MAGNETIC RESONANCE IN CHEMISTRY
Magn. Reson. Chem. **36**, S44–S46 (1998)

ACCORD–HMBC: a superior technique for structural elucidation†

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Received 29 October 1997; revised 29 December 1997; accepted 29 December 1997

ABSTRACT: The standard gradient selected HMBC pulse sequence was modified. With the ACCORDION principle a range of spin coupling constants is sampled which gives a complete set of long-range C,H connectivities. A better low-pass filter allows GARP ^{13}C decoupling. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: HMBC; ACCORDIAN spectroscopy; pulsed field gradient; low-pass *J*-filter

INTRODUCTION

The HMBC method[1] is probably the single most important 2D NMR method for the structural elucidation of organic compounds.[2] It provides correlation signals for C,H spin pairs indicating connectivities over two and three bonds and can therefore be used to build a molecular skeleton. The structures of numerous natural products have been investigated with the help of HMBC spectra, and often these spectra deliver the decisive answer for a structural problem. A breakthrough for the performance of the HMBC spectra was the introduction of pulsed field gradients,[3,4] which allowed the spectroscopist to open the receiver gain of the spectrometer by several orders of magnitude, since the unwanted coherences are already filtered off in the probe head. The gs-HMBC modification is now routine standard in most service laboratories.[5]

Nevertheless, the experiment still suffers from deficiencies. First, the commonly employed low-pass filter[1] consisting of one additional 90° pulse on the ^{13}C channel compared with the HMQC sequence does not work sufficiently well. Depending on the spin coupling constant values, one observes a breakthrough of $^{1}J(C,H)$ signals. Therefore, broadband ^{13}C decoupling is usually not used, since under decoupling conditions a correlation signal arising from $^{2/3}J(C,H)$ cannot be distinguished from one arising from $^{1}J(C,H)$. However, ^{13}C decoupling would be highly desirable because of the sensitivity gain obtainable by sharpening the multiplets in the F_2 dimension.

The second drawback of the experiment is due to the range of $^{2/3}J(C,H)$ spin coupling constants, which can have values from 1 to 25 Hz. Usually HMBC spectra are recorded with a compromise by setting the corresponding delay in the sequence to 60 ms to allow an optimum transfer for correlation signals where the spin coupling constant is about 8 Hz. Under these conditions several important connectivities yield only very small correlation signals or are completely lost. One remedy to this problem would be to record several HMBC spectra with different delays or, as recently proposed by Furihata and Seto[6] to record the HMBC spectra in a 3D manner. In the 3D technique the third dimension is used to increase continuously the polarization delay d_2 of the HMBC sequence, thus all possible long-range spin coupling constants are met during the recording time of a 3D cuboid. After 3D Fourier transformation, a projection of the F_2/F_3 plane is calculated, which yields the final HMBC spectrum as a sum of all planes in the 3D cuboid. The procedure has merit but seems to be unnecessarily time consuming. Here we report our solution to the problem.

EXPERIMENTAL

The spectra shown were measured in 5% solutions of the compounds in $CDCl_3$ or D_2O using a Brukar AMX-500 spectrometer equipped with a multinuclear inverse probe head with additional coils for *z*-gradients. The gradient length was set to 1 ms with a strength in the order of 0.2 $T\ m^{-1}$ using a 10 A BGU gradient unit. The 2D spectra were obtained with two scans for each of 256 FIDs, a relaxation delay of 2 s and a time domain of 2K. The variable long-range HMBC polarization delay *vd* was adjusted from 200 to 20 ms and the dual-stage low-pass filter acted on coupling constants between 140 and 180 Hz. The spectra were processed in

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† Dedicated to Professor John D. Roberts on the occasion of his 80th birthday.

Contract/grant sponsor: Deutsche Forschungsgemeinschaft;
Contract/grant number: SFB-260-B1.
Contract/grant sponsor: Fonds der Chemischen Industrie.

