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Current Trends in Fourier Transform NMR Spectroscopy*†

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Nuclear magnetic resonance is an intrinsically insensitive technique compared to other spectroscopic techniques for two reasons: (i) The population differences between the ground and excited states is small; and (ii) the long lifetimes of excited states place a generally low upper limit on the power that is used to induce transitions.

Frequency-domain Spectrum

In the first and second generation NMR spectrometers, the spectra were obtained by sweeping either the excitation frequency or the field through the region of nuclear precession frequencies. In almost all the cases, the second technique was in vogue. This method was obviously inefficient, since only one line could be observed at any given point in time. Nevertheless, available instrumentation afforded adequate sensitivity to measure NMR spectra of molecules containing nuclei which have favourable natural abundance and magnetogyric ratio, e.g. ^{1}H, ^{19}F, ^{31}P; but for a large number of nuclei, e.g. ^{13}C, ^{15}N, ^{2}H, etc., problems of sensitivity arising from low natural abundance as well as unfavourable magnetic moments, were too forbidding for widespread use of the technique. The sensitivity is directly proportional to the relative natural abundance and to the third power of the magnetic moment. The problem was only incompletely solved by using higher magnetic fields and techniques such as computer averaging and broad band decoupling.

Pulsed Excitation and Fourier Transform (Time-domain Spectrum)

The advent of pulsed excitation combined with Fourier transformation[1,2] marked a crucial breakthrough in NMR spectroscopy and revolutionized several areas. The inefficiency of the frequency domain spectrum was noted earlier. This becomes exacerbated with nuclei like ^{13}C with intrinsically narrow lines of width of 1 Hz or less and covering a total range of up to 600 ppm. Simultaneous excitation of the whole band of frequencies could be a solution to the problem, which has been achieved by the pulsed NMR method. This was being developed all along and in the last few years has reached the point of practical and widespread utility. In this method, a strong pulse of radio frequency energy is used, covering a large band of frequencies. These are capable of exciting all resonances of interest simultaneously. At the end of the pulse period, the nuclei precess freely with their characteristic Larmor frequencies, which are defined (for a given kind) by their chemical environment. The resonance gives rise to a free precession signal which is also denoted as free induction decay (FID). The FID which represents the time development of magnetization is related to the conventional frequency spectrum (frequency-domain) by the mathematical operation known as Fourier transformation[1,2], which is achieved by a computer. By repeating the experiments several times and adding the response of each pulse coherently in the computer memory, useful spectra are obtained.

Apart from ^{1}H NMR spectra for which already adequate frequency-domain instrumentation is available, ^{13}C spectra are of considerable interest to organic chemists[3-7]. Subsequent discussions will be largely concerned with a few illustrative applications in the domain of ^{13}C NMR spectroscopy. NMR spectra of a few other nuclei are also discussed. For more detailed information on the theory and application of Fourier Transform NMR spectroscopy, especially for ^{13}C NMR, several excellent treatises are available[2-7].

^{13}C NMR Spectroscopy

General—Commercial FT NMR spectrometers are now available which give good spectra of 3-300 mg samples. The first spectrum of a given sample is run with broad-band (proton-noise) decoupling. In most cases, all the carbon atoms of the molecule are identified separately.

High resolution spectra of magnetic nuclei like ^{13}C are characterized by the following variables: (i) chemical shift, (ii) line-width, (iii) relative intensity of a line, and (iv) signal multiplicity.

Chemical shifts of carbon atoms are easily measured in natural abundance ^{13}C spectra, since broad band proton decoupling removes heteronuclear couplings. Scalar homonuclear ^{13}C spin-spin coupling does not

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arise because of the low abundance. Each chemically nonequivalent carbon in a molecule is seen as a separate line, whose position is determined accurately with respect to an internal standard such as tetramethylsilane (TMS) with the help of the computer. Line-widths are of the order of 1 Hz. The relative intensities of ^{13}C nuclei cannot be gauged accurately unlike those of protons because of differences in relaxation times and also variation in Nuclear Overhauser Effects that are an inevitable consequence of broad band decoupling. The quaternary carbons exhibit signals of least intensity consistent with longer relaxation times. Signal multiplicities could arise from ^{13}C-^{13}C or ^{13}C-^{1}H couplings. The low natural abundance of ^{13}C nuclei precludes the former. Since ^{13}C spectra are generally run under broad band proton decoupling, the latter is not seen in the first spectra. But such coupling information is obtained by single resonance, off-resonance or gated decoupling modes of scanning spectra.

