NUCLEAR MAGNETIC RESONANCE IN POLYMER STUDIES

STANLEY R. JOHNS
STANLEY ROY JOHNS 1935-1986

Stanley Roy Johns was born in Cessnock, N.S.W. on 8 July, 1935. His secondary education was at Cessnock High School, and then he went to the University of New England, graduating B.Sc. (Hons) in 1957. In 1957 he was a Teaching Fellow at the University of New South Wales, then he proceeded to the University of Sydney as a NSW State Cancer Council Research Student (1958-1960) to work for his Ph.D. degree under Professor C.W. Shoppee. After graduating Ph.D. in 1961, he was a Burroughs Wellcome Research Fellow for the period 1961-1962 at the Department of Pharmaceutical Chemistry, University of Sydney where he worked in collaboration with Professor S.E. Wright. During 1962-1964 he was a Postdoctoral Fellow at the NRC (National Research Council) Ottawa, Canada where he worked with Dr. Leo Marion. In 1964 he was appointed as Research Scientist at the CSIRO Division of Organic Chemistry that later became the Division of Chemicals and Polymers. He was a Visiting Associate at the California Institute of Technology for six months during 1972 with J.D. Roberts. In a highly productive collaboration with Dr. J.A. Lamberton on the Chemistry of Australian plant extractives, Stan Johns' contribution was mainly in the applications of 1H and 13C n.m.r. spectroscopy in structure determination. He also applied 13C n.m.r. spectroscopy in a study of the structure and shape changes in biomembranes, and used various n.m.r. spectroscopic techniques in studies of the structure and stereochemistry of polymers. In 1983 he was one of six Australian scientists invited to participate in a joint Australian, West German Science Agreement Workshop on the "Biophysics and Biochemistry of Photosynthesis" held at Osnabruck in West Germany.

Stan Johns was very active in affairs of the Royal Australian Chemical Institute (RACI) - from 1978 to 1984 he was a member of the Executive Council, and in 1979 he was President of the Victorian Branch. His death from cancer at the relatively young age of 51 deprived CSIRO of an excellent chemist, and a popular colleague.

Honours and Awards

1980 Liversidge Research Lecture, Royal Society of New South Wales

Biographical Source


(2) Willing, R.I., personal communication.

Scientific Publications of S.R. Johns

A list of 157 publications by S.R. Johns is given here. A large number of these were on the structure determination of alkaloids from Australian plants, carried out in collaboration with his CSIRO colleague Dr. J.A. Lamberton.
Scientific Publications of S.R. Johns

(A) Pre-CSIRO


(B) CSIRO - Papers on Natural Products Chemistry


The following list of papers on nuclear magnetic resonance studies of polymers was kindly supplied by Mr Ian Willing.


148. Coddington JM, Johns SR, Willing RI, Kenrick JR and Bishop DG. Monolayer and 
$^{13}$C NMR studies on the interaction between Melittin and chloroplast lipids. 

149. Johns SR, Rizzardo E, Solomon DH, and Willing RI. Identification of end groups in 

150. Moad G, Solomon DH, Johns SR, and Willing RI. Fate of the initiator in the 
azobis(isobutyronitrile)-initiated polymerization of styrene. Macromolecules, 1984, 
17, 1904-9.

151. Moad G, Rizzardo E, Solomon DH Johns SR, and Willing RI. Application of 
$^{13}$C-labelled initiators and $^{13}$C NMR to the study of kinetics and efficiency of 
793-798.

152. Johns SR, and Willing RI. $^{13}$C Chemical shift and $^{13}$C,$^{13}$C coupling constant 
assignment of quaternary carbons by double quantum coherence. Use of the doubtful 
pulse sequence in natural abundance $^{13}$C NMR spectra. Magn. Reson. in Chemistry, 
1985, 23, 16-17.

The detection of preferred conformation in oligomers of methyl methacrylate in 
1985, 1355.

154. Moad G, Solomon DH, Spurling TH, Johns SR and Willing RI. Tacticity of 
poly(methyl methacrylate). Evidence for a penultimate group effect in free-

155. Moad G, Rizzardo E, Solomon DH, Johns SR, and Willing RI. 
$^{13}$C-$^{1}$H Heteronuclear chemical shift correlation spectroscopy applied to 
poly(methyl(carbonyl-$^{13}$C) methacrylate: An unambiguous method for assigning 

156. Johns SR, Willing RI, and Winkler DA. Conformational studies of methyl 
methacrylate by NMR and potential energy calculations: The origin of the acyclic 

NMR studies of proanthocyanidin polymers (condensed tannins). Magn. Reson. in 
NUCLEAR MAGNETIC RESONANCE
IN POLYMER STUDIES*

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ABSTRACT. $^{13}$C nmr spectroscopy is now extensively used in both synthetic and biological polymer studies. Structural and dynamic properties of a polymer can be determined from the $^{13}$C nmr chemical shift and relaxation time parameters. Examples from both the synthetic and biological fields are presented together with descriptions of new techniques for the analysis of end groups and the determination of tacticity in synthetic polymers.

Introduction

In his will the late Professor A. Liversidge requested that this lecture should be "for the encouragement of research in Chemistry.... and shall not be such as are termed popular lectures dealing with generalities.... but shall be such as will primarily encourage research and stimulate the lecturer and the public to think and acquire new knowledge by research instead of merely giving instructions". Further he directed that "the lecture shall be upon recent researches and discoveries and the most important part of the lecturers' duty shall be to point in which direction further researches are necessary and how he thinks they can best be carried out".

