NON-PLANAR DISTORTIONS IN A PEPTIDE UNIT AS STUDIED BY NMR IN LIQUID CRYSTAL SOLVENTS*

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ABSTRACT

A brief account of the results on non-planar distortions around the nitrogen atom in a peptide unit as studied by NMR in liquid crystal solvents is given. The studies are confined to simple model compounds namely, formamide, N-methyl formamide and its ^{13}C *and* ^{15}N *isotopically enriched analogues and N-methyl acetamide. Under the assumption of reasonable geometry and the neglect of the influences of molecular vibrations, it is observed that the non-planar distortions around the nitrogen atom are nearly* 10° ± 5°.

Keywords: Peptide unit, chemical shift, indirect spin-spin coupling constants, direct dipolar couplings, order parameter, nematic phase, lyotropic phase.

1. INTRODUCTION

The spatial arrangement of atoms in the peptide unit (C_1^{α}–C′O–NH–C_2^{α}) is of great importance for studies connected with the conformation of polypeptides and proteins. In most of the work, it is generally believed that the peptide unit is rigidly planar, *i.e.*, the six atoms namely C_1^{α}, C′, O, N, H and C_2^{α} lie in one plane, though it has been realised that significant distortions from planarity in a peptide unit can occur, and hence the necessity of considering a non-planar peptide unit has been suggested [1]. Subsequently, there has been a considerable interest to study non-planar distortions in model systems [2–6]. In view of the great importance, the problem has been investigated theoretically using *ab initio* and semi-empirical molecular orbital approaches, and experimentally, using NMR spectroscopy of molecules dissolved in nematic liquid crystal solvents, and by X-ray crystal structure analysis. It may be pointed out that the NMR studies are in the

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liquid phase. It is the aim of this review to give a brief account of the NMR results on this problem.

2. PRINCIPLES OF THE NMR METHOD

Though the basic principles involved in such studies have been discussed earlier in this journal [7] and several monographs on the subject are available [8–11], still, in order to make this article self-contained, it may not be out of place to mention them briefly again. The high resolution NMR spectra of nuclei (i, j) with spin $\frac{1}{2}$ in diamagnetic materials are influenced by chemical shifts (δ_{ij}), indirect spin-spin coupling constants (J_{ij}), and the direct couplings (D_{ij}). Due to rapid random molecular motions, the *average* values of these parameters, proportional to the traces of these second rank tensors, are the ones that influence the spectra in ‘ isotropic ’ media. On the one hand, such a situation simplifies the NMR spectra; on the other, the information about the anisotropy of these parameters is lost in this averaging process. Of particular mention in this connection is the fact that the direct dipolar couplings, which are proportional to the inverse cube of the distances between interacting nuclei, vanish in isotropic media and hence the information about the internuclear distances carried by them is lost. However, in the nematic phase of liquid crystals, the motion of dissolved molecules is anisotropic due to the inherent anisotropy of the solvent. This results in non-zero values for the intramolecular direct dipolar couplings in such media and hence valuable information about internuclear distances may be derived from the NMR spectra of the molecules dissolved therein. The direct dipolar coupling D_{ij} between nuclei i and j is given by equation (2.1)

$$D_{ij} = -\frac{h\gamma_i\gamma_j}{4\pi^2}\cdot\frac{1}{2}\left\langle\frac{3\cos^2\theta_{ij}-1}{r_{ij}^3}\right\rangle \tag{2.1}$$

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, equation (2.1) reduces to (2.2)

$$D_{ij} = -\frac{h\gamma_i\gamma_j}{4\pi^2 r_{ij}^3}\cdot S_{ij} \tag{2.2}$$

where S_{ij} defines the ‘ degree of order ’ of the axis ij. It may be mentioned that the equation (2.2) neglects influences of all types of molecular vibrations and takes into account only the ‘ average ’ molecular order.

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 mooecule 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)

$$S_{pq} = \tfrac{1}{2}(3\cos\theta_p \cos\theta_q - \delta_{pq}) \tag{2.3}$$

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 $\alpha_x{}^a$, $\alpha_y{}^a$ and $\alpha_z{}^a$ with the molecule fixed coordinate system according to equation (2.4),

$$S_a = \sum_{p,\,q} \cos\alpha_p{}^a \cdot \cos\alpha_q{}^a \, S_{pq}. \tag{2.4}$$

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 molecule 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 the Z-axis perpendicular to this plane leaves S_{xx}, S_{yy} and S_{xy} as the only independent elements of the S-matrix.