Chemical shifts of ^{13}C nuclei are determined by the nature of the chemical environment and depend upon factors such as hybridization state of the observed nucleus, inductive effects of substituents, van der Waals and steric effects between closely spaced nuclei, electric fields originating from molecular dipoles, hyperconjugation, delocalization effects, diamagnetic shielding due to heavy substituents, neighbouring anisotropy effects and isotope effects. Although the general trend, $\delta(sp^3) > \delta(sp) > \delta(sp^2)$, parallels the order found in ^{1}H NMR spectra, ^{13}C shifts which spread over several hundred ppm are more complexly affected by various factors than protons; nevertheless they are still reasonably well correlated qualitatively and quantitatively. Tabulations and spectral collections are available which help the interpretation[8,9]. In fact, computer assisted matching of the spectrum of an unknown sample with a literature collection of known ones and interpretation is now possible[9].

The ^{13}C couplings with like nuclei are not encountered, except when working with enriched specimens. Apart from the chemical shifts, CH coupling information is most valuable in structural interpretation. Empirically, one-bond coupling constant $^1J_{CH}$ can be approximately related to the % S character of the carbon by the equation

$$^1J_{CH} = 5 \times (\%\ S)\,[\mathrm{Hz}]$$

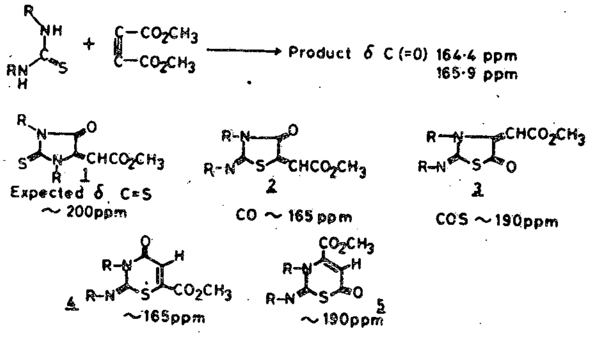
and in the case of hydrocarbons, lies in the region +120 to +200 Hz. Substituent effects on $^1J_{CH}$ are roughly additive. Two bond coupling constants $^2J_{CH}$ are small and not very well correlated with structural parameters. Three bond couplings $^3J_{CH}$ are in the range 0-12 Hz and depend on the dihedral angle φ. A Karplus curve similar to the one for $^3J_{H-H}$ can be drawn for this purpose. Large variations are caused by heteroatoms in the coupling path, or by the type and geometry of substituents. Information about molecular geometry obtained from $^3J_{CH}$ must, therefore, be assessed cautiously.

Some applications of ^{13}C NMR spectroscopy are discussed in the sequel.

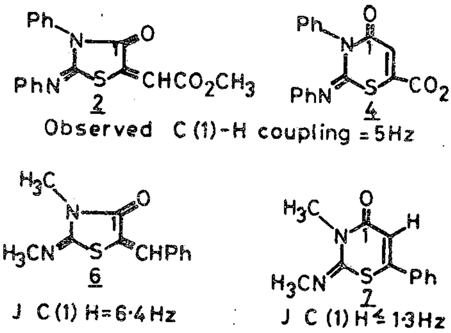
Structure Determination

Enormous literature is available in this field. Excellent illustrations are already mentioned in standard treatises[3-7]. The following work from the author's laboratory is cited.

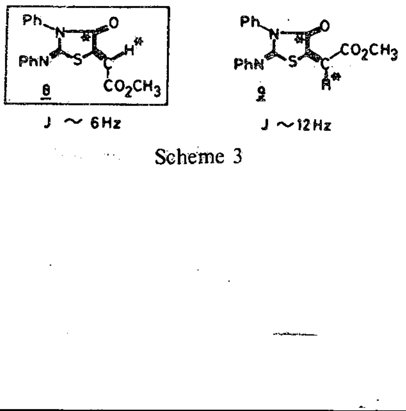
Structure of acetylenedicarboxylic esters to dinucleophiles[10]—There has been a longstanding confusion about the structures of such additions to thiourea substrates. The products obtained have molecular compositions representing addition of the two constituents and loss of the elements of alcohol. In principle, three 5-ring and two 6-ring structures are possible (Scheme 1). The ^{13}C chemical shift (δ, ppm) values obtained for the C=O groups of the products were 164.4 and 165.9, ruling out imidazolinethione (1) and thiolactone (3 and 5) structures. The choice narrowed to thiazoline (2) and thiazine (4), and was made readily on the basis of $^3J_{CH}$ coupling of lactam C=O with the vinyl H (Scheme 2). Model compounds (6) and (7) with unambiguous structures had values of 6.4 and $\leqslant$ 1.9 Hz respectively, allowing the elimination of thiazine structure (4). Two possibilities still existed for thiazoline (2), namely 8 and 9, due to geometrical isomerism around the exocyclic double bond (Scheme 3). The choice in favour of 8 was readily made, because it was known from earlier work[11] that $^3J^{cis}_{CH}$ is



