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Application of HSQC to the measurement of homonuclear coupling constants, *J*(H,H)

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ABSTRACT: It is demonstrated that HSQC gives considerably simpler multiplets than HMQC, allowing precise extraction of proton–proton coupling constants. Application of a spin-locking pulse prior to acquisition yields pure-phase ^{13}C decoupled multiplets. The combined use of coupled and refocused HSQC allows second-order structure of the proton multiplets to be avoided in favourable cases. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; HSQC; HMQC; coupling constants; second-order effects; borneol

INTRODUCTION

Since its discovery in the early 1950s, it has been realized that indirect scalar spin–spin coupling (*J* coupling) is an important structural parameter.[1] As the coupling is mediated by bonding electrons, it provides valuable information about the constitution of molecules in terms of the connectivity of the coupled nuclei. The size of the coupling often depends also on dihedral angles and is therefore an important source of conformational information.[2] Application of high-resolution proton NMR spectroscopy is often less limited by sensitivity or resolution requirements than by our inability to disentangle a forest of overlapping spin multiplets, frequently complicated by second-order effects. One method that has been demonstrated for alleviating the problem of spectral overlap is to use ^{13}C (or another heteronucleus) which has a larger chemical shift dispersion than its proton counterpart.[3] Owing to their considerably higher sensitivity, the proton detected heterocorrelation experiments provide a more convenient method for extraction of proton multiplets. Yang *et al.*[4] used HMQC successfully for the measurement of homonuclear couplings.[5] The HSQC sequence[6] was reported to give better results.[7,8]

In the present paper, we demonstrate that, as predicted theoretically,[9,10] the HSQC pulse sequence is the method of choice for extracting clean ^{1}H multiplets and consequently the corresponding coupling constants in spectra with overlapping signals. Optimal resolution and suppression of unwanted signals using a spectrometer not equipped with pulse field gradients is obtained by the simultaneous use of a BIRD-nulling delay and two spin-locking pulses.[11,12] It is also shown that the use of ^{13}C-coupled HSQC spectra could, in favourable cases, eliminate second-order effects and thus facilitate the determination of coupling constants in strongly coupled systems as represented by the example of borneol (2-*endo*-hydroxybornane).

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RESULTS AND DISCUSSION

Refocused HSQC for measurement of homonuclear coupling constants

It has been shown[9] that, for a single proton–carbon pair, the initial ^{1}H magnetization I_z is modulated by the angular carbon-13 offset frequency Ω^C in inverse correlation experiments (HMQC and HSQC) and could be detected as a single absorptive correlation in the 2D phase-sensitive spectrum. The magnetization transfer process can be summarized by

$$I_z^H \xrightarrow{\text{HMQC}} -I_y \cos \Omega^C t_1$$

and

$$I_z^H \xrightarrow{\text{HSQC}} -I_x \cos \Omega^C t_1$$

Both excitation schemes give different multiplet structures in the presence of homonuclear couplings. Most often these are the proton–proton couplings. Figure 1 displays typical HSQC and HMQC multiplets, acquired with high ^{13}C resolution (0.12 Hz per point after fourfold zero-filling). The pure phase nature of the HSQC multiplets of approximately equal linewidths in both dimensions is clearly visible.

Extraction of traces from the HSQC spectrum at the carbon frequency allows the collection of proton multiplets with high resolution (nearly equal to the conventional proton spectrum), making this method clearly superior for extraction of coupling constants. In contrast, the HMQC multiplets are complicated both by the presence of homonuclear couplings in the indirect dimension and by impure phases. During the evolution period in the HMQC experiment, refocusing only of chemical shifts and heteronuclear couplings occurs. Homonuclear couplings evolve and are not refocused,[9]