For the study of the spatial arrangement of atoms in a peptide unit, expressions for the dipolar couplings in terms of the geometrical and the order parameters are written. For an over-determined system, *i.e.*, when the number of dipolar couplings exceeds that of the sum of the geometrical and the order parameters, the internal consistency of the dipolar couplings is checked. For example, if all the geometrical parameters are known, the 'best-fit' order parameters using a suitable 'least-squares fit' procedure (such as the SHAPE programme [18]) can be determined from the dipolar couplings by solving linear simultaneous equations, the number of which equals that of the different dipolar couplings. If the dipolar couplings are internally self-consistent with three S-values, a plane of symmetry in the molecule is indicated. On the other hand, if the results are self-consistent only with five order parameters, the molecule is non-planar. The plane of symmetry can be 'real' or 'effective' introduced by a rapid interconversion of energetically equivalent bent conformations. The angles of bend may be determined from the dipolar couplings as the additional parameters.

3. DESCRIPTION OF NON-PLANARITY IN THE PEPTIDE UNIT

The non-planar distortions around the nitrogen atom in a peptide unit are defined according to standard conventions by dihedral angles $\triangle\omega$ and θ_N [2]. ω is the dihedral angle ($C_1^\alpha C' NC_2^\alpha$) between the planes $C_1^\alpha C'N$ and $C'NC_2^\alpha$. It is 0° for the *cis* and 180° for the *trans* peptide units. $\triangle\omega$ is the variation in ω (positive in the clockwise direction). θ_N defines the dihedral angle between the planes containing the atoms $C'NC_2^\alpha$ and C'NH (positive in the clockwise direction when looking from C' towards N). It is 0° for the *trans* planar unit.

It has been shown from quantum chemical calculations on model compounds that there are significant non-planar distortions around the nitrogen atom. Further, it has been noticed that $\triangle\omega$ and θ_N are correlated approximately through the relation

$$\theta_N = -2\triangle\omega. \qquad (3\cdot1)$$

Values for $|\triangle\omega|$ upto 15° and those of $|\theta_N|$ upto 30° are quite probable [3].

4. MODEL SYSTEMS STUDIED BY NMR

The model systems studied so far by the NMR method and reported in the present review are the following:

(*a*) ^{15}N-enriched formamide in a lyotropic solvent [12].

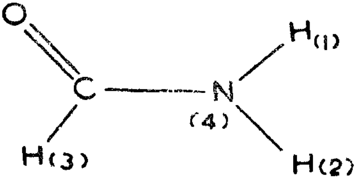
(*b*) N-methyl formamide in lyotropic [13] and thermotropic solvents [14].

(*c*) N-methyl acetamide [15].

(*d*) ^{13}C and ^{15}N isotopically enriched N-methyl formamide in a thermotropic solvent [16].

(*a*) ^{15}*N-enriched formamide in a lyotropic solvent*

A PMR study of ^{15}N-formamide (structure 1) oriented in the lyotropic



(Structure 1)

nematic solvent formed by a mixture of 7 per cent sodium sulphate, 36 per cent sodium decyl sulphate, 7 per cent decanol and 50 per cent water by weight has been reported [12]. By a suitable adjustment of the pH of water used to prepare the solution, the exchange of the NH proton was slowed down and all the 6 dipolar couplings between the four nuclei 1, 2, 3, 4 were derived as given in Table I. The self-consistency of the 6-dipolar couplings was checked against 3 or 5 S-values depending upon whether the molecule is (i) planar due to rapid inversion through the nitrogen atom, or (ii) nonplanar such that the inversion is slow on the NMR time scale. In case (i), 3 dipolar couplings were used to compute the three S-values using the microwave data on the molecular geometry [17]. The remaining couplings were computed for comparison with their experimental values. All the 20 possibilities for such calculations were repeated. Very large deviations between the observed and the calculated D-values were thus found, *e.g.*, calculated D_{23} was found to vary from $-111 \cdot 9$ to to $+35 \cdot 5$ Hz in these 20 calculations.

Later on, we made the 'best-fit' calculations of the three S-values from the 6 dipolar couplings using the same microwave geometry. For such a purpose, a version of the SHAPE programme [18] which was modified so as to take into account the inversion through the nitrogen atom was used. In another calculation, we used the same microwave data on bond lengths and bond angles and iterated upon the three S-values and the two dihedral angles OCNH(1) and OCNH(2). Calculations were also undertaken using recent microwave data [19]. The results are reproduced in Table I. It is seen from the table that the r.m.s. error between the observed and the calculated dipolar couplings is acceptable except for the case where only three S-values are iterated upon, for which it is $1 \cdot 7$ Hz. Furthermore, values of the dihedral angles OCNH(1) and OCNH(2) agree fairly well with the microwave data within the reported experimental error ($\pm 5°$) of the dihedral angles [17].

(*b*) *N-Methyl formamide in a thermotropic and a lyotropic solvent*

The spectra are shown in figures 1 and 2 respectively together with the experimental details.

