# Observation of a High Resolution Heteronuclear NMR Spectrum in an Inhomogeneous Magnetic Field

**Suzanne L. Duce, Laurance D. Hall, and Timothy J. Norwood**†

*Herchel Smith Laboratory of Medicinal Chemistry, Cambridge University School of Clinical Medicine, Forvie Site, Robinson Way, Cambridge CB2 2PZ, U.K.*

A pulse sequence is described that produces a well resolved heteronuclear spectrum of pregnenolone using an inhomogeneous magnetic field, which is easily assigned since it closely resembles the conventional $^{1}$H-decoupled $^{13}$C spectrum.

Conventional high resolution nuclear magnetic resonance (NMR) spectroscopy requires magnets which have a homogeneity of the static magnetic field across the sample of about 1 part in $10^{8}$. Producing a highly homogeneous magnetic field is a severe technical problem when building magnets which have a wide bore, operate at ultra high field strength, or are sufficiently cheap to be used for various on-line measurements in industry. A number of experiments have been designed that produce high resolution spectra even from highly inhomogeneous magnetic fields; one promising family is based upon the observation of either homonuclear[1—4] or heteronuclear[5,6] coherence transfer echoes.[7] The aim of this communication is to demonstrate that meaningful high resolution heteronuclear spectra can be obtained from a quite large organic molecule even in an inhomogeneous magnetic field. Such spectra can be easily assigned since they closely resemble the corresponding conventional $^{1}$H-decoupled $^{13}$C spectrum, once a scaling factor has been taken into account.

The pulse sequence used is represented in Figure 1(A). A $^{1}$H–$^{13}$C single quantum coherence transfer echo is observed and the evolution periods of the proton and carbon spins are balanced in a ratio of 1 : 4 to reflect the relative magnitudes of their gyromagnetic ratios. Thus any dephasing due to magnetic field inhomogeneities that occurs during the $^{1}$H evolution period will be refocused during the $^{13}$C evolution period. All heteronuclear scalar couplings are removed from the $F_1$ spectrum; the homonuclear $^{1}$H scalar couplings are normally unresolved due to limited digitisation as they are scaled down by one fifth.

The $F_1$ spectrum produced by this 2D experiment contains peaks whose linewidths are independent of magnetic field inhomogeneities. Each peak in the $F_1$ spectrum originates

---

† *Present address*: Department of Biochemistry, University of Oxford, South Parks Road, Oxford, U.K.