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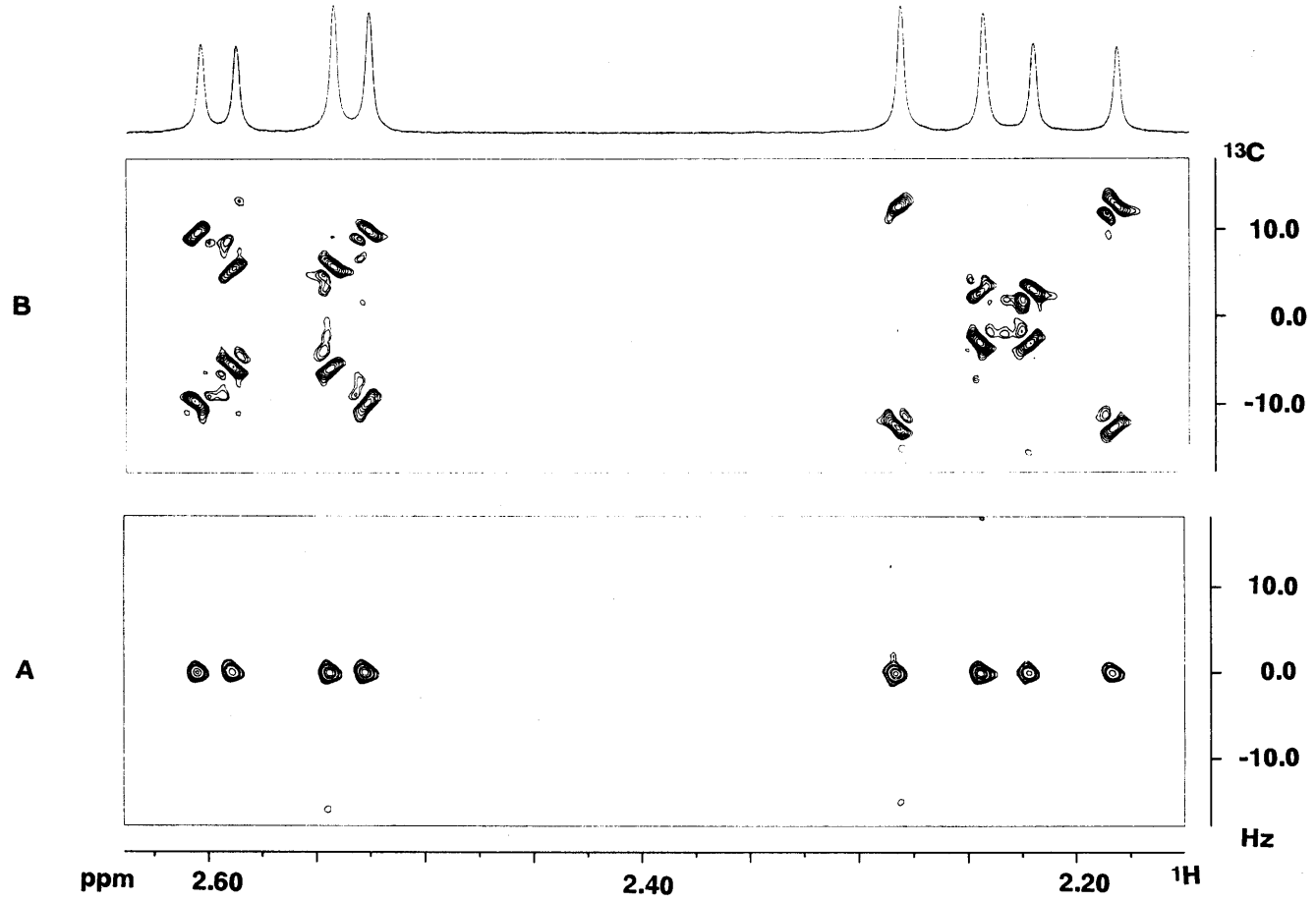

Figure 1. (A) HSQC and (B) HMQC multiplets for the methylene protons of C-β of the dianion of L-asparatic acid using the pulse sequences depicted in Fig. 2.

thus giving rise to antiphase dispersive components in the phase-sensitive 2D spectrum. The multiplets presented in Fig. 1 originate from the two geminal protons of C-β in the dianion of L-asparatic acid in D_2O.