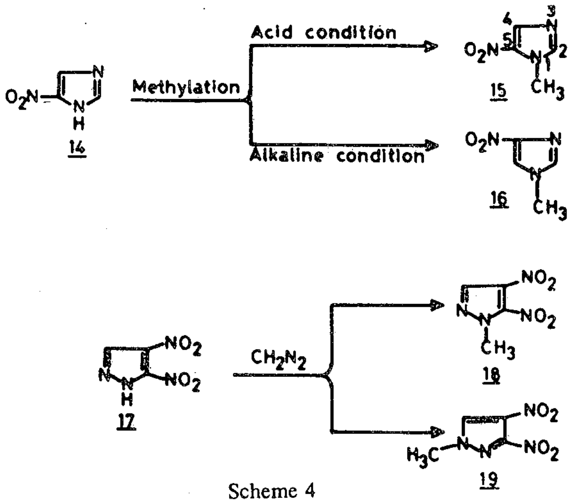
Scheme 1



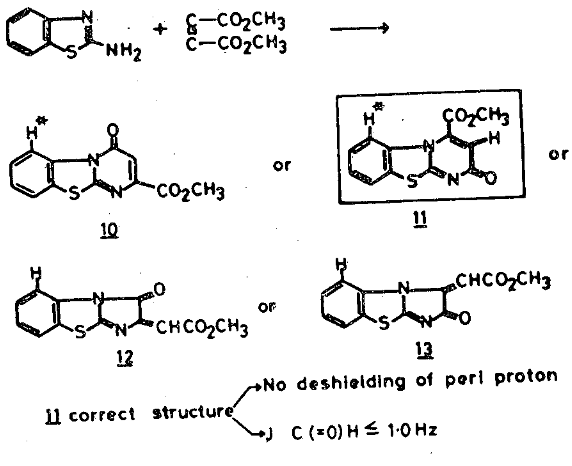
Scheme 2



Scheme 3



Scheme 4



Scheme 5

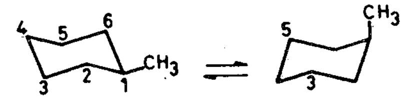
of the order of 4-6 Hz, while ${}^3J^{trans}_{CH}$ ranges around 12 Hz. Such an unambiguous structural and stereochemical assignment was not possible earlier with other tools.

Condensed two 6-ring structures, however, arose from the addition of molecules like 2-aminobenzothiazole to acetylene-dicarboxylic esters (Scheme 4). A very small value for ${}^3J_{CH}$ of C=O precluded condensed imidazoline structures (**12**) and (**13**) in favour of pyrimido-benzothiazoles (**10**) and (**11**). ^{13}C NMR did not provide the choice between the last two structures, but the proton spectrum allowed selection of **11**, since it did not exhibit the typical low field signal for the starred *peri*-proton expected from the powerful deshielding influence of the proximate C=O group.

Structures of alkylation products of nitroimidazoles and nitropyrazoles—We have been successful in using ${}^3J_{CH}$ coupling for differentiating between isomeric nitroimidazoles and pyrazoles (unpublished work from the author's laboratory). It has been known that methylation of 4-nitroimidazole (**14**) can give rise to 4- or 5-nitro-1-methylimidazole (**16** and **15**) respectively or a mixture, depending upon the reaction conditions (Scheme 5). The two structures could be differentiated by the differences in UV absorption and chemical shifts of the proton at C-4 or C-5. These were small, necessitating the availability of both isomers for guaranteed assignment. However, the problem was solved by ^{13}C NMR. It was observed that in this pair, as in many other such pairs, C-5 in **16** exhibited an unmistakable coupling (${}^3J_{CH}$) of ~ 7 Hz with the CH_3 group, in addition to the large ${}^1J_{CH}$ coupling, while it did not do so in the spectrum of **15**, wherein C-5, being quaternary, did not give rise to an analyzable signal. C-4 did give rise to a prominent signal, but did not couple with the CH_3 protons since four-bond C-H couplings are too weak to be measurable.

