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Improved Performance Accordion Heteronuclear Multiple-Bond Correlation Spectroscopy—IMPEACH-MBC

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A modification of the recently reported ACCORD-HMBC long-range heteronuclear shift correlation experiment is described. The new experiment, IMPEACH-MBC (*im*proved *pe*rformance *ac*cordion *h*eteronuclear *m*ultiple-*b*ond *c*orrelation), introduces a new pulse sequence element, a constant time variable delay. The incorporation of the constant time variable delay into the IMPEACH-MBC sequence suppresses ^{1}H–^{1}H coupling modulation inherent to the utilization of the accordion principle to sample a broad range of potential long-range heteronuclear couplings. ^{1}H–^{1}H coupling modulation, which introduces an F_1 modulation or a "skew" of responses in the second frequency domain of the ACCORD-HMBC experiment, is suppressed in the IMPEACH-MBC experiment. Results of identically optimized IMPEACH-MBC and ACCORD-HMBC experiments performed on a sample of strychnine are compared.

Key Words: **IMPEACH-MBC; ACCORD-HMBC; long-range heteronuclear shift correlation; constant time variable delay; accordion principle.**

INTRODUCTION

During the early 1980s, following the demonstration of the feasibility of long-range heteronuclear shift correlation in the seminal report of Reynolds and co-workers (*1*), there was a surge of interest in the development and refinement of long-range correlation techniques which was examined in the 1988 review of Martin and Zektzer (*2*). The 1986 report of the proton-detected HMBC experiment by Bax and Summers (*3*) brought the development of new long-range heteronuclear correlation experiments to a close for a number of years, aside from the incorporation of gradients into these experiments in the early 1990s (*4*). More recently, however, there has been a surge of interest in the development of new, inverse-detected gradient long-range correlation experiments, which is reviewed briefly below.

The first reported modification of the HMBC experiment was the D-HMBC experiment of Furihata and Seto (*5*). Rather than initiating acquisition immediately following the last 90° ^{13}C pulse, they instead inserted a fixed delay with a duration equivalent to the delay used to prepare long-range couplings for conversion to heteronuclear multiple-quantum coherence. Then, by "refocusing" long-range couplings, broadband heteronuclear decoupling could be applied during acquisition. While D-HMBC does offer improved sensitivity, the improvement for a given response is a function of the congruence of the individual long-range coupling with the optimization of the long-range delay, which can lead to variable results.

Furihata and Seto (*6*) followed their report of the D-HMBC experiment with a pseudo-3D experiment, 3D-HMBC. We refer to this experiment as "pseudo-3D" in that the data are normally used as a projection of the F_2/F_3 plane after transformation. The basic idea was to acquire a series of HMBC spectra differing in optimization. Thus, successive planes in the 3D-HMBC experiment differ in the long-range coupling for which they are optimized. In this fashion, the projection of the F_2/F_3 plane gives the means of sampling a range of potential long-range coupling optimizations *via* the third frequency domain. Unfortunately, this approach has the inherent drawback of any 3D experiment in that it is the cumulative result of performing a series of 2D experiments. Consequently, for weak samples, the time required to perform this experiment would likely be prohibitive.

In early 1997, Marek *et al.* (*7*) reported a long-range heteronuclear correlation experiment derived from the GHSQC rather than the GHMQC experiment. Their experiment, GSQMBC, provides phase-sensitive data, allowing the extraction of the heteronuclear couplings from the antiphase multiplets. In early 1998, Sheng and van Halbeek (*8*) reported a phase-sensitive variant of the GHMBC experiment that was also developed for the purpose of extracting long-range heteronuclear coupling constants from the data using the shift method of Titman and co-workers (*9*).