TABLE I

NMR data on ^{15}N formamide oriented in a lyotropic nematic phase

		Calculated values			
		Using the earlier bond length and bond angle data [17]			Using recent geometrical data [19] on bond length and angles
Parameters	Experimental values	Iterations: 3 S-values	Iterations: 3 S-values and two dihedral angles	Iterations on 5 S-values	Iterations on 3 S-values and two dihedral angles
D_{12}	—289·8 Hz	—288·7 Hz	—289·8 Hz	—289·8 Hz	—289·8 Hz
D_{13}	— 45·2 Hz	— 42·5 Hz	— 45·4 Hz	— 45·3 Hz	— 45·3 Hz
D_{14}	130·0 Hz	132·4 Hz	130·0 Hz	130·0 Hz	130·0 Hz
D_{23}	— 58·3 Hz	— 58·3 Hz	— 58·3 Hz	— 58·3 Hz	— 58·3 Hz
D_{24}	116·5 Hz	117·5 Hz	116·5 Hz	116·5 Hz	116·5 Hz
D_{34}	12·6 Hz	11·1 Hz	11·8 Hz	12·2 Hz	12·3 Hz
S_{zz}*		0·00312	0·0030	0·00319	0·0028
S_{xx}*		—0·0095	—0·00942	—0·00949	—0·00927
S_{xz}*				0·00078	
S_{xy}*		0·0002	0·0006	0·00062	0·00053
S_{yz}*				0·0168	
r.m.s. error		1·7 Hz	0·3 Hz	0·2 Hz	0·1 Hz
OCNH (1)		7°	12·8°	7°	11·1°
OCNH (2)		12°	7·3°	12°	1·2°

* The indexing of the S-values corresponds to a Cartesian coordinate system in which the 'effective' plane of symmetry is the xy plane with x-axis along the C–N bond.

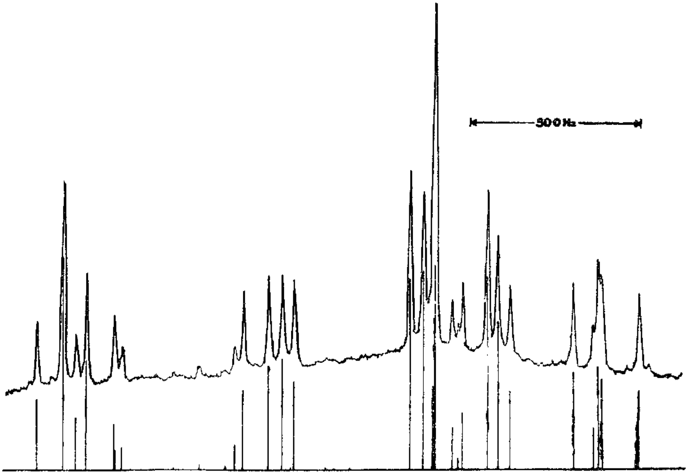
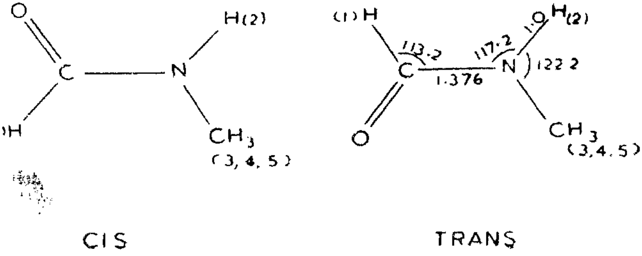


FIG. 1. Observed and calculated PMR spectra of N-methyl formamide dissolved in the nematic phase of 4-methoxybenzylidene-4-amino-α-methyl cinnamic acid *n*-propyl ester (A).

Solute concentration: 16·7 mole per cent.
Temperature: 30° C.
Spectrometer frequency: 100 MHz.

The calculated spectrum corresponds to the *trans* species. Some of the small lines in the observed spectrum which do not appear in the calculated one may arise from the *cis* species.

In the lyotropic solvent, the spectrum due to the *cis* species (structure 2)



(Structure 2)

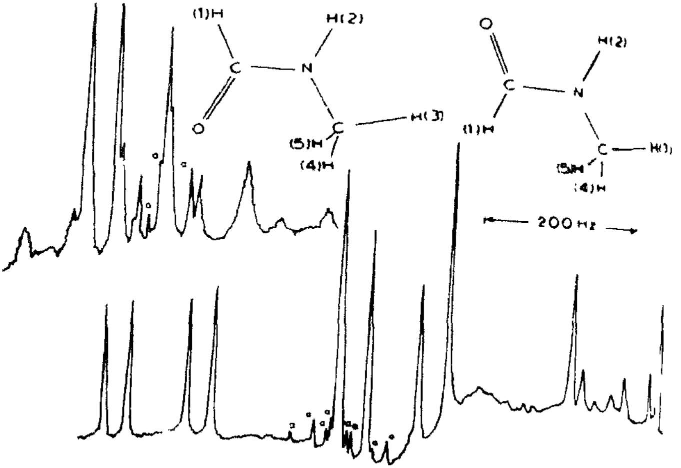


FIG. 2. PMR spectrum of N-methyl formamide oriented in the lyotropic phase formed by neutral decylammonium sulphate, decanol, water and ammonium sulphate (B).

Solute concentration: 2·5 per cent by weight.

