

159. *Proton-Proton Spin Decoupling by Single Sideband Modulation.*

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Application of the technique of proton spin decoupling to the nuclear magnetic resonance spectroscopy of complex organic compounds is discussed. Examples are given in which the technique assists complete analysis of the spectra or permits exact location of protons whose resonance is obscured by overlying absorption. Apparatus is described which enables the required two-line radiofrequency spectrum to be generated relatively simply.

WHEN a sample containing two groups of nuclei that have different chemical shifts and are spin-coupled is irradiated with a strong radiofrequency field at the resonance frequency (f_1) of one group whilst the resonance of the other with a weak radiofrequency field of frequency f_0 is observed, the resonance pattern of the latter is simplified, in favourable cases to a single line. The effect was predicted by Bloch¹ and discussed by Bloom and Shoolery² who gave an example where the two nuclei were different (P and F). Royston³ also carried out double irradiation of a C¹³-proton system to suppress the proton resonance splitting. It was observed first for the case of two hydrogen nuclei by Anderson,⁴ and later demonstrated with simplified apparatus by Itoh and Sato,⁵ Kaiser,⁶ and Freeman.⁷ The procedure, now called spin decoupling, was early recognised as valuable for simplification of complex resonance spectra. However, in all of the cases cited above, only spectra for simple compounds containing relatively few lines were shown.

For proton-proton spin decoupling to be a generally useful technique to the organic chemist, the frequency of the strong decoupling radiofrequency field must be uniquely determined and should not interfere with the recording of the resonance spectrum; and, further, no additional resonance lines due to unwanted sideband responses should appear; ambiguity from either cause could make the interpretation of a complex spectrum harder rather than easier.

These requirements considerably reduce the utility of those modulation techniques which produce symmetrical radiation frequency patterns, whether field or radiofrequency modulation is used. The ideal radiation pattern, one decoupling frequency and one search frequency, was generated by Anderson,⁴ using two separate oscillators whose frequency difference was controlled, but the requirements of exceptional stability in two oscillators and the complexity of the system employed to lock them together with a known frequency difference seemed prohibitive when an apparatus for routine application was under consideration.

This radiation pattern is arrived at in the technique described here by single sideband,

¹ Bloch, *Phys. Rev.*, 1956, **102**, 104.

² Bloom and Shoolery, *Phys. Rev.*, 1955, **97**, 1261.

³ Royston, *Phys. Rev.*, 1954, **98**, 543.

⁴ Anderson, *Phys. Rev.*, 1956, **102**, 151.

⁵ Itoh and Sato, *J. Phys. Soc. Japan*, 1959, **14**, 851.

⁶ Kaiser, *Rev. Sci. Instr.*, 1960, **31**, 963.

⁷ Freeman, *Mol. Phys.*, 1960, **3**, 435.

suppressed carrier modulation of a stable radiofrequency transmitter; the apparatus proved relatively simple and the method readily applicable to complex spectra. Moreover, it is easy to add the modulator to commercial nuclear magnetic resonance apparatus without modification if the latter includes a radiofrequency phase-sensitive detector.

Theory.—Magnetic field scanning with fixed modulating frequency corresponds to the second of the three experimental approaches considered theoretically by Bloom and Shoolery.² The resonance frequencies of the first multiplet traverse the frequency f_1 of the sideband while those of the second traverse the frequency of the carrier f_0 , and resonance at f_0 is recorded. When, as here, the sideband is strong enough to cause decoupling, spin transitions take place rapidly in relation to J , the coupling constant; the discrete multiplet pattern of the first absorption is absent and, instead, absorption takes place over the whole region that it originally covered. Thus decoupling is most effective when f_1 corresponds to the centre of gravity of the multiplet. Bloom and Shoolery showed that if $H_{1(a)}$ was sufficiently large ($>2\pi J/\gamma$) a simple AX system underwent decoupling to a single line provided that f_1 was at the correct value for double resonance; an error in either direction gave two lines closer together than the undecoupled doublet. However, when