A novel approach to sample a range of potential long-range couplings was advanced by Wagner and Berger (*10*) in the ACCORD-HMBC experiment. ACCORD-HMBC employs the "accordion" principle (*11*) in which the delay used for long-range heteronuclear couplings is systematically decremented from τ_{max} to τ_{min} in steps of $(\tau_{max} - \tau_{min})/ni$, where ni is the total number of t_1 increments, in concert with the incrementation of the evolution time, t_1. The net result is the equivalent of "integrating" across the selected range of possible long-range couplings in a single experiment. In addition, the symmetrical constitution of the ACCORD-HMBC pulse sequence allows

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the application of heteronuclear decoupling during acquisition. Beneficially, the experiment affords considerably more long-range coupling information as noted by Wagner and Berger in their initial report (*10*) and as discussed in the more comprehensive recent study of Martin *et al.* (*12*). As in HMBC experiments, cross peaks are frequency modulated in F_1 due to homonuclear coupling. The unfortunate drawback of using accordion optimization is that these modulations are "scaled" by a scaling factor, N, which is defined by

$$N = 2\tau/\Delta t_1,$$

where

$$\tau = (\tau_{max} - \tau_{min})/ni$$

and where τ_{max} and τ_{min} are the limits of the accordion optimization range; ni is the number of increments of the evolution time in the second frequency domain; and Δt_1 is the dwell time in F_1 (*12*). The adverse effects of F_1 modulation can be ameliorated somewhat by adjusting the range of long-range couplings being sampled ($\tau_{max} - \tau_{min}$) or by increasing the number of increments (ni) used to digitize the second frequency domain. F_1 modulation, however, cannot be suppressed in the original version of the ACCORD-HMBC experiment (*10, 12*) irrespective of parameter selection.

Most recently, in late 1998, Furihata and Seto (*13*) reported a pair of constant evolution time GHMBC experiments. The concept of a constant time two-dimensional NMR experiment was first used from homonuclear correlation spectroscopy (*14, 15*). More recently, there have been various applications of the idea in heteronucleus-detected (*16–20*) and inverse-detected experiments (*21–25*). Furihata and Seto's first experiment, CT-HMBC-1, employs a conventional "static" long-range optimization and then incorporates a decremented delay,

$$(\Delta_3 - t_1/2),$$

before and after the evolution period (*13*). The decrementable delay is systematically decremented in concert with the incrementation of the evolution period, keeping the overall time of evolution constant. This modification keeps the time during which ^{1}H–^{1}H J modulation can occur constant. As a result, the splitting of peaks by scalar ^{1}H–^{1}H coupling in F_1 is suppressed, improving F_1 resolution. Unfortunately, at the same time the ^{1}H–^{1}H J modulation is removed ^{1}H–^{13}C J modulation is being introduced. Broadband decoupling is not an option with the CT-HMBC-1 pulse sequence. The CT-HMBC-2 sequence precedes evolution, t_1, by an echo constant time interval of the form

$$(\Delta_3 - t_1/2) - 180^\circ_x(^1\mathrm{H}) - (\Delta_3 - t_1/2).$$

The authors demonstrate that this modification suppresses not only scalar ^{1}H–^{1}H coupling modulation in F_1 as in the CT-HMBC-1 sequence, but also the range of ^{1}H–^{13}C coupling modulation in F_1 introduced by the "variable" long-range transfer delay by the modification of CT-HMBC-1. Again, no broadband heteronuclear decoupling is possible during acquisition although the authors comment in one of the footnotes to their work that such a sequence is under development.

