

PROTON DOUBLE QUANTUM AND RELAYED PROTON DOUBLE QUANTUM COHERENCE TWO-DIMENSIONAL NMR MAPPING OF PROTON-PROTON CONNECTIVITY NETWORKS IN NATURAL PRODUCTS: A MODEL STUDY OF STRYCHNINE

DOUGLAS A. CRAIG and GARY E. MARTIN*

Department of Medicinal Chemistry, College of Pharmacy,
University of Houston-University Park, Houston, Texas 77004

ABSTRACT.—The utilization of two-dimensional proton double quantum coherence in mapping proton-proton connectivity networks of complex natural products is demonstrated using the alkaloid strychnine as a model compound. The proton double quantum experiment, in addition to providing the vicinal proton connectivities, also provides evidence for the existence of several previously unreported coupling pathways (H14-H22 and H20b-H22). The sensitivity of the proton double quantum responses to the optimization for various size coupling constants also suggests that a recent report which addressed the nature of the H17a/b geminal couplings may not be entirely correct. Characteristics of the proton double quantum experiment are also compared to those obtained using the relayed proton double quantum coherence experiment.

The task of piecing together the structures of complex, unknown natural products has benefited significantly from the development of two-dimensional nmr techniques. Two-dimensional proton double quantum coherence (¹H-2D-INADEQUATE) (1,2) is among the newest of such techniques and has recently been employed in the elucidation of spin coupling pathways involving small, long-range couplings in plumericin (3) and in the assignment of the ¹H-nmr spectra of the antibiotic ristocetin (4) and the cembranoid diterpene jeunicin (5). Thus, we were interested in further evaluating the abilities of the proton double quantum experiment to elucidate complex proton connectivity networks. As a model system, strychnine, which contains seventeen nonequivalent protons within the region from 4.3 to 1.2 ppm, was chosen. In addition to providing a sufficiently complex model, the examination of Dreiding models also suggested that several long-range coupling pathways which have not been previously reported (6,7) should exist for strychnine. Finally, since response intensity in the proton double quantum experiment is governed by the optimization of the delay, τ , employed when the double quantum coherence is created, the proton double quantum experiment also provides an independent means of probing the size of the geminal coupling between the H17a-H17b pair. This coupling has variously been reported to be as small as 0.02 Hz in one study (6) and as large as -15.5 Hz in a more recent study (7).

RESULTS AND DISCUSSION

PROTON DOUBLE QUANTUM COHERENCE.—The proton double quantum coherence experiment employs the pulse sequence shown below

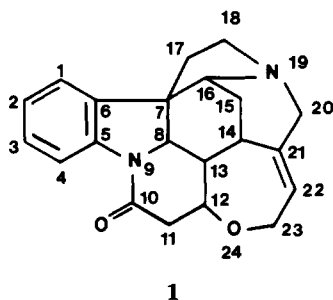
$$90^\circ-\tau-90^\circ/180^\circ/90^\circ-\tau-90^\circ-t_1-45^\circ\text{-acquire}(t_2) \quad [1]$$

in which the duration of the delay, τ , is normally set equal to $\frac{1}{4}J_{\text{HH}}$ or $\frac{1}{8}J_{\text{HH}}$. Thus, it is possible, to an extent, to determine the nature of the responses which will optimally appear in the data matrix. Fesik *et al.* (4), in assigning the spectrum of ristocetin, have exploited the effects of variation of the τ delay. The location and nature of responses in the proton double quantum experiment has been treated briefly in our previous work (3,5) and much more rigorously in the work of Braunschweiler *et al.* (2) and will therefore not be discussed here.

Numerous entry points exist from which the interpretation of the proton double

quantum spectrum may be begun. One of the most convenient starting points is the H22 vinyl proton which appears as a broadened "triplet" resonating at 5.88 ppm. Broadening of the multiplet may be attributed to long-range coupling for which several possibilities exist. Chazin *et al.* (7) in a recent study reversed the assignments of the H20a/H20b geminal pair on the basis of nOe data. During the course of their study, Chazin *et al.* (7) were able to account for a long range coupling H22-H20a (0.9 Hz) on the basis of the H22-H20a dihedral angle, but they discounted the possibilities of an H22-H20b coupling pathway since the dihedral angle between these protons was near 0° (7,8). Beyond long-range couplings to the H20a/b pair, the possibility also exists for a coupling between the H22 vinyl proton and the H14 methine proton although this coupling pathway has never previously been considered.

