

19. *The High-resolution Proton Resonance Spectra of Glycerides.*

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Interpretation and discussion of the nuclear magnetic resonance spectra of some simple glycerides, particularly simple mono-acid triglycerides, are made by comparison of model systems such as 1,2,3-trichloro- and 2-acetoxy-1,3-dichloro-propane, and by use of AB_4 theoretical systems. The use of these spectra in obtaining structural information is pointed out. The spectra of diglyceride and monoglyceride isomers are also discussed, and the way in which the isomers can be distinguished by means of their spectra is shown.

DETAILED interpretation of high-resolution nuclear magnetic resonance spectra usually involves considerable computation. In the present paper the basic grouping of glycerides, $CH_2 \cdot CH \cdot CH_2$, is discussed on the basis of an approximate or pseudo- AB_4 system. The AB_4 system has been studied theoretically by a number of authors,¹ using the symmetry properties to simplify the Hamiltonian, and has been applied to genuine AB_4 systems such as the perfluoroalkyl derivatives containing the SF_5 group.² Analysis of this system is made relatively easy without recourse to computers by using the composite-particle technique³ (see Appendix). It is here applied to the spectra of 1,2,3-trichloro- and 2-acetoxy-1,3-dichloro-propane, triacetin, and long-chain triglycerides. The use of nuclear magnetic resonance spectroscopy for distinguishing various glyceride isomers is also pointed out.

Experimental.—The long-chain glycerides were of high purity. However, owing to acyl migration, our 1-monoacetin contained some of the 2-isomer, and our 1,3-diacetin contained some of the 1,2-isomer. Concentrations were as shown; otherwise optimum concentrations were obtained to give adequate signal to noise ratio and good resolution.

The spectra were obtained at 40 Mc./sec., by using a Varian Associates V4300A nuclear magnetic resonance spectrometer and 12'' electromagnet with flux stabilisation, sample spinning, and a Varian V4365 field homogeneity control unit. The samples were examined in Pyrex tubes of 5 mm. diameter with tetramethylsilane as an internal reference compound, and the separations between the lines were measured by using side bands of the reference compound generated by a Muirhead-Wigan D695A decade oscillator. The sweep rate was somewhat variable and average values were determined. Positions of the lines are expressed as chemical

¹ Banerjee, Das, and Saha, *Proc. Roy. Soc.*, 1954, **226**, A, 490; Corio, *Chem. Rev.*, 1960, **60**, 363.

² Muller, Lauterbur, and Svatos, *J. Amer. Chem. Soc.*, 1957, **79**, 1043.

³ Whitman, Onsager, Saunders, and Dubb, *J. Chem. Phys.*, 1960, **32**, 67.

shifts on the τ scale (τ for $\text{SiMe}_4 = 10$), and distances between lines are expressed as cycles per sec. For 1,2,3-trichloropropane particular care was taken to remove dissolved oxygen from the sample.

Discussion.—The glycerol residue in $\text{X}\cdot\text{CH}_2\cdot\text{CHY}\cdot\text{CH}_2\text{Z}$ involves five non-equivalent protons; hence five chemical shift values and ten coupling constants are required to characterise it. The system is essentially a substituted propane and rotational isomerism can occur as with the substituted ethane system. However, with the substituted ethanes there are only three staggered rotational configurations, corresponding to three potential-energy minima, the difference in energy between the configurations and the barrier heights depending on the nature of the substituents. With substituted propanes rotation is possible about two positions in the molecule, and nine isomers are possible. Steric interactions, of course, become important and some of the isomers formally described are expected to be unstable. Nevertheless, the system is of some complexity.