CCC 0749-1581/98/SI0044–03 $17.50

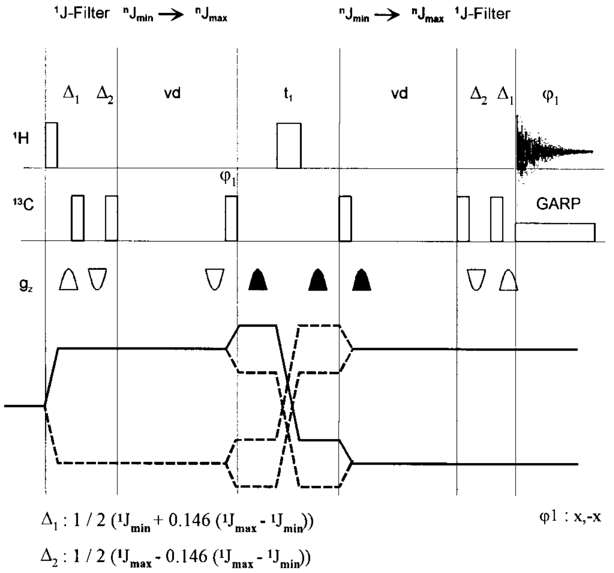


Figure 1. Pulse sequence for the ACCORD–HMBC technique. Small bars 90° and thick bars 180° pulses. Selected coherence transfer is given as thick line. Gradient length 1 ms in the ratios 15 : −10 : −5 : 50 : 30 : 40 : −5 : 5. Variable delay *vd* decremented during incrementing t_1. Equations for Δ_1 and Δ_2 and pulse phases are shown.

the quadrature-off mode using an exponential filtering in F_2 with $lb = 2.5$ Hz and a squared cosine window in F_1. The total time required for the spectra shown was 21 min for sucrose and 26 min for strychnine.

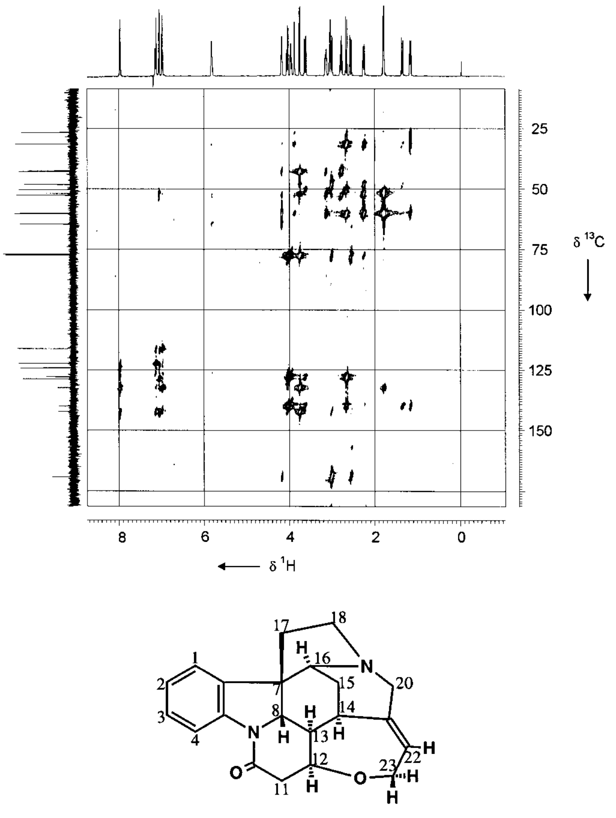


Figure 2. ACCORD–HMBC spectrum of strychnine. For parameters, see Experimental; *vd* decremented from 100 to 20 ms.

