*NATURAL-ABUNDANCE C^{13} NUCLEAR MAGNETIC RESONANCE SPECTRA OF MEDIUM-MOLECULAR-WEIGHT ORGANIC COMPOUNDS**

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The difficulties in detection of C^{13} magnetic resonance signals in organic compounds which arise from the low natural abundance and the small magnetic moment of C^{13} are well known.[1] For complex substances of biochemical interest, further reduction in signal-to-noise ratio occurs because of extensive carbon-proton spin-spin interactions. Considerable relief from these difficulties is possible in principle with complete proton decoupling that enhances the signals by removing the proton splittings and contributing a favorable Overhauser effect.[2]

The problem with proton decoupling as ordinarily practiced is the necessity for extensive variations in the decoupling frequency to locate the proper values for sharp decoupling of all of the carbon resonances. If the C^{13} signals are already so weak as to require enhancement by time averaging with repetitive scans,[3] excessively long scanning times may be necessary to obtain satisfactory spectra. Some idea of what is involved, in a rather favorable case, may be seen from Figure 1, which shows a series of C^{13} spectra of a mixture of α- and β-pinenes run with four different proton decoupling frequencies with the use of the DFS spectrometer previously described.[3, 4] Although the power input at the decoupling frequencies chosen was close to the maximum that could be supplied to the oscillator coil, no one frequency gave sharp proton decouplings for all the carbons. Furthermore, if the intensities of the resonances are to be maximized, quite fine adjustments of the decoupling frequencies are required; this may well lead to prohibitive scanning times.

Much more general proton decoupling can be achieved with noise modulation of an average proton frequency with a random-noise generator,[5] and this technique has now been found to be highly useful for C^{13} spectra. The hookup is shown in Figure 2, and sample noise-decoupled spectra for the same pinene sample used to obtain Figure 1 are given in Figure 3. It will be seen that spectral quality is relatively independent of the center-band frequency.

Other spectra which illustrate the value of noise decoupling for C^{13} spectra of medium-molecular-weight organic compounds have been obtained. One example is cholesterol $C_{27}H_{46}O$ which has such a variety of carbons and so many proton couplings as to provide little hope for detailed structural information by ordinary C^{13} spectroscopy. Even with 1000 scans, the undecoupled spectrum shows only few easily discernible peaks (Fig. 4). With noise decoupling, a dramatic change is evident (Fig. 5); with narrower sweeps, fully 26 of the 27 carbon atoms of cholesterol have large enough chemical-shift differences to be easily distinguishable. The assignment of these resonances to specific carbons will not be an easy task but is straightforward in principle at least. Two further

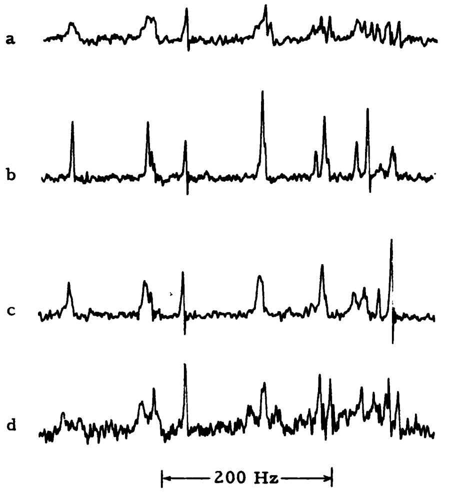


FIG. 1.—Partial single-frequency decoupled C^{13} spectra of a mixture of pinenes with center frequencies at 60,004,000 Hz plus: (*a*) 700 Hz; (*b*) 560 Hz; (*c*) 520 Hz; (*d*) 340 Hz. All the spectra were time-averaged over 12 scans and were taken with a sweep width of 500 Hz and a sweep time of 50 sec.

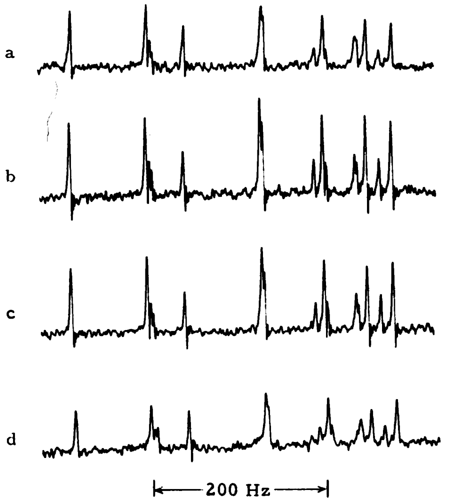


FIG. 3.—Noise-modulated C^{13} spectra of a mixture of pinenes with center frequency at 60,004,000 Hz plus: (*a*) 700 Hz; (*b*) 560 Hz; (*c*) 520 Hz; (*d*) 340 Hz. The noise bandwidth was set at 300 Hz. The assignment of the various carbon resonances has not yet been made, but the large peaks arise from α-pinene and the smaller ones from β-pinene.

