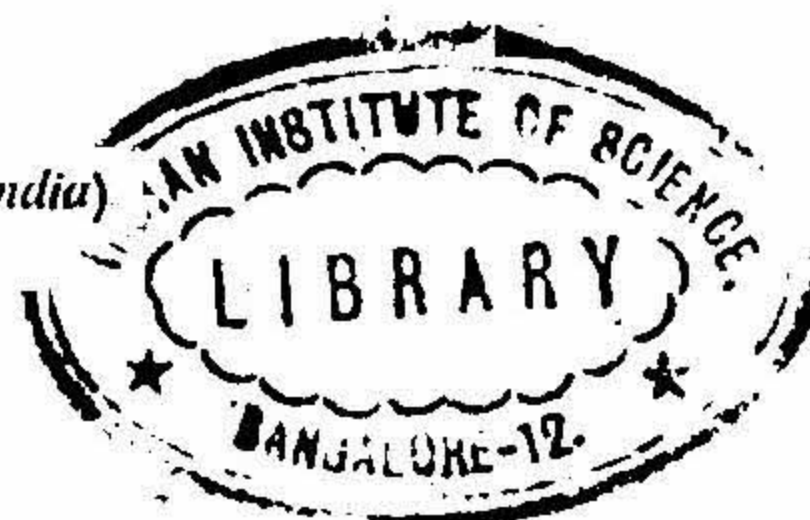


NMR SPECTROSCOPY OF MOLECULES DISSOLVED IN LIQUID CRYSTAL SOLVENTS

C. L. KHETRAPAL

(Raman Research Institute, Bangalore 560006, India)

Received on February, 26, 1974



ABSTRACT

Basic principles and the scope of the method of determination of molecular structure using liquid crystal solvents are discussed. Utility of the method is demonstrated with the help of some recent studies.

Key words: Liquid crystals, nematic phase, resonance frequency, chemical shift, indirect spin-spin coupling constant, direct dipolar couplings, magnetogyric ratio, anisotropy of the chemical shift and the indirect coupling tensors, orientation parameter, order matrix, peptide unit.

1. INTRODUCTION

As early as in 1963, Saupe and Englert [1] observed that the ^1H -nuclear magnetic resonance (NMR) spectrum of benzene in a liquid crystalline nematic phase [2] has a relatively complex appearance compared to a single line spectrum in an isotropic phase. The interpretation of this spectrum led to the discovery of a new method for the determination of molecular geometries. It provides the only available technique for the precise determination of the relative arrangement of nuclei in the liquid phase and is the most recent addition to the earlier existing list of methods for the determination of molecular structure, *e.g.*, X-ray, neutron and electron diffractions and the microwave spectroscopy.

During the span of about a decade from its discovery, the method has been extensively used [3-6]. Initial investigations were mainly centred round the understanding of the scope and the limitations of the method and hence only simple molecules of which the structure was already known were studied and the results compared with those obtained by other methods. During the past few years, considerable progress has been made towards the experimental and the theoretical aspects, resulting in a considerably broader applicability of the method.

2. BASIC PRINCIPLES

2.1. *Parameters affecting the high resolution NMR spectra.*—The entire discussion in the present article is restricted to high resolution NMR investigations in diamagnetic materials for nuclei with spin $\frac{1}{2}$ (in units of $h/2\pi$). Effects due to 'relaxation' phenomenon are not considered.

In an NMR experiment, transitions between adjacent Zeeman nuclear energy levels are introduced by an alternating field of frequency ν such that the energy gap between the two levels is $h\nu$. ν is called the resonance frequency and is given by

$$\nu = \frac{\gamma H}{2\pi} \quad (2.1)$$

where γ is the magnetogyric ratio and H the magnetic field at the site of the nucleus. Since nuclei are not isolated, the field at the site of the nucleus is the applied field modified by small contributions, depending upon the molecular structure. These effects are of the following type:

- (i) *The chemical shift.*—The electron cloud in an atom or molecule produces a magnetic field at the site of a nucleus and consequently the field at the nuclear site $((H_{\text{site}})_i)$ differs from the applied field (H_{applied}) and is given by

$$(H_{\text{site}})_i = H_{\text{applied}} (1 - \sigma_i) \quad (2.2)$$

where the dimensionless quantity σ_i is called the 'screening constant' for the nucleus i and depends upon the nuclear environments. The difference between the screening constants of two nuclei is called the 'chemical shift'. The magnitude of 'chemical shift' for protons may be of the order of 100 Hz when the operating frequencies are in the range of several MHz.

- (ii) *Indirect spin-spin coupling constant.*—An effect in which the nuclear spins interact *via* bonding electrons alters the field at the nuclear site. The magnetic moment of one nucleus induces polarisation in the surrounding electrons which in turn interact with the magnetic moment of the other nucleus through the electrons. For liquids and gases, the interaction energy between nuclei i and j with nuclear spins \vec{I}_i and \vec{I}_j is proportional to the scalar product $J_{ij} \cdot \vec{I}_i \cdot \vec{I}_j$. J_{ij} is called the nuclear spin-spin coupling constant.

It has a magnitude of the order of 10 Hz for protons. Its value increases for heavy nuclei but decreases with the number of intervening bonds between the nuclei.

- (iii) *Direct dipolar coupling constant.*—Nuclear spins, which behave like tiny magnetic dipoles, modify the field at the site of other nuclei by an interaction which operates through space. The magnetic field of a proton at the site of another proton separated by 1 Å may be several gauss, depending upon the angle which the inter-nuclear axis makes with the direction of the applied magnetic field. Such interactions (known as the direct dipolar interactions) are dominant in solids where due to little mobility, a given nucleus experiences effects from a large number of neighbours; the interaction is inter- or intra-molecular.

If we consider an isolated molecule with nuclei i and j separated by a distance r_{ij} and if θ_{ij} is the angle which the internuclear vector makes with the direction of the magnetic field, the nucleus i which can orient parallel or antiparallel to H_0 (the applied magnetic field) produces a field proportional to $(3\cos^2\theta_{ij} - 1)/r_{ij}^3$ at the site of j . Thus, the field at the site of j will be $H_0 \pm (K/r_{ij}^3)(3\cos^2\theta_{ij} - 1)$ where K is a constant for the pair of interacting nuclei; its resonance will be a doublet with spacing equal to $(2K/r_{ij}^3)(3\cos^2\theta_{ij} - 1)$. A similar effect will be observed on the resonance of i due to the presence of j .

In liquids and gases where the high resolution NMR spectroscopy is usually applied, all θ_{ij} values are equally probable due to rapid molecular motions and then the average value of $(3\cos^2\theta_{ij} - 1)$ vanishes. Consequently, normal high resolution NMR spectra in isotropic solutions are not influenced by the direct dipolar interactions and are, therefore, interpretable in terms of the first two parameters alone. This simplifies the situation considerably but at the same time results in the loss of information carried by the direct dipolar interactions. Furthermore, one cannot obtain information on the anisotropy of the chemical shift and the indirect coupling tensors from the high resolution NMR spectroscopy in isotropic media since the spectra are affected only by the trace of the tensors.

If the motion of the molecules is not isotropic, one gets non-vanishing values for the direct-dipolar couplings. By far, the nematic liquid crystals have served as the most widely and conveniently usable media for orienting the molecules.