RESULTS AND DISCUSSION

Before the advent of 3D NMR spectroscopy, Bodenhausen and Ernst[7] showed that a 2D NMR spectrum may be recorded with three different time variables. They termed their approach ACCORDION spectroscopy and applied it to EXSY- and NOESY-type spectra. Despite the two traditional time variables t_1 and t_2, they varied in the ACCORDION experiment the mixing time τ_m. Their original experiment has found several applications.[8–11] Recently, the basic idea was transferred to 2D-TOCSY spectra by Kontaxis and Keeler,[12] allowing for a variable TOCSY spin-lock length. In principle it should be possible to vary the HMBC delay in the same manner. Instead, however, of increasing the HMBC delay with increasing t_1 time, we chose to decrease it, thus keeping the overall delay after the first excitation and final detection to a minimum. As seen in the pulse sequence in Fig. 1, we keep the whole approach symmetric and decrease the refocusing delay after the last pulse and the acquisition accordingly. The parameters of the pulse sequence can be adjusted in such a manner that spin coupling values corresponding to 25–2 Hz are sampled, with the smaller values of the delay *vd* at the initial t_1 increments and the higher values towards the end of the t_1 evolution. To keep phase distortions and frequency offsets in F_1 to a minimum, the procedure is repeated after the back transfer from carbons to protons, thus the 180° proton pulse is always exactly in the middle of the sequence. Nevertheless, we observe a phase modulation of the signals in F_1 due to the ACCORDION principle, as can be seen by close inspection of Fig. 3 in comparison with Fig. 4. In all practical cases measured so far, however, the observation of additional cross peaks outweighs these effects.

Like long-range spin coupling constants, $^1J(\mathrm{C,H})$ spin coupling constants also span a range of values of typically from 130 to 160 Hz. This variation is too large for the simple single-pulse low-pass filter commonly employed in the HMBC sequence. Sørensen and coworkers proposed a dual-stage low-pass filter[13] and recently demonstrated a gradient selected version of this procedure[14] for similar purposes in their 2Q-HMBC sequence. We have programmed this two-pulse low-pass filter into our ACCORD–HMBC sequence for both the initial and the reverse transfer and can report that it does indeed filter off the undesired coherences to such a level that GARP broadband ^{13}C decoupling is feasible. Higher order filters, as originally proposed by Kogler *et al.*,[15] should also be possible, but have not been tested by us. The significant increase in sensitivity in decoupled HMBC spectra has already been demonstrated by Furihata and Seto[16] in the case of natural product chemistry, but without employing a low-pass filter.

The final sequence as given in Fig. 1 was tested on strychnine, being a typical organic application, and subsequently on sucrose, having shorter relaxation times. The results are given in Figs 2 and 3 and demonstrate by comparison with the standard HMBC version given

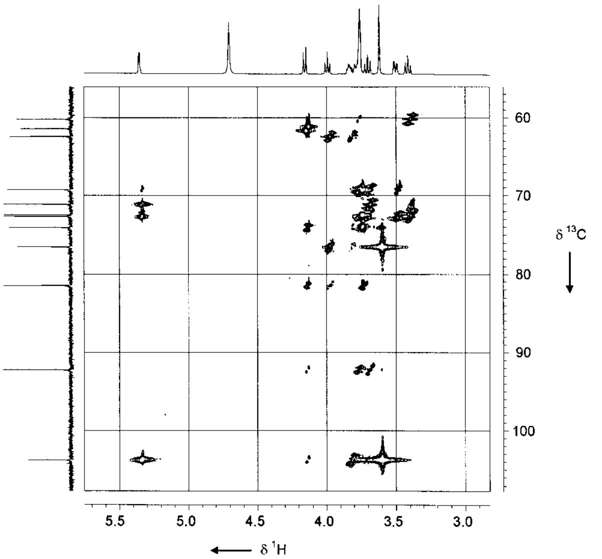
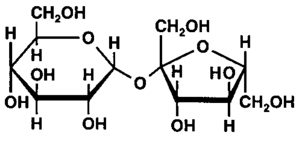


Figure 3. ACCORD–HMBC spectrum of sucrose. For parameters, see Experimental; *vd* decremented from 200 to 20 ms.