Similar techniques helped to assign structures to the isomeric pair of dinitromethylpyrazoles (**18**) and (**19**) obtained from **17**.

Conformational studies—The ^{13}C spectra have been of diagnostic help both qualitatively and quantitatively in studying the conformation of rigid and flexible molecules. Methylcyclohexane has the methyl group preferentially in the equatorial position at room temperature. At −110°, the ^{13}C NMR spectrum (Fig. 1) reveals in addition to the strong lines due to the



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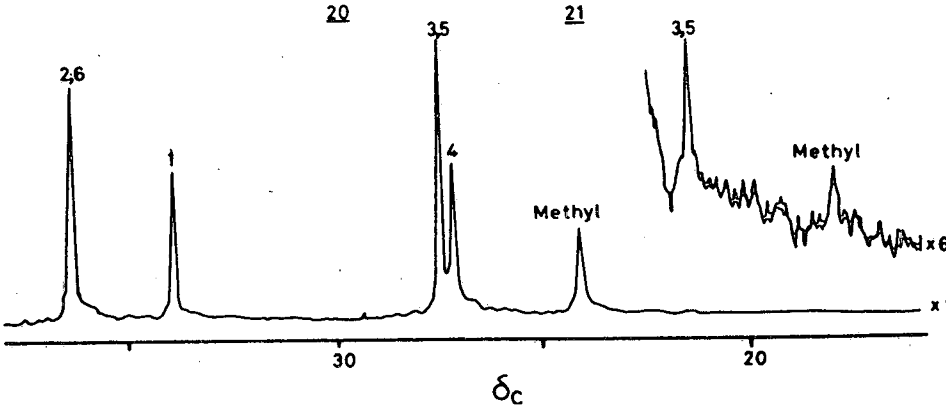
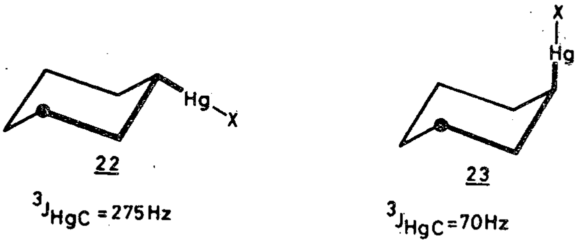


Fig. 1—63.1 MHz proton noise decoupled ^{13}C spectrum of neat methylcyclohexane at −110°C

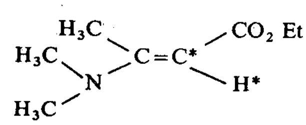
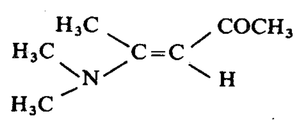
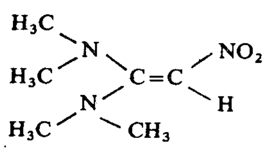
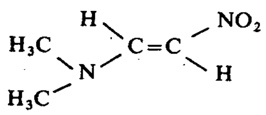
equatorial conformer (**20**), weak resonances at the high field of the spectrum, with chemical shifts in agreement with those expected for C-3, C-5 and CH_3 in the axial form (**21**). An equilibrium constant of about 100 was computed which was in agreement with the energy difference of 1.6 kcal/mol, between the equatorial and axial conformations[12]. The ^{13}C NMR spectroscopy shows that in cyclohexyl mercuric derivatives, the axial conformer (**23**) predominates over the equatorial one (**22**) (ref. 13). Assignments are made on the basis of vicinal ^{13}C-^{199}Hg coupling constants which obey the Karplus relationship (Scheme 6).



Scheme 6

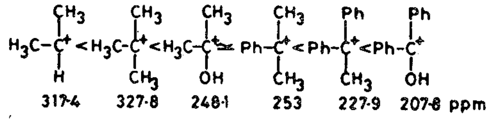
Chemical reactivity studies—We had correlated some years back the chemical shifts of enaminic protons with reactivity in a number of cycloalkanones[14]. Recently, their ^{13}C NMR spectra were studied[15,16] and the work was extended to ^{13}C NMR spectra of a number of amino-nitro and amino-acyl ethylenes[17,18]. The data given in Table 1 show that these correlate well with each other and also with relative reactivities and electron densities obtained by extended Huckel calculations. The more reactive enamines show smaller chemical shifts (from TMS) for the enaminic carbon and proton in keeping with greater electron availability at the carbon centre.