2.2. *The Hamiltonian.*—It is clear from discussions of section 2.1 that the Hamiltonian for ‘oriented’ systems differs from that for the ‘isotropic’ cases in having additional terms due to direct couplings and the anisotropy of the chemical shift. The anisotropy of the indirect couplings has the same functional dependence as the direct couplings and hence is included in the direct coupling itself. Fortunately, this contribution has been shown to be negligible [3] for *HH* couplings and hence whatever ‘direct-dipolar couplings’ one obtains from the spectra are the ‘true dipolar couplings’ for protons. On the other hand, significant contributions of the anisotropy of the indirect *FF* couplings [7, 3, 5] have been observed. They will not be described in this article any more.

The Hamiltonian for the ‘oriented’ systems can, therefore, be written as follows:

$$H = - \sum (1 - \sigma_i - \sigma_{ia}) \nu_0 \cdot I_{zi} + \sum_{i < j} \sum (J_{ij} + 2D_{ij}) I_{zi} \cdot I_{zj} \\ + \frac{1}{2} \sum_{i < j} \sum (J_{ij} - D_{ij}) (I_i^+ \cdot I_j^- + I_i^- \cdot I_j^+). \quad (2.2.1)$$

In equation (2.2.1), σ_i and J_{ij} represent one-third of the traces of the corresponding tensors and are identical to the chemical shifts and the indirect spin-spin couplings in the normal high resolution NMR in isotropic media. σ_{ia} is the anisotropy of the chemical shift and D_{ij} is the direct dipolar coupling constant.

Equation (2.2.1) transforms to the Hamiltonian for an ‘isotropic’ case if D_{ij} and σ_{ia} are set zero. Conversely, the Hamiltonian of an ‘isotropic’ case transforms into that for an ‘oriented’ system if in the diagonal and the off-diagonal contributions to all the elements of the matrix, J_{ij} is replaced by $(J_{ij} + 2D_{ij})$ and $(J_{ij} - D_{ij})$ respectively and $(1 - \sigma_i - \sigma_{ia})$ substituted for $(1 - \sigma_i)$. Therefore, the method for the analysis of the spectra of ‘oriented’ molecules is essentially the same as the one for the ‘isotropic’ cases.

Several computer programmes are now available which can be used for the iterative analysis of spectra. Of these, LAOCOONOR [8] is the one which is used most commonly.

2.3. *The direct coupling and the ‘degree of order’.*—Theoretical treatment of the direct couplings in terms of the inter-nuclear distances and the

order parameters has been reported in the literature [9, 10]. The direct dipolar coupling D_{ij} between nuclei i and j is given by equation (2.3.1).

$$D_{ij} = -\frac{h\gamma_i\gamma_j}{4\pi^2} \cdot \frac{1}{2} \left\langle \frac{3 \cos^2 \theta_{ij} - 1}{r_{ij}^3} \right\rangle \quad (2.3.1)$$

where θ_{ij} is the angle between the magnetic field direction and the axis connecting nuclei i and j separated by r_{ij} and γ is the magnetogyric ratio. The average is taken over inter- and intra-molecular motion. If i and j belong to the same rigid part of the molecule, r_{ij} can be taken out of the angular brackets since it is then constant. In such a case the equation (2.3.1) reduces to (2.3.2)

$$D_{ij} = -\frac{h\gamma_i\gamma_j}{4\pi^2 r_{ij}^3} \cdot S_{ij} \quad (2.3.2)$$

where S_{ij} defines the degree of order of the axis ij . It may be mentioned that the equation (2.3.2) neglects influences of all types of molecular vibrations and takes into account only the 'average' molecular order. It may sometimes be an over-simplified picture of the real situation (*see*, for example, references [11–15]) but in most of the cases studied so far, it provides reasonably satisfactory results. In the present article, we will not consider problems arising out of such a simplification.

The S -values of different axes in the molecule are interdependent and the average order of a rigid molecule is given by a symmetric and traceless matrix $\{S\}$ with five independent elements. If X , Y and Z are the axes of a Cartesian coordinate system fixed within the molecule and θ_x , θ_y and θ_z are the angles between these axes and the magnetic field direction, the elements (S_{pq}) of the S -matrix are given by equation (2.3.3)

$$S_{pq} = \frac{1}{2} \langle 3 \cos \theta_p \cos \theta_q - \delta_{pq} \rangle \quad (2.3.3)$$

where $p, q = X, Y, Z$ and $\delta_{pq} = 1$ for $p = q$ and $\delta_{pq} = 0$ for $p \neq q$. The matrix elements S_{pq} are related to the S -values of an axis (S_a) forming angles α_x^a , α_y^a and α_z^a with the molecule fixed coordinate system according to equation (2.3.4).

$$S_a = \sum_{p,q} \cos \alpha_p^a \cdot \cos \alpha_q^a S_{pq} \quad (2.3.4)$$

By a suitable choice of molecular axes, the number of independent S -values necessary for the description of the orientation can be reduced from five to one depending upon the symmetry of the molecule. If the mole-

cule has a 3-fold or greater axis of symmetry, its selection as the Z-axis leaves S_{ZZ} to be the only independent orientation parameter. If there are two perpendicular planes of symmetry in the molecule both containing the Z-axis, with X and Y axes being in these planes, only two independent S-values are needed for the complete description of the molecular orientation. If the molecule possesses only one plane of symmetry, a selection of Z-axis perpendicular to this plane leaves S_{XX} , S_{YY} and S_{XY} as the only independent elements of the S-matrix.

2.4. *The chemical shift anisotropy.*—The anisotropy of the chemical shift (σ_{ia}) may be expressed in terms of the tensor components by equation (2.4.1).

$$\sigma_{ia} = \frac{1}{3} \sum_{p=X,Y,Z} \sum_{q=X,Y,Z} S_{pq} (\sigma_{pqi} + \sigma_{qpi}). \quad (2.4.1)$$

Molecular symmetry reduces the number of non-zero elements of the chemical shift tensor as reported in the literature [3].

2.5. *Anisotropy of the indirect spin-spin couplings (D_{ij}^{ind}).*—A substitution of J for σ in equation (2.4.1) provides the relations for anisotropic indirect coupling constants. J_{ia} thus obtained corresponds to $2D_{ij}^{ind}$.

In the rest of this article, we will concentrate on some structural results derived from the NMR spectra of 'oriented' molecules and hence we will not be discussing the chemical shift and the indirect coupling anisotropies.

2.6. *Obtainable structural information from the spectra of oriented molecules.*—The amount of information obtainable from the spectra of oriented molecules depends critically upon the molecular symmetry and the number of interacting nuclei. The number of relations which can be derived for the molecular geometry is equal to the difference between the number of independent direct couplings and the parameters needed to specify the molecular order. Thus, a system of less than 4 interacting nuclei does not provide any new geometry information. On the other hand, the spectrum becomes rapidly complex with the increase in the number of interacting nuclei. Systems containing 7 to 8 interacting nuclei can be handled but the time of computation drastically increases. It imposes a serious limitation on the wider applicability of the method. However, recently a novel technique [14, 16] (which effectively reduces the number of interacting nuclei) involving a selective deuteration of the molecule followed by heteronuclear spin decoupling has been suggested and used. Development of such

a technique is expected to make this method of determination of molecular geometries applicable for larger molecules also.

Equation (2.3.2) shows that if S_{ij} and R_{ij}^3 are changed by a constant factor, D_{ij} remains unchanged. Since no method is yet available for the precise determination of S_{ij} without the knowledge of an internuclear distance, NMR studies of oriented molecules do not allow the measurements of absolute distances. Only the relative distances and the molecular shapes can be determined.