Temperature: 25° C.

Spectrometer frequency: 360 MHz.

The lines due to the *cis* species are marked (*a*). The upper trace (at lower field) is due to the formyl and the amide protons and the lower one arises from the methyl protons.

could also be analysed in addition to that due to the predominant *trans* species (structure 2). However, in the thermotropic solvent, the spectrum due to the *trans* species only could be analysed. Values of the dipolar couplings and the chemical shifts derived from the analyses are given in Table II; the values of the indirect spin-spin coupling constants used were taken from the literature on the spectra in isotropic media [20] and are included in the table. Signs of the direct couplings in Table II are based on the assumption of the known signs of indirect spin-spin couplings.

It is seen from the magnitudes of the direct dipolar couplings given in Table II that the molecular orientation for the *cis* species is much lower than that in the *trans*. Furthermore, conditions of 'weak' couplings [8] are satisfied for the chemically shifted protons in the *cis* species and hence only the magnitudes of the corresponding $(J + 2D)$ could be derived from the spectrum. Moreover, D_{12} in the *cis* compound is so small that even minor errors in J_{12} may produce significant errors in D_{12}. Due to these uncertainties, no attempt was made to study the conformation around the nitrogen atom in the *cis* species. For the *trans* species in the lyotropic phase, though the condition of ' weak ' coupling is satisfied for protons 1 and 3, the values reported in the table correspond to those which give results consistent with studies in the thermotropic solvent.

It may be mentioned that the chemical shifts (in ppm) of the CH and the CH_3 protons in the lyotropic and thermotropic solvents do not differ appreciably for the *trans* species, and, even in the *cis* species, $\nu_3 - \nu_1$ differs only by 0·16 ppm. On the other hand, the difference between chemical shifts of the NH proton in the thermotropic and the lyotropic solvent is

TABLE II

Spectral parameters for N-methyl formamide dissolved in the nematic phase of (A) and in the lyotropic phase (B). Numbering of protons refers to that given in structure 2

Parameters	Values (Hz)		
	Thermotropic solvent	In lyotropic solvent	
	trans species	*trans* species	*cis* species
J_{12}	1·9	1·9	13·8
$J_{13} = J_{14} = J_{15}$	—0·8	— 0·8	— 0·8
$J_{23} = J_{24} = J_{25}$	4·9	4·9	4·9
$J_{34} = J_{35} = J_{45}$	does not affect	does not affect	does not affect
D_{12}	—376·96	—28·79	— 1·565
$D_{13} = D_{14} = D_{15}$	— 44·39	—13·76	23·50
$D_{23} = D_{24} = D_{25}$	— 36·35	—51·00	16·70
$D_{34} = D_{35} = D_{45}$	163·20	91·58	8·43
$\nu_2 - \nu_1$	123·30†	57·93*	48·14*
$\nu_3 - \nu_1$	533·10†	1919·60*	1861·09*

† at 100 MHz; * at 360 MHz.

nearly 1·1 ppm. This certainly is not due to the chemical shift anisotropy but is indicative of a weak molecular interaction either like the one in a pi complex between the NH proton and the pi electrons of the benzene ring [21] in the thermotropic solvent and/or as in a stronger hydrogen-bonded complex between the NH protons and the lyotropic phase. In absence of the data on the NH proton chemical shift for the *cis* species in a thermotropic solvent, it is difficult to rule out one or the other of the above explanations.

The results on the conformation around the nitrogen atom for the *trans* species in the thermotropic solvent are discussed below:

The 4 direct dipolar HH couplings in N-Methyl formamide do not even provide the *S*-matrix if the molecule is 'rigidly' non-planar. If, on the other hand, the molecule possesses a plane of symmetry, only 3 direct couplings suffice to describe the molecular order. As in formamide, a known geometry for the molecule [17] shown in structure 2 together with the HCH bond angle and the CH bond lengths as 109·5° and 1·1 Å respectively, were used to find out whether the 4 direct dipolar couplings are internally self-consistent. The 3 best-fit *S*-values were derived from the 4 direct couplings using a version of the SHAPE program modified so as to include the rotation of the methyl group about the N–C bond. The following possibilities were considered:

Case 1. A rigid planar conformation for the fragment H.CO.HN.C; and

Case 2. Two energetically equivalent rapidly interconverting non-planar conformations.

Case 1: *A rigid planar conformation.*—In this case, the coupling constants D_{13} and D_{23} are influenced by the mode of rotation of the methyl group about the N–C bond. The following possibilities about the mode of rotation of the methyl group were considered:

(*a*) A hindered rotation in a '3-fold' potential such that one of the C–H bonds of the methyl group lies in the plane containing C′, H(1), O and N. In this case, both the possibilities, *i.e.*, (1) when the methyl C–H bond in the plane is pointing away from the N–H bond (referred to as 'staggered' hereafter) or (2) it is towards the N–H bond (referred to as 'eclipsed' hereafter), were considered.