Table 1—Correlation of Reactivity of Amino-nitro and Amino-acyl Ethylenes with ^{13}C and ^1H Chemical Shifts

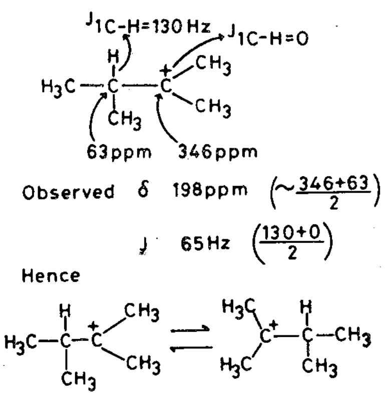
	δ_C	δ_H	$J_{C,H}$
	84	4.53	152
	93.7	5.03	151.5
	100.9	6.30	186
	111.4	6.64	188.3

Recently, an interesting ^{15}N study of enamines has been reported[19].

Carbonium ions and rearrangements—Carbonium ions carrying (formal) positive charges are expected to show carbon resonances at very low yields. Further, their relative stabilities can be guessed from the chemical shifts, since the stabler ones will have more extensive delocalization. Data given in Scheme 7 bear this out generally[20]. Rapidly equilibrating carbonium ions can be nicely studied by ^{13}C NMR. The spectrum of dimethylisopropyl carbonium ion shows two resonances, one for the methyl groups and the other for the central carbon atom at 198 ppm, with a $^1J_{CH}$ of 65 Hz. This is interpreted in terms of two species in a fast equilibrium (Scheme 8). Empirical calculations show that the central carbon atoms should show shifts of 63 ppm for the non-charged and 346 ppm for the charged ones. Rapid degenerate equilibrium should lead to an average value of 204.5 ppm. The coupling constant of 65 Hz is also a result of averaging of 130 Hz and 0 for the two ionic sites[21]. The ^{13}C NMR spectra have been used to confirm that arenium ions postulated as intermediates in electrophilic substitution are σ complexes of type (**24**) (ref. 22), and to vindicate the existence of bromonium ions of the type (**25**) (ref. 23).

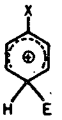


Scheme 7


J_{1C-H}=130 Hz
J_{1C-H}=0
63 ppm
346 ppm
Observed δ 198 ppm $\left(\sim\frac{346+63}{2}\right)$
J 65 Hz $\left(\frac{130+0}{2}\right)$
Hence

Scheme 8

Studies with ^{13}C-enriched molecules—The advent of ^{13}C NMR spectroscopy and the availability of ^{13}C-enriched organic substrates have simplified the elucidation of reaction mechanisms in synthetic and biogenetic studies. The technique is facile and non-destructive and avoids completely, laborious and painful chemical degradation studies which ^{14}C labelling engenders.



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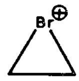
(E=electrophile)



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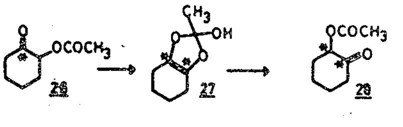
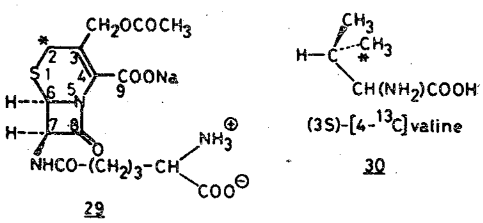
Reaction mechanism studies—The labelling of C-1 of 2-acetoxycyclohexanone (26) with ^{13}C has helped to establish its degenerate rearrangement on heating with acetic acid and potassium acetate to 142°C. The ^{13}C spectrum reveals equal distribution of the label between positions 1 and 2. The symmetrical intermediate (27) becomes thus implicated (Scheme 9) (ref. 24).



Scheme 9

Application in biosynthetic studies—Valine is known to be a substrate in the biosynthesis of β-lactam antibiotics. It was of interest to study its incorporation in the biosynthesis of cephalosporin (29), since it was speculated that one of the isopropyl methyl groups ended up as C-2 of the antibiotic. Incubation of the appropriate culture with (2*RS*, 3*S*)-[4-^{13}C] valine (30) afforded cephalosporin whose ^{13}C NMR spectrum showed C-2 to be the exclusive site of enrichment (Scheme 10) (ref. 25).