3. EXPERIMENTAL DETAILS

3.1. *Preparation of the solution.*—If one uses a thermotropic nematic phase as the solvent, about 8–20 mole per cent solution of the solute is usually made. The temperature of the mixture is raised above the clearing point so as to make the solution homogeneous and then the mixture is brought back to the room temperature or the operating temperature. The solution thus prepared in the usual NMR sample tube is stored horizontally for a few days before recording the spectra. In the ‘conventional NMR spectrometers’ where the direction of the magnetic field and the axis of spinning of the sample are orthogonal to each other, spinning of the sample destroys the orientation. In such cases, the samples are, therefore, usually not spun although sometimes spinning at slow speeds helps to improve the line-widths [17]. Line-widths upto 3 Hz have been reported in such cases.

If one uses a lyotropic mesophase [3, 18, 19] about (1–3) % (by weight) of the solute is added to the ingredients of the phase and the mixture centrifuged for about an hour. The solutions thus prepared are transferred to the NMR sample tubes and kept for spinning in the magnetic field for several hours (≈ 10 hours) before the spectra are finally recorded. In such a case, the orientation of the phase is such that spinning of the sample about an axis perpendicular to the direction of the magnetic field does not destroy the molecular order. In this phase, lines of about (1–2) Hz width have been obtained.

Relative merits and demerits of the thermotropic and the lyotropic phases have been discussed in the literature [19].

3.2. *The spectra of the dissolved molecules.*—The solute molecules dissolved in a thermotropic or a lyotropic mesophase have free translational motion but the rotational motion is anisotropic. This results in averaging to zero of the intermolecular dipolar couplings but the intramolecular ones have still non-zero average value. The NMR spectrum of such a solution,

therefore, consists of relatively sharp lines (widths may be around 1 Hz in some cases) due to protons of the solute, superimposed over a broad background due to the solvent protons. The reason for the broad background of the solvent lies in the large number of interacting nuclei it contains. They give rise to an enormous number of allowed transitions such that the intensity of each individual line is too small to be observed, if a large number of lines is degenerate or nearly so, one gets a broad background. Sometimes, the background may be so broad that one can hardly see it. Consequently, the protons due to liquid crystal molecules either do not 'show up' or 'appear as broad background' which does not interfere with the spectrum due to solute molecules.

3.3. *Analysis of the spectra.*—In general, particularly for spectra obtained in a thermotropic mesophase, it is difficult to make a reasonably accurate guess on the magnitude of the various dipolar couplings. This is contrary to the spectra in the isotropic media where values of the indirect coupling constants can be guessed fairly satisfactorily. In the analysis of such spectra, the following steps are usually followed:

- (i) Equations relating the D -values, the molecular geometry and the order parameters are first written.
- (ii) A reasonable guess of the molecular geometry is then made and the values substituted in the equations written in step (i). The equations relating all the D -values and the independent number of S -values are thus obtained.
- (iii) One can then select an arbitrary value for one of the dipolar couplings and compute the others. If one is dealing with a one S -value case, the spectrum is essentially a linear contraction or expansion of the selected D -value (this assumes that the effects of the indirect couplings and the chemical shifts are much smaller than those of the dipolar couplings; it is usually a reasonable approximation for protons in thermotropic solvents) and hence the analysis becomes trivial if one uses the LAOCOONOR programme [8]. If one is dealing with a two S -value case, one has to calculate several spectra using Part I of the LAOCOONOR programme keeping one of the S -values fixed and varying the other. The observed and the calculated spectra are then compared. A calculated spectrum which resembles the observed one is usually found. Such a spectrum is then selected as the starting point for the final iterative analysis using the Part II of the LAOCOONOR programme.

For a 3 or more S -value case, the problem becomes more complicated and one may have to calculate quite a large number of trial spectra before one finds a spectrum resembling the observed one.

- (iv) Final analysis is made where the chemical shifts and direct couplings are all iterated upon. The indirect couplings are sometimes iterated upon but most of the time they are given the same values as those obtained from the analysis of the spectra in the isotropic media.

3.4. *Interpretation of the dipolar couplings.*—Having derived the dipolar couplings from the analysis of the spectra of 'oriented' molecules, the next step is to interpret them in terms of molecular geometry and the order parameters. If the number of independent dipolar couplings equals the sum of the geometrical informations and the independent order parameters to be derived, one can solve the simultaneous equations connecting the various parameters. If, however, the number of the independent dipolar couplings is less than the sum as defined above, one has to make assumptions on the molecular geometry; the number of such assumptions equals the number by which the direct couplings fall short of the 'sum' as defined earlier. If the number of the dipolar couplings exceeds the sum of the geometrical and the orientational parameters, an iterative procedure which computes the geometry and the order parameters by a 'weighted least square fit' method is used. The iterative programme SHAPE [20] has been most commonly used for such a purpose.

4. APPLICATIONS

During the span of a decade from the discovery, this method has so widely been applied to various structural and conformational problems that it is impossible to cover them all in this short review. However, an up-to-date picture of the work done can be obtained from references [3-6]. In the present chapter, the use of the method to some simple and recent cases which do not appear in references [3-6] is illustrated. The following problems are discussed:

1. Structure of pyridazine, pyrimidine, pyrazine, pyridine and pyridine-N-oxide dissolved in a lyotropic mesophase [19, 21].
2. The conformation 2, 2'-bithiophene [22].

3. ^{13}C -acetylene, its effective structure, shrinkage effects and apparent temperature dependent geometry [11].
4. Ring puckering vibrations in trimethylene oxide and trimethylene sulphide [23].
5. Conformation of the peptide unit [24].

1. *Structure of pyridazine, pyrimidine, pyrazine, pyridine and pyridine-N-oxide dissolved in a lyotropic mesophase.*—Spectra of these compounds have been studied in the lyotropic mesophase formed by a 14:1:1:20 mixture (by weight) of sodium dodecyl sulphate, sodium sulphate, decanol and heavy water. A typical spectrum of pyrimidine is shown in Fig. 1. Values