(*b*) A hindered rotation in a '6-fold' potential such that one C–H bond of the methyl group is in the plane of symmetry as described above, This is equivalent to having both the 'eclipsed' and the 'staggered' configurations with equal probability.

(*c*) A free rotation of the methyl group.

In all these calculations, the methyl group itself was considered rigid, and influences of all types of molecular vibrations on the dipolar couplings were neglected. values of the 'best-fit' parameters obtained using the 'SHAPE' programme are given in Table III. Errors of the S-values were estimated to be 0·0003 in each case, using the errors of the observed *D*-values. The 'r.m.s. error' given in Table III is the 'weighted root mean square error' between the observed and the 'best-fit' calculated *D*-values using the modified 'SHAPE' programme, where all the coupling constants were given equal weights.

It was found that there is no significant difference between the '6-fold' and the 'free rotation' cases and hence they are included in the same column in Table III.

TABLE III

'Best-fit' parameters obtained with the help of the modified 'SHAPE' programme for N-methyl formamide oriented in the nematic phase of (A). Numbering of the protons refers to that given in structure 2

Parameter	'eclipsed'	'staggered'	'6-fold' or 'free rotation'
D_{12}	—376·90 Hz	—377·01 Hz	—376·97 Hz
$D_{13} = D_{14} = D_{15}$	— 46·42 Hz	— 41·75 Hz	— 44·00 Hz
$D_{23} = D_{24} = D_{25}$	— 36·91 Hz	— 35·67 Hz	— 36·25 Hz
$D_{34} = D_{35} = D_{45}$	162·63 Hz	163·90 Hz	163·31 Hz
S_{xx}*	0·0153	0·0154	0·0153
S_{yy}*	0·0177	0·0283	0·0229
S_{zz}*	— 0·0330	— 0·0437	— 0·0382
S_{xy}*	0·0214	0·0134	0·0175
r.m.s. error	1·09 Hz	1·41 Hz	0·21 Hz

* Refers to the Cartesian coordinate system where the *x* and *y* axes lie in the plane of the symmetry with *x*-axis as the N–C axis. The *S*-values are calculated using a distance between the methyl protons equal to 1·78 Å.

Table III shows that the coupling constant D_{13} is the one which is most significantly influenced by the mode of rotation of the methyl group. It is also seen from the table that the magnitude of this coupling constant is larger than the observed value (Table II) for the 'eclipsed' and smaller for the 'staggered'. It is, therefore, logical to expect the values for the '6-fold' or 'free rotation' cases (which contain both the '3-fold' cases considered) to be closer to those observed. It is actually found to be so, as seen from Table III. The r.m.s. error of about 0·2 Hz for the '6-fold' or the 'free rotation' cases seems to support the assumption of the plane of symmetry in the molecule. Table III also shows that the data are strongly dependent upon the mode of rotation of the methyl group. The r.m.s. errors of 1·09 and 1·41 Hz obtained for the 'eclipsed' and the 'staggered' cases are certainly outside the experimental errors.

Case 2: *Two energetically equivalent rapidly interconverting non-planar conformations.*—A conformation of this type, where the molecular reorientation is slower than the rate of interconversion, also needs three *S*-values to describe the molecular order. Let us assume that $\theta_N = -2\triangle\omega$. In such a case, three *S*-values and an independent dihedral angle are to be determined from the four dipolar coupling constants. One, therefore, normally expects a 'perfect-fit' between the observed and the 'best-fit' calculated dipolar coupling constants. It was actually found to be so for the various modes of rotations of the methyl group discussed in case 1, except for the 'eclipsed' case, for which the minimum r.m.s. error between the observed and calculated dipolar couplings was obtained for the 'planar' structure. The resulting *S*-values and the dihedral angles are given in Table IV for the various possibilities considered, except for the 'eclipsed' case for which the values given in Table III are valid. In this case also, no significant differences were observed for the 'free rotation' and the '6-fold' cases and hence the data for these are given in the same column in Table IV.

Table IV shows that, for the two rapidly interconverting bent conformations of N-methyl formamide, the NMR results are in conformity, provided the dihedral angle $\triangle\omega$ is less than 10°. The value of $\triangle\omega$ in fact varies from 3° to 8° depending upon the mode of rotation of the methyl group. An error of about 5° is estimated for the value of $\triangle\omega$ given in Table IV. It may be emphasized that more definite and reliable information on the conformation of N-methyl formamide is expected to be derived from the isotopically enriched (^{13}C and ^{15}N) species as discussed in section (*d*).