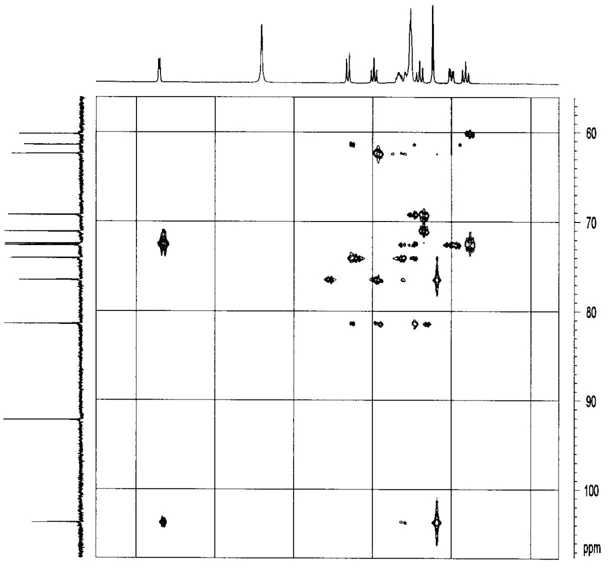


Figure 4. Standard gradient selected HMBC spectrum[5] of sucrose using an HMBC delay of 50 ms.

in Fig. 4 for sucrose as an example (a comparison spectrum for strychnine can be found in Ref. 17) that more long-range connectivities may be seen without spending significantly more measurement time compared with the standard HMBC experiment.

CONCLUSION

ACCORD–HMBC is a new and useful variant of the gradient selected HMBC technique and allows the sampling of long-range C,H connectivities acting over a range of spin coupling constants. An effective $^{1}J(\mathrm{C,H})$ low-pass filter allows for GARP decoupling; thus we feel that the sequence proposed here might attract attention for standard routine service operations in analytical laboratories.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (SFB-260-B1) and the Fonds der Chemischen Industrie.

REFERENCES

1. A. Bax and M. F. Summers, *J. Am. Chem. Soc.* **108**, 2093 (1986).
2. G. E. Martin and A. S. Zektzer, *Two Dimensional Methods for Establishing Molecular Connectivity*. VCH, Weinheim (1988).
3. J. Ruiz-Cabello, G. W. Vuister, C. T. W. Moonen, P. van Gelderen, J. S. Cohen and P. C. M. van Zijl, *J. Magn. Reson.* **100**, 282 (1992).
4. W. Wilker, D. Leibfritz, R. Kerssebaum and W. Bermel, *Magn. Reson. Chem.* **31**, 287 (1993).
5. S. Braun, H. O. Kalinowski and S. Berger, *100 and More Basic NMR Experiments*. VCH, Weinheim (1996).
6. K. Furihata and H. Seto, *Tetrahedron Lett.* **37**, 8901 (1996).
7. G. Bodenhausen and R. R. Ernst, *J. Am. Chem. Soc.* **104**, 1304 (1982).
8. J. Boyd, K. M. Brindle, I. D. Campbell and G. K. Radda., *J. Magn. Reson.* **60**, 149 (1984).
9. L. E. Kay and J. H. Prestegard, *J. Magn. Reson.* **77**, 599 (1988).
10. V. L. Yarnykh and Y. A. Ustynyuk, *J. Magn. Reson. A* **102**, 131 (1993).
11. A. M. Mandel and A. G. Palmer III, *J. Magn. Reson. A* **110**, 62 (1994).
12. G. Kontaxis and J. Keeler, *J. Magn. Reson. A* **115**, 35 (1995).
13. O. W. Sørensen, N. C. Nielsen, H. Bildsø and H. J. Jakobsen, *J. Magn. Reson.* **70**, 54 (1986).
14. A. Meissner, D. Moskau, N. C. Nielsen and O. W. Sørensen, *J. Magn. Reson.* **124**, 245 (1997).
15. H. Kogler, O. W. Sørensen, G. Bodenhausen and R. R. Ernst, *J. Magn. Reson.* **55**, 157 (1983).
16. K. Furihata and H. Seto, *Tetrahedron Lett.* **36**, 2817 (1995).
17. S. Braun, H. O. Kalinowski and S. Berger, *100 and More Basic NMR Experiments*, p. 366. VCH, Weinheim (1996).