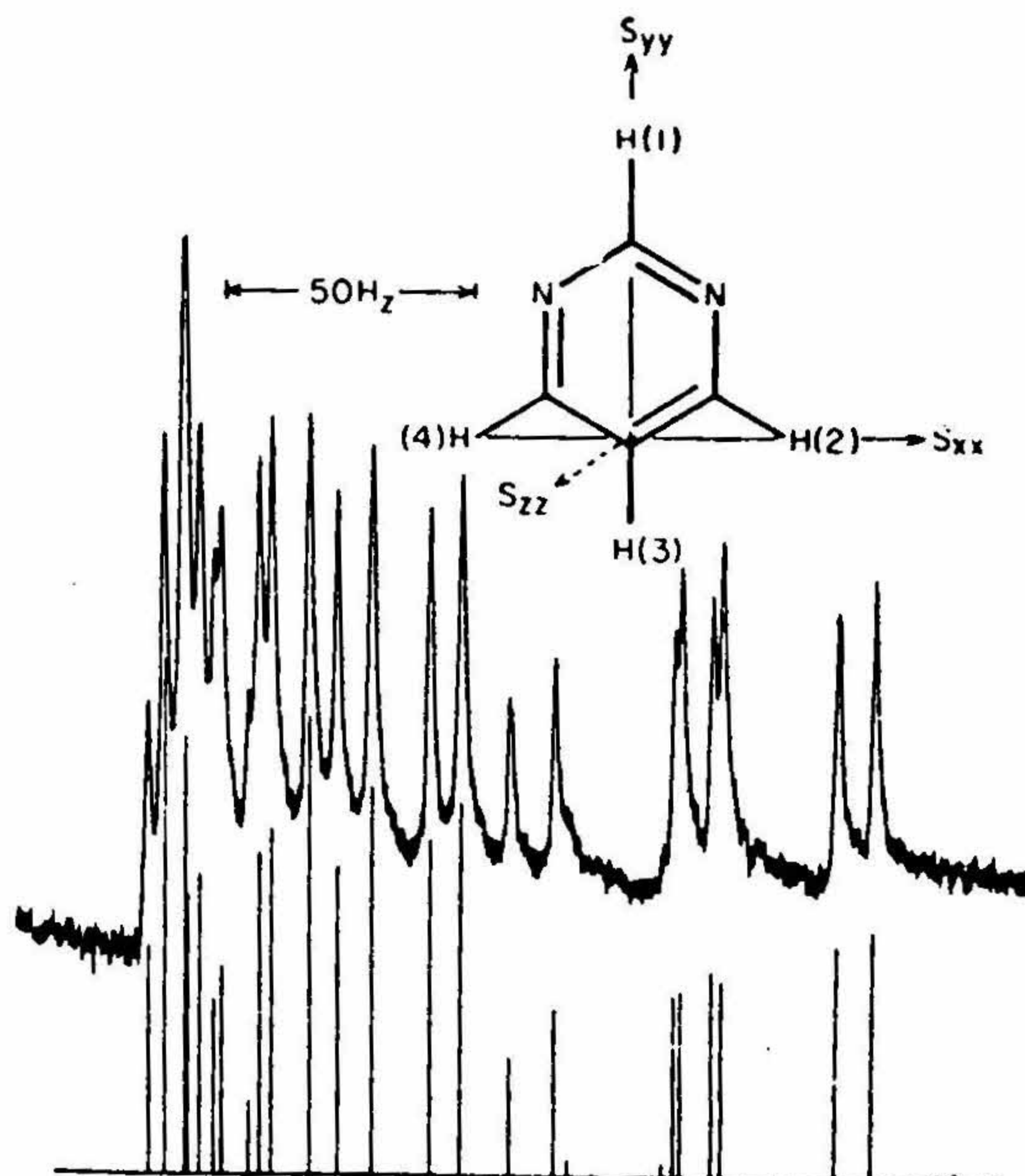


FIG. 1. Observed and calculated PMR spectra of pyrimidine oriented in a lyotropic mesophase. Composition of the phase: 14:1:1:20 weight mixture of sodium dodecyl sulphate, sodium sulphate, decanol and heavy water.

Solute concentration: 2% (by weight)

Spectrometer: A-60.

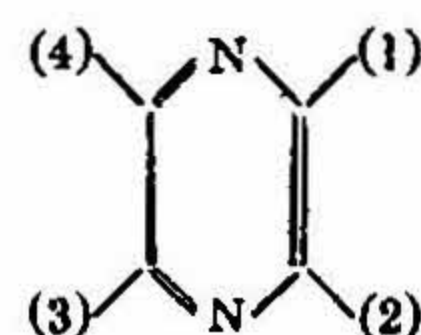
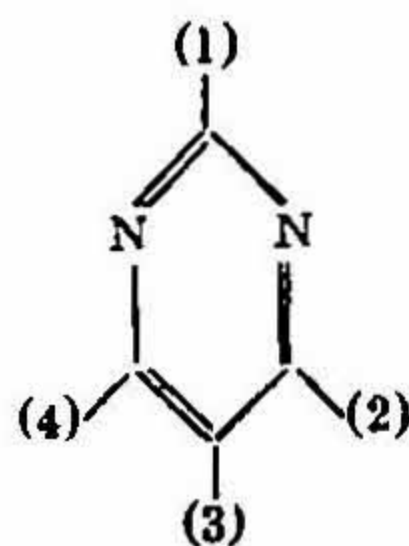
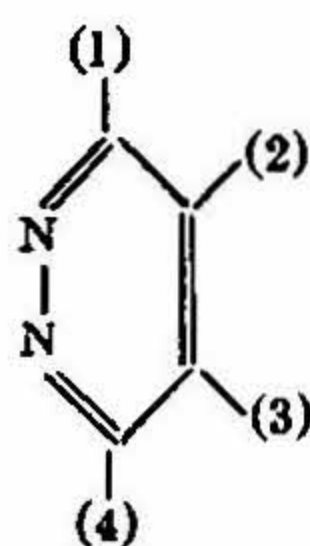
Temperature: 45°C.

of the dipolar coupling constants and the chemical shifts derived from the iterative analyses of the spectra are given in Tables 4.1 and 4.2 respectively for the 4- and the 5-spin cases. For pyridine and pyridine-*N*-oxide, even the indirect spin-spin couplings were derived and are included in Table 4.2.

TABLE 4.1

Spectral parameters in pyridazine, pyrimidine and pyrazine oriented in a lyotropic mesophase

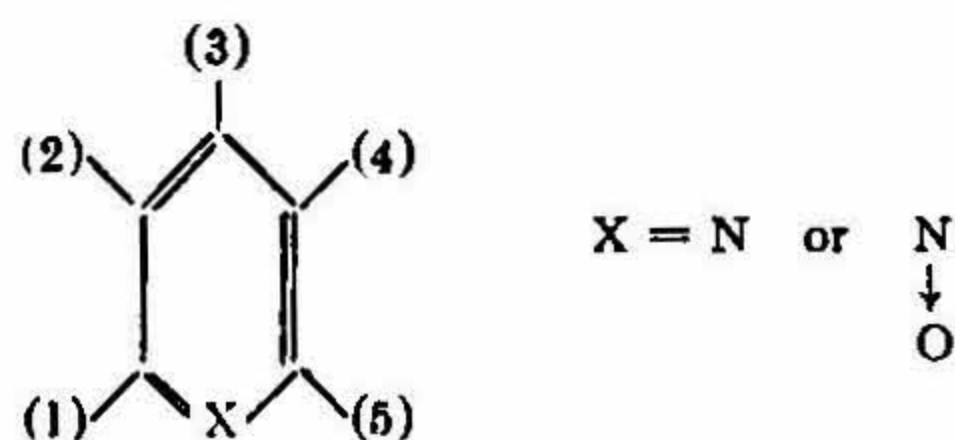
Pyridazine		Pyrimidine		Pyrazine	
Parameter	Value (Hz)	Parameter	Value (Hz)	Parameter	Value (Hz)
$D_{12} (= D_{34})$	-16.49 ± 0.09	$D_{12} (= D_{14})$	-3.44 ± 0.05	$D_{12} (= D_{34})$	103.72 ± 0.03
$D_{13} (= D_{24})$	-7.41 ± 0.11	D_{13}	-4.70 ± 0.05	$D_{13} (= D_{24})$	-1.28 ± 0.03
D_{14}	-6.39 ± 0.05	$D_{23} (= D_{34})$	13.96 ± 0.04	$D_{14} (= D_{23})$	-11.35 ± 0.03
D_{23}	-44.38 ± 0.07	D_{24}	6.53 ± 0.05		
$\nu_2 - \nu_1$	88.58 ± 0.10	$\nu_1 - \nu_2$	-23.53 ± 0.17		
		$\nu_3 - \nu_2$	75.65 ± 0.08		