TABLE IV

'Best fit' parameters for N-methyl formamide considering two rapidly interconverting configurations as described in 'case 2' in the text

Parameter	Using the data given in structure 2	
	'staggered'	'6-fold' or 'free rotation'
$S_{zz}{}^{a}$	·0166	·0155
$S_{yy}{}^{a}$	·0265	·0225
$S_{xx}{}^{a}$	—·0431	—·0380
$S_{xy}{}^{a}$	·0147	·0177
θ (in degrees)	8·3	3·2

[a] Refers to the Cartesian coordinate system where the axes x and y lie in the 'effective' plane of symmetry [containing atoms C′, H (1), O, N]. The x-axis is along the axis of rotation of the methyl group in a 'rigid-planar' configuration as in case 1. The S-values are calculated using the scaling distance of 1·78 Å between the protons of the methyl group.

(*c*) *N-Methyl acetamide.*—The PMR spectrum of N-methyl acetamide is shown in Fig. 3. Values of the NMR parameters derived from the analysis of the spectrum are given in Table V.

The proton NMR spectrum of N-methyl acetamide provides 5 distinct HH direct couplings which were used to study the planarity of the peptide unit as discussed for N-methyl formamide. The standard bond length and the bond angle data [22–25] are given in Fig. 4 (data 1 to 4). Data 1 refer to the standard Pauling-Corey geometry of the peptide unit. Data 2 give the average values from some recent X-ray and neutron diffraction studies on several peptides. Data 3 correspond to the X-ray results on N-methyl acetamide with N-H bond length equal to 1·00 Å and the ∠ CNH = 123° (Pauling-Corey values). The electron diffraction values, which are not very accurate, are given in data 4. The C-H bond length was assumed as 1·10 Å and the HCH bond angle was given the tetrahedral value (109·48°). In order to obtain an estimate of the errors due to uncertainty in the geometrical data, the computations were carried out using all the four data given

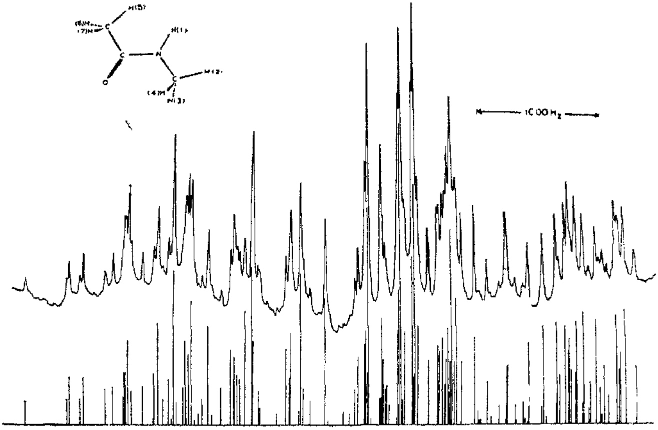


FIG. 3. Observed and calculated PMR spectra of N-methyl acetamide oriented in the nematic phase of (A).

Solute concentration: 9 mole per cent

Temperature: 25° C.

Spectrometer frequency: 360 MHz.

TABLE V

Parameters derived from the analysis of the PMR spectrum of N-methyl acetamide oriented in the nematic phase of (A). Numbering of the protons refers to that given in Fig. 3

Nuclear pair (i, j)	Coupling constants (Hz)		Chemical shifts (Hz)
	Indirect (J_{ij})	Direct dipolar (D_{ij})	$\nu_j - \nu_i$
(1, 2)	$4 \cdot 7 \pm 0 \cdot 1$	$-174 \cdot 3 \pm 0 \cdot 1$	$1598 \cdot 0 \pm 0 \cdot 2$
(1, 5)	$-0 \cdot 5 \pm 0 \cdot 1$	$-227 \cdot 5 \pm 0 \cdot 1$	$1878 \cdot 3 \pm 0 \cdot 2$
(2, 3)	..	$436 \cdot 8 \pm 0 \cdot 1$	
(2, 5)	$0 \cdot 3 \pm 0 \cdot 1$	$-48 \cdot 1 \pm 0 \cdot 1$	
(5, 6)	..	$419 \cdot 5 \pm 0 \cdot 1$	

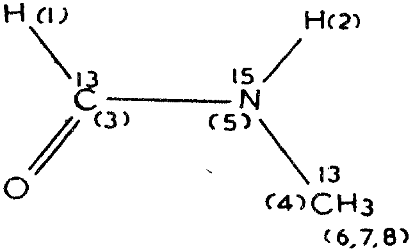
in Fig. 4. As in N-methyl formamide, the following possibilities were considered:

(*a*) Free rotation of the methyl groups.

(*b*) Rotations of the methyl groups hindered in 3-fold potentials such that the methyl groups ($C_2{}^{\alpha}H_3$ and $C_1{}^{\alpha}H_3$) are 'staggered' or 'eclipsed' about the N-H or the C=O bonds respectively. The methyl groups themselves were assumed rigid and no coupled motion between the two methyl groups was considered.

The mininum r.m.s. error between the observed and the calculated dipolar couplings was obtained when the dihedral angles were 10 ± 4° depending upon the geometrical data for the peptide unit.