The proton double quantum spectrum of strychnine (**1**) optimized for 7 Hz coupling ($\tau=35.71$ msec) is shown in Figure 1. Responses due to the large vicinal and some of the geminal couplings are readily discernible. Unfortunately, however, a relatively large number of the vicinal couplings in strychnine are in the range of 2.0-4.9 Hz (6,7) and are thus either only weakly visible or absent entirely. With a 7 Hz optimization, the H12-H13 (3.1 Hz) and H11b-H12 (3.3 Hz) coupling responses are relatively weak. Responses for the H13-H14 (3.1 Hz), H14-H15a (4.0 Hz), H14-H15b (1.0 Hz), H15a-H16 (4.9 Hz), and H15b-H16 (2.0 Hz) are entirely absent in the contour plot shown in Figure 1. Beginning to utilize the spectrum, the H22-H23a and H22-H23b coupling responses are observed in the double quantum frequency domain (F_1 or ω_1) at $F_1 = -2142$ and -2164 Hz, respectively. In cases where signal to noise ratios are sufficiently high, weak responses due to small couplings may still be visible if sufficient contour levels are plotted. This is not, however, a reliable approach, and thus more dependable means of observing long-range couplings are necessary.



Our efforts have shown that the widest range of responses are observed when the duration of the τ delay is optimized at 1.75 Hz (142.85 msec), with the resulting spectrum shown in Figure 2. Under these conditions, long-range couplings corresponding to H22-H20a, H22-H14, and H22-H20b are observed at $F_1 = \approx -2568$, -2442 , and -2287 Hz, respectively. The latter two coupling responses have not been previously reported or observed. As will be noted from the 90° projection of the data matrix ($J_{HH} = 1.75$ Hz, $\tau = 142.85$ msec), responses for these long-range couplings (denoted by arrows in Figure 3A) have good intensity. In contrast, when the experiment is optimized for larger couplings ($J_{HH} = 14$ Hz, $\tau = 17.85$ msec), responses due to the long range couplings are not visible at all while those for the geminal couplings are significantly intensified (Figure 3B).

Given the identification of the F_2 (ω_2) frequency of the H14 resonance via the H22-H14 double quantum response (Figure 2), we may begin to identify the responses arising from the complex structural fragment represented by **2**. Thus, H14 will be vicinally coupled to H13 and to the anisochronous geminal methylene pair, H15a/b. These