I hope to fulfill these requirements in this lecture by describing some of our recent work in the field of synthetic and biological polymers using the versatile physical technique, nuclear magnetic resonance (nmr) spectroscopy. In the past 35 years the nmr technique has crossed many discipline barriers. After its discovery and initial description by physicists in the mid 1940's (Block, 1946; Purcell, 1946), chemists and particularly organic chemists, have developed the technique over the next 30 years into a powerful tool for the study of structure and motion within organic molecules. The method is now used extensively in the fields of polymer and biological sciences and it is some of these applications I wish to discuss today.

I shall concentrate on $^{13}$C nmr and after an initial introduction to the technique and a description of the two most important parameters of chemical shift and relaxation time, I shall discuss some of our recent research. Firstly I shall describe our work dealing with structural studies of synthetic polymers, and with new methods for the analysis of end groups and for the determination of the tacticity of synthetic polymers. I shall then turn to our studies of biological polymers, and in particular to the use of relaxation times in the study of motion and interaction in lipid bilayers. We expect that this work should lead to an understanding of lipid/protein interactions and the overall function of biological membranes.

\textbf{13C Nmr Theory}

The $^{13}\text{C}$ nucleus has a quantum spin number of $1/2$ and when a nucleus is placed in an applied magnetic field ($H_0$) it precesses about the field with a characteristic frequency, $\omega_0$, (its Larmor frequency) such that $\omega_0 = -\gamma H_0$ where $\gamma$ is the gyromagnetic ratio of the $^{13}\text{C}$ nucleus (Figure 1).

![Figure 1. Precession of a magnetic moment $\mu$ about a fixed magnetic field $H_0$.](image1)

In practice we never study a single nucleus but rather a collection of nuclei. Some nuclei precess in the direction of the applied field and some opposed to the field. At thermal equilibrium there are more aligned in the direction of the field (the lower energy state) than opposed which results in a net magnetisation ($M$) along the direction of $H_0$ (conventionally the z axis). (Figure 2).

The resonance condition in an nmr experiment is achieved by applying a second oscillating radio-frequency (rf) field ($H_1$) at right angles to $H_0$. Resonance occurs with an absorption of energy when the frequency of $H_1$ equals the Larmor frequency of the nucleus.

![Figure 2. Precession of a collection of identical nuclei with net macroscopic magnetisation ($M$) along the z axis.](image2)

The motion of the net magnetisation under the influence of two different fields, $H_0$ and $H_1$, is extremely complicated in the normal laboratory frame of reference and to simplify the discussion of pulsed nmr and relaxation times, it is convenient to consider the motion of $M$ in a co-ordinate system rotating about $H_0$ at the angular frequency $\omega_0$. This new co-ordinate system, designated $x'$, $y'$, $z'$, is called the rotating frame. In the laboratory frame, $H_1$ is rotating in the $xy$ plane but in the rotating frame this second field is fixed and, by convention, fixed along the $x'$ axis. Again, by convention, the receiver is placed at right angles to the transmitter i.e. along the $y'$ axis.

When an intense rf pulse ($H_1$) is applied for time $t_p$ the magnetisation $M_0$ along $z'$ will begin to precess about the new field ($H_1$) and in fact rotate through an angle $\theta$ radians.
where $\theta = \gamma H_1 t_p$ (Figure 3). By varying $t_p$ it is possible to rotate the magnetisation through any desired angle.

![Diagram of rotation of magnetisation (M_0) around H_1 field.](image)

**FIGURE 3.** Rotation of magnetisation ($M_0$) around $H_1$ field.

After the pulse is turned off the spin system will relax back to its equilibrium position ($M_0$) according to two rate equations.

$$\frac{dM_z'}{dt} = -\frac{(M_z' - M_0)}{T_1}$$

$$\frac{dM_y'}{dt} = -\frac{M_y'}{T_2^*}$$

where $T_1$ and $T_2^*$ are the longitudinal and transverse relaxation times respectively.

The signal in the Pulsed Fourier Transform (PFT) experiment is detected in the $y'$ axis and is given by

$$M_y' = M_0 \sin \theta e^{-t/T_2^*}$$

Obviously, the maximum signal is observed when $\theta = \pi/2$ (90°) i.e. when $\sin \theta = 1$. If we plot the signal strength versus time we have a free induction decay (FID) or time domain spectrum (Figure 4), which on Fourier transformation gives a frequency domain spectrum. This is the case for a collection of nuclei irradiated at the Larmor frequency ($\omega_0$).

![Experimental FID of magnetisation along y' axis](image)

**FIGURE 4.** Experimental FID of magnetisation along $y'$ axis

However, if the pulse frequency is slightly off-resonance by $\Delta \nu$ (as is the usual case) the FID will be modulated by a term $\cos 2\pi \Delta \nu t$ which gives a modulated FID (Figure 5) given by

$$M_y' = M_0 \sin \theta e^{-t/T_2^*} \cos 2\pi \Delta \nu t$$

![Modulated FID for off-resonance signal.](image)

**FIGURE 5.** Modulated FID for off-resonance signal.

Because of the non-uniform distribution of electrons within an organic molecule, nuclei in different chemical environments within the molecule resonate at slightly different frequencies.
Therefore, in a normal nmr experiment, we obtain a series of modulated FID's i.e. an interferrogram and this signal is given by

\[ M_y = \sum_i M_0 \sin \theta_i e^{-i\gamma \tau^2} \cos 2\pi \Delta \nu_i t \]

Fourier transformation of such an interferrogram gives a frequency domain spectrum made up of a number of resonance signals. These chemically induced differences in frequency, the chemical shift, are not recorded in absolute frequency units, but rather, in the form of a dimensionless quantity (\( \delta \)) defined with respect to the observing frequency

\[ \delta = \frac{H_C - H_R}{H_0} \times 10^6 \text{ ppm} \]

The reference standard is usually tetramethylsilane, arbitrarily set at \( \delta = 0.00 \text{ ppm} \).

