

PROTON ZERO QUANTUM TWO-DIMENSIONAL NMR SPECTROSCOPY: AN ALTERNATIVE TO PROTON DOUBLE QUANTUM INADEQUATE FOR MAPPING COMPLEX PROTON SPIN SYSTEMS OF NATURAL PRODUCTS

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ABSTRACT.—Despite numerous potential advantages, proton zero quantum coherence (HZQC) two-dimensional (2D)-nmr spectroscopy has been totally ignored by the natural products chemists engaged in structure elucidation. The HZQC experiment is described. Application of this highly sensitive 2D-nmr experiment to strychnine as a model system is presented. Comparisons are drawn between the HZQC and proton double quantum INADEQUATE(HDQC) experiment. HZQC offers considerable advantages in time and resolution but does not appear to have the abilities of HDQC in elucidating coupling pathways where $^1J_{\text{HH}}$ is very small.

Burgeoning growth in the area of two-dimensional (2D)-nmr spectroscopy has seen the development of new experiments at a rate far greater than they can be assimilated and brought into general practice in natural products structure elucidation. Such has been the case with the proton double quantum INADEQUATE (HDQC) experiment. After the initial report by Mareci and Freeman (1) and the comprehensive treatment of Braunschweiler, Bodenhausen, and Ernst (2), applications of the HDQC experiment were initially limited to groups investigating protein structure (3-12). It was not until 1985, when the experiment was applied to plumericin, that the technique was actually introduced to the field of natural product structure elucidation (13). Subsequently, the HDQC experiment has been applied to ristocetin (14), the total assignment of the ^1H -nmr spectrum of the cembranoid diterpene jeunicin (15) and the novel related C_{21} analog planaxool (16), and the elucidation of new long range coupling pathways in strychnine (17). Doubtless, HDQC applications will begin to increase in numbers as more natural product chemists begin to appreciate the capabilities of this rather powerful, albeit somewhat "exotic," experiment. Indeed, there are some applications where HDQC may be the method of choice, for instance in the elucidation of very weak couplings where $J \approx 0$ Hz (2,4,6,17). However, more recent work in this laboratory prompted by the work of Müller (18) and the much earlier work of Wokaun and Ernst (19) suggests that for general use, 2D-proton zero quantum coherence (HZQC) nmr may prove to be a superior and much simpler technique to implement than the HDQC experiment.

Surprisingly, despite very favorable comments regarding HZQC nmr spectroscopy made by Wokaun and Ernst (19) and more recently by Turner (20), HZQC has been virtually ignored as an experimental technique. Regarding specific advantages, zero quantum transitions (ZQT's) are insensitive to magnetic field inhomogeneity and, thus, permit the recording of high resolution spectra in inhomogeneous magnetic fields (19). In addition, because zero quantum coherence (ZQC) can be considered as a rotating monopole, single quantum coherence (SQC) as a dipole, and double quantum coherence (DQC) as a quadrupole and so on, ZQC can thus be seen to be relatively insensitive to the phase of pulses applied to the spin system (20) thereby allowing a simplified four step phase cycle to be utilized (18) in combination with a homogeneity spoiling pulse that can be shown to suppress all higher order coherences (19). Thus, the four-step phase cycle employed in the HZQC nmr experiment shown in Figure 1 represents a considerable economy of time over the much more complex 32-step phase cycle required for the successful performance of the HDQC nmr experiment (13,14). The potential major drawbacks to ZQC, dependence on both spin-spin coupling con-