(*d*) *^{13}C and ^{15}N isotopically enriched N-methyl formamide in a thermotropic solvent.* In this case (structure 3), the isotropic ^{1}H and ^{13}C NMR spectra were also analysed in addition to the PMR spectrum in a nematic solvent. The analysis of the spectra in the isotropic media was essential since some of the indirect spin-spin couplings could only be determined from such spectra. The various spectra of a sample from Merck, Sharp and Dohme of Canada are shown in Figs. 5–8.



(Structure 3)

The groups of lines marked A, B, and C in Fig. 5–8 arise from *cis* ^{15}N-methyl (^{13}C) formamide (^{13}C), *cis* and *trans* ^{15}N-methyl formamide (^{13}C) and *trans* ^{15}N-methyl (^{13}C) formamide. The other major lines arise from the predominant *trans* ^{15}N-methyl (^{13}C) formamide (^{13}C). The PMR spectrum in the nematic solvent is shown in Fig. 9. The detailed analyses of the spectra were performed only for the predominant species and the

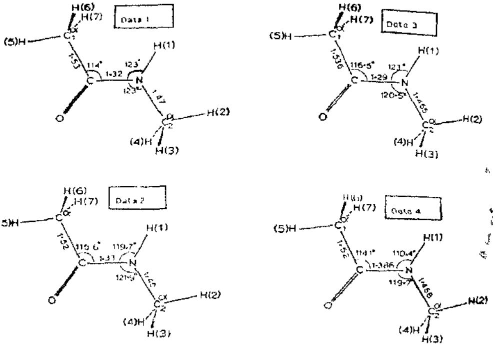


FIG. 4. Bond length (in Å) and bond angle data used for N-methyl acetamide. The four data correspond to those reported in the literature. [22–25].

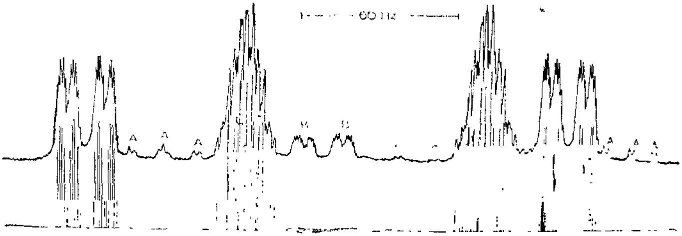


FIG. 5. PMR spectra of the formyl and the amide protons of ^{13}C and ^{15}N isotopically enriched N-methyl formamide at 270 MHz. The calculated spectrum is for the *trans* species of ^{15}N-methyl (^{13}C) formamide (^{13}C). The group of lines marked (A) arises from the *cis* species of ^{15}N-methyl (^{13}C) formamide (^{13}C). B and C are due to the *trans* and the *cis* respectively which do not have ^{13}C at the formyl carbon.

calculated spectra both in the isotropic and the nematic solvents are shown only for them. Values of the derived parameters are given in Table VI.

Signs of the HH and the ^{15}NH indirect couplings (J_{ij}'s) in Table VI were assumed from the earlier study on ^{15}N-methyl formamide. The indirect couplings between ^{13}C and the directly bonded ^{1}H were assumed positive. The signs of the other indirect couplings could not be determined. However, a combination of these studies with those in the nematic solvent together

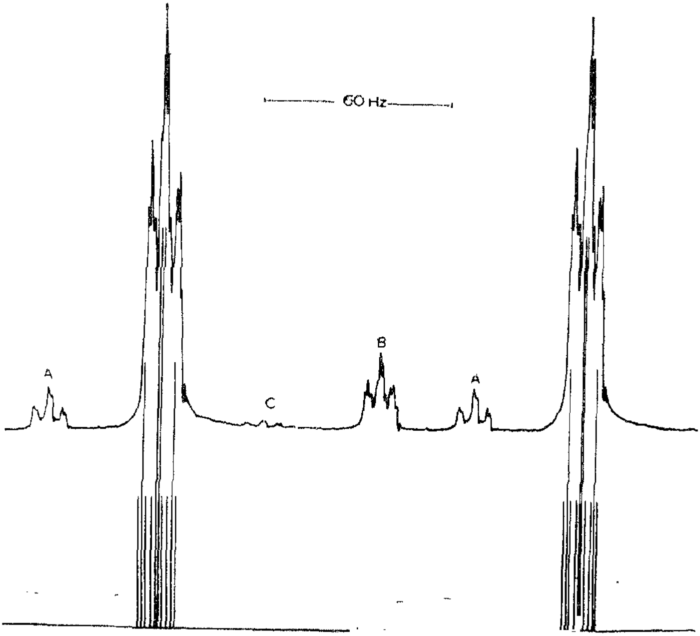


FIG. 6. PMR spectra of the methyl protons of ^{13}C and ^{15}N isotopically enriched N-methyl formamide at 270 MHz. The calculated spectrum is for the *trans* species of ^{15}N-methyl (^{13}Cr) formamide (^{13}C). The A group of lines arises from the corresponding *cis* species. B and C are due to the *trans* and the *cis* species respectively which do not have ^{13}C at the methyl carbon.

