant proton pair.[3,4] The RF amplitude during the doubly-selective irradiation period was about 55 Hz for each sideband. Typical data acquisition times were 42 min for each soft-COSY and 1.5 h for each PICSY multiplet. No window function was applied before Fourier transformation in any of the spectra, except for the DQF-COSY shown in Fig. 2.

The algorithms for convolution and deconvolution were implemented in C language on a Silicon Graphics Instruments Power Challenge with 75 MHz R8000 MIPS processors. Before the calculations, a suitable window was excised, depending on the width of the multiplets, and the digital resolution of the multiplets was increased to 0.05 Hz per point by trigonometric interpolation. The trial coupling constants J^* were typically varied from 0.05 to 18 Hz in steps of 0.05 Hz. Both convolution and deconvolution methods allow one to obtain active coupling constants in less than 1 min of CPU time. For the determination of passive coupling constants with the deconvolution method, two trial coupling constants must be varied systematically in the two orthogonal frequency dimensions. To make this process less time consuming, a first search was conducted using multiplets with a modest digital resolution of 0.5 Hz per point to obtain rough estimates of the coordinates of the minima in the two-dimensional global magnitude functions. The digital resolution of the multiplets was then increased by trigonometric interpolation to 0.05 Hz per point, and the two trial coupling constants were then varied systematically in smaller steps of 0.05 Hz to refine the estimates of the coordinates of the minima. The typical CPU time needed for this refinement process was about 2 h for each multiplet.

RESULTS AND DISCUSSION

The ^1H and ^{13}C resonances of paclitaxel have been completely assigned[18–20] and the J_{HH} coupling constants have been estimated from conventional 1D and 2D NMR data.[20–23] Table 1 lists the homonuclear scalar couplings J_{HH} that were re-investigated in this work. Couplings between aromatic nuclei have not been included. In the DQF-COSY of paclitaxel (Fig. 2), cross-peak multiplets could be observed for three geminal pairs, 11 vicinal pairs and two long-range couplings (13H–18Me and 10H–18Me). For ten of these 16 spin pairs, it was possible to acquire both soft-COSY and PICSY spectra of good quality. The soft-COSY and PICSY multiplets that were obtained for $14H^{\alpha}$–$14H^{\beta}$, 13H–$14H^{\alpha}$ and 13H–$14H^{\beta}$ were distorted because of strong coupling[24] between $14H^{\alpha}$–$14H^{\beta}$. These multiplets could not be used for analysis. Although the resonances of 13H and 10H overlap, a good soft-COSY spectrum could be obtained with two partially overlapping multiplets for 13H–18Me and 10H–18Me, but we failed to obtain a satisfactory PICSY spectrum for these two multiplets. The chemical shifts of $20H^{\alpha}$ and $20H^{\beta}$ are close (separated by a mere 34 Hz at 300 MHz), so that it is difficult to excite one of the two spins selectively to obtain useful PICSY and soft-COSY spectra.

If the threshold is low enough, five cross peaks with very low intensities could be observed in DQF-COSY. These are due to weak $^4J_{HH}$ couplings (3H–$20H^{\alpha}$, 5H–$20H^{\beta}$, 2H–$14H^{\beta}$ and 16Me–17Me). For these cross peaks, we obtained only two good soft-COSY spectra for 3H–$20H^{\alpha}$ and 5H–$20H^{\beta}$ (circled cross peaks in Fig. 2), but no satisfactory PICSY spectrum could be obtained because the active couplings had a magnitude less than 2 Hz.