For pyrazine, pyrimidine and pyridazine, the number of *HH* direct dipolar couplings is equal to the sum of the numbers of the geometrical and the orientational parameters. Consequently, equations reported in the literature [3] for these cases have been used to determine the ratios of the inter-proton distances and the order parameters. The values are given in Table 4.3. The distance ratios obtained from the studies in the thermotropic phase [3, 25, 26] are included within parentheses in Table 4.3. The table indicates that the agreement between the distance ratios obtained in the lyotropic and the thermotropic mesophase is satisfactory for pyrimidine

TABLE 4.2

Spectral parameters in pyridine and pyridine-N-oxide oriented in a lyotropic mesophase



Nuclei (i, j)	Parameters in pyridine			Parameters in pyridine-N-oxide		
	J_{ij} Hz	D_{ij} Hz	$(\nu_j - \nu_i)$ Hz	J_{ij} Hz	D_{ij} Hz	$(\nu_j - \nu_i)$ Hz
(1, 2)=(5, 4)	4.59 ± 0.25	196.91 ± 0.12	127.13 ± 0.39	7.98 ± 0.64	28.89 ± 0.32	78.48 ± 0.21
(1, 3)=(5, 3)	1.68 ± 0.19	27.83 ± 0.12	98.08 ± 0.28	0.90 ± 0.15	2.56 ± 0.05	73.20 ± 0.16
(1, 4)=(5, 2)	0.94 ± 0.06	2.97 ± 0.04		0.58 ± 0.09	—	3.16 ± 0.05
(1, 5)	0.19 ± 0.15	—	8.54 ± 0.06	2.03 ± 0.11	—	9.41 ± 0.04
(2, 3)=(4, 3)	7.80 ± 0.37	22.78 ± 0.20		7.63 ± 0.04	—	22.80 ± 0.05
(2, 4)	1.17 ± 0.13	—	7.16 ± 0.05	2.06 ± 0.08	—	8.26 ± 0.04

and pyrazine. However, there are significant deviations between the values for pyridazine. It may at least partly arise from the formation of the hydrogen bonds involving the non-bonding electrons of nitrogen of pyridazine and the protons of water.

In pyridine and pyridine-N-oxide, there are six direct dipolar proton-proton couplings. Of these, only three suffice to describe the shape of the proton skeleton and only two independent S -values are needed to specify the molecular orientation. The system is, therefore, over-determined by one coupling constant as far as the determination of molecular geometry and orientation from the direct dipolar couplings is concerned. The iterative programme SHAPE has, therefore, been used for obtaining the 'best-fit' geometry information and the order parameters given in Table 4.4. Values of the relative interproton distances obtained for pyridine from NMR studies in a thermotropic mesophase [8] are included within parentheses in Table 4.4. No such data for pyridine-N-oxide are available,

TABLE 4.3

Distance ratios for pyridazine, pyrimidine and pyrazine as determined from PMR studies in a lyotropic mesophase

Numbering of nuclei refers to that in Table 4.1.

Distance ratio	Pyridazine	Pyrimidine	Pyrazine
r_{12}/r_{23}	0.943 ± 0.014 (0.988 ± 0.010) ^μ	1.611 ± 0.018 (1.62 ± 0.01) [*]	0.607 ± 0.002 (0.602 ± 0.008) [†]
r_{13}/r_{23}	1.673 ± 0.010 (1.693 ± 0.007) ^μ	1.898 ± 0.017 (1.90 ± 0.02) [*]	1.170
r_{14}/r_{23}	1.908 ± 0.006 (1.890 ± 0.004) ^μ	1.611	1.000
r_{24}/r_{23}	1.673	1.697 ± 0.004 (1.706 ± 0.004) [*]	1.170

Values within parentheses are those obtained from studies in a thermotropic mesophase.

^μ reference [25].

^{*} reference [26].

[†] reference [3].

A comparison of the structural data for pyridine obtained from the lyotropic and the thermotropic mesophases reveals that the distance ratio r_{15}/r_{24} is about 2% shorter when obtained from the spectrum in the lyotropic phase, like in pyridazine. However, it must be emphasized that a variety of reasons, *e.g.*, the neglect of the influence of 'molecular vibrations', 'solvent effects', etc., may contribute to deviations of this magnitude and hence it is difficult to pinpoint exactly how these discrepancies arise.

2. *Conformation of 2, 2'-bithiophene.*—The conformation of 2, 2'-bithiophene (Fig. 2) has been studied with the help of electron diffraction in the gas phase [27] and X-ray diffraction in the solid state [28]. The former investigations show that the molecule is non-planar with a twist around the central C-C bond; the average value of the twist angle between the two thiophene ring planes is about 34°. The X-ray diffraction studies, on the other hand, show that the molecule exists in a *trans*-like planar form. ESR studies in solution on the radical anion of the molecule [29] show the presence of the two species in the ratio 4:1. From similarities in the ESR spectra and

the hyperfine coupling constants in these two species, it has been derived that they should be *cis*- and the *trans*-like rotamers.

TABLE 4.4

Structural and order parameters for pyridine and pyridine-N-oxide oriented in a lyotropic mesophase

Numbering of nuclei refers to that in Table 4.2.

The indexing of the S -values refers to a Cartesian coordinate system in which x and y axes are in the plane of the ring, with y axis being the C_2 -axis of symmetry.

Parameter	Value in pyridine	Value in pyridine-N-oxide
r_{12}/r_{24}	0.573 ± 0.002 (0.579)*	0.583 ± 0.006
r_{15}/r_{24}	0.943 ± 0.005 (0.962)*	0.957 ± 0.002
r_{23}/r_{24}	0.583 ± 0.002 (0.587)*	0.584 ± 0.003
S_{zz}	0.00466 ± 0.00003 (0.0265)*	0.00537 ± 0.00002
S_{yy}	-0.02412 ± 0.00056 (0.0484)*	-0.00373 ± 0.00055
S_{xx}	0.01946 (-0.0749)*	-0.00164

* Values from the thermotropic mesophase [8].

The NMR spectrum of the compound dissolved in a thermotropic mesophase is shown in Fig. 3. The calculated spectrum with the help of the parameters given in Table 4.5 is shown below the observed one in Fig. 3.

An interpretation of the observed dipolar couplings shows that the compound in the nematic solution does not exist in any of the following forms:

- (a) Planar *cis* structure
- (b) Planar *trans* structure
- (c) The non-planar conformation.