Suppression of the strong, unmodulated signals arising from protons bound to ^{12}C is of prime importance for obtaining good-quality spectra. The HSQC pulse sequence used [Fig. 2(A)] incorporates a BIRD-relaxation preparation period and two spin locking (SL)

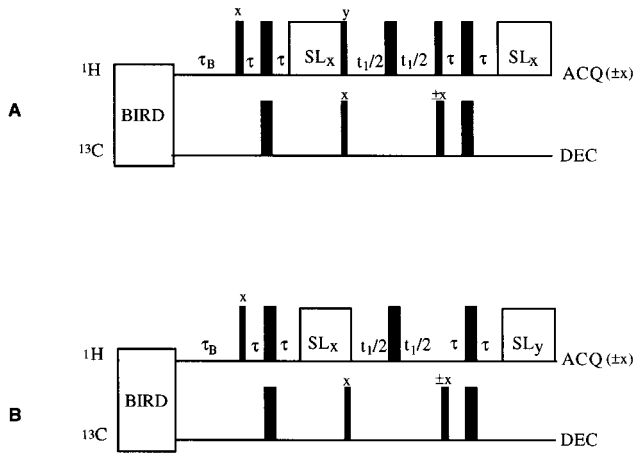

Figure 2. (A) HSQC and (B) HMQC pulse sequences including a BIRD nulling delay and two spin-locking pulses.

purge pulses. The first SL pulse serves for elimination of undesired residual ^{12}C-bound proton signals left after the BIRD sandwich. The second pulse has the additional effect of removing unwanted magnetization which gives rise to phase distortions of the refocused in-phase signals prior to acquisition (due to differences in the individual heteronuclear proton–carbon one bond coupling constants, evolution of homonuclear couplings during the refocusing delay and/or to pulse imperfections). For comparison, the corresponding HMQC pulse sequence [Fig. 2(B)] was also applied. The effect of the last SL pulse to purge antiphase magnetizations in this case is especially pronounced (see Fig. 4). It should be noted that the HMQC multiplets presented in Fig. 1(B) were acquired with this pulse sequence, so the antiphase components in the original scheme are actually larger.

We used borneol as a model compound. Its conventional 250 MHz spectrum (shown in part in Fig. 3) contains both proton multiplets with clearly expressed first-order character (H-3*exo*) and a second-order coupled spin system comprising H-5*exo*, H-5*endo*, H-6*exo* and H-6*endo*, due to the accidental near coincidence of the chemical shifts of H-5*endo* and H-6*exo*.

Comparison of the multiplets in the proton spectra and in traces through the peaks of different correlation experiments allows one to evaluate how useful they will be for extracting coupling constants in crowded spectra.

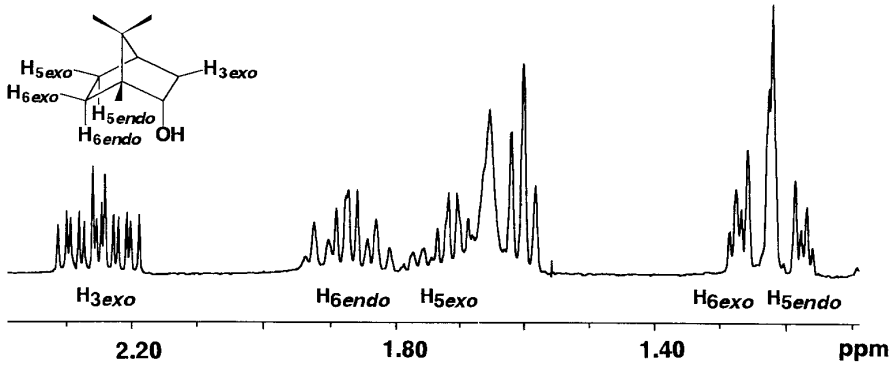

Figure 3. Part of the proton spectrum from 1.08 to 2.40 ppm of borneol in $CDCl_3$.