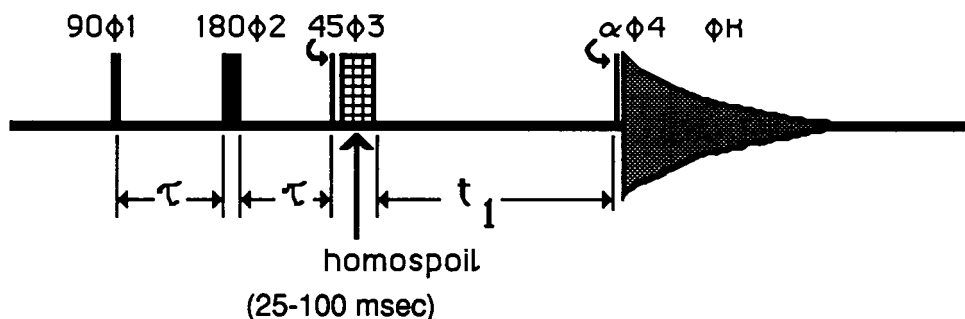


FIGURE 1. Homonuclear proton zero quantum coherence (HZQC) pulse sequence. The α -pulse may be set to 90° or 45° , although the latter was preferentially used in this work to simplify the spectra as described by Müller (18). The duration of the homogeneity spoiling pulse may be varied, as all coherences other than ZQC are destroyed in 100 msec or less (19). Phases (ϕ_1 - ϕ_4) are given in Table 1.

stant size and chemical shift (18,20), are conveniently overcome using the pulse sequence developed by Müller (18) shown in Figure 1, thus elevating the ZQC from the ignoble status of a quantum mechanical nuisance in some experiments to that of a highly useful experimental technique.

Applications of ZQC thus far are severely limited in number. Bolton (21,22) has utilized heteronuclear zero quantum coherence as a conformational probe. Blümich and Kaiser have examined magnetization exchange in zero quantum spectra (23); Ruessink, De Kanter, and MacLean (24) have used ZQC to determine ^2H and ^{14}N quadrupolar coupling constants by taking advantage of the abilities of the technique to produce high resolution spectra in the presence of inhomogeneity. Blumich (25) has more recently discussed the representation of zero quantum spectra, while Bolton (26) has considered the effects of flip angle filters on longitudinal magnetization arising during the creation of zero quantum coherence. Müller and Pardi (27) have devised a proton zero quantum relay pulse sequence, while Turner (20) has reported a heteronuclear zero quantum relayed coherence technique. Finally, Pouzard, Sukumar, and Hall have reported a spectral analysis of simple zero quantum spin systems (28) while Hall and Norwood have further demonstrated the capabilities of zero quantum coherence in instances of inhomogeneous magnetic fields (29). There have, however, been no applications of HZQC in natural product structure elucidation. Thus, to demonstrate the power of the HZQC technique, we would like to present its application to strychnine, which was chosen to allow direct comparisons to be made to the HDQC spectra of strychnine reported in our preceding paper (17).

RESULTS AND DISCUSSION

The pulse sequence¹ and one-phase cycling scheme used for the proton zero quantum coherence nmr study reported are shown in Figure 1 and Table 1, respectively. Importantly, the utilization of the homogeneity spoiling pulse in the pulse sequence allows the phase cycling scheme to be compressed to a four step cycle. Relative to the

¹The Nicolet 293-C pulse program used for the HZQC nmr experiment may be obtained from the authors upon request. Authors interested in implementing this experiment on instruments which either do not possess homospoil capabilities or which exhibit unacceptable deuterium lock channel instabilities when a homogeneity spoiling pulse is applied can utilize the pulse sequence shown in Figure 1 by simply deleting the homogeneity spoiling pulse and increasing the phase cycle to sixteen steps as suggested by Müller (18).