Experimentally, it is easier to measure the longitudinal relaxation time, \( T_1 \) than the transverse relaxation time \( T_2^* \). A number of multiple pulse sequences can be used but the most common is the so-called 'inversion/recovery' method (Vold, 1968) which involves a 180° - \( \tau \) - 90° pulse sequence (Figure 6).

The first 180° pulse applied along the \( x' \) axis inverts the magnetisation into the \(-z'\) axis. Longitudinal relaxation occurs causing \( M_{z'} \) to change from a value of \(-M_0\) through zero to the equilibrium value \( M_0 \) with time. If at a time \( t \), after the 180° pulse, a 90° pulse is applied along \( x' \), \( M_{z'}(t) \) is rotated into the \( y' \) axis and a FID is detected. For signal averaging purposes, relaxation must be complete before the pulse train is repeated. Usually a time \( T \) is set such that \( T = 5 \times T_1 \) and the pulse train \((180°-\tau-90°-T)_n\) is used. The equation of motion of \( M_{z'} \) is given by

\[ \frac{dM_{z'}}{dt} = \frac{(M_{z'} - M_0)}{T_1} \]

At time \( t = 0 \), \( M_{z'} = -M_0 \)

Integration yields

\[ M_{z'}(t) = M_0 (1 - 2e^{-t/T_1}) \]

This equation is usually rewritten in the form

\[ \ln(A_\infty - A_t) = \ln 2 A_\infty - t/T_1 \]

where \( A_t = \) signal amplitude at time \( t \) and \( A_\infty = \) limiting amplitude at time \( 5 \times T_1 \)

\( T_1 \) is determined from a series of spectra (Figure 7) recorded at different \( t \) settings using either an exponential fit (Figure 8) or a linear fit and measurement of slope (Figure 9). It is possible to measure \( T_1 \) values of all the different carbon nuclei in a molecule by careful selection of \( t \) values.
FIGURE 7. $^{13}$C nmr (180° - $\tau$ - 90°) spectra of $n$-propanol for $t = 0.4$ to 13.0 sec. in 0.6 sec. steps.

FIGURE 8. Exponential plot of signal intensity versus time in (180° - $\tau$ - 90°) sequence.

FIGURE 9. Linear plot of $\ln(A_\infty - A_t)$ versus time in (180° - $\tau$ - 90°) sequence.

In many cases the $T_1$ values, as measured, are inversely proportional to a correlation time ($\tau_c$) which is defined as the time a particular nucleus spends in a particular position before moving through one radian. The correlation time is therefore a direct measurement of motion of the nucleus.

$$\frac{1}{T_1} = \frac{N\gamma_C^2\gamma_H^2}{r^6} \cdot \tau_c$$

where $N$ is the number of directly bound hydrogen atoms, and $r$ is the C-H interatomic distance.

The $^{13}$C chemical shift and longitudinal relaxation time parameters will both be used in the subsequent discussions on the nmr of polymers.

**Structure of Polymers**

An organic molecule can be represented by a single structure and this structure can be readily determined by nmr measurements. In a polymer, the nmr spectrum represents an 'average molecule' from which we can determine overall structural features and also a monomer distribution e.g., the percentages of monomers as structural entities in homopolymers and a ratio of monomer A to monomer B in a copolymer (Randall, 1977).
As an example I wish to describe our work on the cycopolymers used in the SIROTHERM process for the desalination of brackish water. Polymers for this process were derived by the cyanoisopropyl radical induced cyclopolymerization of diallylamines. Depending upon the initial position of attack and upon the subsequent cyclisation five, six or even seven membered ring structures are possible (Diagram 1). $^{13}$C nmr can be used successfully to distinguish between these polymer possibilities. The proton decoupled $^{13}$C nmr spectrum of the cycopolymer from $N$-methyl-$N,N$-diallylamine (Diagram 1, $R_1 = R_2 = H$) is shown in Figure 10, (Johns, 1976).

Comparison of this spectrum with those of a series of $\beta,\beta$-substituted pyrrolidines and piperidines (Hawthorne, 1976a) shows that the cyclic moieties in the polymer structure are of the pyrrolidine type (4a, 4b) and no piperidine type units (5a, 5b) are present. Specifically, $N$-methyl signals in the region 42.5 - 42.8 ppm can be assigned to $N$-methyls of cis- and trans-pyrrolidine units and no signals are observed in the 47 ppm region for cis- and trans-piperidine. The overall structure is therefore that of a polypyrrolidine and not a polypiperidine.

A full assignment of the spectrum indicates a 5:1 ratio of cis- and trans-polypyrrolidine. The downfield peak at 62.7 ppm can be assigned to the C2,5 methylene carbons of both cis- and trans-pyrrolidine rings while the two peaks at 41.9 and 41.5 ppm can be assigned to the C3,4 methine carbons of the cis-substituted rings and the peaks at 45.9 and 45.5 ppm to the trans-substituted rings. Similarly the remaining broadened peaks at 28.4 and 34.4 ppm can be assigned to the methylene carbons of the ethylene groups in the cis- and trans-pyrrolidines.
respectively. The splitting of the C3,4 methine signals into pairs of signals in the *cis*- and *trans*-structures depends upon the different dyad structures (Diagram 2) possible.