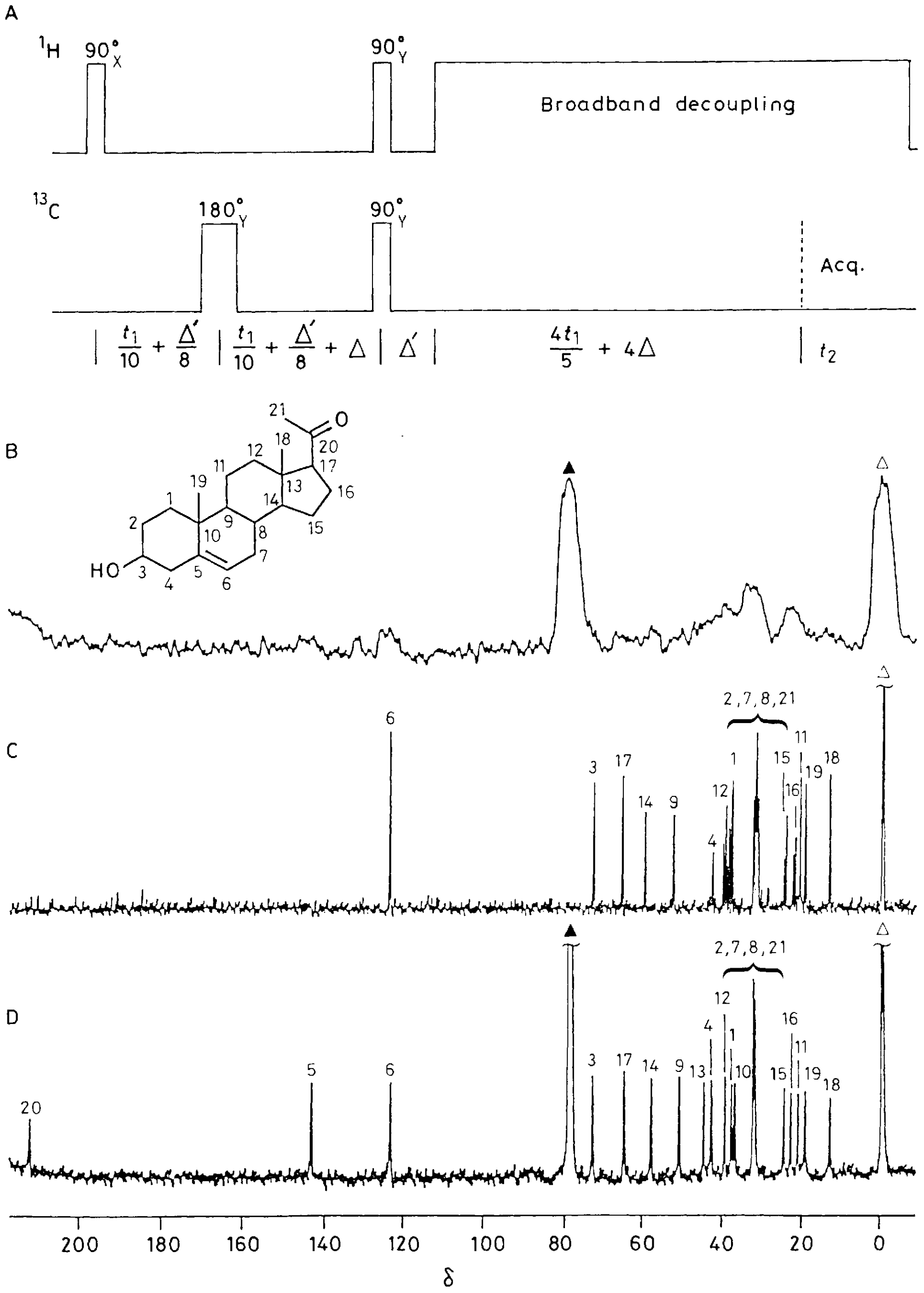

**Figure 1.** Spectra of pregnenolone (0.5 M in $CDCl_3$). (A) Pulse sequence for producing high resolution heteronuclear $^1$H–$^{13}$C spectra in an inhomogeneous magnetic field. $\Delta = 1/(2\,J)$; $\Delta' = 1/(3.3\,J)$. (B) Conventional broadband $^1$H-decoupled $^{13}$C spectrum, obtained in an inhomogeneous magnetic field. (C) Heteronuclear spectrum corresponding to an $F_1$ projection produced with the pulse sequence given in (D) in the same inhomogeneous magnetic field as (B). (D) Conventional broadband $^1$H-decoupled $^{13}$C spectrum obtained in a homogeneous magnetic field. The frequency scale is correct for spectra (B) and (D); however, for comparison purposes the spectrum (C) has been scaled up by 1.32 with respect to the normal $^{13}$C spectral dispersion, to compensate for the inherent scaling factor of 0.76 (equation 2). For (C) $\Delta = 4$ ms; $\Delta' = 2.3$ ms; number $t_1$ increments = 2048; number of scan = 1; experimental time = 1.33 h. The $t_1$-FIDs were zero filled by a factor of two and a pseudo echo weighting function was applied before Fourier transformation. All experiments were performed on a Varian VXR-300 spectrometer operating at 300 MHz for $^1$H and 75 MHz for $^{13}$C spins.