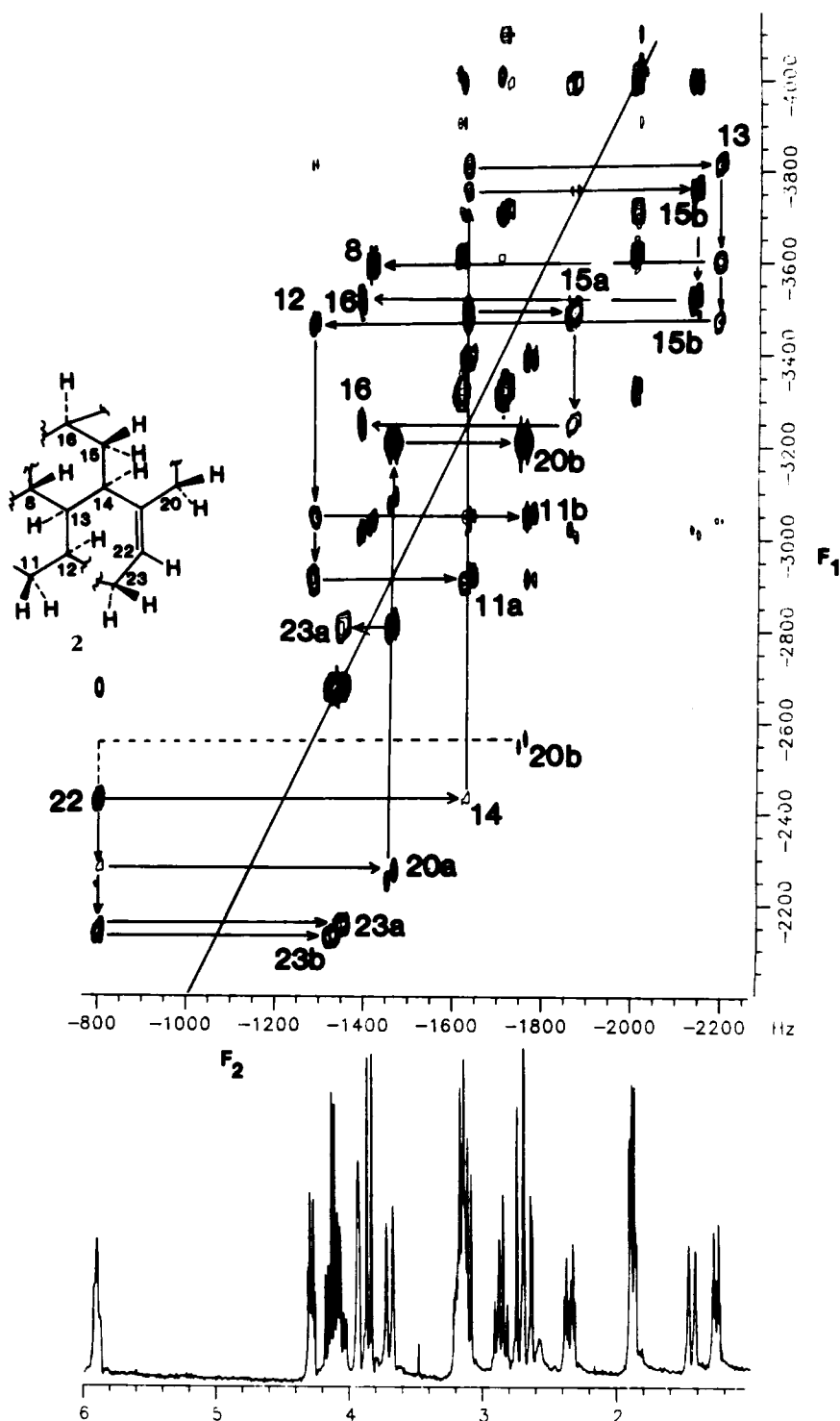


FIGURE 2. Proton double quantum coherence spectrum of strychnine (1) optimized for $J_{\text{HH}} = 1.75$ Hz ($\tau = 142.85$ msec). Usable response intensity is obtained under these conditions for couplings ranging in size from ≈ 0.9 Hz (H20a-H22) to 17.4 Hz (H11a-H11b). Coupling responses tracked in this experiment allow the assembly of the complete structural fragment shown by 2.