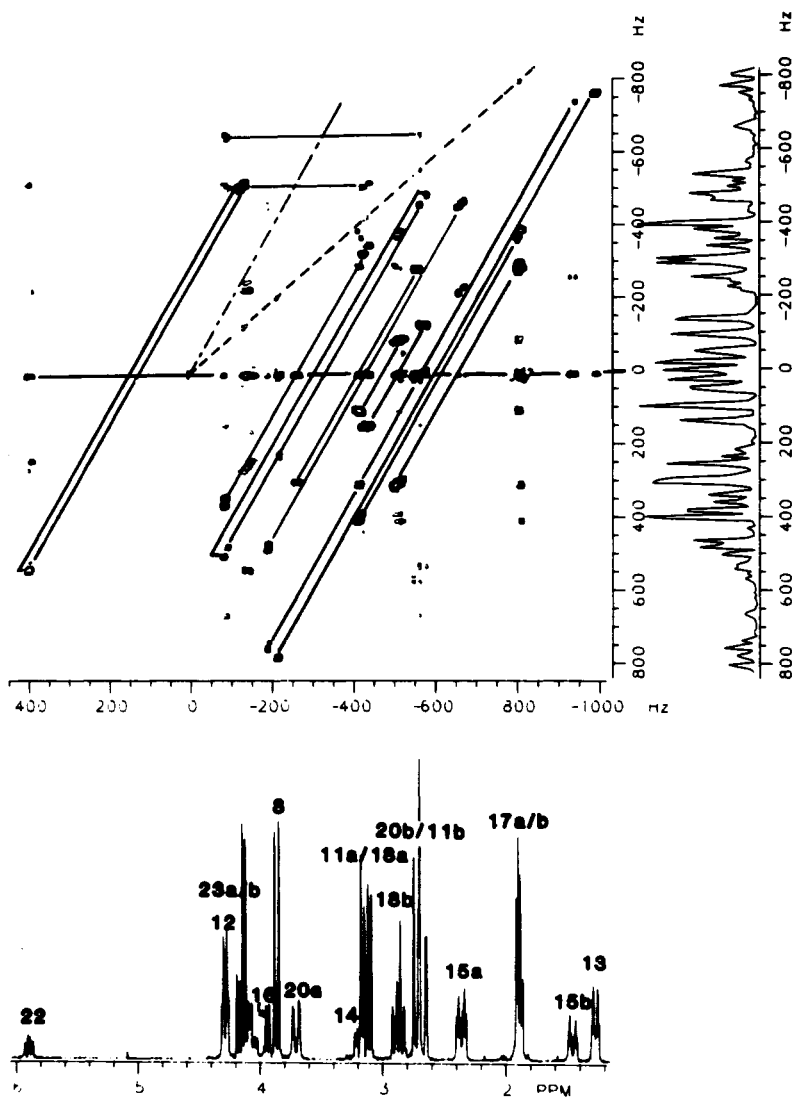


FIGURE 2. Homonuclear zero quantum spectrum of strychnine [1] in CDCl_3 acquired using $\tau=35.7$ msec. Connectivities representing zero quantum transitions are shown by the solid lines, $2F_2 = +F_1$. The axis along which minimal residual single quantum coherence appears is shown by ----. Incompletely canceled double quantum coherence is shown by responses along the horizontal lines which are symmetrically disposed about the "skew" diagonal (— — —) which parallels the zero quantum connectivity responses. The former is easy to recognize, however, since it originates at $F_2 = F_1 = 0$ Hz. Some residual signal is also observed along the axis $F_1 = 0$ Hz (18).

much more elaborate 32-step phase cycle necessary with the HDQC nmr experiment, the simpler phase cycling requirements of the HZQC nmr experiment represents an eight fold savings in time simply in satisfying the phase cycling requirements. The 7 Hz optimized ($\tau = 1/[4J_{\text{HH}}] = 35.7$ msec) HZQC nmr spectrum of strychnine is shown in Figure 2. The spectrum was acquired with the final recovery pulse $\alpha = 45^\circ$ to simplify the spectrum by suppressing half of the zero quantum responses (18), thereby providing a spectrum resembling a SECSY spectrum (30).

TABLE 1. Phase Cycling Scheme for 2D Proton Zero Quantum Coherence Spectroscopy

Acq.	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_R
1	x	y	y	y	x
2	x	-y	y	y	x
3	x	y	-y	y	-x
4	x	-y	-y	y	-x

Concerning responses contained in the HZQC contour plot shown in Figure 2, there are several types that the user of this experiment should be aware of. First, ZQTs appear in the zero quantum (F_1) frequency domain at the algebraic difference between the shifts of the coupled resonances relative to the transmitter frequency (frequencies relative to the transmitter in Hz are shown in Figure 2 plotted above the normal high resolution spectrum). Normally, the responses would be paired symmetrically about the $F_1 = 0$ Hz axis if a 90° conversion pulse were to be employed. By using a 45° conversion pulse, however, half of each response pair is suppressed, thereby considerably simplifying the resulting contour plot. Zero quantum correlations are defined along parallel lines with a slope of $2F_2 = F_1$, the responses in the zero quantum frequency domain symmetrically displaced from $F_1 = 0$ Hz by the frequency of the ZQT. In the case of incomplete suppression, there may also be a pair of responses correlated by lines with slope $2F_2 = -F_1$, which will generally be much weaker in intensity.²