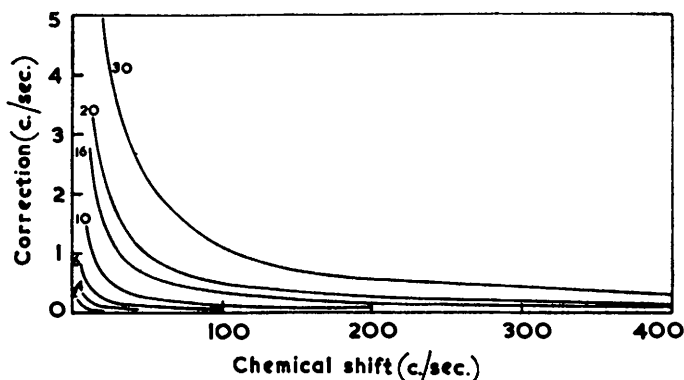


FIG. 1. Curves of (modulation frequency - chemical shift) against chemical shift for various values of J (2—30 c./sec.; see plots) when decoupling is just complete.

$H_{1(a)}$ had the critical value of $\pi J/\gamma$, optimum adjustment of f_1 gave three lines, a central line having about twice the intensity of the weaker vestiges of the original doublet which has expanded. Therefore, in such simple systems and probably also in more complex ones, the onset of decoupling should be evident for the correct frequency settings when the critical value ($\pi J/\gamma$) of $H_{1(a)}$ is used and should rapidly disappear where f_1 is different from the correct double resonance value (as in Figs. 3, 7, and 8). When $H_{1(a)}$ is increased much above $2\pi J/\gamma$, the range of f_1 over which decoupling is effective also increases. The modulating frequency $f_m (= f_0 - f_1)$ is, however, not exactly equal to the chemical shift between the nuclei being decoupled, and the difference increases rapidly as $H_{1(a)}$ increases. According to the condition for double resonance,

$$(2\pi f_m)^2 = (2\pi\delta)^2 + [\gamma H_{1(a)}]^2,$$

the error incurred, $f_m - \delta$ depends on the relative magnitudes of J and δ . In Fig. 1 some values of the correction to be applied are plotted for the field appropriate to critical decoupling ($\pi J/\gamma$) at selected values of J from 2 to 30 c./sec. This shows that for most organic compounds corrections will be commonly not greater than 0.5 and very rarely as large as 3 c./sec. Determination of chemical shifts can therefore be made as accurately as is normally possible by the more usual method of superposition of sidebands, and when a band origin cannot be assigned with certainty owing to complexity of the spectrum the method gives a unique solution.

Examples.—The selective suppression of spin coupling has two important uses in facilitating the application of nuclear magnetic resonance to organic chemical problems. The first is in the analysis of complex spectra, particularly where overlap of multiplet systems leads to ambiguities unresolvable without complex computation; an experimental demonstration of spin coupling has a degree of certainty which even the best theoretical analysis lacks; it is possible directly to determine the relative sign of coupling constants in suitable cases, and for this purpose the double sideband techniques are not applicable. The second is the detection and precise determination of the resonance frequency of protons which, though obscured from direct measurement by strong overlying resonances or by virtue of spin splitting of great complexity, are spin-coupled to other protons whose resonances are observable. This situation is often encountered in the spectra of complex molecules and spin decoupling provides the only way in which the spectrum can be completely understood.

Ethanol and acetaldehyde provide trivial illustrations of the method for spectra where

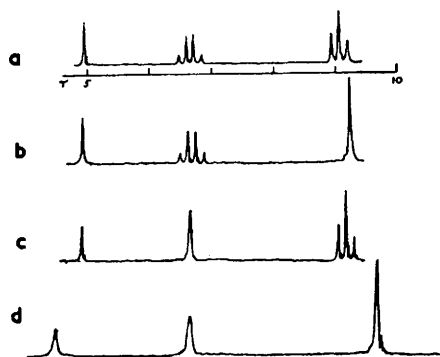


FIG. 2. The spectrum of ethanol (a) showing the effect of irradiating the methylene protons (b), the methyl protons (c), and both (d). (H_0 increases from left to right in all the spectra.)

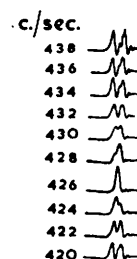


FIG. 3. The acetaldehyde doublet recorded with values of f_m from 424 to 434 c./sec.

no obscuring of resonance occurs, but they show that decoupled spectra can be obtained free from unwanted sideband resonances and audiomodulation.