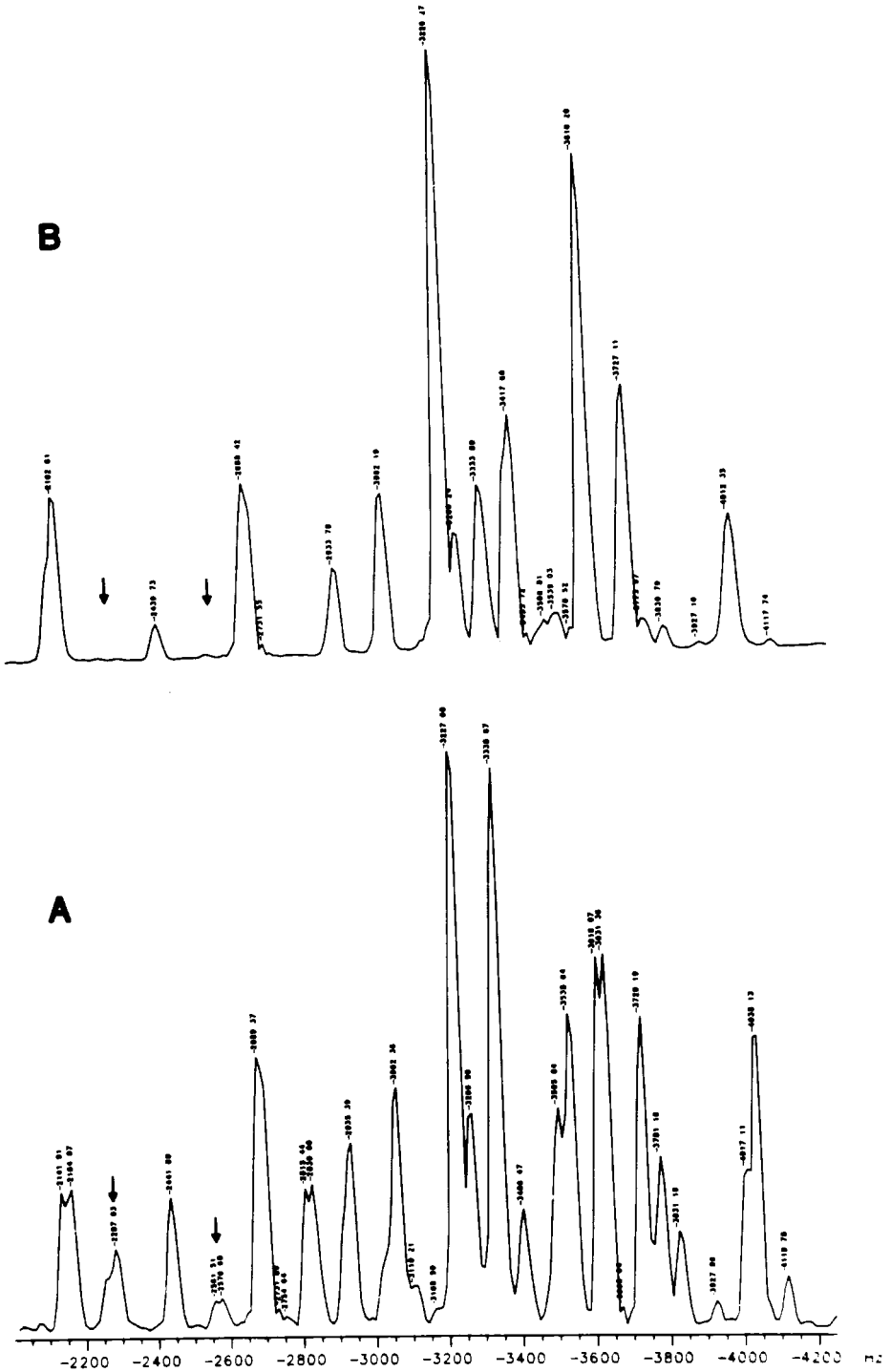


FIGURE 3. 90° Projections of the proton double quantum coherence spectrum optimized for 1.75 Hz (A) and 14 Hz (B). Long-range coupling responses for the previously unreported H14-H22 and H20b-H22 couplings are present with good intensity in the former (position denoted by arrows) but are completely absent in the latter spectrum (see location denoted by arrows in projection B). Simultaneously, the 1.75 Hz optimized experiment (projection A) gives good response intensity for couplings ranging in size from the small long-range couplings through the large geminal couplings.

coupling responses may be readily differentiated since the latter pair will also show a geminal coupling response. The response for the H14-H13 coupling is observed at $F_1 = -3831$ Hz while the responses for the H14-H15a and H14-H15b couplings appeared at $F_1 = -3539$ and -3781 Hz, respectively. The 15a-H15b geminal coupling response was observed at $F_1 = -4012$ Hz.