Figure 4 compares the multiplet of the H-3*exo* proton at 2.25 ppm in the conventional proton spectrum (**A**) with traces through the standard refocused HMQC (**B**)[5] and the HMQC with two additional SL pulses (**C**) as depicted in Fig. 2(B). Multiplets **D** and **E** originate from the refocused and coupled HSQC pulse sequence. Equal resolution in the proton dimension of all five spectra is used; the digital resolution in the indirect dimension of **B**, **C**, **D** and **E** is also the same (5.3 Hz cm^{-1}). It can be clearly seen that undistorted multiplets, closely matching the proton spectrum and suitable for coupling constant determination, could be obtained only from the traces of the HSQC experiments, both refocused (**D**) and coupled (**E**). It is also obvious that the standard refocused HMQC pulse sequence is not suitable for the extraction of coupling constants owing to phase distortion of the individual components of the multiplet. We found differences of up to 0.3 Hz in the values of the coupling constants extracted from **B** when compared with **A**, **D** and **E**. In **C** the dispersive part is removed by the last SL pulse; however, the intensities of the components of the multiplet are distorted. The multiplets in Fig. 1(**B**) suggest that the pattern of the trace will depend on the resolution of the indirect dimension and the phase distortions could vanish only if the resolution is worse than the width of the broadest proton multiplet (in natural products such as steroids, e.g. lower than 50 Hz per point), which in some cases could be insufficient.

Coupled HSQC for avoiding second-order effects

Strong coupling is always a problem when trying to analyze a high-resolution spectrum by inspection. Splittings different from the coupling constants in multiplets with distorted intensities could partly or completely obscure the coupling constants of a proton. Even for a simple compound such as borneol[13] at the highest attainable proton frequency so far, it can be calculated

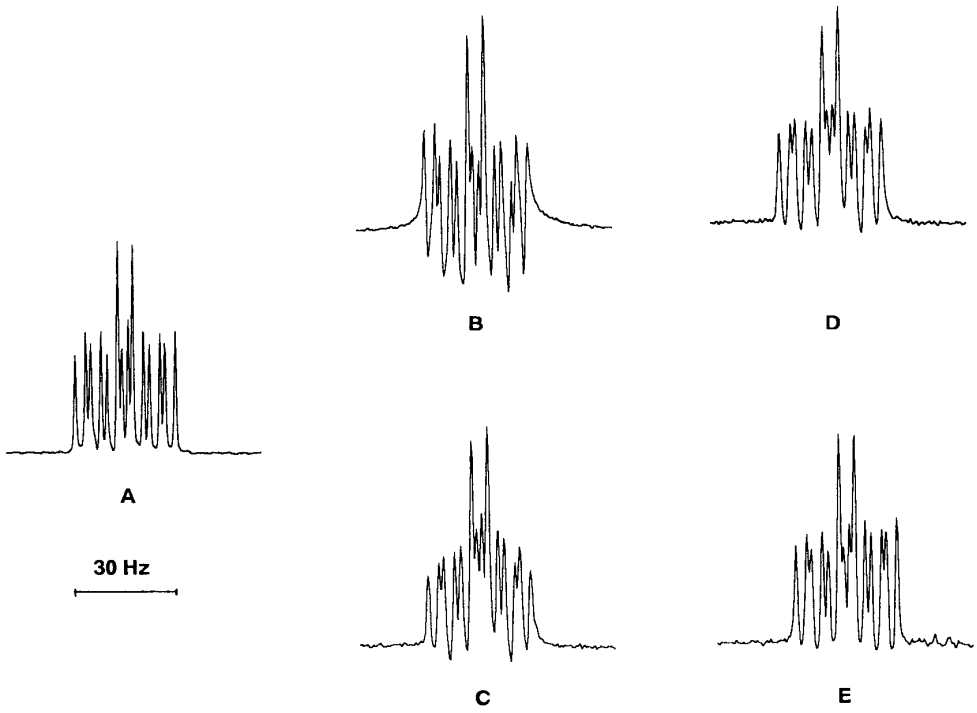

Figure 4. Multiplets for H-3*exo* from the conventional proton spectrum (**A**) and extracted at the ^{13}C chemical shift position from HMQC (**B**), HMQC [Fig. 2(B)] (**C**), refocused HSQC [Fig. 2(A)] (**D**) and coupled HSQC without refocusing (**E**).

that the spectrum will remain second order, thus preventing direct extraction of the coupling constants from the proton multiplets.

