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Assignment of Carbon-13 N.M.R. Spectra by Selective Saturation

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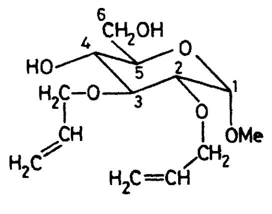
Summary Selective radio frequency saturation allied to gated proton decoupling and Fourier transform difference spectroscopy is used to give separate fully coupled ^{13}C n.m.r. spectra from individual carbon sites, and so aid the assignment of the spectrum of methyl 2,3-di-*O*-allyl-α-D-glucopyranoside.

THE assignment of the Fourier transform ^{13}C n.m.r. spectra of complex molecules is often facilitated by such instrumental techniques as off-resonance[1] and selective proton irradiation[2] and gated decoupling,[3] and more recently '*J*-scaling'[4] and selective excitation with gated decoupling[5] have been applied to a number of model compounds. The last of these methods is especially valuable in the study of molecules with overlapping resonances since a major problem in interpreting proton-coupled ^{13}C spectra arises from overlap of the many resonance lines usually present. In this communication we report an alternative but related approach which yields separate fully coupled ^{13}C spectra from individual carbon sites, and which we have found useful in the study of several types of complex organic molecule.

The essence of the method is to prepare the spin system by proton noise decoupling and the simultaneous application of a weak homonuclear continuous wave ^{13}C radio frequency (r.f.) field (typically $\gamma B_2/2\pi$ *ca.* 0·5 Hz, supplied in our case by a Schlumberger FS-30 frequency synthesizer) such that a single proton-decoupled ^{13}C resonance is selectively saturated. Immediately prior to the normal 90° detecting pulse and acquisition of the free induction decay the two irradiating r.f. fields are gated off, so that the resulting response is that of the undecoupled ^{13}C spectrum except that the (undecoupled) signals from the selected saturated carbon site are absent. Subtraction of this spectrum from a normal [gated to preserve the nuclear Overhauser effect (n.O.e)] undecoupled ^{13}C spectrum then yields a difference spectrum which contains only the resonances of the chosen carbon nucleus, showing appropriate couplings to protons. The sequence is then repeated to generate a satisfactory signal-to-noise ratio (S/N). A selectivity of ±1 Hz is readily attainable in the preliminary saturation part of the experiment, and thus undecoupled ^{13}C spectra from molecules of considerable complexity can be obtained without any problems of overlapping. The short and long range proton couplings to the individual carbon atoms can then be used to aid assignments in the usual way.



(I)

The Figure shows the application of this 'Carbon Assignment by Selective Saturation' (CASS) experiment to the ^{13}C n.m.r. spectrum of the monosaccharide derivative, methyl 2,3-di-*O*-allyl-α-D-glucopyranoside, (I),[6] and it is evident that the various proton splittings can be easily recognised, even in the region of 75 p.p.m. where there is normally much overlap. For example, there is no difficulty in identifying the doublets given by C-4 and C-5, and the triplets given by the allyl CH_2O carbons, and it is further possible to observe that the components of the doublet from C-2 are broader than those from C-3 as a result of the additional coupling of the former to the protons of the OMe group.

