Application of a Simple Technique for the Sole Observation of N.M.R. Resonances of Protons which are Directly Bonded to Nitrogen

David Doddrell,[a] Dudley H. Williams,*[a] David G. Reid,[a] Keith Fox,[b] and Michael J. Waring[b]

[a] *University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, U.K.*
[b] *Department of Pharmacology, Medical School, Hills Road, Cambridge, CB2 2QD, U.K.*

A multi-pulse sequence is employed which permits the selective recording of protons directly bonded to ^{15}N; the sequence should prove useful in probing the nature of hydrogen bonds formed in biologically important complexes of limited molecular weight.

The assignment of the proton resonance spectra of complex molecules is an important task (for purposes of structure elucidation); it is often tedious and sometimes difficult. Aids to this assignment process are therefore extremely useful. An aid for the assignment of NH-protons would be particularly useful since such protons are of paramount importance in interactions between many biologically active molecules. We now report the application of a method for the sole observation of NH-proton resonances.

A number of multipulse techniques for editing a heteronuclear-coupled multiplet relative to the normal proton spectrum have been reported recently.[1] The simplest method uses the multi-pulse sequence (1).

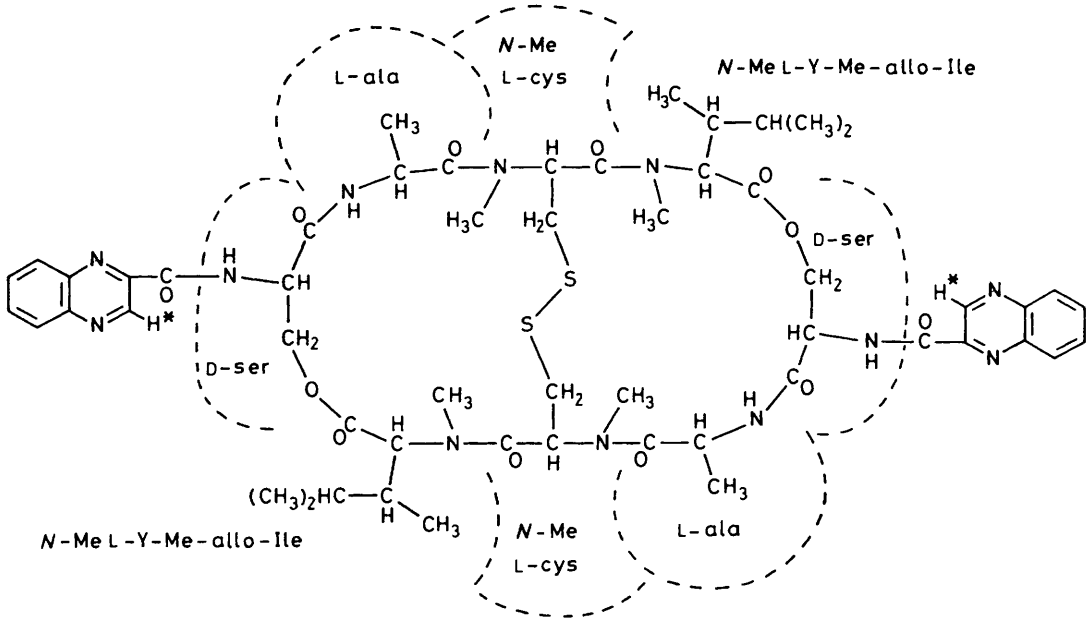
$$\frac{\pi}{2}(\mathrm{H})_x - \frac{1}{2J} - \pi(\mathrm{H})_x - \frac{1}{2J} - \text{acquire (H)} \qquad (1)$$
$$\pi(^{15}\mathrm{N};\ 0,\ 1)$$