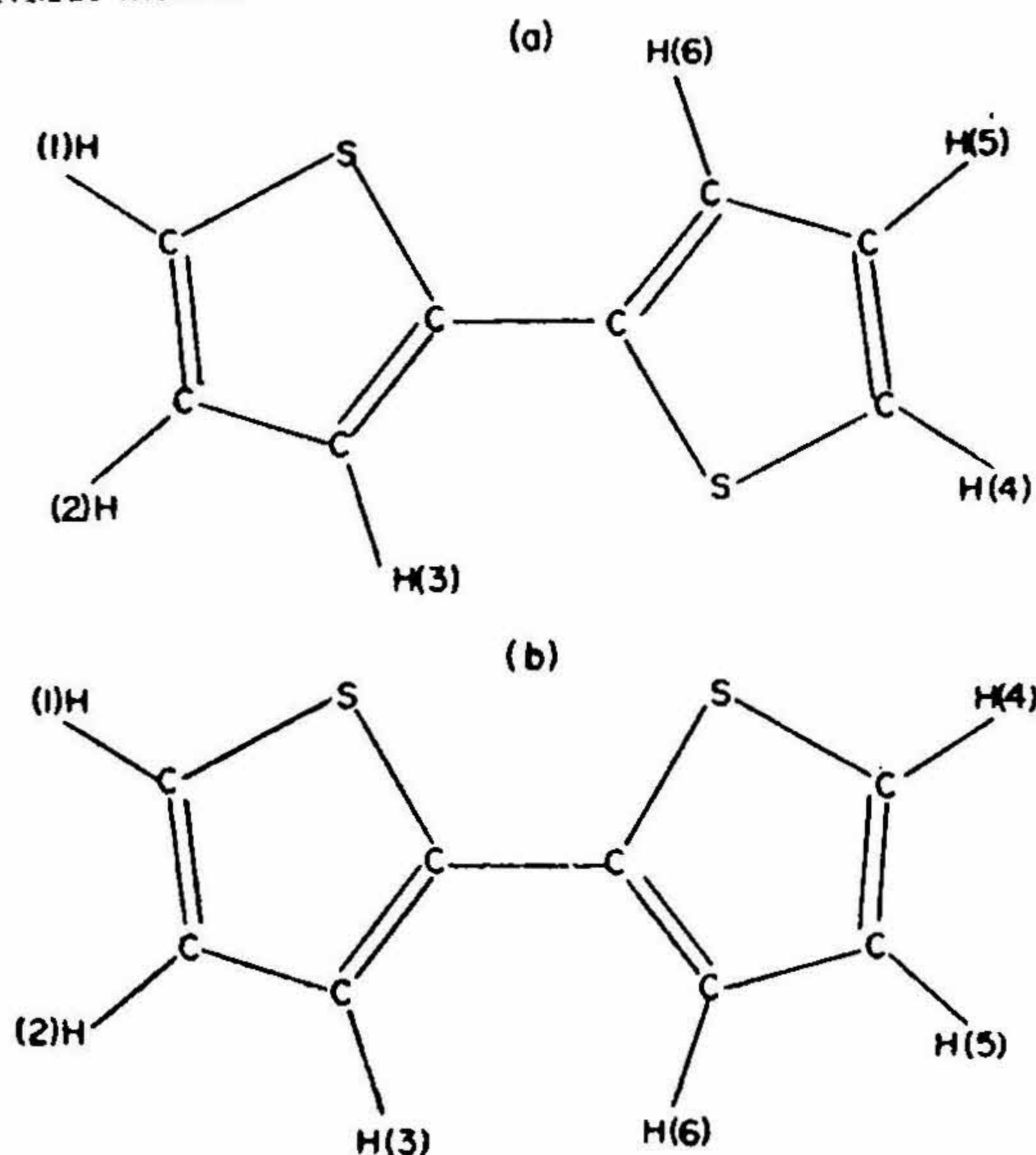


FIG. 2. Structure of 2, 2'-bithiophene (a) *trans*-like (b) *cis*-like.

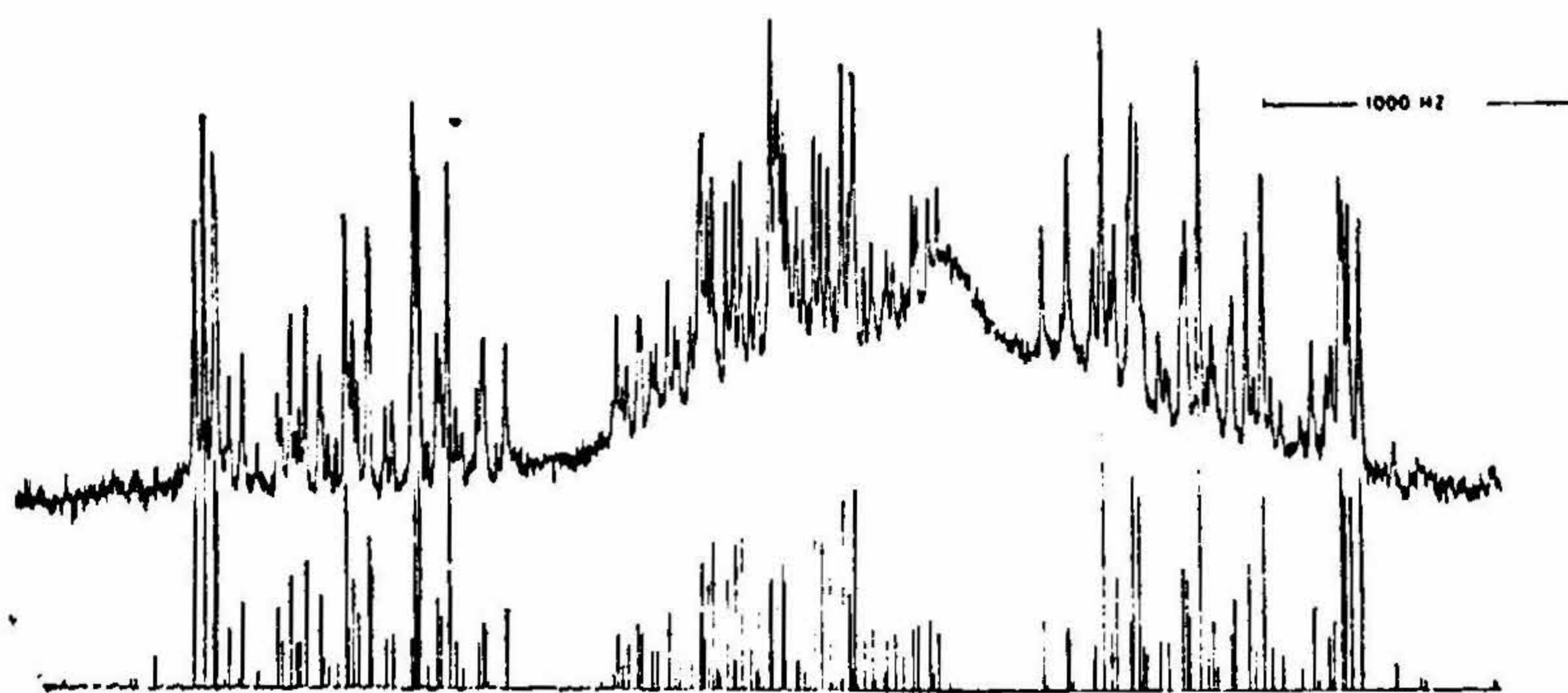


FIG. 3. PMR spectrum of 2, 2'-bithiophene dissolved in a nematic solvent.

Solvent: 80% (by weight) 4-ethoxybenzylidene-4-*n*-butylaniline + 20% (by weight) *O*-carboboxy-4-oxybenzoic acid ethoxy phenyl ester.

Solute concentration: 14 mole per cent.

Temperature: 28° C.

Spectrometer: XL-100.

■ : "Lock-signal."

However, it has been shown that 2, 2'-bithiophene exists as a mixture of *cis* and *trans* species with the fractional weight of the *trans* species lying between 35% and 80%.