THE IMPEACH-MBC PULSE SEQUENCE

The ACCORD-HMBC pulse sequence (*10, 12*) employs a variable delay, vd, which is systematically decremented from τ_{max} to τ_{min} as the evolution time, t_1, is incremented. As noted in our previous work (*12*) and by Furihata and Seto (*13*), ^{1}H–^{1}H coupling modulation occurs during the variable delay, which serves as a pseudo-evolution time for this process. Suppressing modulation problems is hardly a new idea in long-range heteronuclear shift correlation experiments (*26–33*). The idea of suppressing one-bond ($^1J_{CH}$) amplitude modulations, for example, has been extensively exploited during the development of numerous heteronucleus-detected long-range correlation pulse sequences (*2*). To suppress ^{1}H–^{1}H coupling modulation, in the present case we have developed an alternative to the variable delay used for the accordion optimization in the ACCORD-HMBC sequence, which we term a constant time variable delay. Despite the rather oxymoronic name for this pulse sequence element, it is conceptually rather simple, consisting of two delays [D and vd] embedded in a constant time delay of the form

$$D/2 - 180^\circ(^{13}\mathrm{C}) - D/2 - \mathrm{vd}.$$

As in the ACCORD-HMBC experiment, the variable delay, vd, is decremented in a systematic fashion by $(\tau_{max} - \tau_{min})/ni$, where ni corresponds to the number of increments of the evolution time being used to digitize the second frequency domain as the evolution periods, $t_1/2$, are incremented by Δt_1. In contrast, when vd is decremented by $(\tau_{max} - \tau_{min})/ni$, the other delay, D, is incremented by $(\tau_{max} - \tau_{min})/ni$, maintaining the constancy of the overall pulse sequence element. The only variability when this pulse sequence element is employed is associated with the incrementation of the evolution time itself (*34*). In a fashion analogous to the ACCORD-HMBC experiment, the variable delay, vd, allows sampling across the range of potential long-range couplings defined by τ_{max} and τ_{min}. During the variable incremented delay, D, the heteronuclear coupling is refocused by the 180° ^{13}C pulse. However, the ^{1}H–^{1}H homonuclear coupling evolves during the constant time variable delay, D + vd. The net result of incorporating this pulse sequence element *in lieu* of the simple variable delay into an accordion-optimized long-range heteronuclear shift correlation experiment is to suppress F_1 modulation due to the evolution of ^{1}H–^{1}H scalar couplings during the pseudo-evolution time provided by the variable delay interval in the conventional ACCORD-HMBC experiment (*10, 12*).

