Saturation Transfer in N.M.R. *via* Low-amplitude Spinning Sidebands

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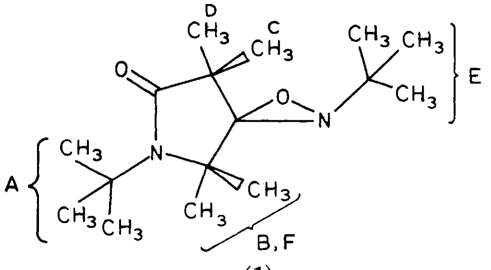
Major artefacts are shown to arise in n.m.r. saturation transfer and nuclear Overhauser difference spectroscopy experiments through the agency of low-amplitude spinning sidebands.

The techniques of saturation transfer and nuclear Overhauser enhancement (n.O.e.) difference spectroscopy[1,2] are widely used. We here identify one source of the artefacts which add to the problems commonly experienced with this experiment.

Compound (**1**) gives the expected six-line spectrum (Figure 1a) in which the amplitudes of the spinning sidebands are all negligible (<0.5%). A n.O.e. difference experiment (Figure 1b) irradiating peak A, with the deoxygenated sample not spinning, gives the expected modest (6%) positive n.O.e. at peaks B and F. No other resonance position shows a significant integral. The assignments were confirmed *via* further n.O.e. difference experiments.

However, when the sample was spun normally (19—20 Hz on the meter, Figure 1c) a very large (36%) apparent negative n.O.e. was detected at resonance C, which lies at 38.6 Hz, or twice the spinning frequency, from A. The reverse effect from C to A was also observed separately. Furthermore, when the spin rate was changed to 18—19 Hz (Figure 1d) the enhancement at C largely disappeared, but a similar 5% negative enhancement was seen at E, 110.8 Hz or six times the spinning frequency from A. In all these experiments the irradiation power was sufficiently low to confine direct irradiation to peak A.



(**1**)

That the enhancement is negative for a small molecule, and depends on the spinning rate, implies a mechanism involving saturation transfer *via* hidden spinning sidebands. This mechanism has a ready classical explanation.[3] Stimulating a resonance at frequency ν in the presence of a magnetic field sinusoidally modulated at frequency ν_m and amplitude γH_m produces components of magnetization rotating at $\nu \pm p\nu_m$, of amplitude $J_p^2(\beta)$, where p is integral, $J_p(\beta)$ the appropriate