If we attempt to simplify the system and let the substituents $\text{X} = \text{Y} = \text{Z}$ we make the number of formal isomers less, but the theoretical system is just as complex and of the $\text{AA}'\text{BB}'\text{C}$ type. Even if we regard this system as an AB_2C_2 type (where B and C refer to protons of the same methylene group) the system still requires fairly considerable computation. The final stage of approximation is to an AB_4 type where we regard the chemical-shift values for each proton as equivalent and only one coupling constant is needed. This is a gross approximation but has the virtue of being comparatively easy to calculate by the composite-particle technique³ (see Appendix). The procedure adopted is, therefore, first to examine the spectra of some simple substituted propanes (1,2,3-trichloro- and 2-acetoxy-1,3-dichloro-propane), to see whether and under what conditions a good fit to an AB_4 type pattern can be expected; this provides some insight into the spectra of glycerides, particularly of the monoacid triglycerides. The 1,3-diglycerides and 2-monoglycerides correspond to the system where $\text{X} = \text{Z} \neq \text{Y}$ and will also be discussed on this basis. The spectra of the 1,2-diglycerides and 1-monoglycerides are contrasted with those of the other glycerides. A feature of the AB_4 theoretical system is that the spectrum is completely determined by R , the ratio of the coupling constant (J) to the chemical-shift difference(s).

1,2,3-Trichloropropane. Although chlorine is a magnetic nucleus it gives no spin-spin interaction effects because of rapid electric quadrupole relaxation.

The 40 Mc./sec. spectrum of 1,2,3-trichloropropane in the liquid state or in solution is rather complex, showing that the difference in chemical shift between the CH and CH_2 protons is not large (see Fig. 1). The states from which the lines originate are shown and designated DT and DS. Details concerning this are given in the Appendix. Attempts to fit the spectrum of the liquid are not particularly good. Whilst good agreement is possible between a calculated spectrum with either the A (CH) group of lines or the B (2CH_2) group of lines, agreement is not satisfactory with both together. The spectrum of the material dissolved in carbon tetrachloride can be fitted better by a ratio $R = 0.4$, although even here agreement is poor. However, a very good fit is obtained with the material dissolved in acetonitrile for a ratio $R = 0.29$. Having fitted the spectrum we can measure directly the chemical-shift difference between the CH and CH_2 protons ($\delta 19.7$ c./sec.) and the averaged coupling constant ($J 5.70$ c./sec.). From the experimental spectra, assuming an AB_4 fit, we can measure the chemical shift difference for the liquid ($\delta 17.3$ c./sec.) and for the solution in carbon tetrachloride (15.1 c./sec.). The reason for the sensitivity of the system to solvent is probably that for rotational isomers of varying dipole moment ΔE , the energy barrier between different isomers, varies with the dielectric constant of the medium, *i.e.*, the relative abundances of the isomers vary with the solvent.⁴ This substituted propane is a good example of an intermediate value of R .

⁴ Mizushima, "Structure of Molecules and Internal Rotations," Academic Press Inc., New York, 1954.

2-Acetoxy-1,3-dichloropropane. When an acetate group instead of a chlorine is placed on the central carbon atom of propane, the chemical shift between the protons of the CH and the CH₂ groups is so large that the system approximates to an AX₄ system. The A lines give rise to a quintet and the B lines a doublet. The values for δ and J can be readily measured from the experimental spectrum and the ratio $R = 0.095$ is readily obtained (see Fig. 2). The values are: δ 57.4, J 5.15 c./sec.

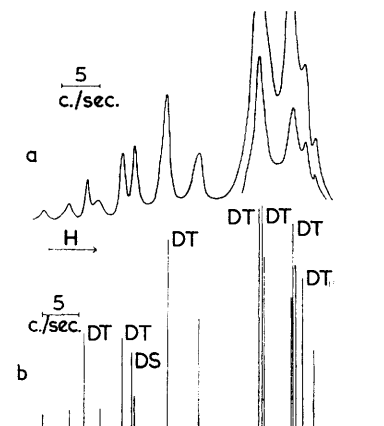


FIG. 1. (a) Experimental and (b) calculated spectrum of 1,2,3-trichloropropane 50 : 50 (v/v in acetonitrile). $R = 0.29$.