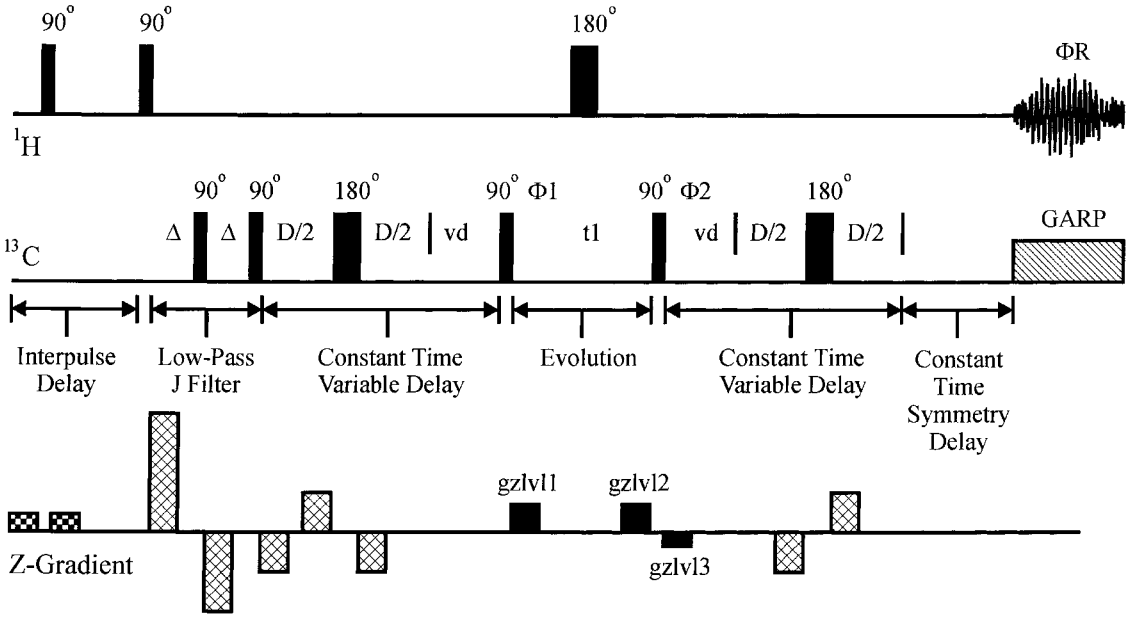


FIG. 1. IMPEACH-MBC (*im*proved *pe*rformance *ac*cordion long-range *h*eteronuclear shift correlation spectroscopy) pulse sequence incorporating a constant time variable delay in place of the normal, accordion variable delay used in the ACCORD-HMBC experiment (*10, 12*). Ratios of the gradients in the IMPEACH-MBC experiment were 2:2:27:−18:−9:9:−9:2:2:−1:−9:9 G cm^{-1}. The sequence employs the gradient double low-pass *J* filter described by Sørensen and co-workers (*35*) as used in the ACCORD-HMBC experiment (*10, 12*); delays for the dual low-pass *J* filter were set to cover the range of one-bond couplings from 140 to 180 Hz. Following evolution, the dual low-pass *J* filter is replaced by a delay of equal length (constant time symmetry delay) to maintain time symmetry for broadband heteronuclear decoupling during acquisition. The constant time variable delay was optimized for 2 to 25 Hz for the IMPEACH-MBC spectrum shown in Fig. 2. The phase of the unlabeled pulses in the sequence was held constant at 0. The cycled phases were $\Phi_1 = 02$, $\Phi_2 = 0202$, and $\Phi_R = 0220$.

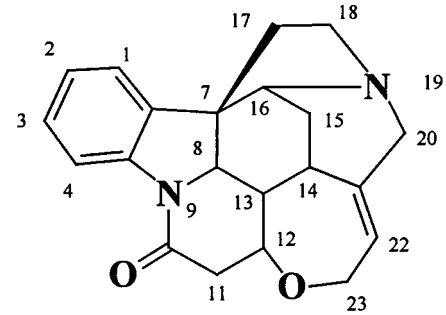
The constant time variable delay is incorporated flanking the evolution period, as shown in the pulse sequence schematic presented in Fig. 1. We have given this experiment the acronym IMPEACH-MBC (*im*proved *pe*rformance *ac*cordion *h*eteronuclear *m*ultiple-*b*ond *c*orrelation). Constant time variable delays flank the evolution period in the experiment, permitting the application of broadband heteronuclear (GARP) decoupling during acquisition in a fashion analogous to the D-HMBC (*5*) and ACCORD-HMBC (*10, 12*) experiments. The experiment employs the gradient dual stage low-pass *J* filter described by Sørensen and co-workers (*35*), which was optimized to exclude direct correlation responses for heteronuclide pairs with one-bond couplings ranging from 140 to 180 Hz. A delay equivalent to the length of the *J* filter (constant time symmetry delay) is introduced prior to acquisition to maintain time symmetry of the sequence (see Fig. 1). A four-step phase cycle was employed as specified in the figure legend. The ratios of the gradient pulses (G cm^{-1}) used in the experiment are also specified in the figure legend. The three gradients shown in solid black were nominally set in a 2:2:−1 ratio for ^{13}C, although a 5:3:4 ratio can equally well be employed. The

gradient–90° ^{1}H–gradient

pulse sequence element used to begin the experiment served to randomize magnetization.