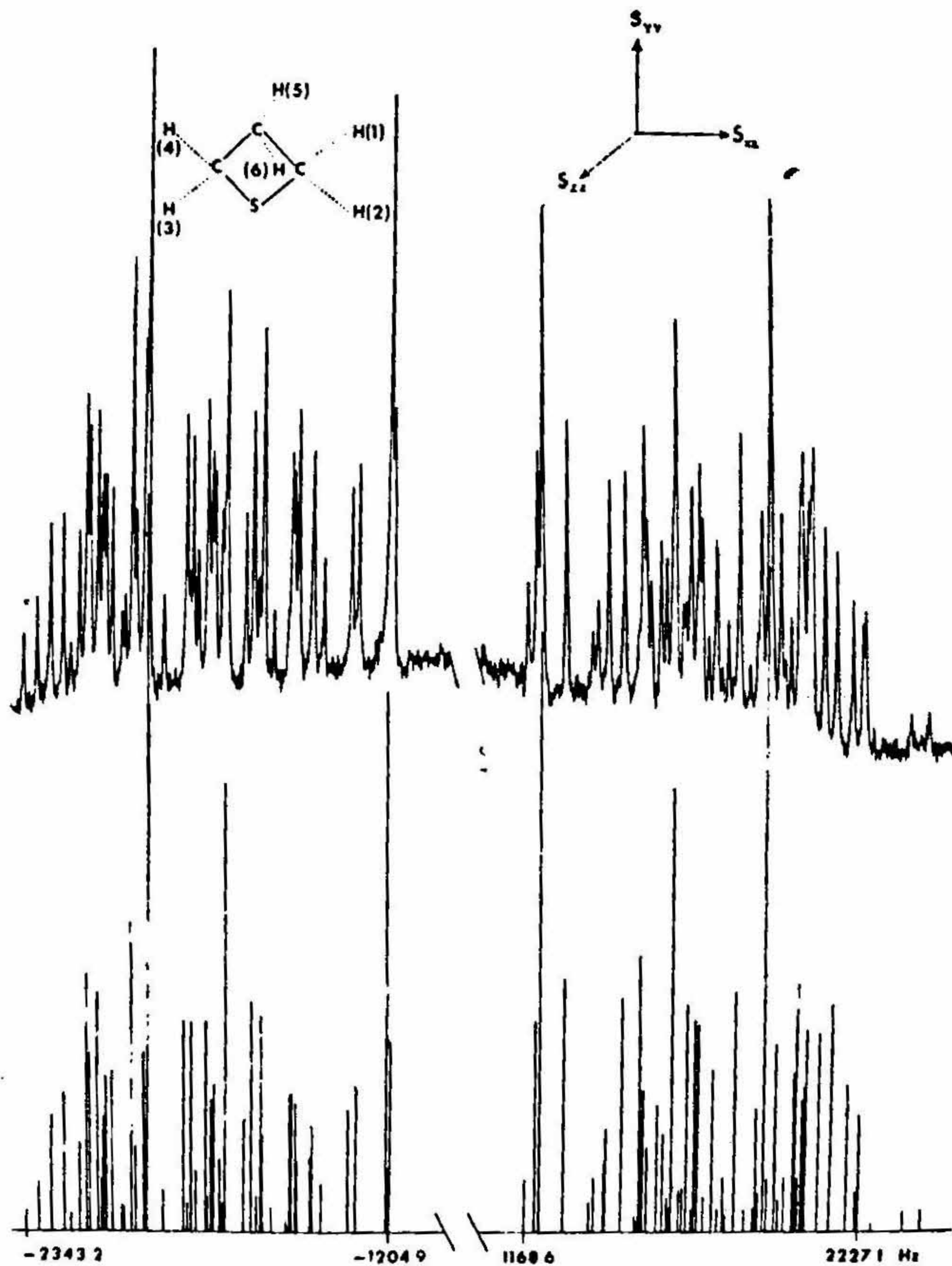


FIG. 4. Observed and calculated PMR spectra of trimethylene sulphide dissolved in the same nematic phase as given in Fig. 3.

Solute concentration: 9 mole per cent.

Temperature: 30° C.

Spectrometer: XL-100.

TABLE 4.5

Results of spectral analysis of 2, 2'-bithiophene oriented in a nematic phase

Numbering of protons refers to that given in fig. 2.

Parameter	Value (Hz)	Parameter	Value (Hz)
$D_{12} = D_{45}$	291.6 ± 0.3	D_{25}	-34.9 ± 0.2
$D_{13} = D_{46}$	-43.6 ± 0.2	$D_{26} = D_{35}$	-81.6 ± 0.2
D_{14}	-28.9 ± 0.2	D_{36}	-332.9 ± 0.2
$D_{15} = D_{24}$	-34.5 ± 0.2	$(\nu_1 - \nu_2) = (\nu_4 - \nu_5)$	12.3 ± 0.6
$D_{16} = D_{34}$	-88.9 ± 0.3	$(\nu_1 - \nu_3) = (\nu_4 - \nu_6)$	9.2 ± 0.5
$D_{23} = D_{56}$	-866.2 ± 0.4		

Indirect coupling constants used are: $J_{12} = 5.0$ Hz; $J_{13} = 1.2$ Hz; $J_{23} = 3.4$ Hz and the inter-ring indirect *HH* couplings are all zero.

3. *Structure of ^{13}C substituted acetylene.*—The ^{13}C -NMR spectra of acetylene have been analysed earlier by Spiesecke [30] and recently by Diehl *et al.* [11]. Diehl *et al.* have observed that in a particular liquid crystal there is an apparent change in molecular geometry of about 24% in a temperature range of 18° C to 44° C, *i.e.*, $r_{\text{CH}}/r_{\text{CC}}$ changes from 0.826 to 0.631 accompanied by an unusual drop in orientation. Since such changes are very improbable, the results have been interpreted in terms of acetylene exchanging between two orientations with order parameters of opposite signs.

4. *Ring puckering vibrations in trimethylene oxide and trimethylene sulphide.*—Studies on the ring puckering vibrations in several 4 and 5 membered ring systems have been a subject of considerable interest [23, 31–33]. It has been shown by infrared and microwave spectroscopic methods that whereas the five membered rings of the type 2:5 dihydrofuran [31] or 2:5 dihydrothiophene [31] are planar, the four membered rings can be non-planar. Trimethylene oxide in the ground state has a nearly planar structure [34] although the ring puckering potential has a double minimum. In

this case, the potential barrier is smaller than the zero point energy of the vibration. On the hand, trimethylene sulphide is puckered in the ground state and has a barrier, about 18 times larger than that of trimethylene oxide [35, 36].

NMR spectra of these compounds have been studied [23] in the nematic phase in order to obtain information on the ring puckering vibrations in these cases. A typical spectrum of the sulphide is shown in Fig. 4. Values of the direct dipolar couplings and the chemical shifts derived are given in Table 4.6. The indirect couplings used have been taken from the literature [37].

For discussion of the results, two cases, namely, (1) the rigid planar structure and (2) the interconverting bent conformations have been considered. It has been shown that the NMR results are not consistent with the rigid

TABLE 4.6

Spectral parameters for trimethylene oxide and trimethylene sulphide oriented in a nematic phase

Numbering of protons refers to that given in fig. 4.

Parameter	Value (Hz)	
	Trimethylene oxide	Trimethylene sulphide
$D_{12} = D_{34}$	946.1 ± 0.3	1174.3 ± 0.2
$D_{13} = D_{24}$	$- 46.9 \pm 0.4$	$- 2.5 \pm 0.3$
$D_{23} = D_{14}$	$- 144.7 \pm 0.3$	$- 51.0 \pm 0.2$
$D_{15} = D_{28} = D_{36}$ $= D_{46}$	$- 183.1 \pm 0.3$	$- 211.2 \pm 0.2$
$D_{16} = D_{25} =$ $D_{35} = D_{46}$	6.4 ± 0.2	7.1 ± 0.1
D_{56}	1019.9 ± 0.3	1133.9 ± 0.2
$\nu_5 - \nu_1$	181.4 ± 0.4	47.3 ± 0.2

The indirect couplings used were taken from the literature [37].

planar structures of trimethylene and trimethylene sulphide. The dihedral angles for the puckered configuration have been estimated.