![Diagram 2: Dyad structures of polypyrrolidines (4a, 4b).](image)

In considering the chemical shifts of the methine carbons in these different structures, it is convenient to focus attention on the "inner" methine carbons of each dyad (labelled 3 and 3' in each case). We propose that the chemical shift of any particular one of these carbons is determined primarily by the stereochemistry of the pyrrolidine unit in which it resides and secondarily by the relative chiralities of C3 and C3' of the appropriate dyad (Johns, 1976). On this basis, the methine carbons fall into two main groups, each of which has two sub-groups.

**Group 1: Methine of *cis*-pyrrolidine units.**

(a) those in which there is a *meso* relationship between C3 and C3'

C3 of (6), C3' of (6), and C3 of (8)

(b) those in which there is a *racemic* relationship between

C3 of (7), C3' of (7), and C3 of (9)

**Group 2: Methine of *trans*-pyrrolidine units.**

(a) *meso* relationships between C3 and C3'

C3' of (8), C3 of (10), and C3' of (10)

(b) *racemic* relationships between C3 and C3'

C3' of (9), C3 of (11), and C3' of (11)

Two doublets are thus expected for the C3 signals in the average polymer spectrum.

A similar analysis of the spectrum (Figure 11) of the cyclopolymer from *N*-methyl-*N*,*N*-di-(2-methylallyl)amine (I, R1 = R2 = Me) reveals a mixture of both
polypyrrolidine and polypiperidine structures in approximately equal amounts with signals from \(N\)-methyl groups at both 44.2 and 47.1 ppm (Johns, 1976). The complexity of this spectrum can be explained and the different signals assigned when the possible combinations of the two structural types are considered.

Further studies (Hawthorne, 1975; Hawthorne, 1976b), on a series of polymers from \(N\)-methyl-\(N\)-allyl-\(N\)-(2-substituted allyl)amines have been made to determine the preferred site of initial radical addition to the diallylamine and the subsequent direction of cyclization. Steric interactions induced by \(\beta\)-substituents tend to favour attack at the unsubstituted allyl group whereas conjugated substituents favour attack at the substituted group with the consequent formation of conjugate-stabilized radicals. In all monosubstituted diallylamines, cyclization to the polypyrrolidine occurs except in the case of the \(t\)-butyl derivative where steric interactions induce cyclizations to both pyrrolidine and piperidine structures.

**End Group Analysis**

The initiation and termination (end) groups can have a significant effect on the properties of a polymer. This is particularly so if the groups are reactive and can be involved in polymer degradation. However, the identification and estimation of the end groups is difficult because of their low overall concentration in our 'average' molecule as detected by nmr.

We have used an inversion/recovery technique similar to that employed in the determination of longitudinal relaxation times, to assign signals to those carbons of the initiation and termination groups (Hawthorne, 1979). Because of the restriction of motion along a polymer chain, the \(T_1\) values of the backbone carbons of a cyclopolymer such as that from \(N\)-methyl-\(N\)-diallylamine (Figure 10) are expected to be quite short. By contrast the \(T_1\) values of the carbons of the end groups of the polymer chain will be much longer because of their greater mobility.

By choosing a time \(t\) in an inversion/recovery, 180° - \(t\) - 90°, pulse sequence such that the backbone carbons with short \(T_1\)'s have recovered their magnetisation in the positive \(z'\) axis, those signals will appear upright, while the signals from the carbons with the longer \(T_1\) values i.e. those of the initiation and termination groups, will retain magnetisation in the negative \(z'\) axis and give inverted signals.
A comparison of the spectra of the cyanoisopropyl radical induced cyclopolymer from N-methyl-N,N-diallylamine (MW ~ 2000, i.e. about 20 units) after t = 20 s. and t = 0.2 s. (Figures 12a and 12b) shows a number of inverted signals in Figure 12b that can be assigned to carbon atoms of the initiation and termination groups. Specifically, the signal at 31.7 ppm can be assigned to the methyl group of the initiating cyanoisopropyl radical and the signals at 14.6 ppm and 19.3 ppm to cis- and trans-methyl groups of terminating 3-methylpyrrolidine residues. The presence of the C-methyl groups on the terminating pyrrolidine indicates that termination occurs by an abstraction of hydrogen, probably from the solvent. These particular polymers are of low molecular weight but with state-of-the-art spectrometers that have greater dynamic range than our own the same approach should be possible with higher molecular weight polymers.

**Tactility Studies**

In attempts to determine the sequencing of monomer units (tactility) in a polymer it has been observed that the chemical shift sensitivity of a particular carbon may depend upon the configuration of from two (dyad) to seven (heptad) monomer units (Randall, 1977). The ensuing nmr signal from such a carbon can therefore consist of as many as thirty-six distinct resonance lines. The analysis of tactility is important in relating polymer structure to polymer properties but is not easy since line shape analyses are unreliable. An example is the signal from the substituted aromatic carbon of polystyrene (Figure 13) which is obviously made up of a number of closely spaced individual lines.
I wish to describe a two pulse, inversion/recovery type \((\alpha - \tau - \delta - 5T_1)_n\) \(^{13}\)C nmr sequence which induces different phase and intensity variations in the individual lines within such peaks and which, by varying the value of \(\tau\) can produce a set of different peak shapes all of which must fit the same parameter set of chemical shifts and intensities (Johns, 1980).