RESULTS AND DISCUSSION

A direct comparison of the results obtained for 2–25 Hz accordion-optimized ACCORD-HMBC and IMPEACH-MBC experiments is presented in Fig. 2. The experiments were performed at 500 MHz on a Varian *INOVA* 500 equipped with a Nalorac Z · SPEC MIDTG-500-3 micro inverse 3-mm gradient triple-resonance probe using a sample prepared by dissolving 45 mg of strychnine (**1**)



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in 150 μL of $CDCl_3$ in a 3-mm NMR tube, which was sealed. The second frequency domain in both experiments was digitized with 512 increments of the evolution period to minimize F_1 modulation in the case of the ACCORD-HMBC spectrum

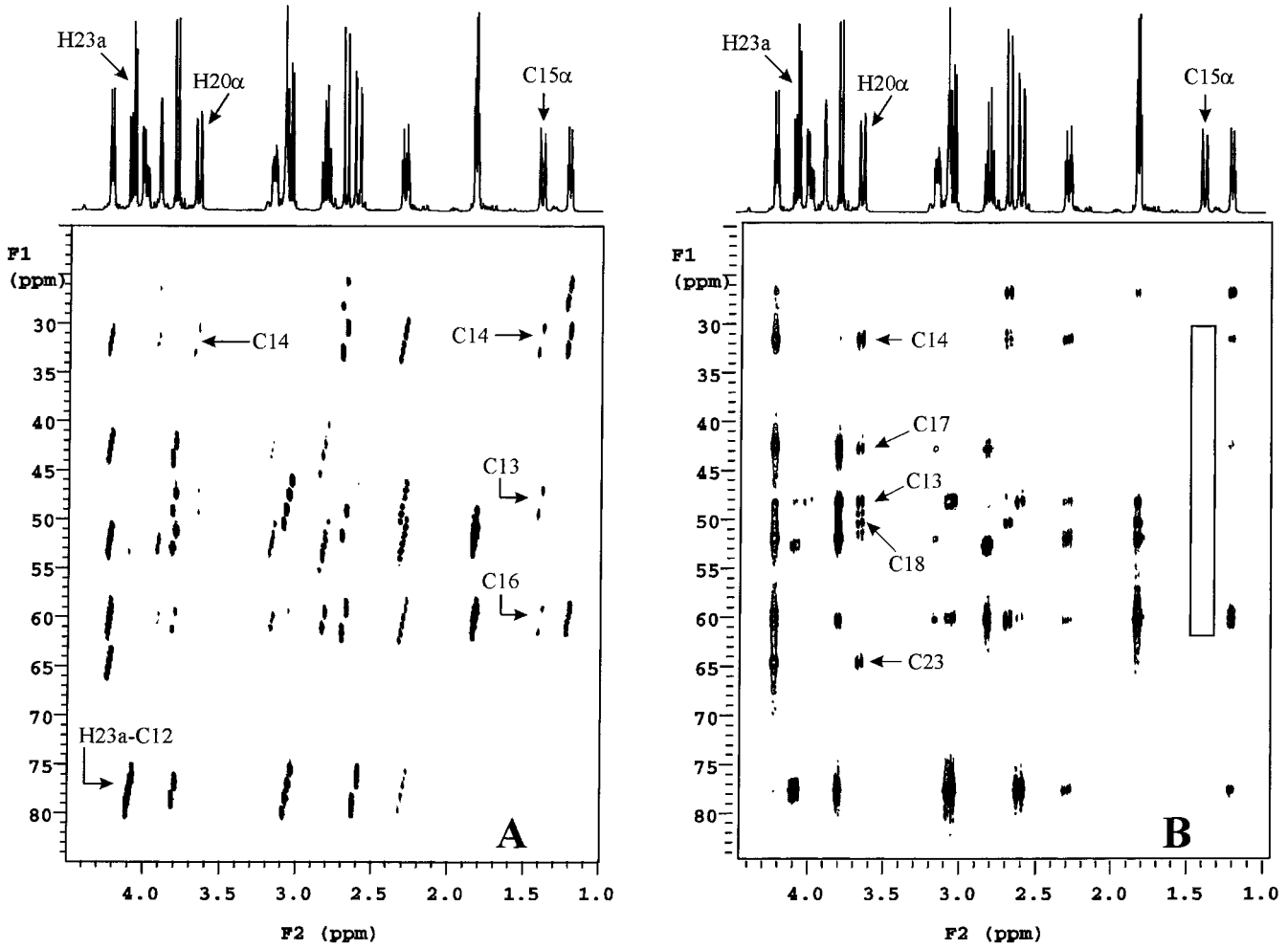

FIG. 2. Comparison of the results obtained with the ACCORD-HMBC (*10, 12*) (A) and IMPEACH-MBC (B) pulse sequences. Both experiments were performed using a sample of 45 mg of strychnine in 150 μL $CDCl_3$ in a sealed tube at 27°C. The experiments were performed on a Varian *INOVA* 500 NMR spectrometer equipped with a Nalorac Z · SPEC MIDTG-500-3 micro inverse 3-mm triple-resonance probe. Both experiments were optimized to cover the range of potential long-range couplings from 2 (250 ms) to 25 (20 ms) Hz. Both experiments were performed by taking four transients for each of 512 increments of the evolution period. The F_2 dimension was digitized with 2048 points, giving an acquisition time of 224 ms. GARP decoupling was applied during acquisition in both experiments. The interpulse delay was 1.5 s. Data were processed using sinebell and cosine multiplication prior to the first and second Fourier transforms, respectively; the weighting factors were matched to the evolution time in each frequency domain. No linear prediction or zero-filling was employed in either frequency domain during the