5. *The conformation of the peptide unit.*—In most of the work on the theoretical calculations related to the conformation of peptides and proteins, a completely planar peptide unit has been assumed [38, 39], though the possibility of the non-planar peptide unit has been suggested [40]. In view of the importance of such studies, it is essential to establish results experimentally. NMR studies in a nematic solvent have been undertaken for this purpose. Only N-methyl formamide, which is the simplest compound in the series has so far been studied. The PMR spectrum of the compound in the nematic solvent 4-methoxybenzylidene 4-amino- α -methyl cinnamic acid-*n*-propyl ester has been investigated. The results indicate that the molecule has a plane of symmetry. This may either arise from the atoms of the fragment H.CO.NH.C lying in one plane or if the fragment H.CO.N is assumed planar, this plane of symmetry may 'effectively' arise also from two rapidly interconverting conformations in which the remaining two bonds meeting at the nitrogen atom are bent either below or above this plane; the angles of bend found are less than 10° . Studies on ^{13}C and ^{15}N enriched species are expected to provide more reliable information on the conformation of the peptide unit. The method can be extended to higher members of the series.

5. CONCLUSIONS

The method of determination of molecular structure with the help of NMR studies in liquid crystal solvents has a very potential future. It is a very versatile method and can be applied to problems of biological importance provided suitable experimental facilities are available. The method is likely to find enormous such applications in years ahead.

6. ACKNOWLEDGEMENT

I am grateful to Dr. A. C. Kunwar for helpful discussions.

REFERENCES

- [1] Saupe, A. and Englert, G. *Physical Review Letters*, 1963, 11, 462.
- [2] Gray, G. W. .. *Molecular Structure and Properties of Liquid Crystals*, Academic Press, Inc., New York, 1962.
- [3] Diehl, P. and Khetrapal, C. L. *NMR—Basic Principles and Progress*, Edited by P. Diehl, E. Fluck and R. Kosfeld. Springer Verlag (Berlin, Heidelberg, New York), Vol. 1, 1969.

- [4] Diehl, P. and Henrichs, P. M. Contribution to Specialist Periodical Report No. 28—NMR 1971.
- [5] Bulthuis, J., Hilbers, C. W. and Maclean, C. *MTP International Review of Science*, Butterworths, University Park Press, Physical Chemistry, 1972, 4, 201.
- [6] Diehl, P. and Niederberger, W. Contribution to Specialist Periodical Report, 1973.
- [7] Snyder, L. C. and Anderson, E. W. *Journal of Chemical Physics*, 1965, 42, 3336.
- [8] Diehl, P., Khetrapal, C. L. and Kellerhals, H. P. *Molecular Physics*, 1968, 15, 333.
- [9] Saupe, A. .. *Zeitschrift fuer Naturforschung*, 1965, 20 A, 572.
- [10] Snyder, L. C. .. *Journal of Chemical Physics*, 1965, 43, 4041.
- [11] Diehl, P., Niederberger, W., Sykora, S. and Burnell, E. Paper presented at the Fifth International Conference on Magnetic Resonance, Bombay, January 1974 and *Journal of Magnetic Resonance* (in the press).
- [12] Bulthuis, J. and Maclean, C. *Chemical Physics Letters*, 1972, 7, 242.
- [13] Lucas, N. J. D. .. *Molecular Physics*, 1972, 23, 825.
- [14] Meiboom, S. and Snyder L. C. *Accounts of Chemical Research*, 1971, 4, 81.
- [15] Diehl, W. P. and Niederberger, W. *Journal of Magnetic Resonance*, 1973, 9, 495.
- [16] (a) Snyder, L. C. and Meiboom, S. *Journal of Chemical Physics*, 1973, 58, 5096.
- (b) Hewitt, R. C., Meiboom, S. and Snyder, L. C. *Journal of Chemical Physics*, 1973, 58, 5089.
- (c) Luz, Z. and Meiboom, S. *Journal of Chemical Physics*, 1973, 59, 1077.
- [17] Diehl, P. and Khetrapal, C. L. *Molecular Physics*, 1967, 14, 283.
- [18] Lawson, K. D. and Flautt, T. J. *Journal of the American Chemical Society*, 1967, 89, 5489.
- [19] Khetrapal, C. L., Kunwar, A. C. and Patankar, A. V. *Proceedings of the International Conference on Liquid Crystals*, Bangalore, 1973.
- [20] Diehl, P., Henrich, P. M. and Niederberger, W. *Molecular Physics*, 1971, 20, 139.
- [21] Khetrapal, C. L., Kunwar, A. C. and Patankar, A. V. *Journal of Magnetic Resonance* (submitted).
- [22] — and Kunwar, A. C. *Molecular Physics* (in the press).

- [23] Khetrpal, C. L., Kunwar, A. C. and Saupe, A. *Molecular Physics*, 1973, 25, 1405, and the references therein.
- [24] —, — and Easwaran, K. R. K. *Proceedings of the International Conference on Liquid Crystals*, Bangalore 1973.
- [25] Burnell, E. E. and De Lange, C. A. *Molecular Physics*, 1969, 16, 95.
- [26] Khetrpal, C. L., Patankar, A. V. and Diehl, P. *Organic Magnetic Resonance*, 1970, 2, 405.
- [27] Almenninger, A., Bastiansen, O. and Svendsen, P. *Acta Chemica Scandinavica*, 1958, 12, 1671.
- [28] Visser, G. J., Heeres, G. J., Wolters, J. and Aafje, V. *Acta Crystallographica*, 1968, 24 B, 467.
- [29] Cavalieri d'Oro, P., Mangini, A., Pedulli, G. J., Spagnolo, P. and Tiecco, M. *Tetrahedron Letters*, 1969, 48, 4179.
- [30] Spiess, H. *Liquid Crystals and Ordered Fluids*, Plenum Press, New York, 1970.
- [31] Green, W. H. and Harvey, A. B. *Journal of Chemical Physics*, 1968, 49, 177, and the references therein.
- [32] Khetrpal, C. L., Kunwar, A. C. and Saupe, A. *Journal of Magnetic Resonance*, 1972, 7, 18.
- [33] Chan, S. I., Borgers, T. R., Russell, J. W., Strauss, H. L. and Gwinn, W. D. *Journal of Chemical Physics*, 1966, 44, 1103.
- [34] —, Zinn, J. and Gwinn, W. D. *Journal of Chemical Physics*, 1961, 34, 1319.
- [35] Rogers, T. R. and Strauss, H. L. *Journal of Chemical Physics*, 1966, 45, 947.
- [36] Harris, D. O., Harrington, H. W., Luntz, A. C. and Gwinn, W. D. *Journal of Chemical Physics*, 1966, 44, 3467.
- [37] Ferretti, J. A. *Ph.D. Thesis*, Berkeley, 1965.
- [38] Ramachandran, G. N. and Sasisekharan, V. *Advances in Protein Chemistry*, 1968, 23, 283.
- [39] Govil, G. and Saran, A. *Journal of the Chemical Society, A*, 1971, p. 3624.
- [40] Ramachandran, G. N., Lakshminarayanan, A. V. and Kolaskar, A. S. *Biochimica et Biophysica Acta*, 1973, 8, 303, and the related references therein.