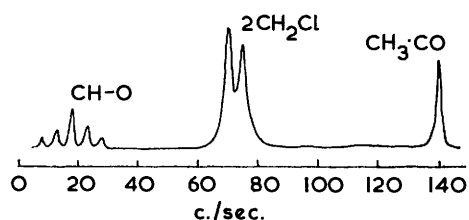


FIG. 2. Experimental spectrum of 2-acetoxy-1,3-dichloropropane (50 : 50 v/v in carbon tetrachloride). $R = 0.095$.

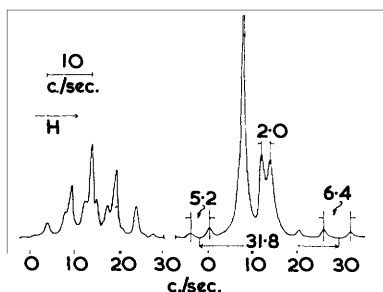


FIG. 3. Experimental spectrum of triacetin (50 : 50 v/v in chloroform).

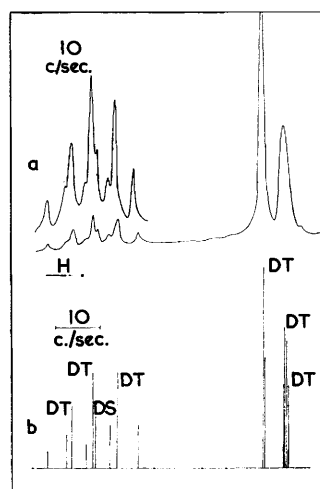


FIG. 4. (a) Experimental and (b) calculated spectrum of triacetin (50 : 50 v/v in acetonitrile). $R = 0.13$.

The chemical-shift values for the different protons in the system on the τ scale are: CH₃ 7.93; CH₂ 6.24; CH 4.83. This molecule is a good example of a large chemical-shift difference between the interacting groups.

Triglycerides. Triacetin in chloroform gives a spectrum (Fig. 3) where the line corresponding to the methyl protons is single, showing that these nuclei are equivalent. It is not possible to fit the remainder of the spectrum with a calculated AB₄ system. Whilst the

quintet structure of the A line can be fitted, the spacing of the three strong B lines cannot. However, when the spectrum is obtained for an acetonitrile solution a good fit to a calculated AB_4 spectrum can be made corresponding to a ratio $R = 0.13$, and the values $\delta = 40.9$ and $J = 5.3$ c./sec. are readily obtained (Fig. 4). This sensitivity to solvent may be analogous to that observed with 1,2,3-trichloropropane and related to the effect of the solvent on the distribution of the rotational isomers. We expect however steric effects to be more predominant with the glycerides because of the large bulky substituent groups present.

Nuclear magnetic resonance spectrum of tributyrin.

| Group | Chemical shift | Theor. multiplicity (first order) | Group | Chemical shift | Theor. multiplicity (first order) |
|------------------------------|----------------|-----------------------------------|----------------|----------------|-----------------------------------|
| $CH_2 \cdot CH_3$ | 9.05 | Triplet | $O-C-CH_2$... | 7.75 | Triplet |
| $CH_2 \cdot CH_2 \cdot CH_3$ | 8.45 | { Quartet | H_2C-O-C ... | 5.77 | Doublet |
| | | Triplet | $HC-O-C$ | 4.75 | Quintet |

The glyceroyl residue can also be regarded as an XB_2C_2 system where X is the H-C proton. If we consider the four weak lines situated about the strong lines of the CH_2 groups, two