Convolution analysis of soft-COSY and PICSY

Ten pairs of soft-COSY and PICSY multiplets were analyzed successfully by 2D convolution, leading to estimates of ten J-coupling constants. Figure 3 shows four examples of increasing complexity. The multiplets obtained by selective methods provide better lineshapes and improved digital resolution compared with multi-

Table 1. Homonuclear J_{HH} coupling constants in paclitaxel (taxol) determined by various methods in this work and in earlier studies

	Convolution		Deconvolution (from Table 2)					
Spin pair	S	χ^2	Active	Passive[a]	Ref. 20	Ref. 21	Ref. 22	Ref. 23
J(NH,2′H)				−0.2, −0.15				
J(NH,3′H)	8.60	8.60	9.10	+8.75	8.82	8.8	8.9	8.7
J(2′H,3′H)	2.75	2.70	(3.00)	2.65, +2.75	2.73	2.6	2.8	2.5
J(2′H,2′OH)	5.20	5.20	5.45	+5.25	5.22	2.0	5.4	5
J(2′OH,3′H)				+0.40, +0.45				
J(2H,14H$^{\beta}$)						<0.7		
J(14H$^{\alpha}$,14H$^{\beta}$)						16	15.4	15.4
J(13H,14H$^{\alpha}$)				9.20[b]	∼9.1	8.8	9.0	8.9
J(13H,14H$^{\beta}$)				9.20[b]	∼9.1	8.8	9.0	8.9
J(13H,18Me)			(2.50)			1.5	1.5	
J(10H,18Me)			(∼2.5[c])					
J(2H,3H)	6.90	6.90	7.30	7.45	6.72	7	7.1	
J(3H,20H$^{\alpha}$)			(2.10)			∼0.7	1.1	
J(5H,20H$^{\alpha}$)				−0.75			0.8	
J(20H$^{\alpha}$,20H$^{\beta}$)				8.90, −9.5	8.43	8.8	8.5	
J(5H,20H$^{\beta}$)			(2.6[d])			1.1	1.0	
J(5H,7H)				−0.5, −0.25				
J(5H,6H$^{\alpha}$)	9.60	9.65	10.00	+9.70, +9.70, 9.80, 9.80	9.75	9.6	9.7	10
J(5H,6H$^{\beta}$)	2.45	2.35	(2.75)	+2.35, +2.40, 2.30, 2.45	2.13	2.5	2.3	2.3
J(6H$^{\alpha}$,6H$^{\beta}$)	14.50	14.45	15.20	−15.10, −15.10, −14.85, −14.75	14.5	15	14.8	14.6
J(6H$^{\alpha}$,7H)	6.65	6.60	7.10	+7.20, +6.55, 6.45, +6.25	6.6	6.8	6.7	6.5
J(6H$^{\beta}$,7H)	10.90	10.90	11.25	+11.20, +11.20, 10.45, +10.80	11.0	11	11.0	11
J(7H,7OH)	4.20	4.20	4.30	4.20, 4.05	4.5	<0.7	4.4	4.1

[a] The signs were determined using the relative signs derived from the multiplets, the knowledge that geminal couplings in aliphatic systems are negative and that vicinal couplings $^3J_{HH}$ must be positive if their absolute value exceeds 2 Hz. Numbers in parentheses are believed to be overestimated (see text).
[b] Two passive couplings could not be resolved.
[c] Estimate from poorly separated overlapping multiplets.
[d] Estimate from irregular multiplet structure obtained after deconvolution of all passive couplings.

plets extracted from the non-selective DQF-COSY spectrum. When a passive spin is coupled to both active spins, i.e. when the coupling network is 'cyclic,' a simplified multiplet structure is obtained in selective experiments, as evident in the fourth example. This is not obvious in the other examples in Fig. 3 because the long-range coupling constants (thin lines) are small.

When the ten pairs of soft-COSY and PICSY multiplets were convoluted with suitable arrays of delta functions, the comparison of the resulting matrices gave the similarity functions plotted in Fig. 4. The extrema of these functions yield the J couplings that are given in Table 1. Note that the agreement between the extrema in the two similarity functions is generally excellent. We believe these results to be reliable and accurate.