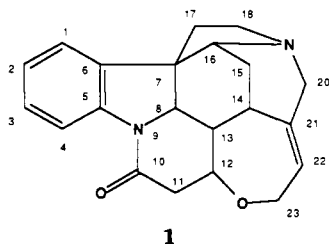
As with any sophisticated nmr experiment, situations will occasionally arise where only one member of the coupled pair being observed shows a response in the data matrix. The existence of a ZQT correlating a pair of protons can be safely inferred, in most cases, by simply considering the frequency characteristics of the ZQT. For example, the H15a/H15b geminal pair shown in Figure 2 is not correlated by a pair of responses. Rather, there is a single response at -255 Hz in the zero quantum frequency domain (F_1) observed at the chemical shift of H15b.³ Its counterpart at $+255$ Hz in F_1 at the chemical shift of H15a, which would normally establish the correlation between this geminal pair is absent. However, the correlation can still be safely inferred from the F_1 and F_2 frequencies associated with the response observed inasmuch as the F_1 frequency must be the algebraic difference of the offsets of the coupled spins relative to the transmitter if it arises due to a ZQT. Thus, given the F_1 and F_2 frequencies of a response, the coupling partner can be located in F_2 at the F_2 frequency observed for the response plus or minus the F_1 frequency as appropriate. In the case at hand, using the operation just described, the H15a response is located in the F_2 domain 255 Hz downfield of the H15b response. This observation confirms the identity of the single response observed under the conditions of the experiment as a zero quantum response correlating the pair of resonances. Care must, however, be taken to insure that inferences such as that just described are indeed due to ZQTs and not due to either single or double quantum transitions. This requires a knowledge of the location of residual single and double quantum frequency responses which are described below.

²The intensity of the mirror image peak is generally below the threshold of the contour plot since its intensity will be proportional to $(\tan \alpha/2)^2$. It is important, however, to be aware of this relationship since the appearance of these peaks, even though weak, can be confusing to those attempting to use HZQC for the first time.

³Determination of zero quantum and other response frequencies contained in the F_1 domain can be accomplished from the F_1 (90°) projection of the data matrix. Other than providing the means to determine frequencies in F_1 , the projection has no interpretive utility and need not be computed in every case. Rather, it is generally sufficient to simply plot the F_1 frequency axis (in Hz) flanking the contour plot.

Under the conditions employed in the generation of the spectrum shown in Figure 2 (homogeneity spoiling pulse duration = 25 msec), some residual DQC remained. As would be expected (1, 2, 13, 17), these responses are symmetric about the axis $2F_2 = F_1$, which parallels the axis correlating ZQTs. Importantly, however, the skew diagonal axes ($2F_2 = \pm F_1$) originate from the transmitter location $F_2 = 0$ Hz and $F_1 = 0$ Hz in the zero quantum frequency domain thereby making it simple to locate any double quantum responses contained in the spectrum (the skew diagonal is denoted by —·—·— in Figure 2). As will be noted in Figure 2, there are a scant few double quantum responses shown that are symmetrically disposed about the skew diagonal axis. The properties of the DQC should also be kept in mind (13, 17), recalling that the DQT appears at the algebraic sum of the offsets relative to the transmitter, and, hence, it is possible for folding of the double quantum skew diagonal to occur in ZQC spectra. Unfortunately, when only one member of a pair of the double quantum responses is observed, there exists that chance that it could mistakenly be interpreted as an isolated zero quantum response. Finally, although extremely weak, the SQC axis can also be seen in this case, $F_2 = F_1$ (denoted by --- in Figure 2). Under conditions of extremely high signal to noise ratios, off-diagonal responses analogous to those which would be observed in a COSY spectrum can be associated with the single quantum coherence axis if sufficiently low threshold levels are utilized in preparing contour plots. Experimentally, phase cycling should be expected to help to eliminate the effects of pulse duration inaccuracies and other factors which could be expected to lead to the observed artifacts in the spectrum shown in Figure 2. Recycling the experiment at an interval shorter than about $1.5 \times T_1$ can be expected to partially offset the benefits gained by phase cycling. The spectrum shown was acquired with a transient time (acquisition + interpulse delay) of approximately 1.2 sec. Increasing the transient time to values in the range of 3-3.3 sec had no beneficial effect on spectral quality. In principle, however, incompletely canceled double and single quantum responses can be suppressed by increasing the power of the homogeneity spoiling pulse (19) from that employed in the acquisition of the spectrum shown in Figure 2.