It is instructive to consider how this technique works. The $\pi/2$ proton pulse, applied along the x-axis rotates the proton magnetisation to the $+y$ axis. After a time-lapse $1/2J$ s, (where J is the directly-bonded NH coupling constant), proton vectors associated with ^{15}N spins in their $+\frac{1}{2}$ z-eigenstate (H_α) have rotated through $\pi/2$ radians and are now directed along the $+x$ axis while those associated with ^{15}N spins in their $-\frac{1}{2}$ eigenstate (H_β) have rotated through $\pi/2$ radians to a position along the $-x$ axis. The ^{15}N π pulse inverts the z-eigenstates of the ^{15}N spins causing a reversal of the direction of precession of H_α and H_β (that is $\alpha \rightarrow \beta$ and $\beta \rightarrow \alpha$ following the ^{15}N pulse). When the ^{15}N π pulse is present, an echo is formed along the $+y$ axis; if it is not applied, the echo is formed along the $-y$ axis. If the computer adds and subtracts data from both experiments, the NH multiplets overall add while the protons not coupled to ^{15}N (which are consequently unaffected by the ^{15}N pulse) cancel. The H π pulse is employed to eliminate chemical shift drift.[2],† The spectral simplification which is possible by combining the alternate $\pi(^{15}N)$ pulse with computer add/subtract does not appear to be widely appreciated.

As an application illustrative of the power of this simple pulse sequence, we have applied it to edit the proton spectrum of the antibiotic triostin C (**1**).[4] The antibiotic was labelled with ^{15}N by feeding the producing strain (*Streptomyces triostinicus* ATCC 21043) with $Na^{15}NO_3$. The normal ^1H n.m.r. spectrum of the product was acquired in C_5D_5N solution (40 mM), Figure 1(A). The enrichment with ^{15}N was *ca.* 85% as indicated by the small residual doublets at δ 8.6 and 9.2 (marked

† *Some experimental aspects:* The setting-up procedure for this pulse sequence is critical if accurate spectral editing is to be obtained. The proton frequency and pulse angle are set in the normal manner [$t_{90}(H) = 5\ \mu s$]. In this case, as is the norm, the ^{15}N π pulse time is initially unknown, as is the ^{15}N excitation frequency. A satisfactory procedure is as follows: the response following the pulse sequence (ref. 3) $(\pi/2)(H) - 1/2J - \theta(^{15}N)$ is recorded. When $\theta = \pi/2$, a null ^1H signal is observed; when $\theta = \pi$, an inverted multiplet (relative to $\theta = 0$) is observed. The ^{15}N excitation frequency can be set to 1 kHz by noting that a good inversion is obtained with $\theta = \pi$ over a range of frequency. In programming the pulse sequence, care must be taken to ensure that the total time periods ($1/2J$) are exactly the same with and without the ^{15}N pulse. That is, the finite time for the ^{15}N π pulse must be allowed for; if this is not done the phase of the uncoupled ^1H signals following the two cycles of the pulse train are not the same and good time-domain editing does not result. In our case, $t_{90}(^{15}N) = 40\ \mu s$.

by vertical bars in Figure 1) due to Ala-^{14}NH and Ser-^{14}NH proton resonances, respectively. The more intense flanking pairs of doublets [J(^{15}N,H) 93 Hz in each case] are due to Ala-^{15}NH and Ser-^{15}NH, with one pair of lines being hidden under a peak due to incompletely deuteriated solvent. The spectrum obtained by application of the described pulse sequence is reproduced in Figure 1(B). The only intense signals remaining are due to the protons directly bonded to ^{15}N. The weak doublet at δ 9.5 in Figure 1(B) (indicated by an arrow) is due to the quinoxaline 3-H proton [H* in (1)] which retains a



(1)