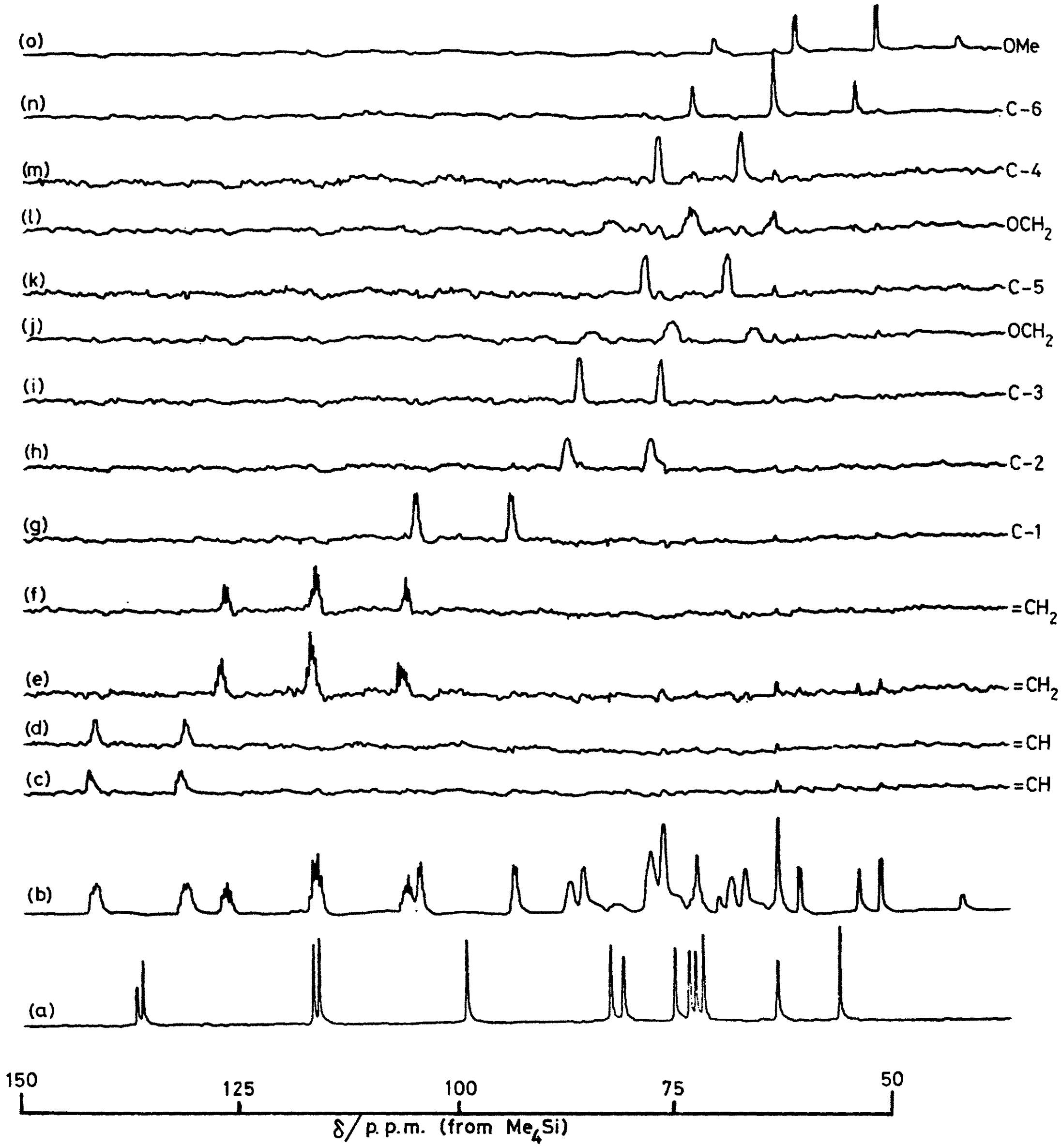


FIGURE. CASS ^{13}C n.m.r. spectra (1000 transients, 1·5 s interval) of (I) recorded at 15·03 MHz on a JEOL FX-60 spectrometer. (a) Normal proton-decoupled spectrum. (b) Undecoupled spectrum with proton irradiation gated to preserve the n.O.e. (c) As for (b) but with selective pre-saturation of the decoupled ^{13}C resonance at highest frequency and subtraction from (b). (d)—(o) As for (c) but successively with pre-saturation of the other twelve decoupled ^{13}C resonances in order; in each case the spectrum contains the response from the chosen carbon site only. From left to right the resonances of the decoupled spectrum are assigned as follows: =CH (2), =CH$_2$ (2), C-1, C-2, C-3, CH$_2$O (1), C-5, CH$_2$O (1), C-4, C-6, and OMe.

The Figure actually shows thirteen experiments, but it would normally be necessary selectively to saturate only those carbons for which there was an assignment problem, so the penalty of reduced sensitivity associated with difference spectroscopy should not be too serious, and could be avoided when many sites were of interest by using more scans to record a single master undecoupled spectrum, which would be used in the subtraction routine.

It is also possible to perform this experiment with continuous saturation by the ^{13}C r.f. field and the time scale is then that of a normal gated undecoupled ^{13}C experiment since it is not necessary to wait between each cycle for the spin system to attain complete equilibrium. In these circumstances, with a selectivity of 1 Hz, the total time required for a given value of S/N is relatively short, and this is especially important when many sites are of interest. In fact, the ease with which high selectivity (as good as 0·2—0·1 Hz if some loss of S/N can be tolerated) can be achieved without risk of multiple excitation is a major advantage of the technique, although the choice between this and direct selective excitation may often depend upon the details of the available instrumentation. Another important feature is that this technique is a quasi steady-state one, and this will affect the behaviour of the system under conditions of further multiple irradiation, *e.g.*, by a selective proton r.f. field. The CASS technique can also be used to study parts of a spectrum at narrow band-width and enhanced resolution without problems of fold-over, and is of course suitable for work at high-field when it should prove especially valuable in the study of biologically important molecules.

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