Owing to the high value of the heteronuclear coupling constants (usually >130 Hz), considerable differences in the effective chemical shifts of the individual protons in the coupled and refocused HSQC spectra are observed. The multiplets extracted from the refocused HSQC spectra closely match the proton spectrum, whereas those from the coupled spectra correspond to the so-called ^{13}C satellite proton multiplets. This difference could be used to avoid strong coupling conditions ($\Delta\nu < 3J$), which is usually sufficient to determine the corresponding coupling constants or at least to restore the first-order multiplet intensities. Figure 5 shows simulated spectra for the simple ABX spin system, where A and B are protons and X is a carbon-13, obtained using the NMRSIM program.[14] Avoiding second-order effects is possible when, e.g., proton A is coupled with a one-bond heteronuclear coupling to the carbon-13 atom whereas proton B is not. As can be seen in Fig. 5(**A**), proton A is strongly coupled to proton B due to the unfavourable $\Delta\nu/J$ ratio. In contrast, both components of ν_A in the coupled case [Fig. 5(**B**)] are virtually weakly coupled with B, displaying first-order multiplet patterns, suitable for extraction of the coupling constants from the splittings. Clearly, more complex spin systems will be found in practice, but this three-spin arrangement has the advantage of showing the relevant features and could be straightforwardly extended to more complicated cases. It should be noted, however, that simplification of the spectrum is possible *only* when the two strongly coupled protons are connected to different carbon atoms.

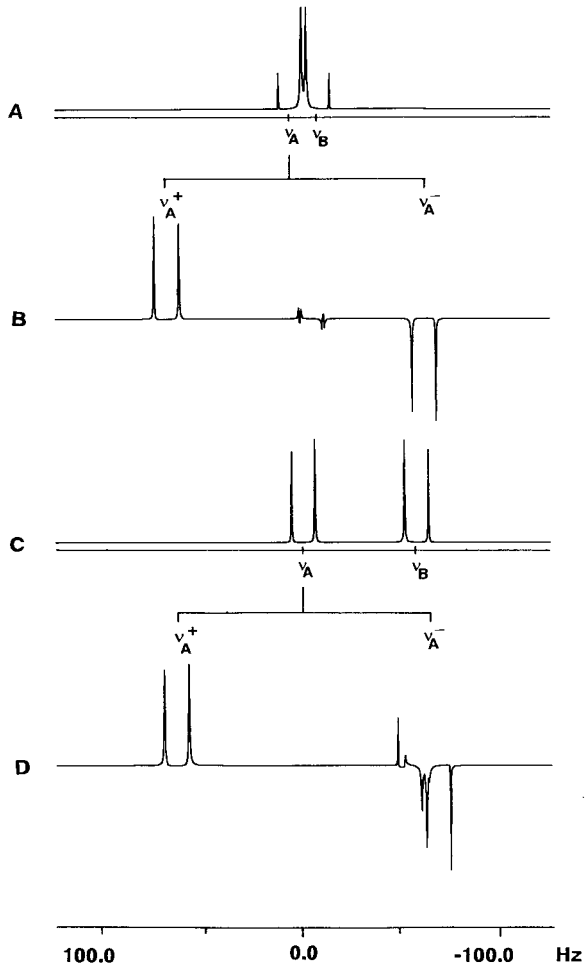

Figure 5. Simulated proton NMR spectra for AB spin system, $J_{AB} = 12$ Hz, $\nu_A - \nu_B = 8$ Hz (**A**) and $J_{AB} = 12$ Hz, $\nu_A - \nu_B = 57$ Hz (**C**), and 1D HSQC spectra (**B** and **D**) for the corresponding ABX spin system with X being carbon-13 coupled to spin A with one-bond heteronuclear coupling of 130 Hz.