processing of either experiment. The spectra in both panels were plotted with identical vertical scaling. Responses discussed in the text are labeled in both panels. The empty boxed region in (B) corresponds to the region of the spectrum where the long-range correlation responses from the H15α to the C14, C13, and C16 resonances were expected to be weakly observed as they were in the ACCORD-HMBC data shown in (A).

shown in Fig. 2A (*12*). Despite this consideration in the acquisition of the data, the responses in the second frequency domain of the ACCORD-HMBC experiment still exhibit considerable F_1 modulation. As an example, the H23a long-range correlation to the C12 resonance in the ACCORD-HMBC spectrum still has a width due to F_1 modulation of ~700 Hz as shown in Fig. 3A. As expected, based on the utilization of the constant time variable delay in the IMPEACH-MBC pulse sequence, F_1 modulation due to ^1H–^1H scalar coupling is suppressed in the IMPEACH-MBC spectrum shown in Fig. 2B and in the expansion of the region showing the H23a–C12 correlation in Fig. 3B. This attribute of the IMPEACH-MBC experiment obviates the possibility of response overlap in F_1 due to modulation when less extensive digitization of the second frequency domain must be employed to accommodate weaker samples and/or time constraints. In contrast, we have recently shown that there is considerable potential for the overlap of long-range correlation responses in the ACCORD-HMBC experiment when broad optimization ranges, e.g., 2–25 Hz, and limited digitization in F_1 are employed (*12*).

Comparing the data content of the two spectra shows them to be largely comparable in terms of the responses which they contain although there are some rather striking and inexplicable differences. For example, correlations from the H20α res-