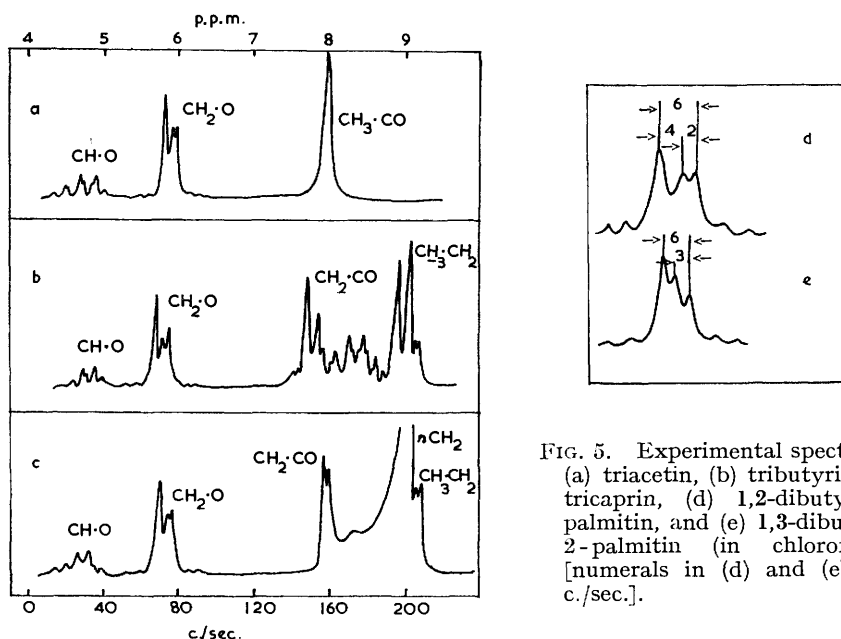


FIG. 5. Experimental spectra of (a) triacetin, (b) tributyrin, (c) tricaprln, (d) 1,2-dibutyro-3-palmitin, and (e) 1,3-dibutyro-2-palmitin (in chloroform) [numerals in (d) and (e) are c./sec.].

may be regarded as arising from the B_2C_2 system. The effect of the X proton is to perturb this system, so that these lines are doubled. The average distance between the two outer lines should correspond to J_{XB} , the coupling constant for the CH and the CH_2 protons. The experimental value is 5.8 c./sec., in fair agreement with the averaged value determined by fitting the AB_4 approximation.

The 40 Mc./sec. spectra of some triglycerides of longer chain length are shown in Fig. 5. That of tributyrin is readily interpreted on the basis of first-order coupling of adjacent groups and chemical shift differences as shown in the Table.

When the chain length increases as it does in tricaprln, the protons present in the methylene chain begin to dominate the spectrum, but the remaining lines can be readily assigned. The practical application of nuclear magnetic resonance spectra to saturated triglycerides is facilitated by the built-in intensity reference system of the two CH_2 and

CH groups of the glyceroyl residue.⁵ Integrating devices for counting the number of groups will be particularly useful. The acetate group is particularly easy to pick out of the spectrum and this suggests that such spectra will be useful for many studies of acetofats.

One would not expect that information on closely related isomers such as 1,2-dibutyro-3-palmitin and 1,3-dibutyro-2-palmitin would be obtainable by nuclear magnetic resonance spectroscopy. The 40 Mc./sec. spectra of chloroform solutions are nearly identical, except for the appearance and separation of the lines due to the CH_2 groups of the glyceroyl residue. This is shown in Fig. 5. This difference is presumably due to the small difference in chemical shift on the two CH_2 groups in the 1,2- as compared with the 1,3-isomer. 2-Butyro-1,3-distearin is similar to 1,3-dibutyro-2-palmitin in appearance in this region of the spectrum, and 1,2-diaceto-3-olein similar in appearance to 1,2-dibutyro-3-palmitin.

As pointed out by Hopkins and Bernstein,⁵ the lines from the protons in the $\text{CH}=\text{CH}$ group occur at approximately the same chemical-shift value as the $\text{H}-\text{C}-\text{O}-\text{C}$ proton. This was also observed in the present investigation with glycerides of the type of 1,2-diaceto-3-olein.