The advantage in digital resolution may be appreciated in the second row of Fig. 3: the splittings due to the passive spin 2′H can easily be seen in soft-COSY and PICSY, but can hardly be recognized in DQF-COSY owing to limited digital resolution. The third row in Fig. 3 illustrates a subsystem with two passive spins 2′OH and NH in addition to the active spins 2′H and 3′H. The improvement in digital resolution obtained with selective methods is striking in this case. The fine structure of the DQF-COSY cross-peak multiplet is not completely resolved, whereas the soft-COSY and PICSY multiplets are very clean and lend themselves to analysis by 2D convolution. The last row in Fig. 3 shows a subsystem where 5H and 6H$^{\beta}$ play the roles of active spins while 6H$^{\alpha}$ and 7H act as passive spins. As may be appreciated in Fig. 4, the extrema of the resulting similarity functions are not as well defined as in the preceding examples. This is due to the poor quality of the PICSY spectrum, which can be attributed in part to the small active coupling constant J(5H,6H$^{\beta}$) $\approx$ 2.4 Hz, and in part to the presence of large passive couplings (9.6, 10.9 and 14.5 Hz). These passive couplings can adversely affect the Hartmann–Hahn transfer during the double-selective irradiation.[25] Selective decoupling of the passive spins during the Hartmann–Hahn magnetization transfer might improve the situation, although preliminary experiments seem to indicate that such experiments are not straightforward to implement. The estimates for J(5H,6H$^{\beta}$) given by the extrema of the scalar product and χ^2 functions are 2.45 and 2.35 Hz, respectively. The discrepancy of 0.10 Hz between these two estimates is the worst of all cases analyzed by convolution (see Table 1).

Deconvolution analysis of soft-COSY multiplets

The determination of coupling constants by deconvolution has been applied successfully to 14 soft-COSY multiplets. The results are given in detail in Table 2 and listed in condensed form in Table 1 for comparison with the estimates obtained by convolution as described in the preceding section.

Two examples will be discussed in some detail to illustrate the principle of deconvolution. In the soft-

