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ASSIGNMENT OF THE STRUCTURE OF DIHYDRODAPHNINE DIACETATE BY NUCLEAR OVERHAUSER EFFECT DIFFERENCE SPECTROSCOPY

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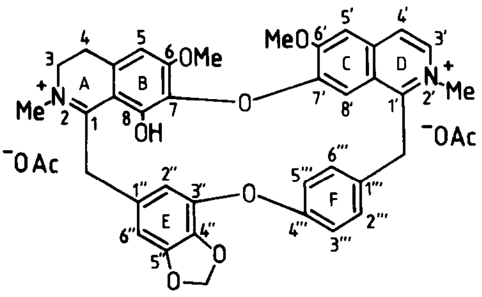
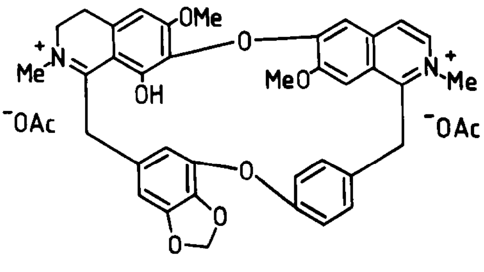
Summary: The structure of the title compound has been resolved using a recent pulsed nmr difference technique to measure small nuclear Overhauser effects between aromatic protons and O- and N-methyl groups.

Nuclear Overhauser effect (nOe) difference spectroscopy is a recent pulsed nmr technique offering a very great improvement in sensitivity over the conventional (CW) nOe experiment.[1,2] We report here its use in resolving a problem of positional isomerism in dihydrodaphnine diacetate, 1a or 1b, a derivative of the bisbenzylisoquinoline alkaloid daphnine, isolated from the Queensland plant Daphnandra repandula. Previous spectroscopic and chemical studies (to be published later) had largely established its structure, but gave no indication of the relative positions of the two ether groups on ring C.



1a



1b

The partially assigned normal spectrum of 1 at 250 MHz in CD$_3$CO$_2$D is shown in the figure (base). Peaks due to H$_{2''}$ and H$_{6''}$ were recognised by their mutual coupling (J$_m$ 0.7 Hz). At this stage, peaks **a**, **b** and **c** remain to be assigned to H$_{5'}$, H$_{8'}$ and H$_5$ and peaks **d** and **e** to the methoxy groups.