Proton-proton connectivities progressing outward from H13 and H15a/b are also easily tracked. The H13-H8 connectivity which leads to a termination point was observed at $F_1 = -3616$ Hz while the H13-H12 coupling response was observed at $F_1 \approx -3460$ Hz. Finally, H12 also shows responses for couplings to the H11a/b pair at $F_1 = -3062$ and -2935 Hz, respectively, the H11a-H11b geminal coupling response observed at $F_1 = -3339$ Hz.

Proceeding outward from the H15a/b pair is considerably less complicated than the case just described as there are only two couplings observed. Coupling between H15a-H16 was observed at $F_1 = -3267$ Hz while that between H15b-H16 was observed at $F_1 = -3539$ Hz.

Remaining to be assigned at this point are the responses for the ethylene bridge protons H17a/b and H18a/b. One particularly interesting point concerning these resonances involves the controversy over the H17a-H17b geminal coupling constant. Early work by Carter *et al.* (6) fixed this coupling constant at 0.02 Hz on the basis of a computer simulation of the spectrum. In contrast, the other geminal coupling constants more normally ranged from 14-17.5 Hz with the exception of the H18a-H18b geminal coupling which was reported to be 9.88 Hz. More recently, Chazin *et al.* (7) reported the results of a 400 MHz study in which a computer simulation of the H17a/b pair gave a better fit when the coupling constant was -15.5 Hz. In the present work, we observe that when the proton double quantum sequence was optimized for $J = 14$ Hz ($\tau = 17.85$ msec), the response for the H17a-H17b coupling was weaker than that of the H18a-H18b geminal coupling which was determined to be 9.88 Hz in the earlier study (6) and -10.1 Hz more recently (7). In contrast, the intensities of all of the geminal coupling responses other than that for H17a/b and H18a/b were observed to be stronger when the timing delay, τ , was optimized to 14 Hz. These observations suggest that the H17a-H17b coupling must thus be comparable to or smaller than the H18a-H18b coupling. Conversely, since the H17a/b coupling response does not appear when the experiment is optimized for 7 Hz (Figure 1), this would suggest that the H17a/b coupling must be larger than those vicinal couplings which do not appear in this experiment. Thus, it would appear that the actual magnitude of the H17a-H17b coupling lies somewhere within the range of 5-10 Hz based upon the double quantum experiment responses. To probe the actual magnitude of the H17a/b coupling, a homonuclear 2DJ spectrum was acquired in which the coupling was determined to be 10.3 Hz, which is in reasonable agreement with the range suggested from the double quantum experiments discussed above. Finally, the coupling responses of the ethylene bridge protons H17a-H17b, H17a-H18a, H17b-H18a, and H18a-H18b are shown in Figure 4.

RELAYED PROTON DOUBLE QUANTUM COHERENCE.—Generally, an effort is made to suppress passive transfer of magnetization (1) in the performance of the proton double quantum coherence experiment by using a non- 90° read pulse (3-5). Instances arise, however, when it becomes advantageous to relay information to remote spins as this can provide considerable insight into the structure of the molecule. Eich *et al.* (9) have shown that magnetization can be "relayed" in the standard COSY experiment by replacing the second 90° pulse in the sequence by a $90^\circ - \tau - 180^\circ - \tau - 90^\circ$ train where $\tau = \frac{1}{4} J_{\text{HH}}$. The relay sequence just described was first incorporated into the proton double quantum experiment by Macura *et al.* (10). A more versatile version of the pro-