Application to the real case of borneol is presented below. The strongly coupled protons of interest form an ABMX spin system (Fig. 3) with some first-order couplings to other protons outside the ABMX group. Strong coupling alters the intensities and frequencies not only of the closely spaced A and B protons (H-5*endo* and H-6*exo*), but also of the distant M and X multiplets (H-5*exo* and H-6*endo*), precluding direct determination of the coupling constants from the conventional proton spectrum.[13] These could be determined from the traces of the coupled HSQC correlation spectrum (best acquired without refocusing) because the A (H-5*endo*) and B (H-6*exo*) protons, strongly coupled in the conventional spectrum, are weakly coupled in the satellite spectra. Figure 6 shows the corresponding multiplets for the protons H-5*endo*, H-6*exo* and H-6*endo* in the proton spectrum of borneol (**A**) and from traces of the coupled HSQC 2D spectrum (**B**). The frequency and intensity symmetry in the traces is evident. Table 1 shows full proton data for borneol, obtained with the use of the HSQC traces.

The values of the coupling constants, being measured from first-order spectra, are more accurate but in agreement with those determined previously from simulation of this spin system at 400 MHz.[13]

Figure 5(**C**) and (**D**) show another case often met when using the coupled HSQC method. In the normal proton spectrum protons A and B could be weakly coupled [Fig. 5(**C**)]. However, in the coupled case, a highly distorted multiplet structure for the high field component ν_{A-} is observed owing to the small shift difference in the effective chemical shifts ($\nu_{A-} - \nu_B < J_{AB}$). In more complicated spin systems, this condition could, in unfavourable cases, be met for both components of the proton of interest. For example, in the case of borneol, no extraction of the multiplet for H-5*exo* was possible, since the low-field component interferes with H-6*exo* and the high field component with the low-field satellite of H-5*endo*. Second-order effects in coupled HSQC have also been discussed.[8]

Our experience so far using refocused and coupled HSQC for the extraction of coupling constants in analysing spectra of small organic compounds of synthetic and natural origin (mainly derivatives of terpenes and steroids) shows a definite preference for the refocused version. The simplicity and the sensitivity are clearly higher and the resolution is comparable, so we recommend the coupled sequence only in cases where the