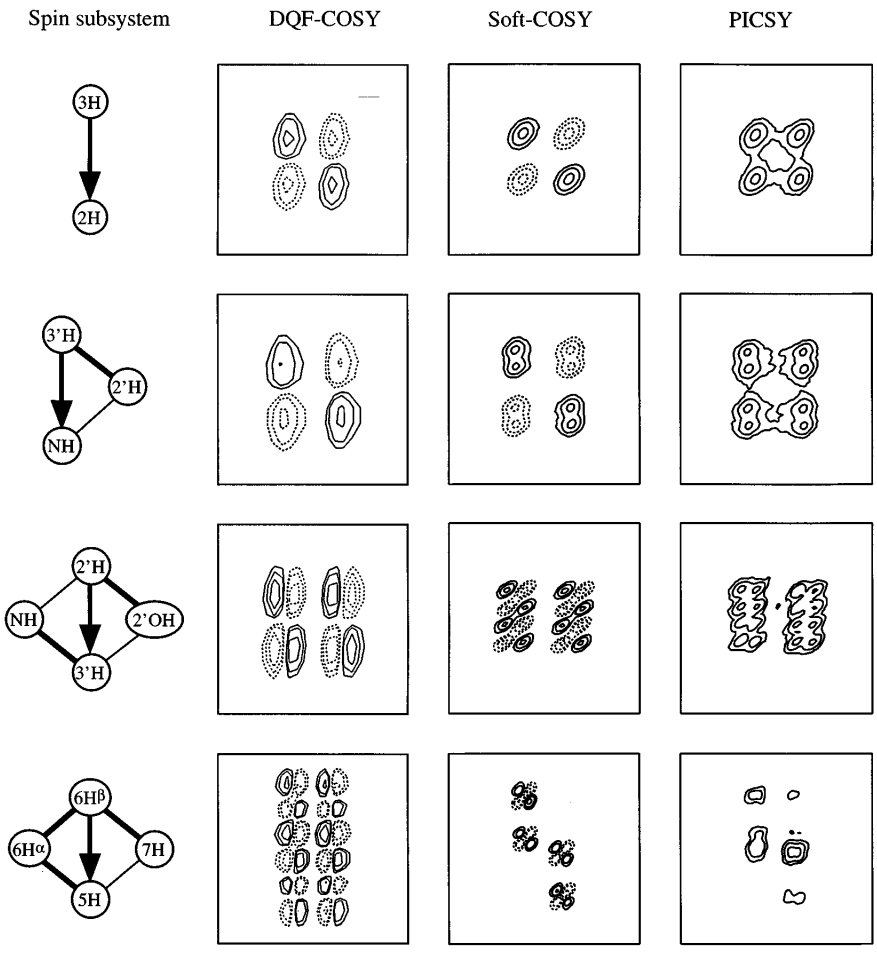

Figure 3. Four subsystems in paclitaxel (taxol) with corresponding multiplets observed in DQF-COSY, soft-COSY and PICSY. The first row shows the isolated two-spin subsystem 2H–3H. The second row shows a three-spin subsystem where 3′H and NH are active spins and 2′H is a passive spin. In the third row, 2′H to 3′H are active spins whereas 2′OH and NH are passive spins. In the fourth row, 5H and $6H^{\beta}$ are active whereas $6H^{\alpha}$ and 7H are passive spins. The arrows drawn in each subsystem indicate the direction of magnetization transfer in the experiments; the thin lines represent weak long-range couplings (see Table 1 for their magnitudes). The spectral windows shown are 30 × 30 Hz in the first three examples and 50 × 50 Hz in the fourth case. Solid and dashed lines represent positive and negative contours, respectively.

COSY multiplet in Fig. 5(b), which is due to magnetization transfer from 2′OH to 2′H, the splittings due to the active coupling can be eliminated by deconvolution. The global magnitude function is plotted in Fig. 5(c) as a function of the trial active coupling constant J^*. The minimum indicated by an arrow corresponds to the active coupling J(2′H,2′OH) = 5.45 Hz. Note the presence of a second (local) minimum which corresponds to a subharmonic of the true coupling. Subharmonics necessarily occur at lower frequencies, so that the global minimum can be identified by approaching from the right-hand side, i.e. by starting with large values of the trial coupling constant J^*. The simplified multiplet, where the active splitting has been removed, is shown in Fig. 5(d). A contour plot of the global magnitude function that is obtained when attempting to deconvolute the passive couplings is shown in Fig. 5(e). The displacement vector has two components corresponding to J(2′OH,3′H) = +0.45 Hz and J(2′H,3′H) = +2.75 Hz. The relative signs of the passive couplings are given by the slope of the corresponding displacement vector. The absolute signs are determined from the knowledge that geminal couplings in aliphatic systems are negative and that vicinal couplings are generally positive, although they could be negative if smaller than 2 Hz. These rules are applied to all cases given in Tables 1 and 2. If the sign cannot be determined (e.g. for active couplings or for non-cyclic passive couplings), neither positive nor negative signs are given in the tables.

Figure 6 shows the stepwise simplification of the soft-COSY multiplet due to magnetization transfer from $6H^{\beta}$ to $6H^{\alpha}$. Deconvolution with respect of the active coupling constant gives the global magnitude function shown in Fig. 6(c), which clearly indicates that $J(6H^{\alpha}, 6H^{\beta})$ = 15.20 Hz. Note the presence of two subharmonics at $J/2$ and $J/3$. By removing the splittings due to the active coupling, the simplified multiplet in Fig. 6(d) is obtained, which features only in-phase components displaced because of passive couplings partners. Deconvolution with respect to these passive couplings gives the two-dimensional global magnitude function of Fig. 6(e). One of the two minima, which corresponds to the coupling to the passive spin 5H, has the coordinates $J(5H,6H^{\beta})$ = +2.40 Hz and $J(5H,6H^{\alpha})$ = +9.70 Hz and the second minimum has the coordinates $J(6H^{\beta},7H)$ = +11.20 Hz and $J(6H^{\alpha},7H)$ = +6.55 Hz.