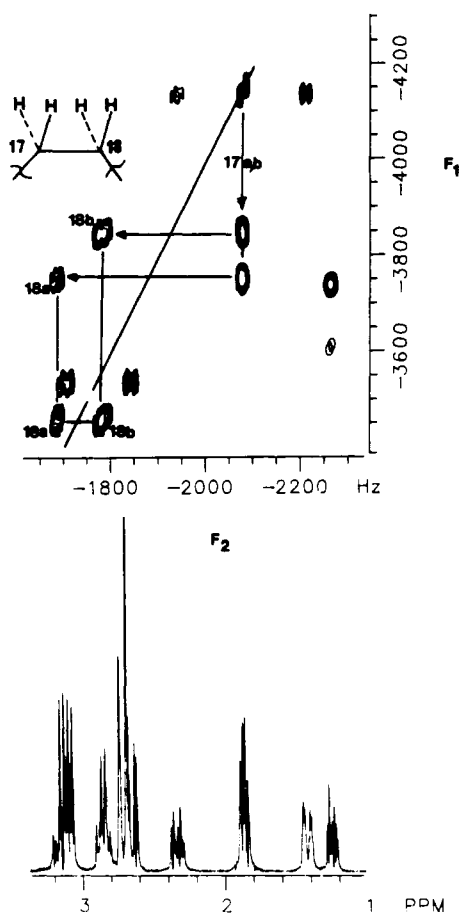


FIGURE 4. Expansion of the 7 Hz optimized proton double quantum coherence experiment (Figure 1) showing the coupling responses for the H17 and H18 ethylene bridge protons.

ton double quantum relay experiment has recently been proposed by Müller and Pardi (11) as shown in expression [2]

$$90^\circ - \tau - 180^\circ - \tau - 45^\circ - t_1 - 90^\circ - \tau' - 180^\circ - \tau' - 90^\circ - \text{acquire} (t_2) \quad [2]$$

We have modified the experiment of Müller and Pardi (11) to employ composite pulses as in expression [1], the modified experiment shown by expression [3]

$$90^\circ - \tau - 90^\circ/180^\circ/90^\circ - \tau - 45^\circ - t_1 - 90^\circ - \tau' - 90^\circ/180^\circ/90^\circ - \tau' - 90^\circ - \text{acquire} (t_2) \quad [3]$$

The difference between the normal proton double quantum coherence experiment [1] and the proton double quantum relay [3] spectrum of strychnine (1) is illustrated by Figure 5. Contrasting the responses for the H22 resonance in Figure 2 with those in Figure 5 readily demonstrates the versatility of the proton double quantum relay experiment. The H22-H23a and H22-H23b responses in Figure 2 ($F_1 = 2142$ and -2164 Hz, respectively) show no hint of passive transfer of magnetization despite the fact that sufficiently low contour levels were plotted to show the weak H22-H14 connectivity ($F_1 = -2442$ Hz). Similarly, none of the other connectivities between H22 and its neighboring protons (H22-H20a, H22-H20b, H22-H14) show any responses due to