The sequence (Figure 14) consists of an initial pulse which rotates the equilibrium magnetisation \((M_0)\) through an angle \(\langle \alpha \rangle\) such that a significant component of the magnetisation exists in the xy plane (we use an angle of 220\(^\circ\)). The time delay \(\langle \tau \rangle\) before the second pulse is set to less than the spin-spin relaxation time \((T_2^*)\) so that a magnetisation component remains in the xy plane at the commencement of the second pulse which rotates the magnetisation through a further angle \(\langle \delta \rangle\). During the time \(\tau\), the xy component of magnetisation precesses about the \(z'\) axis and by varying the time \(\tau\) a series of spectra can be obtained with phase and intensity variations arising from different excess precession angles. A series of different peak shapes can be experimentally produced.

![Figure 14. Two pulse sequence \((\alpha - \tau - \delta - 5T_1)_n\)](image)

The spin magnetisation during the two-pulse sequence can be described by the following equations:

\[
M_{\alpha} = R_y^{-1}(\beta) R_x^{\alpha}(\alpha) R_y(\beta) M_0
\]
\[
M_{\tau} = R_y(\theta K) SM_{\alpha} + (1 - e^{-i\Omega r_{1}}) M_0
\]
\[
M_{\delta} = R_y^{-1}(\beta) R_x^{\delta}(\delta) R_y(\beta) M_{\tau}
\]

where \(M_{\alpha}\) and \(M_{\delta}\) are the magnetisations after the \(\alpha\) and \(\delta\) pulses, \(M_{\tau}\) the magnetisation following the time delay \(\tau\), \(R_y(\beta)\), \(R_x^{\alpha}(\alpha)\) and \(R_x(\theta)\) are rotation matrices which rotate the magnetisation about the \(y'\), \(x''\) and \(z'\) axis respectively. The angle \(\theta\) is the precession angle of the magnetisation during the time \(\tau\) about the \(z'\) axis and \(\Theta\) is the excess precession angle defined as

\[
\Theta = 2n\pi + \theta = (\Omega - \omega_1)r
\]

where \((\Omega - \omega_1)r\) is the offset frequency

\(S\) is the relaxation matrix given by

\[
S = \begin{bmatrix}
0 & 0 & 0 \\
0 & e^{-T_1^{\perp}} & 0 \\
0 & 0 & e^{-T_2^{\perp}}
\end{bmatrix}
\]

Evaluation of \(M_{\delta}\) gives a system of equations which provide the data necessary to define the spin state after the \(\delta\)-pulse and these equations have been adapted to a Fortran programme to calculate a theoretical spectrum.
The simulation of a number of different peak shapes, derived from different $\tau$ values for a single set of chemical shifts and intensities should confirm the analysis of a multiline resonance peak.

Figures (15a, b, c) show the experimental $^{13}$C nmr spectrum of the fully substituted aromatic carbon signal of polystyrene in CDCl$_3$ using a single FT pulse sequence, a $(220^\circ\cdot 0.2\text{ s.} - 110^\circ - 5 \times T_1)_n$ sequence and a $(220^\circ\cdot 0.35\text{ s.} - 110^\circ - 5 \times T_1)_n$ sequence.

![FIGURE 15. $^{13}$C nmr spectrum of the fully substituted aromatic carbon signal of polystyrene in CDCl$_3$ using a](image)

(a) Single FT pulse sequence.
(b) $(220^\circ\cdot 0.2\text{ s.} - 110^\circ - 5 \times T_1)_n$ pulse sequence.
(c) $(220^\circ\cdot 0.35\text{ s.} - 110^\circ - 5 \times T_1)_n$ pulse sequence.

Figures (16a, b, c) show the theoretical $^{13}$C nmr spectrum of the fully substituted aromatic carbon signal of polystyrene using 22 lines which correspond to the different chemical shifts and a $(220^\circ\cdot 20.0\text{ s.} - 110^\circ - 5 \times T_1)_n$ sequence, a $(220^\circ\cdot 0.2\text{ s.} - 110^\circ - 5 \times T_1)_n$ sequence and a $(220^\circ\cdot 0.35\text{ s.} - 110^\circ - 5 \times T_1)_n$ sequence.

![FIGURE 16. Theoretical $^{13}$C nmr spectrum of fully substituted aromatic carbon signal of polystyrene using 22 lines and a](image)

(a) $(220^\circ\cdot 20.0\text{ s.} - 110^\circ - 5 \times T_1)_n$ pulse sequence.
(b) $(220^\circ\cdot 0.2\text{ s.} - 110^\circ - 5 \times T_1)_n$ pulse sequence.
(c) $(220^\circ\cdot 0.35\text{ s.} - 110^\circ - 5 \times T_1)_n$ pulse sequence.

The good agreement between the experimental and simulated spectra confirms a heptad sensitivity to chemical shift for the fully substituted aromatic carbon of polystyrene although 14 of the 36 possible lines are coincident in shift within the experimental resolution.

Of course the complete assignment of the tacticity broadened lines in the $^{13}$C nmr spectrum of polymers will require the analysis of a number of samples prepared under different conditions with different monomer sequences, but the method should prove an excellent technique for the analysis of such spectra.

**Biological Polymers**

The $^{13}$C nmr technique is equally suited to the study of biological polymers. Our own particular interest is in the use of $^{13}$C nmr as a means of probing the structure and function of biological membranes. A membrane is considered to be made up of a mosaic of globular
proteins within a lipid bilayer (Singer, 1972) sometimes described as "icebergs in a lipid sea". The properties of the membrane depend particularly upon the fluidity of the lipid bilayer and the measurement of $^{13}$C longitudinal relaxation times provides an excellent method for the study of motion within these lipid bilayers.

