$^1H/^2H$ COSY: a New N.M.R. Method for locating Deuterium Nuclei in Labelling Studies

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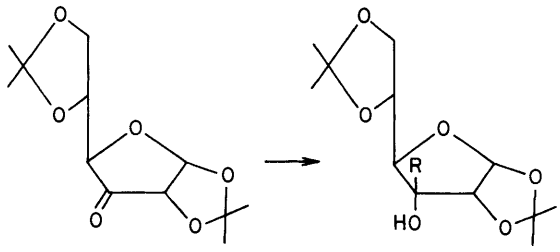
A two-dimensional n.m.r. experiment correlating proton and deuterium chemical shift resonances has been developed to reveal the precise location of deuterium labels; this technique can be used to edit a ^2H n.m.r. spectrum when overlapping resonances prevent assignments of a one-dimensional spectrum.

The ready availability and safety of deuterium often makes it the preferred isotope for hydrogen labelling in biosynthetic and mechanistic studies; tritium is far more expensive, requires special facilities when used in synthesis, and necessitates the special handling associated with radioisotopes. Numerous schemes have been developed for potentially routine detection of deuterium labels by n.m.r. spectroscopy. These include direct detection by ^2H n.m.r. spectroscopy, and indirect detection *via* ^{13}C n.m.r. spectroscopy.[1] The ^{13}C n.m.r. techniques have been devised to overcome the inherent insensitivity of deuterium and the potential difficulty of analysing highly overlapped, broad resonances from multiply-labelled compounds by ^2H n.m.r. spectroscopy. Recent innovations for ^{13}C n.m.r. analysis include ^2H–^{13}C TANDEM-SEFT,[2] ^2H–^{13}C polarization transfer (^2H INEPT),[3] and difference spectroscopy involving ^1H DEPT/^2H spin echo probe sequences.[4] However, each of the latter two techniques requires non-routine hardware (pulse-programming ability for the ^2H-lock channel in the former case and ^{19}F-lock capability in the latter case), and all ^{13}C n.m.r. techniques require the substantial additional expense—and potentially the synthetic effort—of introducing a ^{13}C label, as well.



(2) (1) **a**; R = D
b; R = H

We recently reported general parameters for the execution of proton–deuterium 2-dimensional n.m.r. spectroscopy.[5] These use the three-pulse sequence of Maudsley and Ernst[6] to allow for the variation in proton–deuterium coupling constants; this is modified with the phase programs of Bax and Morris[7] to provide quadrature detection in the first frequency (F_1) dimension as well as to suppress directly excited deuterium signals. Both geminal and vicinal coupling can be detected. The second frequency (F_2) dimension provides the