Ethanol. The group of lines near $\tau 9.1$ (Fig. 2a) is confirmed as a spin multiplet by its collapse to a single line (Fig. 2b) on strong irradiation of the region $\tau 6.5$, and similarly the quadruplet there is shown to collapse (Fig. 2c) on strong irradiation near $\tau 9.1$. Both groups may also be made simpler in the same spectrum (Fig. 2d) by double sideband irradiation with a single-phase audio-oscillator instead of the two-phase oscillator normally used. The precise chemical shift between the centres of these fairly broad groups ($J \approx 10$ c./sec.) could readily be measured (to within 1 c./sec.) by reducing the sideband power to give critical decoupling. The modulation frequency setting was then found to be 137 c./sec. and the correction is expected to be only 0.25 c./sec.

Acetaldehyde. The spectra of Fig. 3 show the doublet of acetaldehyde with various values of modulating frequency and illustrate the sensitivity to small variations in chemical shift which can be expected with critically adjusted sideband power level. The correction in this case would be <0.1 c./sec. In this series and in the series of Figs. 7 and 8 the appearance and increasing separation of the doublet as the frequency departs from the exact double resonance value can be discerned, in agreement with the predictions of Bloom and Shoolery for this type of decoupling experiment.

The diethylthallium cation (sample prepared by Mr. J. P. Maher). In the first two

examples, in which only a single spin-coupled system was present, the ability to use single sideband irradiation conferred no unique advantage. In the diethylthallium cation, however, and in subsequent examples, the complexity of the spectrum makes suppression of the unwanted sideband and harmonics usually desirable and frequently essential for a unique solution. In this case it makes possible (as suggested by Dr. D. F. Evans of this Department) the direct experimental determination of the relative sign of the coupling constants. In the diethylthallium cation, the thallium nucleus causes an extremely large splitting of the methyl and methylene resonances⁸ to give a proton resonance spectrum covering a range of about 600 c./sec. (Fig. 4). If the methyl and methylene coupling constants had the same sign, the groups (A) should be spin-coupled and so should groups (B). If they were of opposite sign, however, CH₃ (A) should be

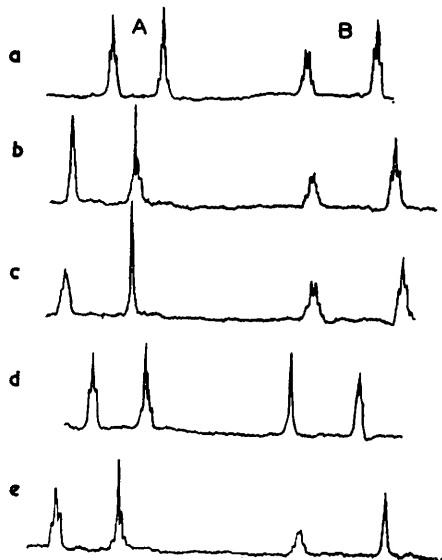


FIG. 4. The spectrum of the diethylthallium ion (20% solution).

$f_m = +1064$ (a), $+464$ (b), $+504$ (c), -464 (d), and -504 (e) c./sec.

coupled to CH₂ (B), and CH₃ (B) to CH₂ (A). Single sideband irradiation distinguishes clearly between these possibilities as Figs. 4b–e show. The Tl–H coupling constants are found to be 340 (Tl–CH₂) and 628 (Tl–CH₃) c./sec. and *opposite in sign*.

Protonated tricarbonylcyclo-octatetraeneiron (sample prepared by Mr. A Davison). In acid solution the single-line resonance of tricarbonylcyclo-octatetraeneiron is replaced by a complex spectrum containing four groups of lines a–d in order of increasing field which have been ascribed by Wilkinson *et al.*⁹ to the four chemically distinct groups in the structure shown in Fig. 5. This would require that the band A (1 proton) should be coupled to the nearest band B (4 protons). However, the fine structure could not be resolved sufficiently to permit detailed analysis since the natural line-width in sulphuric acid is usually rather broad. A spin-decoupling experiment, however, confirmed this assignment unambiguously (Fig. 5), the chemical shift of the group causing the splitting in A being found to be 132 c./sec. on the high-field side, coincident with group B.