The particular membrane on which we have focussed our attention is the inner chloroplast membrane of green plants. This is where the light trapping, energy transfer and energy storage of photosynthesis takes place. The chloroplast membrane system, unlike most biological membranes, consists principally of neutral glycolipids rather than the charged phospholipids of other organelle and cellular membranes. The photosynthetic membranes are composed of approximately 50% protein and 50% lipid, the lipid composition comprising the four types; MGG (40%) (12), DGG (40%) (13), SL (10%) (14), and PG (10%) (15).

The fatty acid composition of the lipids shows a remarkably high concentration of $(9Z,12Z,15Z)$-octadeca-9,12,15-trienoic acid ($\alpha$-linolenic acid) which may comprise over 90% of the acylating acids in DGG and MGG, 50% in SL and up to 30% in PG.

We have isolated and purified these lipids and measurement of their $^{13}$C nmr spectrum in d$_4$-methanol, e.g. that of DGG (Fig. 17) allows the assignment of all carbons in the molecules. We have measured the $^{13}$C longitudinal relaxation times in different solvent systems, calculated the correlation times ($\tau_c$) (Table 1) and interpreted these in terms of different secondary structures in the different solvents (Johns, 1977a). In methanol, correlation times

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**FIGURE 17.** $^{13}$C nmr of DGG in d$_4$-methanol.
**TABLE 1**

$^{13}$C Longitudinal Relaxation Times (sec) and Correlation Times ($x \times 10^{-11}$ sec) in Brackets of DGG in Various Solvents.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>CD$_3$OD</th>
<th>CDCl$_3$</th>
<th>D$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.30 (7.8)</td>
<td>0.09 (26)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.25 (9.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.53 (4.4)</td>
<td>0.26 (9.0)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.72$^a$ (3.2)</td>
<td>0.47$^a$ (5.0)</td>
<td>0.26$^a$ (9.0)</td>
</tr>
<tr>
<td>6</td>
<td>0.72$^a$ (3.2)</td>
<td>0.47$^a$ (5.0)</td>
<td>0.26$^a$ (9.0)</td>
</tr>
<tr>
<td>7</td>
<td>0.91 (2.6)</td>
<td>0.72 (3.2)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.5 (1.6)</td>
<td>0.84 (2.8)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.1 (1.1)</td>
<td>1.2 (1.9)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.1 (1.1)</td>
<td>1.3 (1.8)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2.9$^b$ (0.80)</td>
<td>2.1 (1.1)</td>
<td>0.88$^b$ (2.7)</td>
</tr>
<tr>
<td>12</td>
<td>3.9 (0.60)</td>
<td>2.6 (0.90)</td>
<td>0.83 (5.6)</td>
</tr>
<tr>
<td>13</td>
<td>3.9 (0.60)</td>
<td>2.6 (0.90)</td>
<td>0.83 (5.6)</td>
</tr>
<tr>
<td>14</td>
<td>2.9$^b$ (0.80)</td>
<td>3.0 (0.78)</td>
<td>0.88$^b$ (2.7)</td>
</tr>
<tr>
<td>15</td>
<td>7.6 (0.31)</td>
<td>7.1 (0.33)</td>
<td>2.3 (2.0)</td>
</tr>
<tr>
<td>16</td>
<td>7.4 (0.32)</td>
<td>6.9 (0.34)</td>
<td>2.3 (2.0)</td>
</tr>
<tr>
<td>17</td>
<td>11.1 (0.21)</td>
<td>9.5 (0.25)</td>
<td>3.0 (0.78)</td>
</tr>
<tr>
<td>18</td>
<td>8.9 (0.17)</td>
<td>7.8 (0.20)</td>
<td>3.6 (0.43)</td>
</tr>
</tbody>
</table>

Glycerol chain

<table>
<thead>
<tr>
<th>Carbon</th>
<th>CD$_3$OD</th>
<th>CDCl$_3$</th>
<th>D$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.10 (24)</td>
<td>0.03$^c$ (100)</td>
<td>0.08$^d$ (30)</td>
</tr>
<tr>
<td>2</td>
<td>0.16 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.10 (24)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Galactoside groups

<table>
<thead>
<tr>
<th>Carbon</th>
<th>CD$_3$OD</th>
<th>CDCl$_3$</th>
<th>D$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>1'</td>
<td>0.16 (30)</td>
<td>0.035 (800)</td>
<td></td>
</tr>
<tr>
<td>2'</td>
<td>0.16 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3'</td>
<td>0.15 (32)</td>
<td>0.035 (800)</td>
<td></td>
</tr>
<tr>
<td>4'</td>
<td>0.12 (40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5'</td>
<td>0.15 (32)</td>
<td>0.035 (800)</td>
<td></td>
</tr>
<tr>
<td>6'</td>
<td>0.07 (32)</td>
<td></td>
<td>0.06 (100)</td>
</tr>
<tr>
<td>1''</td>
<td>0.19 (25)</td>
<td>0.035 (800)</td>
<td></td>
</tr>
<tr>
<td>2''</td>
<td>0.17 (28)</td>
<td>0.04$^d$ (200)</td>
<td></td>
</tr>
<tr>
<td>3''</td>
<td>0.14 (34)</td>
<td>0.04$^d$ (200)</td>
<td></td>
</tr>
<tr>
<td>4''</td>
<td>0.18 (26)</td>
<td>0.04$^d$ (200)</td>
<td></td>
</tr>
<tr>
<td>5''</td>
<td>0.17 (28)</td>
<td>0.04$^d$ (200)</td>
<td></td>
</tr>
<tr>
<td>6''</td>
<td>0.19 (12)</td>
<td>0.03$^c$ (100)</td>
<td>0.08$^d$ (30)</td>
</tr>
</tbody>
</table>