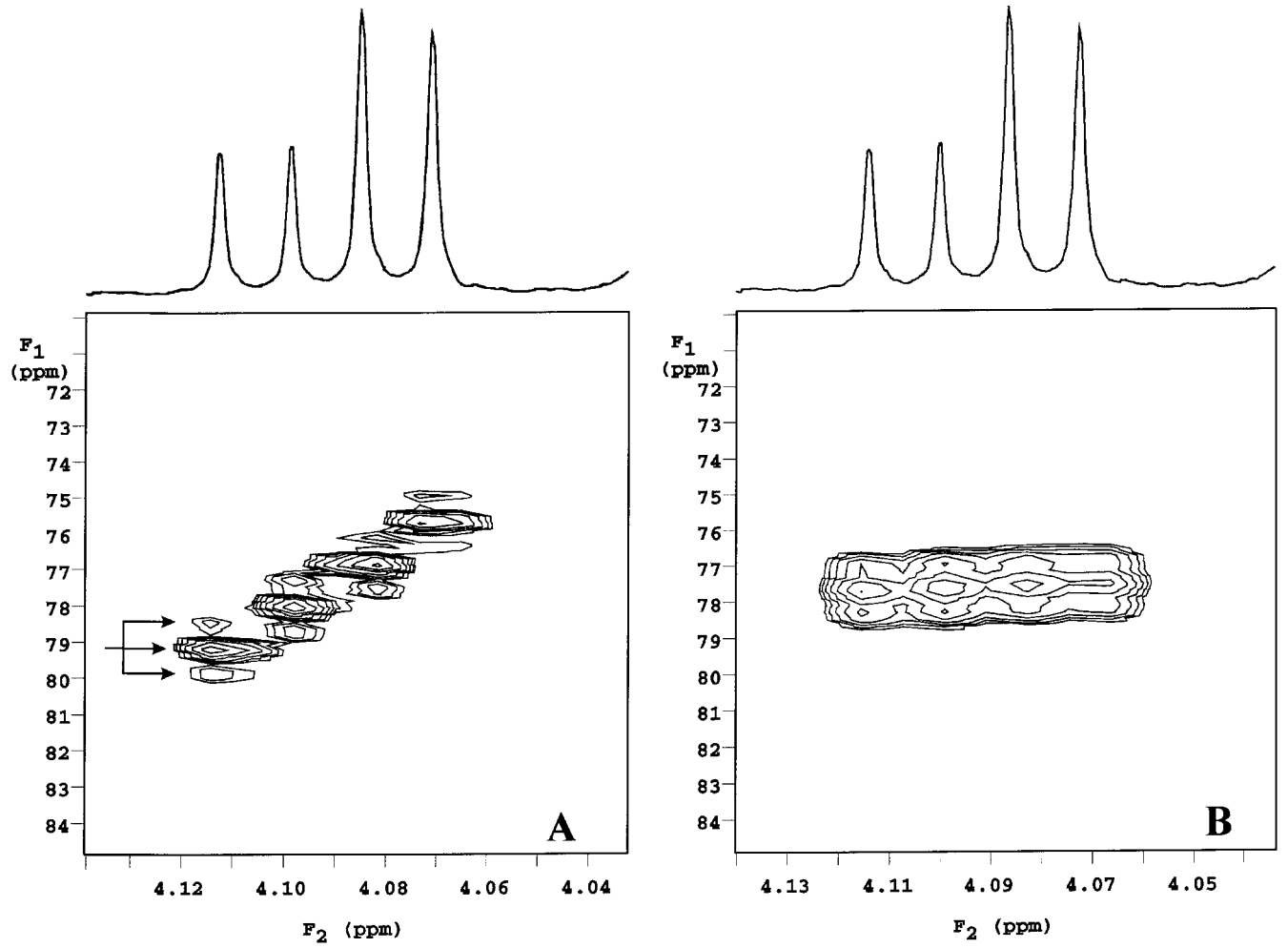

FIG. 3. Expansion of the region of the 2–25 Hz optimized ACCORD-HMBC and IMPEACH-MBC spectra shown in Fig. 2 to illustrate multiplet detail for the $^{3}J_{CH}$ long-range coupling across the oxepin ether linkage of strychnine (**1**) of the H23a resonance to the C12 resonance at 77.6 ppm. The response from the ACCORD-HMBC spectrum is shown in (A). The corresponding response from the IMPEACH-MBC spectrum is shown in (B). The chemical shift axis in F_1 is in ppm relative to TMS in (B). The characteristic "skew" of the response in F_1 in the ACCORD-HMBC experiment, which is caused by ^{1}H–^{1}H homonuclear coupling modulation during the variable delay, vd, is suppressed by the use of the constant time variable delay incorporated into the IMPEACH-MBC sequence (see Fig. 1) in lieu of the simpler vd. There remains an unsuppressed modulation of undetermined origin in both experiments in F_1, leading to the "triplet-like" appearance as designated by the forked arrow in (A).