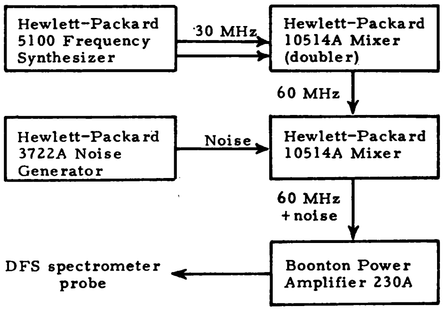


FIG. 2.—Block diagram of apparatus for proton decoupling with pseudo-random noise generator.

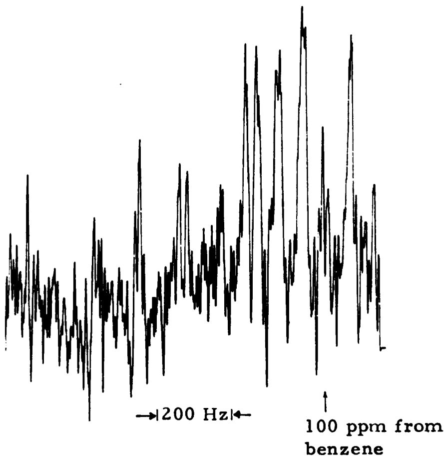
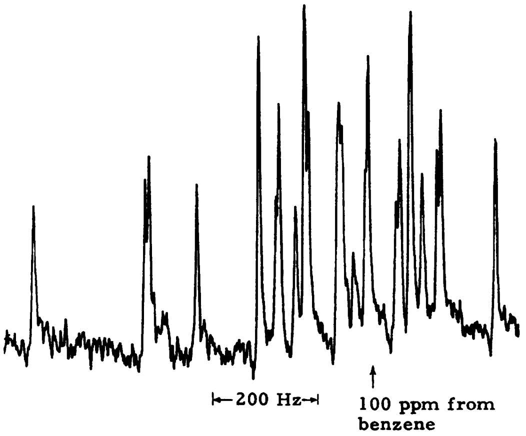


FIG. 4.—Cholesterol (1.1 *M* in CS_2) without decoupling; 1000 scans. The 2-vinyl carbons are 1000 Hz downfield.



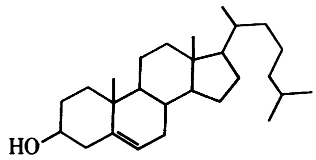


FIG. 5.—Cholesterol (1.1 *M* in CS_2); 1000 scans. The 2-vinyl carbons are 1000 Hz downfield.

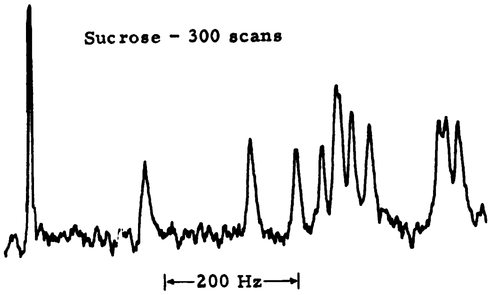


FIG. 6.

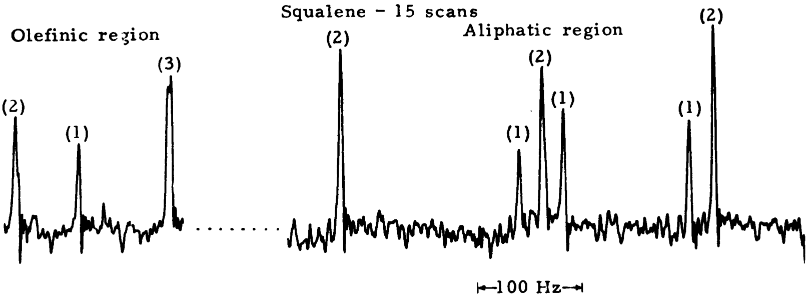


FIG. 7.—The parenthetical numbers are the numbers of carbons contributing to each resonance. On narrower sweeps, the multicarbon resonances are usually resolved to the baseline.

examples are provided by sucrose (Fig. 6) and squalene (Fig. 7), which illustrate the relatively short scanning times needed to obtain satisfactory spectra with complex molecules that can be obtained in relatively high concentrations. Detailed discussion of these and related spectra will be given elsewhere.

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[1] Emsley, J. W., J. Feeney, and L. H. Sutcliffe, ***High Resolution Nuclear Magnetic Resonance Spectroscopy*** (New York: Pergamon Press, 1966), vol. 2, pp. 988–991.

[2] Paul, E. G., and D. M. Grant, *J. Am. Chem. Soc.*, **86**, 2977 (1964).

[3] Weigert, F. J., and J. D. Roberts, *J. Am. Chem. Soc.*, **89**, 2967 (1967).

[4] For proton decoupling, it is necessary to use a lock signal from an external reference which may give considerable drift as compared to the normal mode of operation of the DFS spectrometer with internal reference. This difficulty can be avoided by internal referencing with C^{13} (Johnson, L. F., private communication).

[5] Ernst, R. R., *J. Chem. Phys.*, **45**, 3845 (1966); applications to P^{31} spectra have been made by L. F. Johnson and M. E. Tate in a paper submitted to *Can. J. Chem.* and independently to C^{13} spectra by L. F. Johnson (private communication), to whom we are indebted for helpful suggestions.