The ZQC spectrum of strychnine [1] shown in Figure 2 offers several very attractive features. Perhaps the most remarkable feature of the spectrum is the degree of resolution afforded in the zero quantum frequency domain (F_1). In particular, note that the responses correlating H-22 to H-23a and H-23b protons are both clearly resolved, whereas they were not distinctly resolved in the HDQC spectrum of **1** obtained under the same conditions (17). Similarly improved resolution is also provided in the case of the ZQT's correlating the H-18 and H-17 protons. Despite the optimization of the experiment shown in Figure 2 at 35.7 msec (7 Hz), the range of coupling pathways observed is quite broad. Coupling pathways ranging from the H11b-H12 (3.3 Hz) to the H11a-H11b (17.4 Hz) coupling generally exhibited good intensity.



A second very attractive feature of the HZQC experiment which is not apparent from Figure 2 was the performance time. Because of the differences in the complexity of their respective phase cycles, there are vast differences in the performance times of the

HZQC and HDQC nmr experiments. Thus, the $512 \times 1K$ HZQC spectrum shown in Figure 2 was acquired in approximately 45 min. To obtain comparable digital resolution, the HDQC spectra of strychnine [1] previously reported (17) required the acquisition of a $512 \times 2K$ spectrum with zero filling prior to the second Fourier transform with an acquisition time for the HDQC spectrum of approximately 9 h. The time factor, coupled with the considerably more massive disc storage requirements necessary with the HDQC experiment, may make the HZQC nmr technique a more attractive alternative for the elucidation of the vicinal coupling networks of complex natural products.

Concerning digital resolution in the second frequency domain, the HDQC nmr experiment produces responses in the double quantum or F_1 frequency domain at the algebraic sum of the offsets relative to the transmitter. Because HZQC nmr responses are at the algebraic difference, it might initially be supposed that the inherent resolution in the second frequency domain is lower in the HZQC nmr experiment. However, because the HZQC experiment offers quadrature detection without having to resort to 45° phase shifts, algebraic differences in cases where responses are on opposite sides of the transmitter yield frequencies in F_1 whose separation is identical to that afforded by the HDQC nmr experiment. Furthermore, since the entire data matrix can be used in the HZQC nmr experiment while in the HDQC nmr experiment three quarters of the data matrix is wasted unless provisions for quadrature detection in F_1 (45° phase shifts) are available, there is a considerably more efficient utilization of digitization in the HZQC nmr experiment, thereby affording, in reality, comparable dispersion and the same or better digital resolution.

Despite the numerous potential advantages inherent to the HZQC nmr experiment, there are clearly, however, many occasions when the more time consuming and complex HDQC nmr experiment will be preferred to the HZQC experiment. Specifically, our attempts to probe the capabilities of the HZQC for elucidating very small coupling pathways were disappointingly unsuccessful. For instance, when the HDQC nmr experiment was optimized at 142.85 msec (1.75 Hz), a large number of small coupling pathways were observable, several of which were previously unreported (17). Under the same optimization conditions, the HZQC nmr experiment gave good sensitivity over the range from 3.3-17.4 Hz but failed to provide any information relative to the small coupling pathways, e.g. H22-H20a, H22-H20b, and H22-H14 previously observed using the HDQC nmr experiment. Thus, the abilities of the HDQC nmr experiment when dealing with small coupling pathways where $J \approx 0$ Hz (2,4,6,17) clearly appear to be superior and the HDQC nmr experiment will probably remain the preferred method for establishing this type of information. For establishing vicinal connectivities, however, the HZQC nmr experiment has been demonstrated to be far more efficient in the present study.