onance at the identical threshold level used to prepare the plots are almost completely absent in the ACCORD-HMBC spectrum shown in Fig. 2A. Responses are observed with considerable intensity correlating H20α to C14, C17, C13, C18, and C23 in the IMPEACH-MBC spectrum. The same correlations are observable in the ACCORD-HMBC spectrum albeit only when much deeper thresholds are used in preparing the contour plot. There is no readily apparent explanation for this disparity in response intensity. Conversely, low-intensity responses are observed in the ACCORD-HMBC spectrum which correlate the H15α proton (1.44 ppm) to C14, C13, and C16; in stark contrast, no correlations are observed from the H15α resonance in the IMPEACH-MBC spectrum. Again, there is no ready explanation for the observed differences between the two experiments.

One final feature which both experiments share in common is their facility for the observation of four-bond long-range correlations when optimized for small couplings, e.g., 2 Hz, which is greatly superior to that of the conventional GHMBC experiment (*4*). While optimizing long-range heteronuclear shift correlations for a broad range of potential couplings does afford a wealth of four-bond correlation information (*12*) it is not always advisable to use this as a first-pass approach since the numerous additional couplings can confuse the structure elucidation and/or spectral assignment process. In contrast, however, when dealing with molecules containing proton-deficient regions in their skeletons, or "buried" quaternary carbons, the ability to probe beyond the normal $^{2}J_{XH}$ or $^{3}J_{XH}$ couplings of the conventional GHMBC experiment is a decided advantage of the accordion-optimized long-range experiments.

CONCLUSIONS

The recently reported ACCORD-HMBC (*10, 12*) and the IMPEACH-MBC long-range heteronuclear shift correlation experiments both offer the capability of surveying broad ranges of potential long-range couplings in a convenient, single experiment. The utilization of an accordion variable delay in the former leads to F_1 homonuclear coupling modulation of long-range correlation responses, which can result in response overlap for highly congested spectral regions or when limited digitization of the second frequency domain must be employed (*12*). In contrast, the development of a new pulse sequence element, a constant time variable delay, which we have incorporated into the IMPEACH-MBC pulse sequence shown in Fig. 1, provides the means of suppressing F_1 homonuclear coupling modulation while still allowing broad ranges of potential long-range couplings to be sampled in a single experiment. The use of IMPEACH-MBC offers an advantage over ACCORD-HMBC when limited digitization of the second frequency domain must be employed because of sample limitations or constraints on instrument time. Fewer increments can be used to digitize the second frequency domain of the IMPEACH-MBC experiment without adverse F_1 modulation effects inherent to the ACCORD-HMBC experiment. Finally, both the ACCORD-HMBC and IMPEACH-MBC experiments provide much better experimental access to four-bond long-range couplings which may be important in the elucidation of some molecules having regions in their structures that are relatively proton deficient.

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34. As an alternative, the overall time of the experiment can be maintained precisely constant by incrementing each of the $D/2$ intervals by the quantity

$$\{[(\tau_{max} - \tau_{min})/ni]/2 - t_1/2\},$$

where $t_1/2$ corresponds to the increment of the evolution time, t_1. In this fashion, ^{1}H–^{1}H couplings can be eliminated from the second frequency domain, F_1, in a manner analogous to the CT-HMBC-1 pulse sequence described by Furihata and Seto (see Ref. *13*). This capability, or the option of user-defined J scaling to control F_1 modulation to be used as a determinant of long-range response authenticity, is incorporated into a new pulse sequence, details of which will be reported elsewhere.

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