$^a,b,c,d$ Averaged values obtained from unresolved signals.
of $2.8 \pm 0.4 \times 10^{-10}$ s. for all the glyceryl and galactosyl carbon atoms of DGG indicate that this portion of the lipid molecule undergoes rotation as a unit. The correlation times of the acyl chain carbon atoms however, decrease along the chain from C2 ($8.0 \times 10^{-11}$ s.) to the terminal methyl carbon ($1.7 \times 10^{-12}$ s.). This increase in motion arises from segmental motion in the acyl chain induced by $\beta$-coupled *trans-gauche* rotations about the C-C bonds. The total motion within the chain is derived from a combination of both molecular tumbling and segmental motion.

The longitudinal relaxation times and correlation times of the individual carbon atoms of the galactosyl and glyceryl moieties of DGG in chloroform are an order of magnitude different from the values in methanol. In chloroform, the motion of the carbon atoms in the polar regions of the molecules are severely restricted which indicates that the hydrophilic groups are associated and that the most likely secondary structure of the lipid molecule is one of an inverted micelle. The molecular tumbling of such a polymeric inverted micelle would be extremely slow which is consistent with the short relaxation times. By contrast, the motion of the acyl chain carbon atoms, which increase from C2 to the terminal methyl group carbons, are only slightly less than those of the monomeric form. Segmental motion along the acyl chain can again account for the observed values.

The correlation times of the glyceryl and galactosyl carbons of DGG in water correspond to a packing of the sugar moieties but in a structure in which the motion of the atoms is greater than in the inverted micellar structure. This is reflected in the broadened spectra of DGG in water (Figure 18). The correlation times of the acyl chain carbons reveal a more restricted motion than observed in the inverted micelles but once again segmental motion increases from the carbon atoms adjacent to the polar groups in the terminal methyl group carbons. The dynamic properties of the individual carbon atoms of DGG in water are therefore consistent with a bilayer structure with associated head groups at the water interface and a hydrophobic inner region comprised of acyl chains. Unlike the galactosyl lipids, SL is characterised by the presence of almost equimolar amounts of palmitic and $\alpha$-linolenic acids. Comparison of the longitudinal relaxation times of the carbon atoms in the two chains (Table 2) (Johns, 1978) shows that at all equivalent positions that can be resolved (Figure 19) the motion of the $\alpha$-linolenic acid chain is greater than that in the palmitic acid chain, but less than that of the same acid in DGG which contains two $\alpha$-linolenic acid molecules. The presence of the saturated acyl chain in SL obviously restricts motion in the unsaturated acyl chain to a greater extent than does a second unsaturated chain.
Table 2

\( ^{13}C \) Longitudinal Relaxation Times (sec) and Correlation Times \( \times 10^{11} \) sec in Brackets of SL in Various Solvents.

<table>
<thead>
<tr>
<th>Carbon</th>
<th>CDCl(_3)</th>
<th>CD(_3)OD</th>
<th>D(_2)O</th>
<th>CDCl(_3)</th>
<th>CD(_3)OD</th>
<th>D(_2)O</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \alpha)-Linolenic acid chain</td>
<td>Palmitic acid chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.14(^a)</td>
<td>0.29(^a) (8.0)</td>
<td>0.2(^a) (11.6)</td>
<td>0.14(^a)</td>
<td>0.29(^a) (8.0)</td>
<td>0.2(^a) (11.6)</td>
</tr>
<tr>
<td>3</td>
<td>0.30(^b) (7.8)</td>
<td>0.44(^b) (5.3)</td>
<td></td>
<td>0.30(^b) (7.8)</td>
<td>0.44(^b) (5.3)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.53(^c) (4.4)</td>
<td>0.67(^c) (3.5)</td>
<td>0.3(^c) (7.8)</td>
<td>0.53(^c) (4.4)</td>
<td>0.67(^c) (3.5)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>5</td>
<td>0.53(^c) (4.4)</td>
<td>0.67(^c) (3.5)</td>
<td>0.3(^c) (7.8)</td>
<td>0.63(^d) (3.7)</td>
<td>0.71(^d) (3.3)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>6</td>
<td>0.53(^c) (4.4)</td>
<td>0.67(^c) (3.5)</td>
<td>0.3(^c) (7.8)</td>
<td>0.63(^d) (3.7)</td>
<td>0.71(^d) (3.3)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>7</td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td></td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>8</td>
<td>0.87 (2.7)</td>
<td>1.0 (2.3)</td>
<td>0.3 (7.8)</td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>9</td>
<td>1.2 (3.5)</td>
<td>1.2 (3.5)</td>
<td></td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>10</td>
<td>1.2 (3.5)</td>
<td>1.2 (3.5)</td>
<td></td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>11</td>
<td>1.9(^f) (1.2)</td>
<td>2.6(^f) (0.90)</td>
<td>0.8(^f) (2.9)</td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>12</td>
<td>2.4 (1.7)</td>
<td>2.8 (1.5)</td>
<td>0.6 (7.0)</td>
<td>0.77(^e) (3.0)</td>
<td>0.86(^e) (2.7)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>13</td>
<td>2.4 (1.7)</td>
<td>2.8 (1.5)</td>
<td>0.6 (7.0)</td>
<td>0.63(^d) (3.7)</td>
<td>0.71(^d) (3.3)</td>
<td>0.3(^c) (7.8)</td>
</tr>
<tr>
<td>14</td>
<td>1.9(^f) (1.2)</td>
<td>2.6(^f) (0.90)</td>
<td>0.8(^f) (2.9)</td>
<td>2.4 (0.97)</td>
<td>2.5 (0.93)</td>
<td>0.6 (3.9)</td>
</tr>
<tr>
<td>15</td>
<td>3.6 (1.2)</td>
<td>4.6 (0.91)</td>
<td>1.0 (4.2)</td>
<td>3.6 (0.43)</td>
<td>6.5(^g) (0.24)</td>
<td>2.0(^g) (0.78)</td>
</tr>
<tr>
<td>16</td>
<td>6.0 (0.39)</td>
<td>9.7 (0.24)</td>
<td>2.1 (1.1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5.7 (0.27)</td>
<td>6.5(^g) (0.24)</td>
<td>2.0(^g) (0.78)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycerol chain\(^h\)  
| 1 | 0.03 (2500) | 0.07 (34) |          | 0.06 (2500) | 0.14 (34) | 0.05 (130) |
| 2 | 0.06 (2500) | 0.13 (37) | 0.05 (130) | 0.05 (2000) | 0.14 (34) | 0.05 (130) |
| 3 | 0.03 (2500) | 0.07 (34) |          | 0.05 (2000) | 0.13 (37) | 0.04 (200) |
| 4 | 0.04 (1400) | 0.13 (37) |          | 0.04 (1400) | 0.13 (37) | 0.05 (130) |
| 5 | 0.04 (1400) | 0.13 (37) |          | 0.04 (1400) | 0.13 (37) | 0.05 (130) |
| 6 | 0.03 (2500) | 0.08 (30) |          | 0.03 (2500) | 0.08 (30) | 0.03 (600) |