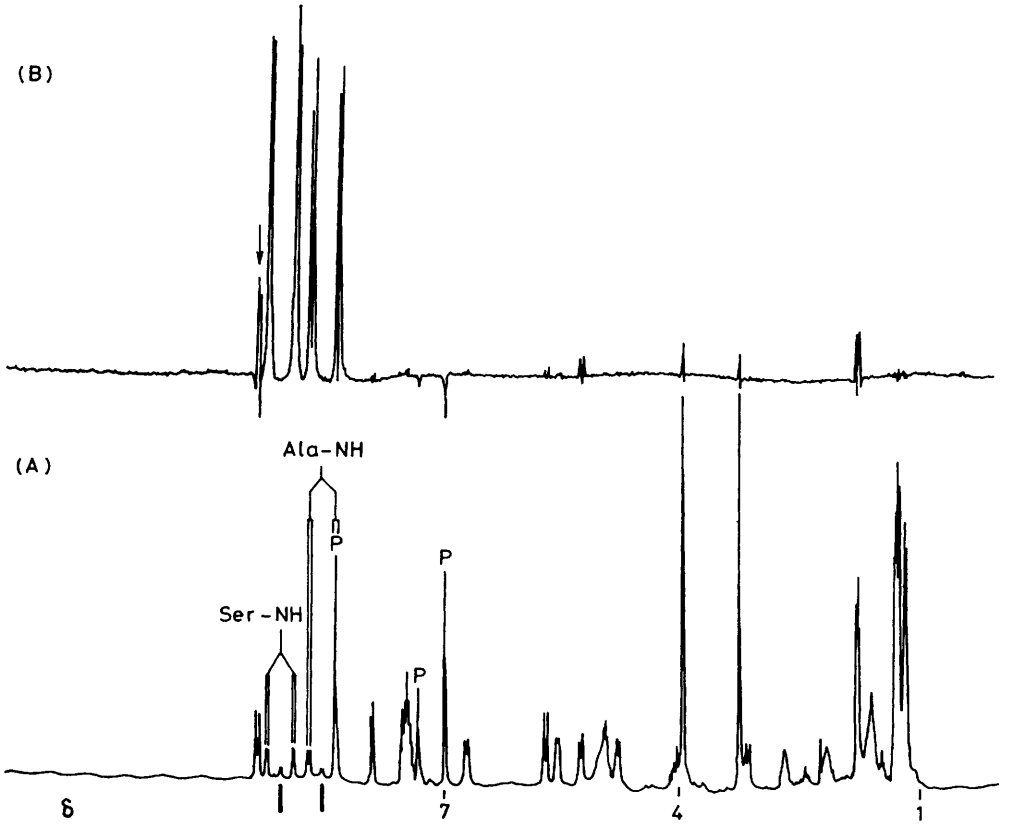


Figure 1. (A) Normal 400 MHz ^{1}H spectrum of *ca.* 85% enriched ^{15}N triostin C in C_5D_5N solution (solvent peaks indicated by 'P'). (B) Spectrum showing ^{15}N–H protons, obtained using the described pulse sequence.

low intensity in the edited spectrum since it is geminally coupled to ^{15}N [$J(^{15}N,H)$ 10 Hz].

The reported pulse sequence appears to have potential in probing the nature of hydrogen bonds which may be formed between physiologically active molecules and their receptors, in those cases where the entities involved are of sufficiently low molecular weight to give at least partially assignable proton spectra. It will be evident, and indeed we have already shown experimentally, that if a $\pi(^{13}C)$ pulse replaces the $\pi(^{15}N)$ pulse, edited spectra showing only protons coupled to ^{13}C will be obtained. In experiments utilising ^{13}C enrichment to elucidate biosynthetic pathways, such edited spectra have the advantage (relative to the observation of the ^{13}C resonance) of (i) much greater sensitivity and (ii) in many cases, greater ease of assignment of the proton spectrum.

We thank S.E.R.C. and Cancer Research Campaign for financial support. D. D. thanks the Royal Society for a Commonwealth Bursary and Churchill College for a Fellow Commonership.

Received, 14th December 1982; Com. 1436

References

1 D. A. Vidusek, M. F. Roberts, and G. Bodenhausen, *J. Am. Chem. Soc.*, 1982, **104**, 5452; M. R. Bendall, D. T. Pegg, D. M. Doddrell, and J. Field; *ibid.*, 1981, **103**, 934; R. Freeman, T. H. Mareci, and G. A. Morris, *J. Magn. Reson.*, 1981, **42**, 341.
2 D. M. Thomas, M. R. Bendall, D. T. Pegg, D. M. Doddrell, and J. Field, *J. Magn. Reson.*, 1981, **42**, 298; M. R. Bendall, D. T. Pegg, and D. M. Doddrell, *ibid.*, **45**, 8.
3 D. T. Pegg, M. R. Bendall, and D. M. Doddrell, *J. Magn. Reson.*, 1981, **44**, 233.
4 H. Otsuka and J. Shoji, *Tetrahedron*, 1965, **21**, 2931.