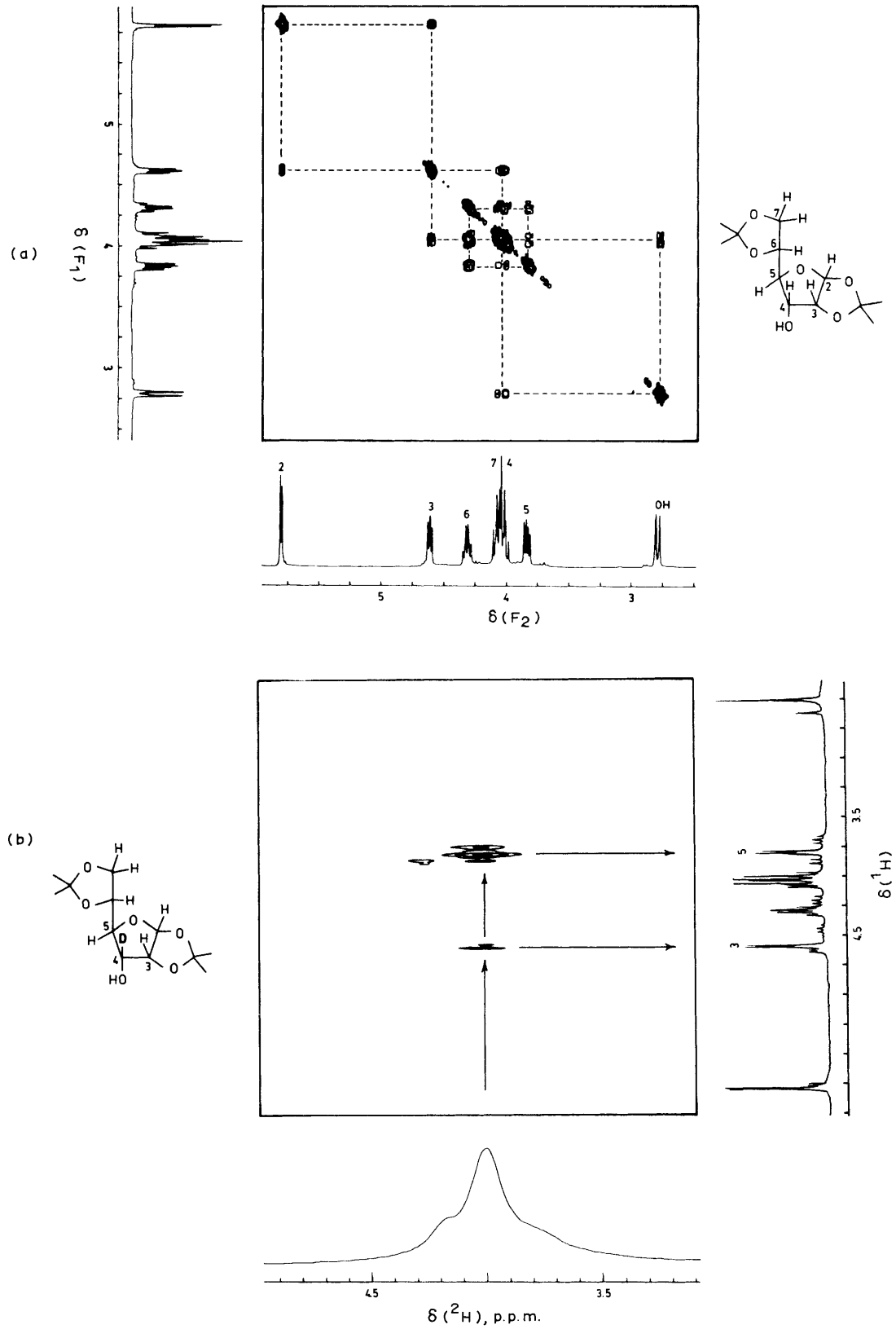


Figure 1. (a) Contour plot of a COSY experiment for allose bis-acetonide (**1b**) in $CDCl_3$. Dashed lines show the correlations of the off-diagonal peaks and the structural assignments are shown. (b) Contour plot of a $^1H/^2H$ COSY experiment for [4-^{2}H]allose bisacetonide (**1a**) in $CHCl_3$ (100 mg/2.5 ml). Spectral acquisition parameters: 200 Hz sweep width in the F_2 (^{2}H) dimension; 256 spectra (64 scans each) were accumulated with 1 ms increments across the interval of 0.080 to 0.336 ms. Resolution was 1.5 Hz/point in the F_2 dimension and 1.9 Hz/point in the F_1 dimension. Both experiments were carried out on an IBM Instrument WP270SY spectrometer operating at 270 MHz for ^{1}H and at 41.45 MHz for ^{2}H.

opportunity to edit overlapped resonances of a 1-dimensional ^2H n.m.r. spectrum and thereby reveal the precise location of each deuterium label through coherence transfer from adjacent spin-coupled protons.

The potential of this proton–deuterium COSY experiment was tested with the deuteriated allose bis-acetonide (**1a**) [prepared by $NaBD_4$ reduction of the corresponding ketone (**2**)] required by us for the synthesis of [3-^2H]-D-glucose.[8] A $^1H/^1H$ COSY spectrum of unlabelled (**1b**), shown in Figure 1(a), allowed the assignment of all the proton resonances. It should be noted that the resonances for 4-H and 7-H completely overlap, even at 270 MHz. The $^1H/^2H$ COSY spectrum of (**1a**), shown in Figure 1(b),† shows cross-peaks between ^2H (F_2 dimension) and the proton resonances for 3-H and 5-H (F_1 dimension), clearly placing the deuterium at C-4.

The $^1H/^2H$ COSY experiment can be run using standard pulse programmers and provides a ready alternative for the analysis of deuterium labelling to the more elaborate approaches previously described. For molecules of higher molecular weight the increasing relaxation rate of deuterium due to T_2 will lead to line broadening and therefore to a loss of sensitivity and resolution. Such sensitivity problems are likely to be encountered to varying degrees with the alternative approaches,[2—4] as well.

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† The sample used was contaminated by a small amount of the epimeric deuteriated glucose bis-acetonide.

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