Sulphoquinovosyl group\(^h\)  
| 1 | 0.03 (2500) | 0.07 (34) |          | 0.06 (2500) | 0.14 (34) | 0.05 (130) |
| 2 | 0.06 (2500) | 0.13 (37) | 0.05 (130) | 0.05 (2000) | 0.14 (34) | 0.05 (130) |
| 3 | 0.03 (2500) | 0.07 (34) |          | 0.05 (2000) | 0.13 (37) | 0.04 (200) |
| 4 | 0.04 (1400) | 0.13 (37) |          | 0.04 (1400) | 0.13 (37) | 0.05 (130) |
| 5 | 0.04 (1400) | 0.13 (37) |          | 0.04 (1400) | 0.13 (37) | 0.05 (130) |
| 6 | 0.03 (2500) | 0.08 (30) |          | 0.03 (2500) | 0.08 (30) | 0.03 (600) |

\(^a\)\(^g\) Averaged values obtained from unresolved signals.  
\(^h\) Correlation times in CDCl\(_3\) are approximate.

This result indicates that the \(^{13}C\) nmr technique can distinguish different motions in carbon atoms which are at the same distance from the polar head group in the different acyl chains of a lipid molecule, unlike the ESR spin label technique which only measures the motion of a reporter molecule.

We are using vesicles prepared from DGG in water and vesicles prepared from the total chloroplast membrane lipids in water to study the effect of added components such as antibiotics, anaesthetics, proteins and peptides to the lipid bilayers. The polyene antibiotic, amphotericin B, has been shown to inhibit a number of membrane-associated reactions in higher plant chloroplasts. That the inhibitions result from effects on membrane fluidity is
clearly indicated by the relaxation time changes on addition of the antibiotic. (Table 3) (Bishop, 1978). The results indicate that the presence of the antibiotic causes a significant restriction in motion of the acyl chain carbons which is consistent with the occurrence of intercalation of the antibiotic between the acyl chains. Conversely, the addition of chloroform to the vesicles results in an increase in motion in the acyl chains.

**TABLE 3**

*Effect of Amphotericin B on the Motion of Acyl chains of Aqueous Multilayers of DGG*

<table>
<thead>
<tr>
<th>Carbon Atom</th>
<th>DGG</th>
<th>DGG plus Amphotericin B (10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/5/6</td>
<td>0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>11/14</td>
<td>0.88</td>
<td>0.91</td>
</tr>
<tr>
<td>12/13</td>
<td>0.83</td>
<td>0.91</td>
</tr>
<tr>
<td>15</td>
<td>2.3</td>
<td>1.2</td>
</tr>
<tr>
<td>16</td>
<td>2.3</td>
<td>1.4</td>
</tr>
<tr>
<td>17</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>18</td>
<td>3.6</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Continuing experimentation on peptides and proteins, specifically membrane associated proteins such as the photosynthetic protein of the chloroplast, should lead to conclusive results on the interaction of proteins and lipids. These will be of importance in a variety of fields ranging from the mechanism of aging to the fundamental processes of colour vision and photosynthesis.

**Future**

$^{13}$C nmr and other magnetic resonance techniques promise an exciting future for study in the synthetic and the biological polymer fields. The examples given have been restricted to
$^{13}$C solution spectroscopy, however, current technological development of instrumentation is such that deuterium and phosphorus nmr now offer great potential in the biological/medical fields and the use of solid state nmr techniques offers unlimited opportunities. I trust that this short overview of our own work in the nmr of polymers might inspire you to use the technique in your own studies and apply the techniques in new areas of research.

Acknowledgements

I would finally like to thank my collaborators in the work described viz, Drs. D.G. Bishop, J.M. Coddington, D.G. Hawthorne, D.H. Solomon and M.A. Yabsley and Messrs. R.I. Willing and D.R. Leslie.

References