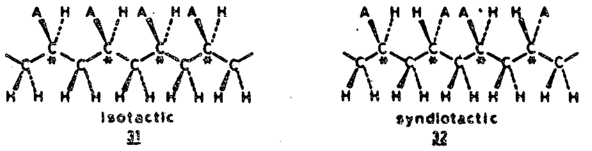


Scheme 10

^{13}C NMR Studies on Macromolecules

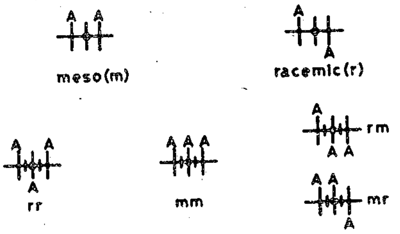
Application in synthetic polymers—^{13}C NMR spectroscopy has been dramatically successful in this area, in terms of detecting and analyzing the variety of isomers which are found in polymer chains. The stereochemical sequence has a direct bearing on the industrially useful physical, chemical and mechanical properties of these molecules. Although earlier a considerable volume of work had been carried out on the ^1H NMR spectra, this was severely limited by three factors: (i) the relative insensitivity of proton shifts to differences in environments which are necessarily small (a problem only incompletely tackled by the use of higher fields), (ii) complication due to spin-spin coupling, and (iii) signal broadening due to slow molecular motion resulting in dipolar spin-spin relaxation. The ^{13}C resonances are not so beset with these problems. With respect to the third difficulty, it is to be specially noted that high resolution ^{13}C spectra can be run on highly viscous solutions and in favourable cases, even on solids.

Polymer tacticity—Many polymers are derivatives of polyethylene. The polymerization of the vinyl unit occurs mostly head to tail, but sometimes head to head attachment also occurs. The stereotacticity of unsymmetrically substituted ethylene polymers is of greater theoretical and practical interest. In such polymers, chiral centres are introduced. 'Pure' stereoregular forms consist of isotactic (31) and syndiotactic (32) ones. In the former, the relative handedness of all asymmetric carbon centres (starred) is the same, while in the latter, it varies alternately (Scheme 11). There could be other degrees of regularity, or the molecule could have no configurational preference in which case it is called atactic.



Scheme 11

The sensitivity of ^{13}C shifts to slight changes in environment was already noted. It can, thus, be expected that in the vinyl polymer $(-CHA-CH_2)_n$, the methylene carbon will have different shieldings, depending on whether the adjacent tertiary carbon atoms have the same or different configurations (31) or (32). Two repeating units, referred to as dyads, can have an *r* or *m* arrangement, while three repeating units or triads can have an *rr*, *mm*, *rm* or *mr* arrangement (Scheme 12). Although the last two cannot be differentiated, they have to be reckoned with for purposes of measurement and analysis of relative intensities.



Scheme 12

The ^{13}C NMR spectrum of a low molecular weight polyvinyl chloride (Fig. 2) shows clearly tetrad and triad fine structures for the methylene and methine resonances respectively. The equation

$$N(n)=2^{n-2}+2^{m-1}$$

gives the number of observationally distinguishable *n*-ads, where $m=n/2$ or $(n-1)/2$, depending upon whether *n* is even or odd. For a triad, we can derive three types of sequences and for a tetrad six. The spectrum of PVC accordingly shows three sequences in the low field region for the methine and six in the high field region for the methylene resonances. The individual assignments within the triad and the tetrad groups are made by fitting the signal intensities to theoretically calculated probabilities[26]. The observation of such fine structures in ^1H NMR spectrum would require very high fields which only superconducting magnets can give. Similar useful insight into the fine structures of many other vinyl polymers such

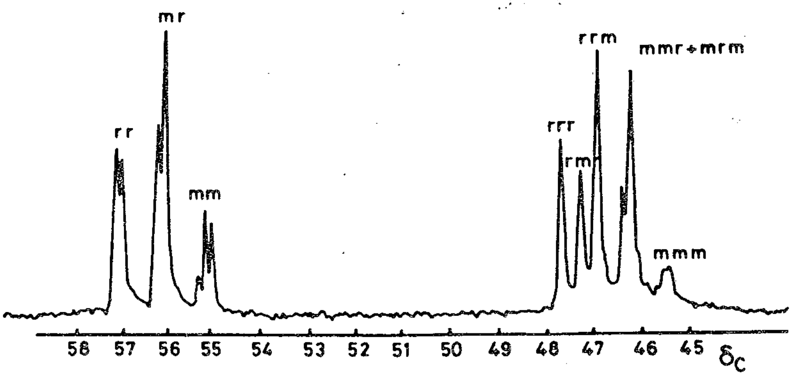
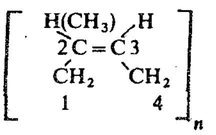