Diglycerides.—With diglycerides greater complexity can occur than with triglycerides, for the following reasons: (a) unless exchange of the hydroxyl proton takes place so rapidly that its presence on the system can be ignored, coupling to it can take place from the protons in the glyceroyl residue and a six-proton interacting system is involved; (b) the effect of hydrogen bonding can vary the chemical shift value of the hydroxyl proton so that its position in the spectrum is uncertain; and (c) acyl migration can occur with the diglycerides⁶ so that some of the 1,2- is present in the 1,3-isomer. Migration is particularly fast with the short-chain acyl groups and it is very difficult to prepare, say, pure 1,3-diacetin. The particular sample examined certainly contained some of the 1,2-isomer, making interpretation of the spectrum difficult.

The spectrum of 1,3-diacetin in chloroform (Fig. 6) contains only two groups of lines, a single line at 7.95 p.p.m. corresponding to the presence of the protons in the $\text{H}_3\text{C}-\text{C}$ group and a group of lines near 6.0 p.p.m. with the strongest line at 5.9 p.p.m. (At higher gain, lines are observed near 5.0 p.p.m., corresponding to protons present in the $\text{H}-\text{C}-\text{O}$ group of the 1,2-isomer.) Empirical data show that the chemical-shift value for the protons in the $\text{H}-\text{C}-\text{OH}$ group will be rather close to that of the protons in the $\text{H}_2\text{C}-\text{O}-\text{C}$ group and hence the effect of interaction between the protons will be large. We see that the lines due to the protons of the HC group and H_2C group are superimposed. We also note that the chemical-shift value for the HC group is slightly greater than that for the CH_2 protons, in contrast to the situation with triacetin. Attempt to fit the experimental spectrum with calculated AB_4 systems of ratio $R = 1.0$ and 2.0 are not successful, although qualitatively similar spectra were obtained. The experimental spectra of solutions in acetonitrile show that shifts occur, but do not clarify the situation. It is not certain whether the crude AB_4 approximation holds at these rather small chemical shift differences. An XB_2C_2 treatment is also not adequate because of the greater interactions of the five protons in the system, and it appears that a much fuller theoretical treatment is required.

The spectra of a number of 1,3-diglycerides are also shown in Fig. 6, for chloroform solutions. In all cases the line due to the $\text{H}_2\text{C}-\text{O}-\text{C}$ groups is single and lacks the doublet structure present in the spectra of the triglycerides. This is due to the small chemical-shift difference of the protons on the glycerol residue. A line near 7 p.p.m. is assigned to the proton of the hydroxyl group; it can shift with concentration and with solvent and is not present in the spectrum given for the chloroform solution of diacetin.

The isomer 1,2-diacetin unfortunately could not be obtained, but 1,2-distearin was examined. The relative proportion of protons in the glycerol residue compared with those

⁵ Hopkins and Bernstein, *Canad. J. Chem.*, 1959, **37**, 775.

⁶ Crossley, Freeman, Hudson, and Pierce, *J.*, 1959, 760.

in the long chain, combined with limits on concentration, hinder good resolution of the lines due to the glycerol protons. With the 1,2-isomer the glycerol residue contains protons in groupings H_2C-O-C , H_2C-OH , and $H-C-OC$, and from empirical data we expect lines near 5.88, 6.44, and 5.0 p.p.m. Lines are observed near these positions in