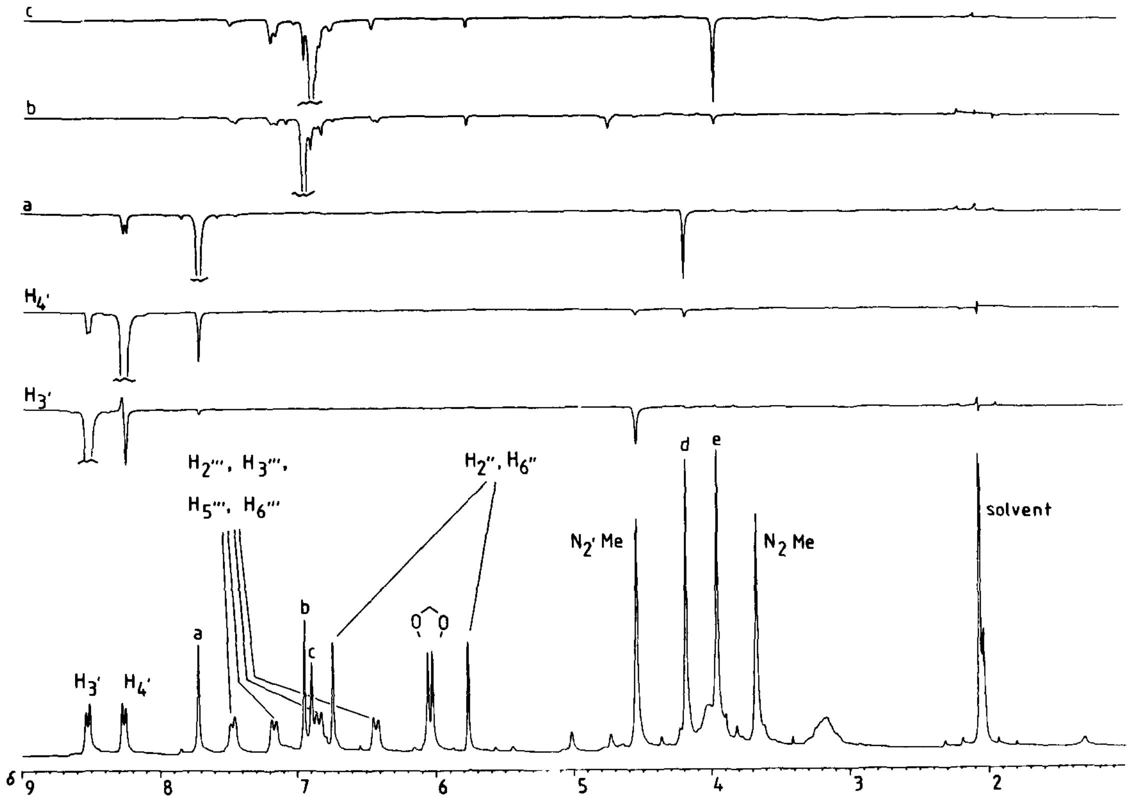


Figure. Normal and nOe difference spectra of 1 at 250MHz in CD$_3$CO$_2$D. Each nOe difference spectrum (upper five traces) is identified using the normal spectral peak which was pre-irradiated in the corresponding experiment. Vertical amplitude is only approximately constant within the difference spectra, being roughly four times that of the normal spectrum.

The results of the nOe experiments are shown in the figure (top). Each difference spectrum is obtained by subtracting the normal spectrum from a spectrum in which the peak specified has been partially pre-saturated. The greatest change resulting from this pre-saturation is a substantial drop in the intensity of the pre-irradiated signal; this is seen in the difference spectrum as a large negative peak (shown truncated in the figure) at the pre-irradiation frequency. Other peaks are essentially all due to genuine nOes induced by the pre-irradiation.[3] As in the conventional experiment, these only occur if the pre-saturated and affected protons are close together in space. In this instance the nOes are negative, presumably due to slow tumbling of the solute ions in acetic acid. In contrast, a less viscous CD_3CO_2D/D_2O solution of dihydrodaphnine dichloride gave negligible nOes, while a related neutral molecule, O-acetylhexahydrodaphnine, showed positive nOes when studied in CD_3Cl solution. The dependence of nOe on tumbling rate has been discussed previously.[4,5]

There are clear nOes between $H_{3'}$ and the $N_{2'}$ methyl group, $H_{3'}$ and $H_{4'}$, $H_{4'}$ and peak **a**, and peaks **a** and **d**. These establish a chain of connectivities around the periphery of rings C and D which leads to assignment of peak **a** to $H_{5'}$ and suggests that $H_{5'}$ is adjacent to a methoxy group. This conclusion is confirmed by the presence of a similarly strong nOe between peak **c** (assigned to H_5) and peak **e** (assigned to the C_6 methoxy group), and by the absence of strong effects involving peak **b** (assigned to $H_{8'}$). The correct structure of dihydrodaphnine diacetate is therefore **1a**.

The magnitude of these nOes is approximately 5% but it must be noted that such results are not *quantitatively* comparable to conventional nOe data.[6] A detailed discussion of nOe difference spectroscopy is given by Hall and Sanders.[1] We used the method of Chapman *et al.*[7] with only minor modifications,[8] and employed a Bruker WM250 spectrometer operating at 250MHz under ASPECT 2000 control. A spectral width of 3500 Hz was used with 8K data points giving an acquisition time of 1.16s. This offered the best compromise between resolution and sensitivity to the nOe. The entire sequence of experiments was executed automatically during a single week-end run, 2400 acquisitions being made for each run. Nulling of unenhanced peaks in the difference spectrum was more than 99.7% efficient and the signal to noise ratio was very high allowing the detection of many very small nOes. Use of a very low pre-irradiation power gave high frequency selectivity; this is shown by the remarkably low degree of crossover between experiments **d** and **e** in which the pre-irradiation frequencies were only 14.6 Hz apart. Use of CD_3CO_2D as solvent simplified the spectra by removing all exchangeable peaks, provided a convenient high field reference, and, by broadening the lines, improved the efficiency with which unenhanced signals were nulled. We also note that our solutions were not degassed, showing that this is not essential in obtaining qualitative results such as these.[9]

References and Notes

1. L. D. Hall and J. K. M Sanders, *J. Amer. Chem. Soc.*, 1980, **102**, 5703 and references therein.
2. G. Kotovytch and G. H. M. Aarts, *Can. J. Chem.*, 1980, **58**, 2649 and references therein.
3. Some minor effects were due to the presence of scalar coupling between the observed and the pre-irradiated protons.
4. J. H.Noggle and R. E. Shirmer, "The Nuclear Overhauser Effect", Academic Press, New York, 1971.
5. D. H. Williams and J. R. Kalman, *J. Amer. Chem. Soc*, 1977, **99**, 2768 and references therein.
6. R. U. Lemieux, K. Bock, L. T. J. Delbaere, S. Koto and V. S. Rao, *Can. J. Chem.*, 1980, **58**, 631 (on p. 649).
7. G. E. Chapman, D. D. Abercrombie, P. D Carey and E. M. Bradbury, *J. Magn. Reson.*, 1978, **31**, 459
8. Subsequently we have found that the method of Hall and Sanders[1] is somewhat more convenient.
9. The complete structure has recently been determined by x-ray crystallography (to be published later).

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