Fig. 2—25.2 MHz proton noise decoupled ^{13}C spectrum of a low molecular weight polyvinyl chloride (10%, w/v) in *o*-dichlorobenzene at 60°C

as polypropylene, polyacrylonitrile and polyvinyl ethers has also been gathered from ^{13}C NMR. ^{13}C NMR has also been a valuable tool in characterizing diene polymers such as polybutadiene and polyisoprene in terms of determining distinguishable double bond sequences. The 1,4-polybutadienes and 1,4-polyisoprenes have been thus examined. Both the *cis* and *trans*-forms are almost exclusively 1,4-polymers with the monomers arranged in a stereoregular, head to tail sequence having essentially all-*cis* or all-*trans* configurations. Solutions as well as bulk samples have been examined. The shifts determined[27] for *cis* and *trans*-1,4-polybutadiene and *cis* and *trans*-1,4-polyisoprene (natural rubber and gutta percha) are listed in Table 2. The differences between the shieldings for the isomeric polybutadienes and polyisoprenes are clearly discernible in terms of those observed for simpler model alkenes. Based upon this experience, even the spectrum of a synthetic polymer containing largely *cis* and *trans*-1,4-units (33 and 53% respectively) with minor amounts of 1,2-units (6%) and 3,4-units (8%) could be interpreted reasonably. Equally important has been the contribution of ^{13}C NMR spectroscopy to the characterization of copolymers such as ethylene-propylene copolymer[28] or methyl methacrylate-methacrylic acid[29] in terms of sequence distribution. Here again the greater sensitivity of carbon chemical shifts to minor environmental changes as compared to protons has been responsible for the popularity of the technique.

Biopolymers—The ^{13}C spectra have played an important role in understanding two- and three-dimensional structures of polypeptides. As is expected, there is a significant *p*H dependence of ^{13}C shifts in amino acids and peptides. Ionization studies have helped structure elucidation of these molecules. Proteins in their native state have a defined helical conformation (α), which is stabilized by intramolecular hydrogen bonds. Lowering of *p*H or raising of temperature results in the disruption of these bonds and the conversion of the α-helix to a random coil. This phenomenon, which is accompanied by loss of biological activity, is reversible within limits and can be followed by ^{13}C NMR spectroscopy. The changes in the appearance of the ^{13}C spectrum of ribonuclease A in going from its native state (*p*H 6.55) to *p*H 1.46 can be seen in Fig. 3 and are interpreted in terms of helix-coil transformation[30].

Table 2—^{13}C Chemical Shifts of 1,4-Polybutadienes and 1,4-Polyisoprenes



Polymer	C-1	C-2	C-3	C-4	C-2 CH$_3$
cis-1,4-Polybutadiene	27.7	129.7	129.7	27.7	
trans-1,4-Polybutadiene	33.1	130.4	130.4	33.1	
cis-1,4-Polyisoprene (natural)	32.5	135.5	125.3	27.0	23.6
trans-1,4-Polyisoprene (natural)	40.0	135.2	124.6	27.1	16.0

^{1}H NMR Spectroscopy

The applications of FT NMR spectroscopy in the study of protons are too numerous to be reviewed. However, the conventional frequency-domain spectra have already provided adequate information. Some advantages of the new technique are considerably decreased sample requirement, (possibly) higher resolution and decreased operational time. Additionally, FT NMR offers the possibility of ready measurement of relaxation times.

An esoteric example is cited here which concerns normal and malignant human tissues. It has been found that malignant tissues exhibit a lower degree of ordering in cell water than is found in normal tissues. The pulse NMR method is ideal for the study of order-disorder phenomena in the cell water of muscle tissues, since the nuclear magnetic relaxation times of the cell water protons are strongly dependent on the freedom of movement of water molecules. An important feature of the NMR technique is that the measurements are non-destructive and cause no deleterious effects on biological tissues. By experience, one knows that a low degree of ordering, i.e. extreme freedom of movement