The remaining soft-COSY multiplets were analyzed in similar fashion. Some difficulties were encountered

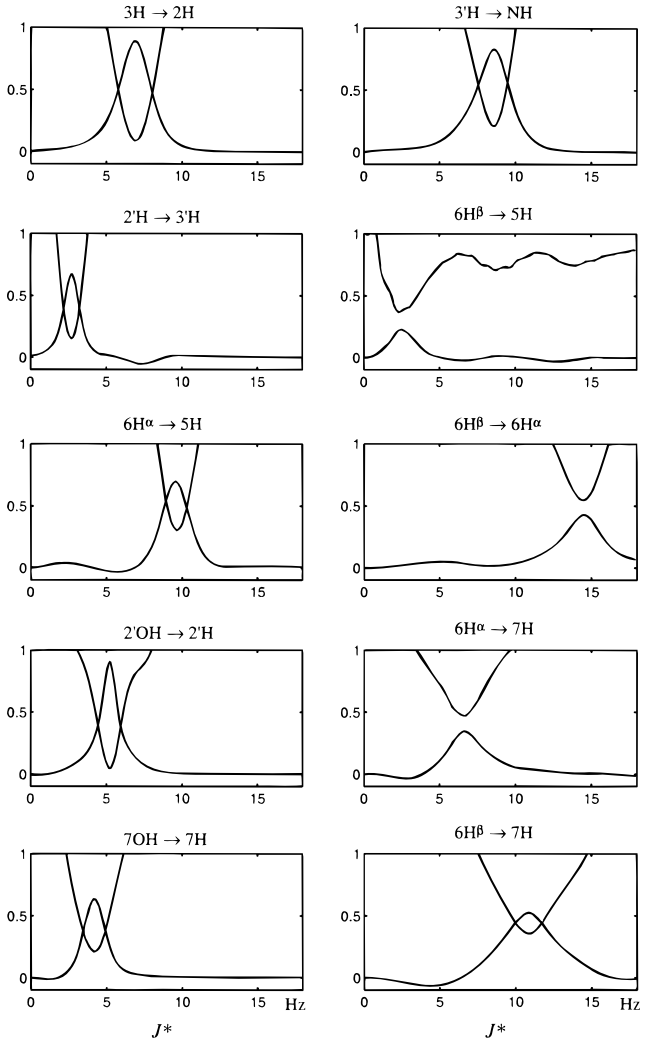

Figure 4. Comparison of ten pairs of soft-COSY and PICSY multiplets by two-dimensional convolution. The similarity functions (see text) that rise like stalagmites were obtained by computing normalized scalar products $S(J^*)$ for different values of the trial coupling constant J^* (abscissa, in Hz) and approach $S = 1$ when the two convoluted multiplets derived from soft-COSY and PICSY are similar. The curves that appear to drop like stalactites are obtained by calculating the χ^2 function to obtain a measure of the discrepancy between the two convoluted multiplets (arbitrary scale). The maxima of $S(J^*)$ and the minima of χ^2 correspond to good estimates of the active scalar coupling constants J (see Table 1).

with the cross-peak multiplets 13H–18H and 10H–18H, which partly overlap, and the multiplets 3H–20H$^{\alpha}$, 5H–20H$^{\beta}$ and 5H–6H$^{\beta}$. The last three have active coupling constants that are comparable to the linewidth (which is about 2 Hz) and are therefore difficult to determine by deconvolution. The values given in parentheses in Table 1, which are larger than the true coupling constants, were obtained by considering the global integrated absolute value as a criterion of simplification. The remaining coupling constants, which are believed to be more accurate, were obtained by integrating over restricted strips of the deconvoluted spectrum (marginal integrated absolute value). Problems with such criteria will be discussed more extensively elsewhere.[9]

An interesting aspect of the deconvolution method is that both the active and passive coupling constants can