*1-Methyl-2-pyridone*¹⁰ (kindly supplied by Dr. J. A. Elvidge). The spectrum of the ring protons consists of a triplet at τ 3.85 (H₅) and a doublet at τ 3.53 (H₃), both of which show further fine structure due to long-range couplings. The remaining ring protons H₄ and H₆ give rise to a band of complex structure near τ 2.7. The spectra in Fig. 6

⁸ Maher and Evans, *Proc. Chem. Soc.*, 1961, 208.

⁹ Davison, McFarlane, Pratt, and Wilkinson, *Chem. and Ind.*, 1961, 553.

¹⁰ Elvidge and Jackman, *J.*, 1961, 859.

confirm these assignments by showing in turn the normal spectrum and the decrease in multiplicity which occurs when:

- (b) H_5 is decoupled by irradiation of H_4 and H_6 ($f_m = -73$ c./sec.)
 (c) H_3 " " " " " " H_4 and H_6 ($f_m = -49$ c./sec.)
 (d) H_4 and H_6 are partly decoupled by irradiation of H_3 ($f_m = +49$ c./sec.)
 (e) " " " " " " " " " H_5 ($f_m = +73$ c./sec.)

In (1) and (2) the multiplicity is reduced to a singlet (apart from long-range residual splittings). In the other two cases the low-field band due to H_4 and H_6 is probably reduced to two different ABX systems, though the chemical shift between protons 3 and 5 (17.5

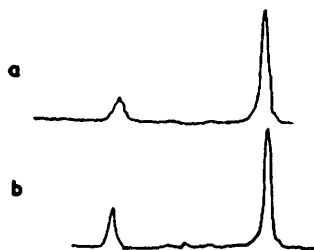


FIG. 5. Tricarbonylcyclo-octatetraeneiron in sulphuric acid.

$f_m = +1032$ (a), $+132$ (b) c/sec. The left-hand peak refers to the H at the position 4 relative to $CH_2 = 1$, and the right-hand peak to the two H at positions 3 and 5.

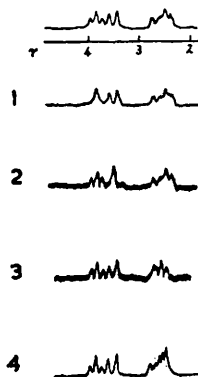
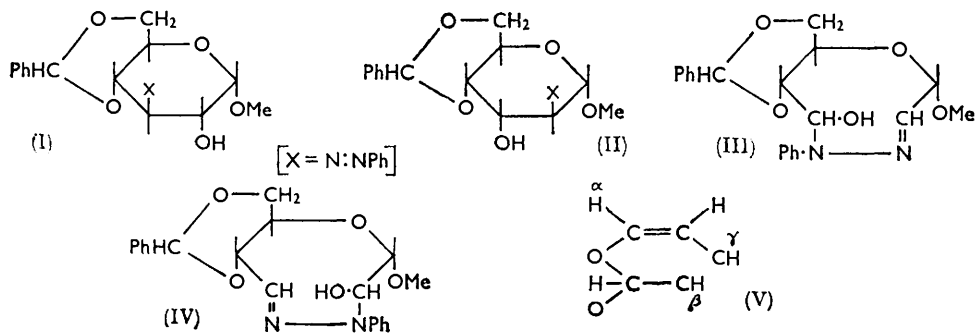


FIG. 6. The ring-proton resonance spectrum of 1-methyl-2-pyridone, without decoupling and decoupled (see text).

c./sec.) may be small enough for some simultaneous decoupling to occur which, if complete would give an AB system. The chemical shift between protons 4 and 6 can thus be shown experimentally to be 12 c./sec., a value which could not be derived from theoretical analysis of the spectrum with the same degree of certainty.

The next two examples illustrate the location of "hidden" protons.

The phenylhydrazine derivative of periodate-oxidised methyl 4,6-O-benzylidene- α -D-glucoside (kindly supplied by Dr. R. D. Guthrie). Four structures (I—IV) have been



considered for this compound on the basis of chemical evidence;^{11,12} nuclear magnetic resonance investigations,¹³ and the more recent chemical investigations favour structure (I).