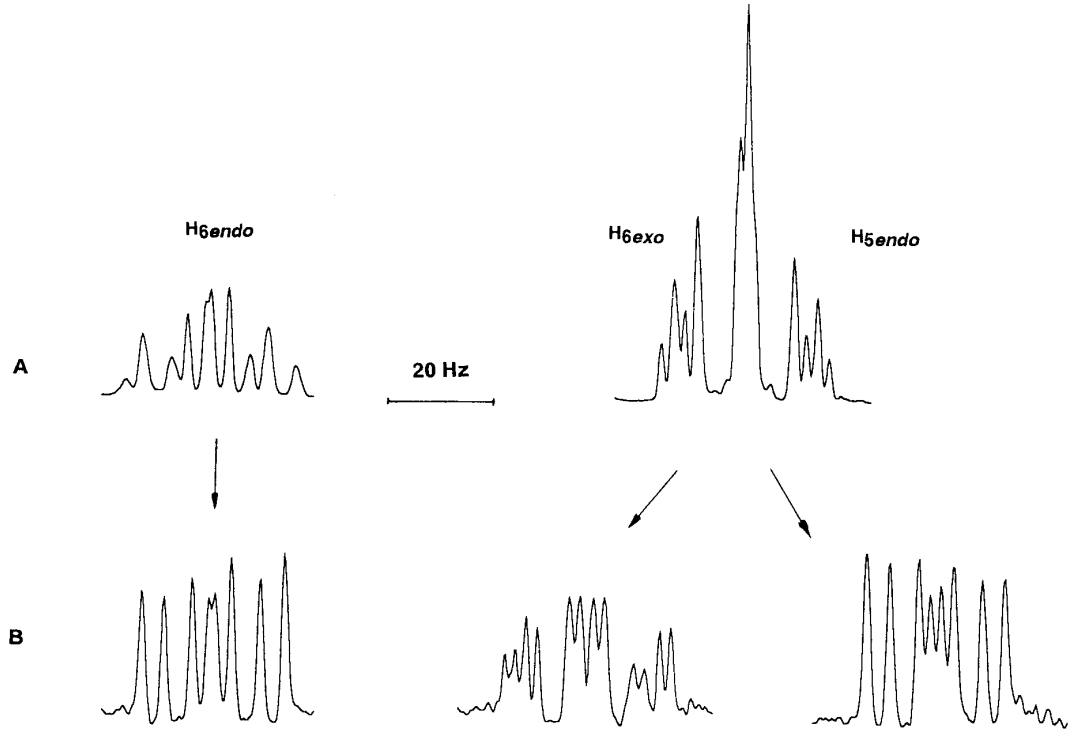

Figure 6. Multiplets for H-6*endo*, H-6*exo* and H-5*endo* of borneol **(A)** in the conventional proton spectrum and **(B)** in traces extracted from the 2D coupled HSQC spectrum at the frequency of the corresponding carbon atoms.

advantage of avoiding second-order effects is sought. It should be noted that we did not find a significant sensitivity difference in HSQC and HMQC, so even for assignment purposes with lower resolution in the detection dimension we prefer the HSQC pulse sequence because of the clearer pattern of the proton multiplets. Additionally, a considerable sensitivity increase in HSQC could be attained when linear prediction is used for processing the data in the indirect (F_1) dimension,[8] as pointed out by one of the referees.

EXPERIMENTAL

A 20 mg amount of L-asparatic acid [$NH_2CH(COOH)CH_2COOH$] was placed in 0.6 ml of D_2O and the suspension was made alkaline until the solid substance was fully dissolved. A solution of 20 mg of borneol in 0.6 ml of $CDCl_3$ was used.

All spectra were obtained on a Bruker Avance DRX-250 NMR spectrometer equipped with a 5 mm normal dual detection probe (^{1}H 90° pulse width 12 μs

Table 1. Proton chemical shifts, δ ppm ($\pm$0.001 ppm), and proton–proton coupling constants, *J*(H,H) Hz ($\pm$0.3 Hz) of borneol in $CDCl_3$ at 300 K

H atom	δ (ppm)	*J*(H,H) (Hz)[a]
H-2*exo*	4.006	10.0 (2*x*3*x*), 3.5 (2*x*3*n*), 2.0 (2*x*6*x*)
H-3*exo*	2.275	10.0 (3*x*2*x*), 13.3 (3*x*3*n*), 4.9 (3*x*4*x*), 3.3 (3*x*, 5*x*)
H-3*endo*	0.943	3.5 (3*n*2*x*), 13.3 (3*n*3*x*)
H-4*exo*	1.624	4.9 (4*x*, 3*x*), 4.4 (4*x*, 5*x*)
H-5*exo*	1.720	3.3 (5*x*3*x*), 4.4 (5*x*4*x*), 12.0 (5*x*5*n*), 12.1 (5*x*6*x*), 4.2 (5*x*6*n*)
H-5*endo*	1.242	12.0 (5*n*5*x*), 4.3 (5*n*6*x*), 9.6 (5*n*6*n*)
H-6*exo*	1.244	2.0 (6*x*2*x*), 12.1 (6*x*5*x*), 4.3 (6*x*5*n*), 12.8 (6*x*6*n*)
H-6*endo*	1.894	4.2 (6*n*5*x*), 9.6 (6*n*5*n*), 12.8 (6*n*6*x*)
H-8	0.868	—[b]
H-9	0.857	—[b]
H-10	0.847	
OH	1.67	