Relative to the more conventional and now widely employed COSY experiment, HZQC would appear to be superior for several reasons. First, quadrature detection with the HZQC experiment is attained with a 4- rather than a 16-step phase cycle thereby providing a significant time savings. Second, diagonal responses in the COSY experiment frequently make it difficult to establish correlations between protons with closely similar chemical shifts. The HZQC experiment, in contrast, provides the means for largely suppressing responses at $F_1 = 0$ Hz through the application of the homogeneity spoiling pulse applied at the beginning of the evolution time, thereby making the correlation of resonances with similar chemical shifts a more facile process. Finally, determining correlations between remote protons in the COSY experiment is also quite difficult when they are correlated to protons with very similar chemical shifts since the resolution in the COSY experiment is governed largely by T_2^* rather than T_2 as in the HZQC spectrum

EXPERIMENTAL

Strychnine, as the free-base used in this study, was obtained from Sigma Chemical Co., St. Louis, Missouri, and was recrystallized once from EtOH prior to use. Samples for the ^1H zero quantum coherence experiments were prepared by dissolving 10 mg of recrystallized strychnine in 0.4 ml of CDCl_3 (Aldrich 99.8% D).

All experiments were performed using a Nicolet NT-300 wide bore spectrometer controlled by a Model 293-C pulse programmer operating at 300.068 MHz for ^1H observation. Spectra were obtained using the decoupler coils of a dual tuned 5mm $^1\text{H}/^{13}\text{C}$ probe at a temperature of 20°. The high resolution reference spectrum plotted below the contour plots was obtained using 32K points with chemical shifts referenced internally to the residual protiochloroform resonance which was taken at 7.24 ppm. ^1H zero quantum coherence spectra acquired for **1** were performed using the pulse sequence and phase cycling shown in Figure 1 and Table 1, respectively (18).² The τ interval utilized in the creation of ZQC was variously optimized at values ranging from 17.85 msec (14 Hz) to 142.85 msec (1.75 Hz). Data were collected as $512 \times 1\text{K}$ complex points and were processed using double exponential apodization prior to both Fourier transforms. The reconversion or "read" pulse employed to convert the evolving ZQC to observable SQC was set to 45° in all cases. The evolution time (τ_1) was set to 0.75* dwell interval, although it may, in some cases, be necessary to use 0.50* dwell if protons at both extremes of the spectral range are coupled to one another. Following the second Fourier transformation, the data were transposed to afford a final F_1F_2 data matrix for convenience in plot presentation. Chemical shifts are given relative to TMS in ppm and relative to the transmitter in Hz on the contour plot. Acquisition times range from 45 min to 2 h depending upon the optimization chosen.

CONCLUSIONS

The ^1H zero quantum coherence nmr experiment has, until now, been largely ignored as a technique for structure elucidation. The properties of the ZQT which make it attractive for establishing molecular connectivity, insensitivity to homogeneity spoiling pulses (18-20), and the ability to provide high resolution spectra in inhomogeneous fields (18,20-22,24,29) make it exceptionally well suited to providing useful connectivity information when dealing with molecules exhibiting highly congested nmr spectra. Clearly the HZQC nmr technique is inferior to the HDQC nmr experiment when attempting to elucidate very small coupling pathways (17), although it is probably a superior method when establishing "normal" vicinal coupling pathways. While it remains to be seen, it is also quite possible that the HZQC nmr experiment has the attributes that will make it preferable to even the now well established COSY experiment. At present, we are working on a number of structural problems in which the advantages of the HZQC nmr experiment are being exploited, and these will serve as the basis of future reports.

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NOTE ADDED IN PROOF: Since the submission of this manuscript, several additional papers have appeared that describe alternative methods of creating an observing homonuclear zero quantum coherence: L.D. Hall and T.J. Norwood, *J. Magn. Reson.*, **69**, 391 (1986); L.D. Hall and T.J. Norwood, *J. Magn. Reson.*, **69**, 397 (1986); L.D. Hall and T.J. Norwood, *J. Magn. Reson.*, **69**, 585 (1986).

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