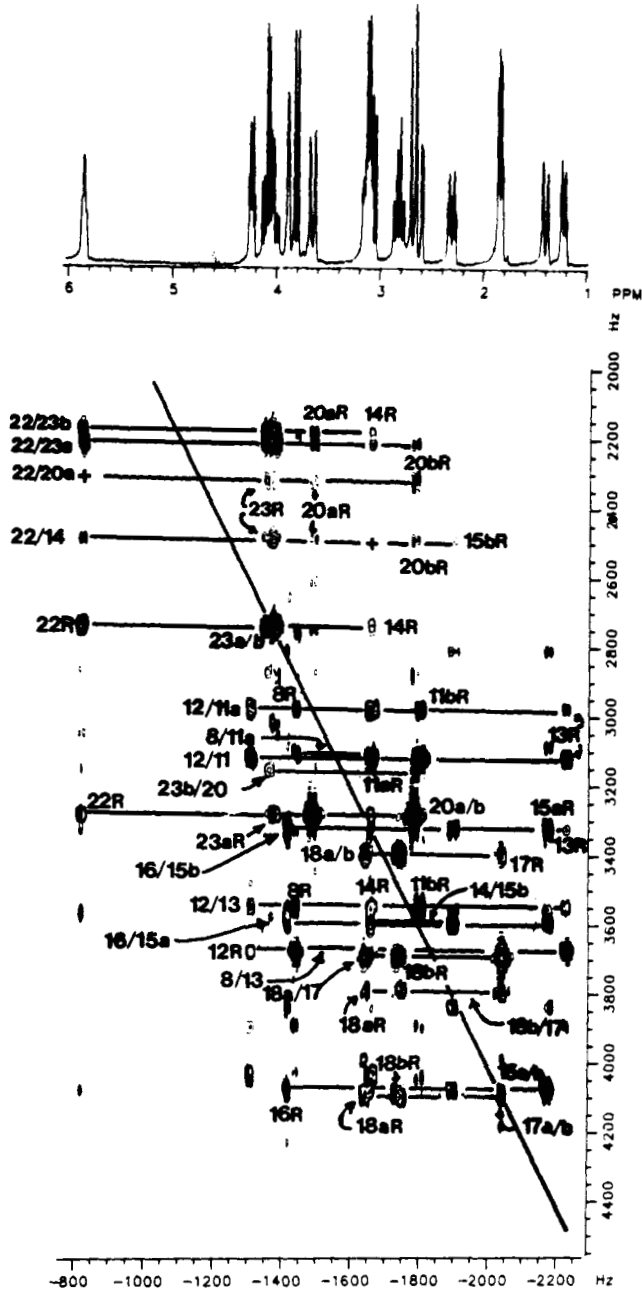


FIGURE 5. Relayed proton double quantum coherence spectrum of strychnine (1) recorded using the pulse sequence shown by expression [3] where $\tau = \tau' = 1/4J_{HH} = 55.5$ msec where J was optimized for 4.5 Hz. The positive F_1 frequency domain is plotted for reasons explained in the experimental with normal responses symmetrically disposed about the skew diagonal as in the proton double quantum spectra shown in Figures 1, 2, and 4. Relayed responses are denoted with the identity of the proton followed by R. Response locations where the response appeared but was not visible at the contour level plotted are denoted by +. The normal, high resolution 32K spectrum of strychnine is plotted above the contour plot with shifts shown in ppm downfield of TMS while the axis below the contour plot shows shifts upfield in Hz relative to the transmitter location.

passive transfer. In contrast, the proton double quantum relay spectrum of strychnine (**1**) shown in Figure 5 shows numerous relayed responses. Returning to the H22-H23a/b responses ($F_1=2167$ and 2202 Hz, respectively, NOTE: the transmitter location was slightly different than that employed for Figure 2) relay responses are observed to the H20b and H14 resonances for both connectivities as well as to the H20a resonance for the H22-H23a connectivity. Similar and numerous relayed responses are observed for a number of the other resonances in the spectrum.

CONCLUSIONS

The two-dimensional proton double quantum experiment provides a versatile method for the elucidation of coupling networks in complex molecules as demonstrated in the present work for the strychnine model system. The proton double quantum experiment also offers significant advantages over the more conventional COSY experiment. Connectivities between resonances with closely similar chemical shifts are more readily visible since there are normally no responses along the "skew" diagonal ($F_1 = +/ - 2F_2$) while the normal spectrum resides along the diagonal ($F_1 = F_2$) in the COSY spectrum. Finally, although it is possible to optimize the proton double quantum experiment for specific types of coupling, in general the optimization for a 1.75 Hz coupling provides very usable signal intensity for couplings ranging from the small, long-range 0.9 Hz H22-H20a coupling to the large 17.4 Hz geminal H11a-H11b coupling.

In those cases where additional information about the environment of a given proton can be used to advantage, magnetization can be relayed in a manner analogous to that employed in the relayed COSY experiment (9). At present, there are two methods available for the performance of this experiment (10, 11), not counting the slight modification of the second in this work as shown by expression [3]. Our experience, although limited, has been better with the pulse sequence reported by Müller and Pardi (11), this experiment having the added advantage of providing access to zero quantum relayed coherence transfer of magnetization when appropriate data storage and data processing is employed.

We are currently using the proton double quantum and relayed proton double quantum coherence experiments in tandem for the elucidation of natural product structures, the results of these efforts forming the subject of a forthcoming report.

EXPERIMENTAL

Strychnine, as the free base used in this study, was obtained from Sigma Chemical Co, St. Louis, Mo., and was recrystallized once from absolute EtOH prior to use. Samples for the proton double quantum experiments were prepared by dissolving 10 mg of recrystallized strychnine in 0.4 ml of CDCl_3 .