▲ Chloroform peak. △ Tetramethyl silane peak.

from a directly bonded $^1$H–$^{13}$C spin-pair and has a frequency $\nu_p$, given by equation (1), where $\nu_C$ and $\nu_H$ are the frequency of the $^{13}$C and $^1$H spins in their respective rotating frames.

$$\nu_p = (4/5)\nu_C - (1/5)\nu_H \qquad (1)$$

For most organic molecules the frequency range of the $^{13}$C spins is approximately five times greater than that of the frequencies observed for the $^1$H spins. Accordingly, if one assumes that the $^1$H and $^{13}$C spins in a spin-pair have the same relative positions in their respective spectra, $\nu_H$ can be replaced by 0.2 $\nu_C$, and equation (1) may be simplified to equation (2). Although this assumption is not rigorous it nevertheless provides a good rule of thumb since directly bonded $^1H$ and $^{13}C$ spins will invariably experience similar electronic environments. Furthermore, it allows a direct comparison between the frequency of peaks observed in the heteronuclear and $^{13}C$ spectra.

$$\nu_P = 0.76\ \nu_C \qquad (2)$$

Figure 1(B) shows the conventional $^1H$-decoupled $^{13}C$ spectrum of pregnenolone acquired in an inhomogeneous magnetic field; clearly, no useful spectral information can be deduced from it. In contrast, in the same inhomogeneous magnetic field, the new pulse sequence produces a well resolved spectrum [Figure 1(C)]. It is quite remarkable how similar this heteronuclear spectrum is to the conventional high resolution $^1H$-decoupled $^{13}C$ spectrum of pregnenolone [Figure 1(D)] after the former has been scaled up by 1/0.76 (equation 2). This allows the spectrum to be assigned. However, the heteronuclear spectrum differs from the conventional $^{13}C$ spectrum in two important respects. First, the quaternary carbons are not observed since they are not directly coupled to any protons; thus carbons 5, 10, 13, and 20 do not give a signal. Second, any carbon that is directly coupled to two inequivalent protons may give rise to a doublet if the difference between the chemical shifts of the protons is sufficiently large; this is the reason the peaks arising from carbons 1, 12, 15, and 16 are split into doublets. Due to a slight overlap of signals the peaks originating from carbons 2, 7, 8, and 21 cannot be individually assigned. Further evidence to confirm spectral assignment was obtained by measuring the normal $^1H$–$^{13}C$ correlated spectrum in a homogeneous field (results not given). Now, the ratio of the heteronuclear peak frequency to the $^{13}C$ frequency peak for the thirteen assigned pregnenolone peaks, both measured relative to the tetramethylsilane reference, had an average value of 0.761 with a standard deviation of 0.014; thus the assumption for equation (2) appears to be justified.

This work demonstrates that it is possible both to acquire and to assign a high resolution heteronuclear spectrum of a complex natural product using an *inhomogeneous* magnetic field. The major disadvantages of this experiment are that its sensitivity is low since it relies on the detection of low natural abundance $^{13}C$ spins and $T_2$* is considerably attentuated by magnetic inhomogeneities. These factors are likely to restrict its typical applications to large or concentrated samples, possibly *in vivo* or industrial fermentation processes, where it is not possible to obtain the high degree of magnetic field homogeneity across the sample necessary to support conventional $^{13}C$ NMR measurements.

We thank the S.E.R.C. for the purchase of the spectrometer used, and Dr. Herchel Smith for an endowment (to L. D. H.) and research studentships (S. L. D. and T. J. N.).

*Received, 16th August 1989; Com. 9/03517F*

## References

1 K. Nagayama, K. Wüthrich, and R. R. Ernst, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 305.
2 K. Nagayama, A. Kumar, K. Wüthrich, and R. R. Ernst, *J. Magn. Reson.*, 1980, **40**, 321.
3 L. D. Hall and T. N. Norwood, *J. Chem. Soc., Chem. Commun.*, 1986, 1508.
4 D. P. Weitekamp, J. R. Garbow, J. B. Murdoch, and A. Pines, *J. Am. Chem. Soc.*, 1981, **103**, 3578.
5 M. Gochin, D. P. Weitekamp, and A. Pines, *J. Magn. Reson.*, 1985, **63**, 431.
6 G. A. Morris and A. Gibbs, *J. Magn. Reson.*, 1988, **78**, 594.
7 A. A. Maudsley, A. Wokaun, and R. R. Ernst, *Chem. Phys. Lett.*, 1978, **55**, 9.