The single proton resonances at τ 4.45 and 5.15 were clearly ascribable to the acetal

¹¹ Guthrie and Honeyman, *J.*, 1959, 2441.

¹² Guthrie, *Proc. Chem. Soc.*, 1960, 387, and personal communication.

¹³ Guthrie, unpublished work.

protons, but the relative intensities of the two components of the doublet (τ 5.15) appeared to require coupling to a less shielded proton, necessarily amongst the lowest-field grouping and therefore vinylic [cf. (III)]. This could not be reconciled with the integrated intensity of this band, which corresponded only with the ten aromatic protons. A double resonance experiment showed that the peak intensity ratio was misleading, and the source of the splitting was found to be a proton resonating 30 c./sec. up-field in the group of lines due to the protons of the sugar skeleton.

After acetylation a very similar spectrum (Fig. 7a) was found, but a one-proton resonance had moved from the group near $\tau = 6$ to $\tau = 4.45$, 30 c./sec. down-field from the doublet (which also suffered a slight shift). This behaviour is characteristic of that of a proton attached to the same carbon atom as the acetylated hydroxyl function. A further double-resonance experiment now demonstrated (Fig. 7b—g) that it was this proton which was coupled to the one showing the doublet resonance (now at τ 4.97) since the sideband was now required to be 30 c./sec. on the low-field side. The structure (I) was thus confirmed.

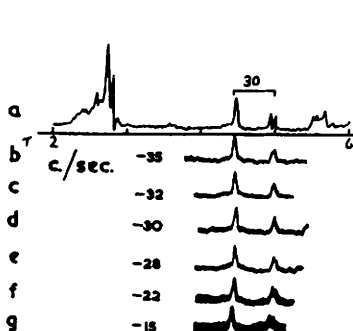


FIG. 7. A portion of the spectrum (from $\tau \sim 2.5$ to $\tau \sim 6$) of the acetylated phenylhydrazine derivative of periodate-oxidised methyl 4,6-*O*-benzylidene-D-glucoside, without decoupling (a) and showing decoupling (b)—(g) for various values of f_m .

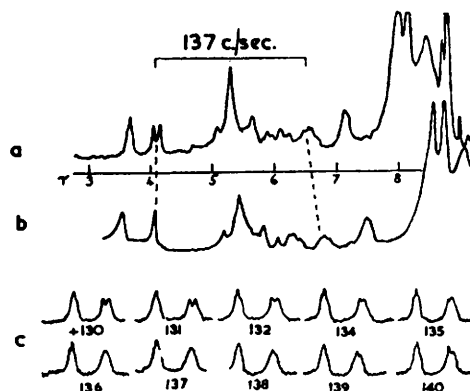


FIG. 8. The spectrum of clerodin with normal coupling (a), decoupled ($f_m = +137$ c/sec.) (b). In (c) the low-field portion of the spectrum has been recorded with f_m varying from 130 to 140 c./sec.

Clerodin. The constitution and stereochemistry of this bitter principle of *Clerodendron infortunatum* has been established by X-ray studies¹⁴ and by chemical and nuclear magnetic resonance investigations.¹⁵ The lowest-field resonance, a narrow triplet (τ 3.60) (unresolved in Fig. 8) could be ascribed to the α -hydrogen of an enol ether, and the second (τ 4.09), a doublet, to the hydrogen flanked by the two oxygen atoms of the partial structure (V). The resonance due to the β -hydrogen would not be distinguished in the complex spectrum but would be expected to appear near or above τ 8 unless this hydrogen atom and the allylic hydrogen (γ) were one and the same, in which case a considerably lower τ value (6—7) could be expected. Its position was found by double resonance, the disappearance of the doublet splitting (Fig. 8c) being noted. The optimum value for f_m 137 (Fig. 8b) gives τ 6.53 for the β -proton and confirms the dihydrofuranoid nature of this portion of the clerodin structure.

EXPERIMENTAL

The Varian 12^m-magnet nuclear magnetic resonance spectrometer used was equipped with the Varian model 4311 56.4 Mc./sec. transmitter-receiver having a radiofrequency phase-sensitive detector. A single sideband suppressed carrier modulator was inserted between

¹⁴ Sim, Hamor, Paul, and J. M. Robertson, *Proc. Chem. Soc.*, 1961, 75.