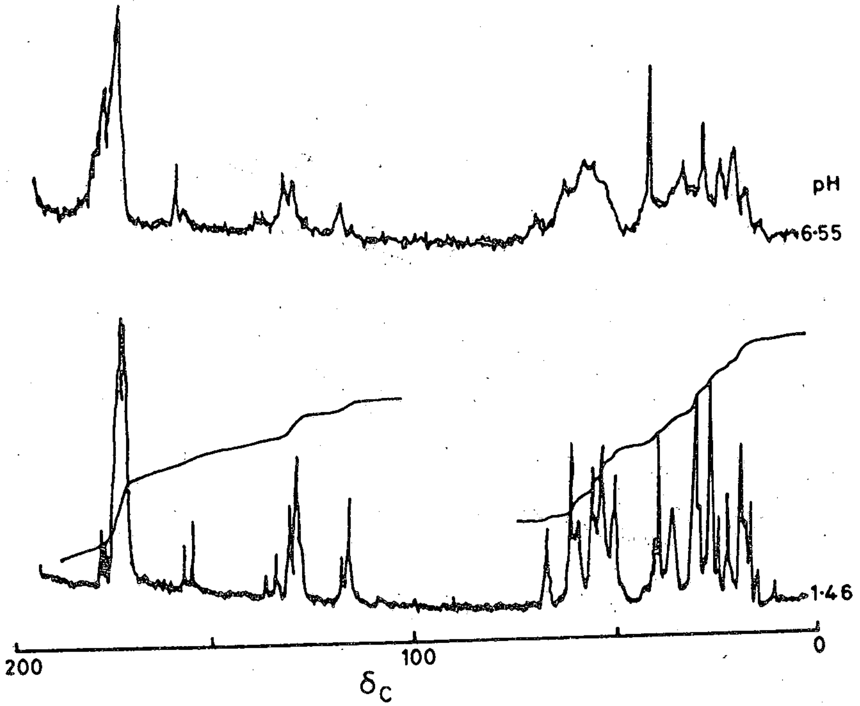


Fig. 3—15.1 MHz proton noise decoupled ^{13}C spectra of ribonuclease A (0.02 mol) in H_2O at various pH values

Table 3—Relaxation Times of Cell Water in Tissues

Tissue and histological results	Relaxation time (T_1) msec
Normal stomach tissue	804
Stomach adenocarcinoma	1060
Normal pectoral gland tissue	380
Cirrhosis mammacarcinoma	1080
Normal thyroid gland tissue	460
Solid thyroid gland carcinoma	770

of the intra- and extra-cellular bound water molecules should result in concomitant increase in the value of the spin-lattice relaxation times T_1 of the cell water protons. The values given in Table 3 bear this out. The tissue samples are all histologically examined before carrying out the NMR experiments in order to compare the accuracy of NMR as a diagnostic technique. A clear distinction can be noted between the relaxation times of water protons in healthy and malignant tissues[31,32].

It may be noted here parenthetically that T_1 data of ^{13}C may be used (i) as an additional criterion for assignments of quaternary carbons, especially in large molecules, (ii) for the assignment of protonated carbons, particularly when off-resonance spectra fail to simplify signal overlaps, and (iii) to differentiate between carbons which undergo internal reorientation and those which belong to the rigid backbone.

A recent publication reports on 'two-dimensional ^1H NMR spectroscopy'. This new technique allows efficient separation of multiplets by spreading the spectra in a second frequency dimension, leading to two-dimensional (2D) J-resolved ^1H NMR spectroscopy[33]. The 2-D spectrum can be thought to arise in the following manner. The conventional 1-D spectrum is placed along the horizontal line ($J = 0$) in the middle of the 2-D plot. Each multiplet is now rotated by 90° about its centre frequency. This process permits complete separation of chemical shifts, determining the centres of the multiplets displaced along the horizontal axis, and of multiplet splittings, determining the peak positions along the horizontal axis.

^{31}P NMR Spectroscopy

The spectra of ^{31}P nuclei can be obtained readily because of favourable natural abundance and magnetic moment. Nevertheless, the relative sensitivity with respect to ^1H is about $\frac{1}{16}$. FT NMR spectroscopy represents a significant advance over the earlier techniques, and makes it easy to study several biochemical phenomena like enzyme-substrate interactions[34].

FT NMR Spectra of Other Nuclei

FT NMR instrumentation has now made it possible to measure the spectra of practically every nucleus with a spin, e.g. of ^2H and ^{17}O in their natural abundance in water[35-37]. Particularly useful have been the possibilities now available for the study of the ^{15}N nucleus which has important biochemical implications.

FT NMR Spectra of Solids

Some of the most useful applications have been in the study of ^1H and ^{13}C spectra of polymers. The spectrum of polyethylene (Fig. 4) shows that both translational and rotational motions are very slow. The spectrum displays the full chemical shift