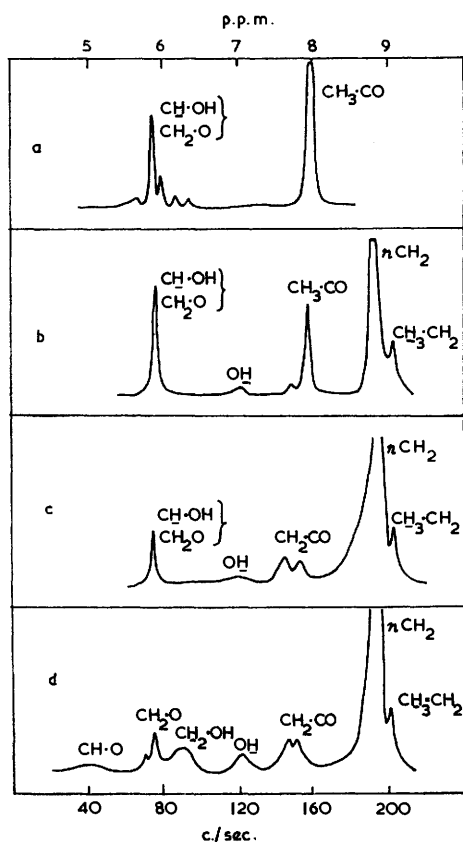


FIG. 6. Experimental spectra of (a) 1,3-diacetin, (b) 1-aceto-3-palmitin, (c) 1,3-distearin, and (d) 1,2-distearin (in chloroform).

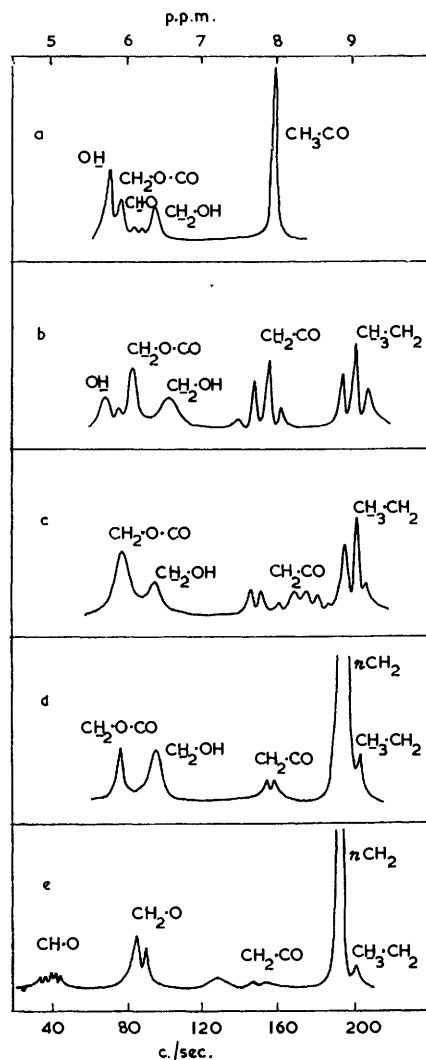


FIG. 7. Experimental spectra of (a) 1-monoacetin, (b) 1-monopropionin, (c) 1-monobutyryn, (d) 1-monostearin, and (e) 2-monostearin (in chloroform).

the spectra, at 5.88, 6.27, and 4.95 p.p.m. Some sort of doublet structure is observed in the first of these lines, poorly resolved structure in the second, and considerable multiplicity in the third. The line at 4.95 p.p.m. is absent for the 1,3-isomers and could help to distinguish the two isomers. However, the high multiplicity makes this signal difficult to detect and may limit its usefulness. The line near 6.4 p.p.m. is probably the best for distinguishing 1,2- from 1,3-isomers and for analysing the two in mixtures, the lines near 4.95 p.p.m. providing confirmation.

Monoglycerides. Many of the factors affecting the spectra of the diglycerides also affect those of the monoglycerides.

With 1-monoglycerides three types of proton grouping occur in the glycerol residue, namely, the $\text{H}_2\text{C}-\text{O}-\text{C}$, $\text{H}-\text{C}-\text{O}$, and $\text{H}_2\text{C}-\text{O}$ groups. Lines are found near 5.95 and 6.39 p.p.m., which are assigned to the CH_2 groups. The lines corresponding to the $\text{H}-\text{C}-\text{O}$ proton appear between these two lines at 6.25 p.p.m. and are partly submerged by these lines. 1-Monoacetin in chloroform gives an additional line at 5.8 p.p.m., which is assigned to the hydroxyl protons: in acetonitrile solution this band does not appear. The longer-chain 1-monoglycerides are similar to 1-monoacetin; the additional lines in the spectra can be accounted for on the basis of chemical shift and first-order coupling (Fig. 7).