All nmr 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 proton observation. Spectra were obtained using the decoupler coils of a dual tuned 5 mm $^1\text{H}/^{13}\text{C}$ probe at an ambient temperature of 17° . The reference spectrum shown plotted below the contour plots was obtained using 32K points, chemical shifts referenced internally to the residual protiochloroform resonance at 7.24 ppm. Proton double quantum experiments were performed using the pulse sequence shown above in expression [1]. Delays for the creation of double quantum coherence were variously optimized for 142.85 msec (1.75 Hz), 35.71 msec (7 Hz), and 17.85 msec (14 Hz). A 32-step phase cycle was used in all of the proton double quantum coherence experiments identical to that described by Fesik and co-workers (4, 12). Relayed proton double quantum coherence was performed using the pulse sequence given by expression [3] with a 64 step phase cycle with appropriate cycling of the phases of the 90° pulses to provide the necessary composite 180° pulses (12). Data were collected in both experiments as $512 \times 2\text{K}$ points with the transmitter located downfield of the aromatic region thus giving proton resonance locations upfield of the transmitter (negative shifts in Hz) because of the lack of quadrature detection in the double quantum frequency domain (F_1 or ω_1). The data was processed using sinusoidal multiplication prior to both Fourier transformations and zero filling prior to the second. The data matrix obtained following the second Fourier transform was transposed to give the F_1F_2 format shown in all of the contour plots. Without quadrature detection in the second frequency domain, the Fourier transformations produce positionally equivalent frequency but not intensity equivalent replicates

symmetric about $F_1 = 0$ Hz. Transposition of the upfield replicate (negative frequencies in F_1) which contains the more intense responses when the read pulse $< 90^\circ$ was employed in the case of the proton double quantum coherence experiment. The downfield replicate (positive frequencies in F_1) which contained the more intense responses was transposed in the case of the relayed proton double quantum coherence experiment. A total of 32 acquisitions were collected for each of the 512 blocks of data taken in the proton double quantum coherence experiment; 64 acquisitions were collected for the relayed proton double quantum experiment, giving acquisition times which ranged from 5 h to 15 h depending upon the duration of τ selected and the experiment.

ACKNOWLEDGMENTS

The authors would like to acknowledge the generous support of the Robert A. Welch Foundation through Grant No. E-792 to G.E.M. and the support of the University of Houston-University Park which provided funds for the acquisition and operation of the NT-300 spectrometer system used in this research.

LITERATURE CITED

1. T.H. Mareci and R. Freeman, *J. Magn. Reson.*, **51**, 531 (1983).
2. L. Braunschweiler, G. Bodenhausen, and R.R. Ernst, *Molec. Phys.*, **48**, 535 (1983).
3. G.E. Martin, R. Sanduja, and M. Alam, *J. Org. Chem.*, **50**, 2383 (1985).
4. S.W. Fesik, T.J. Perun, and A.M. Thomas, *Magn. Reson. Chem.*, **23**, 645 (1985).
5. R. Sanduja, G.S. Linz, E.L. Ezell, M. Alam, A.J. Weinheimer, and G.E. Martin, *J. Heterocyclic Chem.*, **23**, 529 (1986).
6. J.C. Carter, G.W. Luther, III, and T.C. Long, *J. Magn. Reson.*, **15**, 122 (1974).
7. W.J. Chazin, L.D. Colebrok, and J.T. Edward, *Can. J. Chem.*, **61**, 1749 (1983).
8. L.M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy," 2nd ed. Pergamon Press, London, 1969, p. 316.
9. G. Eich, G. Bodenhausen, and R.R. Ernst, *J. Am. Chem. Soc.*, **104**, 3731 (1982).
10. S. Macura, N.G. Kumar, and L.R. Brown, *J. Magn. Reson.*, **60**, 99 (1984).
11. L. Müller and A. Pardi, *J. Am. Chem. Soc.*, **107**, 3484 (1985).
12. The Nicolet 293-C pulse program and phase cycling or phase tables for the proton double quantum and relayed proton double quantum experiments may be obtained from the authors upon request.

Received 8 November 1985