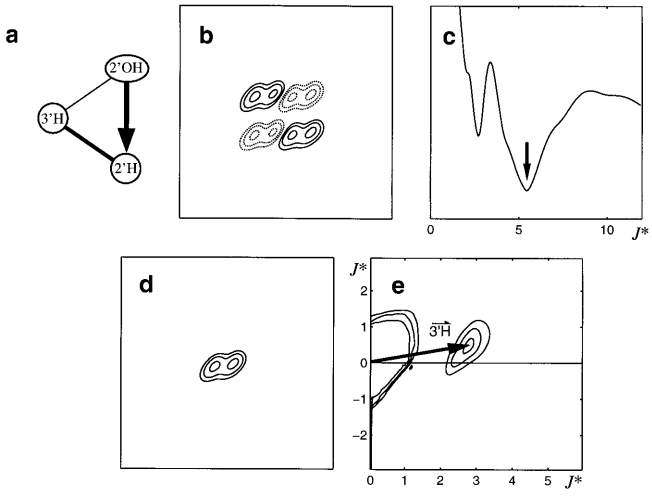

Figure 5. (a) Subsystem where 2′OH and 2′H are active spins and 3′H is a cyclic passive spin. (b) Soft-COSY multiplet due to the transfer of magnetization from 2′OH to 2′H. This pattern consists of two antiphase squares due to the active coupling constant J(2′H,2′OH), displaced by the passive coupling J(2′H,3′H) on the one hand and the long-range coupling J(2′OH,3′H) on the other. (c) One-dimensional global magnitude function obtained when attempting to eliminate the active coupling by deconvolution with an antiphase square array. The minimum gives an estimate of the active coupling constant J(2′H,2′OH) = 5.45 Hz. (d) Multiplet obtained by two-dimensional deconvolution using this estimate. This pattern consists of two positive peaks displaced with respect to each other by passive couplings to 3′H. (e) Two-dimensional global magnitude function obtained when attempting to deconvolute the passive splittings. The coordinates of the minimum correspond to J(2′OH,3′H) = +0.45 Hz in the vertical ω_1 dimension and J(2′H,3′H) = +2.75 Hz in the horizontal ω_2 dimension.

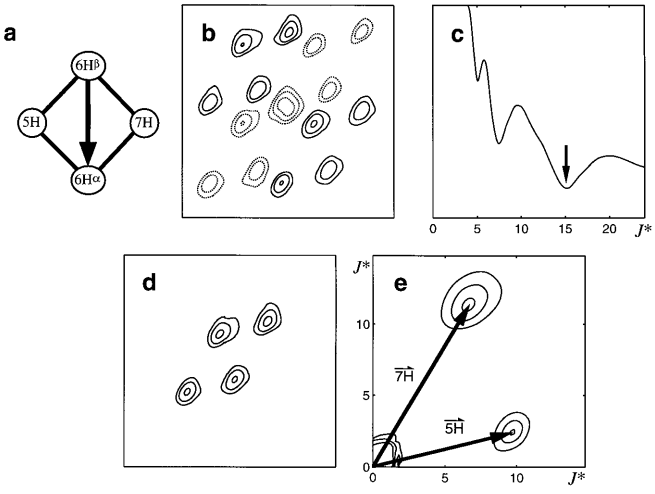

Figure 6. (a) Subsystem where 6H$^{\alpha}$ and 6H$^{\beta}$ are active spins and 5H and 7H are cyclic passive spins. (b) Soft-COSY multiplet due to the transfer of magnetization from 6H$^{\beta}$ to 6H$^{\alpha}$. This pattern consists of four antiphase squares due to J(6H$^{\alpha}$,6H$^{\beta}$), displaced with respect to each other by passive couplings to 5H and 7H. (c) One-dimensional global magnitude function obtained when attempting to eliminate the active coupling by deconvolution with an antiphase square array. The minimum gives an estimate of the active coupling constant J(6H$^{\alpha}$,6H$^{\beta}$) = 15.20 Hz. (d) Multiplet obtained by two-dimensional deconvolution using this estimate. This pattern consists of four positive peaks displaced with respect to each other by passive couplings to 5H and 7H. (e) Two-dimensional global magnitude function obtained when attempting to deconvolute the remaining passive splittings. The coordinates of the two minima correspond to J(5H,6H$^{\beta}$) = 2.40 Hz and J(6H$^{\beta}$, 7H) = 11.20 Hz in the vertical ω_1 dimension and J(6H$^{\alpha}$,7H) = 6.55 Hz and J(5H,6H$^{\alpha}$) = 9.70 Hz in the horizontal ω_2 dimension.