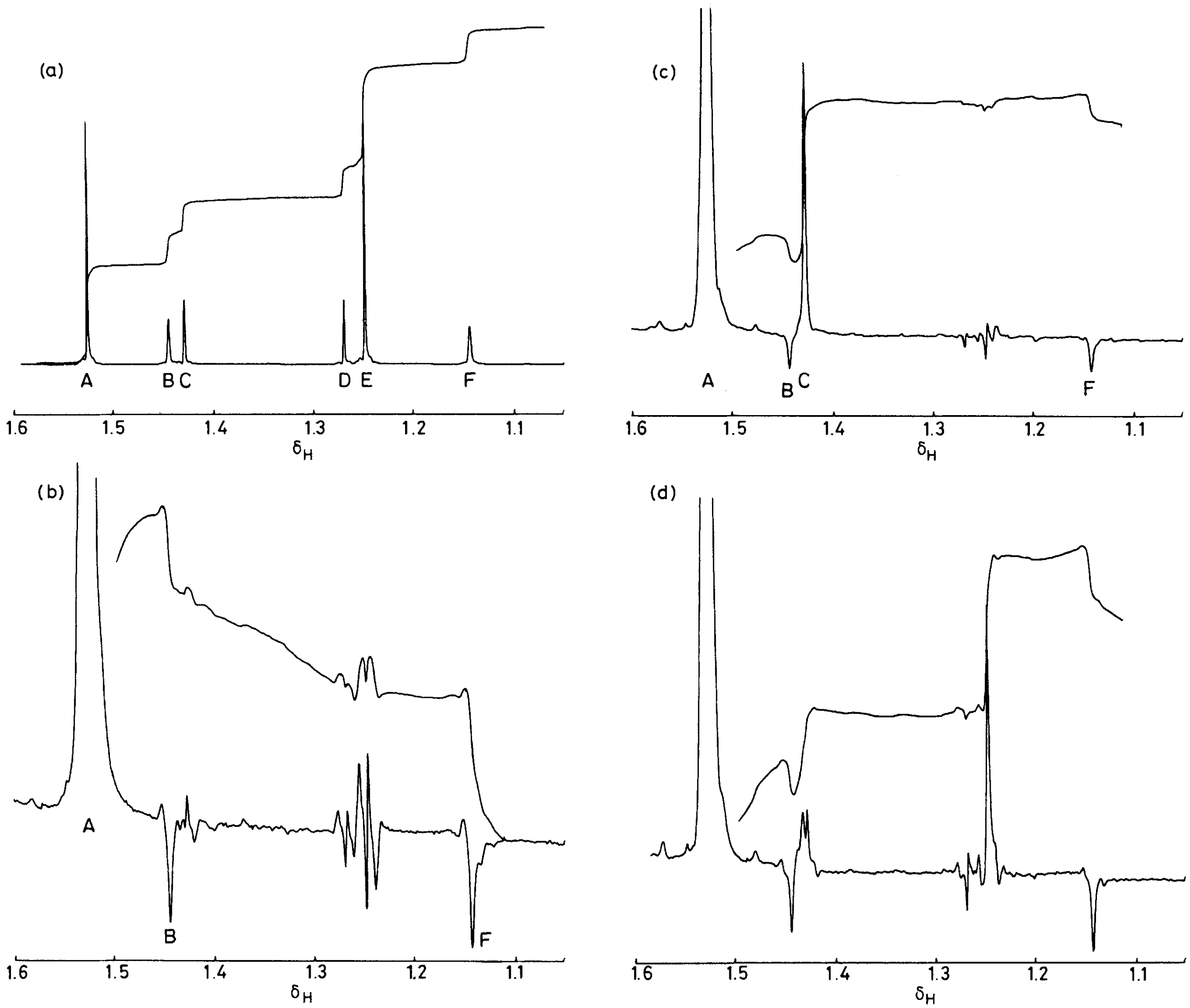


Figure 1. (a) Normal 400.13 MHz ^1H n.m.r. spectrum of (**1**), with assignments. (b)—(d) N.O.e. difference spectra of (**1**) with irradiation alternately at A and a well-separated dummy frequency: (b) non-spinning, (c) spinning at 19—20 Hz, (d) spinning at 18—19 Hz.

Bessel function, and $\beta = \gamma H_m/2\pi\nu_m$. The original peak now has the relative intensity $J_0^2(\beta)$. Sidebands with $p>1$ will be further enhanced by the presence of non-linear field gradients. Thus a small fraction of the total magnetization of the irradiated nuclei precesses at a frequency which can become in resonance with other spatially unrelated nuclei. In the absence of strong relaxation, this resonant perturbation will induce precession of their otherwise static bulk magnetization, and hence transfer saturation fairly efficiently within the 2—3 seconds during which A is normally irradiated.

An alternative explanation might be that on irradiating A one simultaneously irradiates the spinning sideband of C. Saturation could then be transferred from this to the centreband of C. We have tested this alternative by irradiating at the position of the other, non-overlapping sideband, and find that the resulting transfer of saturation is less than 20% of that obtained above. Thus the alternative is of minor importance.

We have observed the same effects in other compounds. As expected, they are reduced when intramolecular spin relaxation is relatively efficient. Our observations explain some of the problems of reproducibility encountered in such experiments, and in their 2D analogues.[4] Evidently a negligible sideband can produce a substantial transfer of magnetization. As such artefacts may be completely eliminated simply by not spinning the sample, we propose that this procedure is desirable in most n.O.e. difference experiments, particuarly at lower fields and involving longer relaxation times. It should be almost mandatory in saturation transfer experiments where the shift separations are not large, because in this case the spurious effect will not be distinguishable by its sign.

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