¹⁵ Barton, Cheung, Cross, Jackman, and Martin-Smith, *Proc. Chem. Soc.*, 1961, 76.

the transmitter output socket and the probe (Fig. 9). The phase-sensitive detector, whose radiofrequency reference phase is provided internally and is undisturbed (Fig. 9), enabled strong signals at the sideband frequency to be rejected completely by the use of a low-pass filter, initially only an extra C,R smoothing section at the output of the unit having a time constant of 1—2 sec. With this, spectra have been recorded in which the modulating frequency

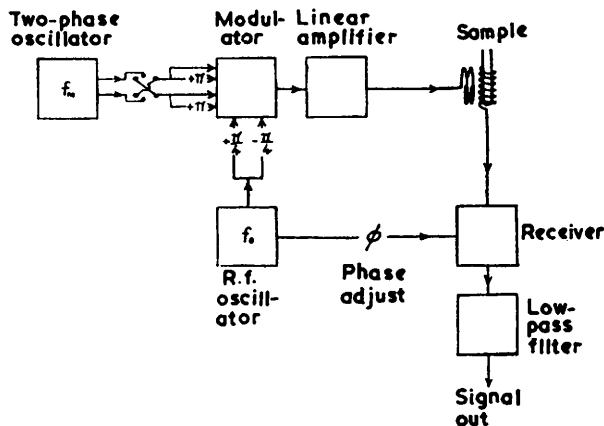


FIG. 9. Block diagram of spin-decoupling spectrometer.

Two-phase oscillator—modulator—linear amplifier—sample; R.-f. oscillator—receiver, low-pass filter, signal output to recorder.

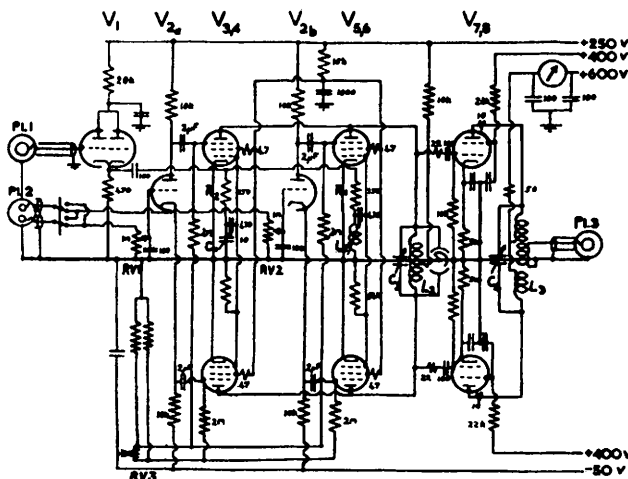


FIG. 10. Modulator and linear amplifier circuit. All heaters 6.3 v a.c. in parallel (V_1 heaters connected through R.f. choke). All resistance and capacitance values in ohms and picofarad except where noted. Pl. 1, R.f. input; Pl. 2, modulation input; Pl. 3, R.f. output to probe.

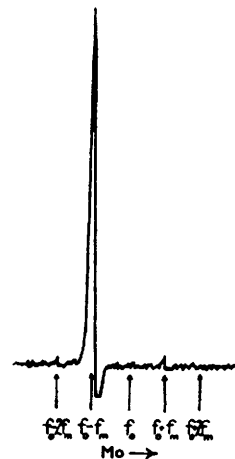


FIG. 11. Radiofrequency spectrum after modulation (ordinate, signal strength, arbitrary units).

has been as low as 16 c./sec. without excessive break-through of the modulating frequency, on the Sanborne recorder which responds to frequencies as high as 50 c./sec. A more satisfactory filter in practice would be one with a sharp cut-off at about 5 c./sec. and high attenuation for all higher frequencies, but the larger strip-chart recorders effectively provide rapid attenuation for frequencies greater than ~ 5 c./sec.