Table 2. Homonuclear J_{HH} coupling constants in paclitaxel (taxol) determined by deconvolution of soft-COSY multiplets[a]

	3′H→NH	2′H→3′H	2′OH→2′H							
^4J(NH,2′H)	−0.20	−0.15								
^3J(NH,3′H)	**9.10**	+8.75								
^3J(2′H,3′H)	2.65	(**3.00**)	+2.75							
^3J(2′H,2′OH)		+5.25	**5.45**							
^4J(2′OH,3′H)		+0.40	+0.45							
	$14H^{\alpha}\rightarrow14H^{\beta}$	$14^{\alpha}H\rightarrow13H$	$14^{\beta}H\rightarrow13H$	18H→10H	18H→13H					
$^2J(14H^{\alpha},14H^{\beta})$	Str. coupl.	Str. coupl.	Str. coupl.							
^3J(13H,14Hα)					9.20[b]					
$^3J(13H,14H^{\beta})$					9.20[b]					
^4J(13H,18Me)					(**2.50**)					
^5J(10H,18Me)				(**∼2.5**[c])						
	3H→2H	$6H^{\beta}\rightarrow5H$	$6H^{\alpha}\rightarrow5H$	$6H^{\beta}\rightarrow6H^{\alpha}$	$3H\rightarrow20H^{\alpha}$	$20H^{\alpha}\rightarrow20H^{\beta}$	$20H^{\beta}\rightarrow5H$	$6H^{\alpha}\rightarrow7H$	$6H^{\beta}\rightarrow7H$	70H→7H
^3J(2H,3H)	**7.30**				7.45	Overlap				
$^4J(3H,20H^{\alpha})$					(**2.10**)					
$^4J(5H,20H^{\alpha})$							−0.75			
$^2J(20H^{\alpha},20H^{\beta})$					8.90		−9.50			
$^4J(5H,20H^{\beta})$							(**∼2.6**[d])			
^4J(5H,7H)		−0.25	−0.25							
$^3J(5H,6H^{\alpha})$		+9.70	**10.00**	+9.70			9.80	9.80		
$^3J(5H,6H^{\beta})$		(**2.75**)	+2.35	+2.40			2.30		2.45	
$^2J(6H^{\alpha},6H^{\beta})$		−15.10	−15.10	**15.20**				−14.85	−14.75	
$^3J(6H^{\alpha},7H)$			+7.20	+6.55				**7.10**	+6.45	6.25
$^3J(6H^{\beta},7H)$		+11.20		+11.20				+10.80	**11.25**	10.45
^3J(7H,7OH)								4.20	4.05	**4.30**

[a] Coupling constants that were determined from multiplets where they appear as active couplings are in bold type and those that were determined from multiplets where they appear as passive couplings in normal type. Numbers in parentheses are believed to be overestimated (see text).
[b] Two passive couplings could not be resolved.
[c] Estimate from poorly separated overlapping multiplets.
[d] Estimate from irregular multiplet structure after deconvolution of all passive couplings.

be determined from a single multiplet (Table 2). This is not only efficient, but also provides valuable long-range coupling constants, which are normally difficult to observe in DQF-COSY spectra because of poor digital resolution. In unfavorable cases (e.g. when the active couplings are very small, when the passive couplings are large, when the multiplets overlap or when the couplings are strong), it may be difficult to record PICSY spectra. In such cases, deconvolution can be used to measure either an active coupling constant from a soft-COSY multiplet or, if this cannot be recorded, a passive coupling in other soft-COSY multiplets.

CONCLUSION

Two-dimensional convolution and deconvolution methods were applied successfully to a variety of multiplets recorded by selective 2D correlation methods (soft COSY and PICSY). Many of the homonuclear proton–proton couplings in paclitaxel could be determined accurately by at least one method. In some cases, as many as seven estimates could be obtained for the same coupling constant. Variations in the numerical estimates for a given coupling constant can be attributed to distortions of the multiplet patterns. The discrepancies in the estimates obtained from different methods (e.g. deconvolution or convolution) appear to be greater than variations observed if the same multiplet is recorded repeatedly in several independent experiments. This work should help to make a more realistic appraisal of the prospects of entirely automated computer-supported analysis of two-dimensional spectra.

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