2-Monoglycerides are distinguished from the 1-isomers by the presence of the $\text{H}-\text{C}-\text{O}-\text{C}$ grouping and the absence of the $\text{H}_2\text{C}-\text{O}-\text{C}$ group. Corresponding with this, lines occur near 5.0 p.p.m. corresponding to the single proton and there are no lines at 5.95 p.p.m. The chemical-shift difference between the $\text{H}_2-\text{C}-\text{O}$ protons near 6.27 p.p.m. and the single proton at 5.0 p.p.m. is quite large (50–60 c./sec.) and hence the glycerol residue gives lines corresponds to an AX_4 type of spectrum, *i.e.*, a doublet occurs for the 2CH_2 protons separated by some 5.1 c./sec., whilst the quintet splitting for the single protons has similar splitting. With 2-monostearin the concentration of the compound in solution in chloroform is such that it is difficult to measure anything other than the first three lines of the quintet. The type of spectrum obtained for the glycerol residue can be compared with that observed with 2-acetoxy-1,3-dichloropropane. The absence of a line at 5.95 p.p.m., along with the presence of lines near 5.0 p.p.m. appears to be the best method for distinguishing these isomers. The presence of unsaturated groupings may also, of course, give lines near the latter region.

APPENDIX: CALCULATION OF THEORETICAL SPECTRA

The composite-particle technique³ does not require determination of the explicit form of the zero-order eigen-functions. The calculations are based on two particles, one containing one proton, the other four protons of fixed total spin. Only one parameter is involved, namely, R , the ratio of the coupling constant J to the chemical shift.

The spin Hamiltonian is

$$\mathcal{H} = -\omega_A \mathbf{I}_{AZ} - \omega_B \mathbf{I}_{BZ} - J \mathbf{I}_A \mathbf{I}_B \quad (1)$$

where A = one proton and B = four equivalent protons, \mathbf{I}_A and \mathbf{I}_B are the vector spin operators of the two groups, \mathbf{I}_{AZ} and \mathbf{I}_{BZ} are the components of these operators along the direction of the magnetic field, and ω_A and ω_B are the resonant frequencies that the two groups would exhibit in the absence of spin coupling. J is the spin-spin coupling between a proton of group A and one of group B. The basis of the method is that wave functions which have different eigen-values of \mathbf{I}_A^2 or \mathbf{I}_B^2 do not mix. The states with $L = 0, \frac{1}{2}, 1, 1\frac{1}{2},$ and 2 (where L is the eigen-value of I) are known as singlet, doublet, triplet, quartet, and quintet states, abbreviated to S, D, T, Q, and Qt, respectively. Here the A group has only the D state, but the B group can occur in S, T, or Qt states. The possible overall states for this system are therefore D_AS_B , D_AT_B , and D_AQt_B . These have degeneracies 2, 3, and 1, respectively.

Once the experimental spectrum is fitted satisfactorily by some value of R , the chemical-shift difference between the two groups can be readily measured from the D_AS_B line as origin to approximately the midpoint of the B group of lines. The value of J , the coupling constant, can then be readily obtained. Details of the calculations with explicit forms for the energies and intensities will be given elsewhere.⁷

The line widths in $(\text{AB})_n$ systems, where n is even, are not the same throughout the spectrum, owing to the different relaxation times associated with the different states. With proton nuclei the lines are not well separated, and in the present spectra no marked variation in line width could be observed owing to this effect.

⁷ Chapman and Harris, *J.*, 1963, 237.

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