The Modulator.—This consisted (Fig. 10) of two balanced modulators, disposed with high symmetry and excited in quadrature to produce cancellation of the unwanted sideband. The modulating elements were suppressor grid-modulated pentodes V_2, V_4, V_5, V_6 (EF 91). The radiofrequency excitation was derived from the transmitter output of the Varian 56.4 Mc./sec. unit through a buffer stage and two adjustable radiofrequency phase-shift circuits L_1R_1 and C_1R_2 .

which were adjusted to a phase difference of 90° between the signal at V_3, V_5 , and V_4, V_6 grids. The modulating signal was applied to each balanced modulator pair by a phase-splitter V_{2a} and V_{2b} (ECC 81), the quadrature signals for which were provided by a two-phase low-frequency oscillator (Solartron). A choice of which sideband was to be reinforced and which cancelled out was provided by the sideband selector switch S which reversed the connections to the low-frequency oscillator.

The common anode load for V_3, V_4, V_5 , and V_6 , is L_2C_3 which is symmetrically coupled to the push-pull class A linear power-amplifier V_7, V_8 (807). High attenuation of the unwanted sideband modulation depends on the linearity of the succeeding output stage; this was therefore designed with much higher power-handling capacity than was in practice required. The output-tuned circuit L_3C_4 is link coupled to the transmitter coil in the Varian probe unit.

Initial Adjustment.—The modulator was adjusted at a high modulating frequency (~ 500 c./sec.) with a stationary water sample and using the "wide line diode" setting of the receiver; this allowed resonance signals to be seen on the oscilloscope at the carrier and sideband frequencies as well. The radiofrequency circuits were tuned for maximum output at the carrier frequency and locked. The suppressor grid-bias potential was then set to -40 v, and the modulation balance (RV1, RV2) and radiofrequency phase controls (L1, C1) were adjusted until the optimum ratio (>40 dB) of wanted to unwanted sideband signal strength was observed for both settings of the sideband selector switch S. The strength of the carrier signal was then progressively reduced by adjustment of the trimming condenser, the "carrier leak" control (RV3), and the suppressor grid bias until a minimum value was obtained estimated to be at least 50 dB below the strongest sideband. (This was difficult to determine exactly since saturation on the sideband signal occurred readily.) The sequence of operations was repeated until a satisfactory frequency spectrum (Fig. 11) was obtained. The unwanted sideband, together with others due to harmonics of the modulation frequency, were observed to be present, but since their strength was >40 dB less than the principal sideband no decoupling action could be detected at these frequencies, and the radiofrequency phase-sensitive detector allowed resonances at them to be rejected completely.

Operating Procedure.—The resonance signal at the carrier frequency was first displayed in the usual way, by using a high modulation frequency (~ 1000 c./sec.). Much more accurate balancing of the radiofrequency leakage field in the probe was found necessary to avoid overloading of the radiofrequency amplifier by strong sideband signal leakage, and it was necessary to carry this out afresh for each sample investigated. The carrier leak control was set to give a radiofrequency signal strength below the saturation level for the sweep desired and the radiofrequency control set finally to show signals in the pure absorption mode. (This was set last since carrier leak adjustment caused radiofrequency phase changes at very low carrier level settings.)

The general location of the source of spin coupling of each multiplet in turn could then be discovered by displaying it on an oscilloscope and adjusting the modulating frequency until the multiplet showed signs of collapse. It was found that, when the maximum available sideband power was used (10 v output from the low-frequency oscillator), its frequency could differ as much as 10 c./sec. from the resonance frequency of the proton to be irradiated and still give rise to largely effective decoupling if the coupling constant were not greater than 10 c./sec. It was adequate, therefore, to adjust the modulating frequency in steps of 10 c./sec. whilst repeated sweeps were made through a multiplet under investigation. This could be facilitated by scanning at a fairly high repetition rate and noting changes in the "wobble-beat" pattern. When the approximate value had been determined, precise adjustments were made at low sweep speed and the multiplet pattern was recorded for every few cycles change in modulating frequency and finally at every 1 c./sec. with the sideband power level reduced. In most cases the frequency at which optimum decoupling took place could be determined to within <1 c./sec. when the sideband power was reduced to narrow its effective spectral range.

I thank those already mentioned for the gifts of samples and for fruitful discussions. I also acknowledge with gratitude much helpful criticism and advice from Dr. L. M. Jackman and Dr. L. Pratt.