[a] *x* denotes the *exo*-position and *n* the *endo*-position of the proton.
[b] Broad signals, couplings smaller than the linewidth.

and ^{13}C 90° pulse width 6 μs) operating at 300 K. In the cases when spin locking was applied, for convenience the hard proton pulse (24 μs) and the spin-locking pulse (2.5–3 ms) had the same power (9 dB below the maximum output of the transmitter). A GARP decoupling power 17 dB below the maximum output for C-13 was used, corresponding to a 60 μs 90° pulse width. The HMQC sequence was used as provided in the Bruker software library. The pulse sequences depicted in Fig. 2 and the coupled HSQC without refocusing (with a BIRD delay and one SL pulse for suppression of ^{12}C magnetization) used a two-step phase cycle.

The following acquisition parameters were used for L-asparatic acid (borneol): spectral width (F_2), 350 Hz (588 Hz); time domain points, 512 (2 K); relaxation delay, 1.5 s; number of scans, 8 (24); and τ delay, 1.923 ms, corresponding to a heteronuclear coupling constant of 130 Hz. The BIRD delay was optimized for each experiment separately and was *ca.* 0.4 s (1.3 s); 90 (128) time increments were acquired in the indirect dimension (F_1) with a ^{13}C spectral window of 60 Hz (2705 Hz). All spectra were acquired in the phase-sensitive mode using TPPI.

Two-fold zero-filling in the detection dimension to 1 K (4 K), corresponding to a digital resolution of 0.34 Hz per point (0.14 Hz per point), and up to eight-fold in the indirect dimension was used (0.12/5.3 Hz per point). Weighting with a sine-squared function was applied prior to Fourier transformation, shifted by $\pi/3$ in F_2 and $\pi/2$ in F_1. No linear prediction was used.

REFERENCES

1. M. Eberstadt, G. Gemmecker, D. Mierke and H. Kessler, *Angew. Chem., Int. Ed. Engl.* **34**, 1671 (1995).
2. M. Karplus, *J. Am. Chem. Soc.* **85**, 2870 (1963).
3. G. A. Morris, *J. Magn. Reson.* **44**, 277 (1981).
4. A. Bax and S. Subramanian, *J. Magn. Reson.* **67**, 565 (1986).
5. D. Yang, X. Xu and C. Ye, *Magn. Reson. Chem.* **30**, 711 (1992).
6. G. Bodenhausen and D. Ruben, *Chem. Phys. Lett.* **69**, 185 (1980).
7. W. Wilson, R. Sumpter, J. Warren, P. Rogers, B. Ruan and G. Schroepfer, Jr, *J. Lipid Res.* **37**, 1529 (1996).
8. W. F. Reynolds, S. McLean, L.-L. Tay, M. Yu, R. G. Enriquez, D. M. Estwick and K. O. Pascoe, *Magn. Reson. Chem.* **35**, 455 (1997).
9. A. Bax, M. Ikura, L. Kay, D. Torchia and R. Tschudin, *J. Magn. Reson.* **86**, 304 (1990).
10. C. Griesinger, H. Schwalbe, J. Scheucher and M. Sattler, in *Two-Dimensional NMR Spectroscopy*, edited by W. R. Croasmun and R. M. K. Carlson, p. 477. VCH Publishers Inc, New York (1994).
11. G. Ottig and K. Wüthrich, *J. Magn. Reson.* **76**, 569 (1988).
12. J.-M. Nuzillard, G. Gasmi and J.-M. Bernassau, *J. Magn. Reson. A* **104**, 83 (1993).
13. S. Simova, H. Sengstschmid and R. Freeman, *J. Magn. Reson.* **124**, 104 (1997).
14. P. Kessler and H. Thiele, *NMR Sim, Version 940131*. Bruker, Karlsruhe (1994).