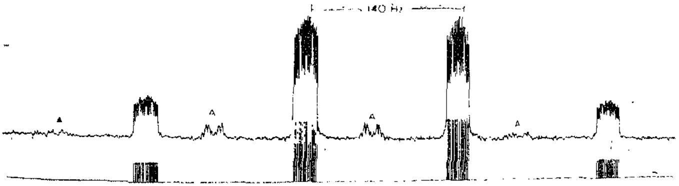


FIG. 7. ^{13}C-NMR spectra of the methyl carbon in ^{13}C- and ^{15}N-isotopically enriched species of N-methyl formamide at 22·63 MHz. The calculated spectrum is for the *trans* species. The spectrum due to the corresponding *cis* species is marked A.

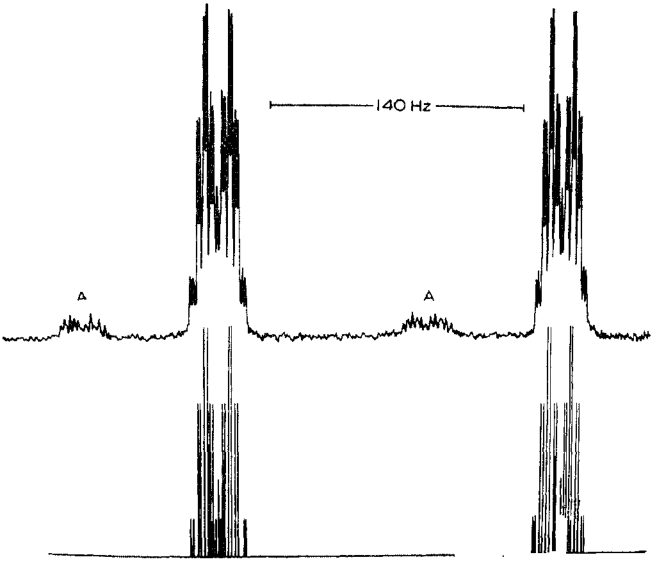


FIG. 8. ^{13}C-NMR spectra of the formyl carbon in ^{13}C and ^{15}N-isotopically enriched N-methyl formamide at 22·63 MHz. The calculated spectrum is for the *trans* species. The spectrum due to the *cis* species is marked A.

with the known geometry may throw some light on the signs of these coupling constants.

During the analysis of the PMR spectrum in the nematic phase, the signs and the magnitudes of the indirect couplings which were determined from the spectrum in the isotropic medium were used. However, since the signs of the indirect couplings J_{14}, J_{23}, J_{24} and J_{36} are not known, the corresponding $(J_{ij} + 2D_{ij})$ values are reported in Table VI. Since only the proton spectrum in a nematic solvent is analysed, it is difficult to label the dipolar couplings to particular heteronuclei. The values of the dipolar couplings given in the table correspond to reasonable bond lengths and bond angles in N-methyl formamide.

The direct dipolar couplings D_{46}, D_{56} and D_{67} provide the HCH bond angle and the angle which the line joining nuclei 5 and 6 makes with the

TABLE VI

Spectral parameters in trans ^{15}*N-methyl* (^{13}*C*) *formamide* (^{13}*C*) (*structure* 3)

^{1}H and ^{13}C spectra in the isotropic phase		PMR spectrum in the nematic phase	
Parameter	Value (Hz)	Parameter	Value (Hz)
J_{12}	1·91	D_{12}	—346·7
J_{13}	191·65	D_{13}	—172·4
J_{14}	± 5·17	$(J_{14} + 2D_{14})$	— 23·2
J_{15}	—15·56	D_{15}	26·2
J_{16}	— 0·94	D_{16}	— 31·2
J_{23}	± 3·94	$(J_{23} + 2D_{23})$	—248·6
J_{24}	± 2·79	$(J_{24} + 2D_{24})$	— 32·3
J_{25}	— 93·46	D_{25}	136·1
J_{26}	4·83	D_{26}	— 33·1
J_{34}	± 1·43	D_{34}	*
J_{35}	±13·84	D_{35}	*
J_{36}	± 3·81	$(J_{36} + 2D_{36})$	— 37·2
J_{45}	±10·57	D_{45}	*
J_{46}	137·83	D_{46}	77·1
J_{56}	0·97	D_{56}	9·65
J_{67}	Does not influence	D_{67}	112·9
$\nu_2 - \nu_1$**	14·69	$\nu_2 - \nu_1$**	240·4
$\nu_6 - \nu_1$**	1463·2	$\nu_6 - \nu_1$**	1424·4
$\nu_4 - \nu_3$†	3143	$\nu_4 - \nu_3$	*

* cannot be determined from the PMR spectrum.

** at 270 MHz; † at 22·63 MHz.

bond connecting nuclei 4 and 5 (structure 3), irrespective of whether the three bonds meeting at the nitrogen atom lie in one plane or not. Neglecting

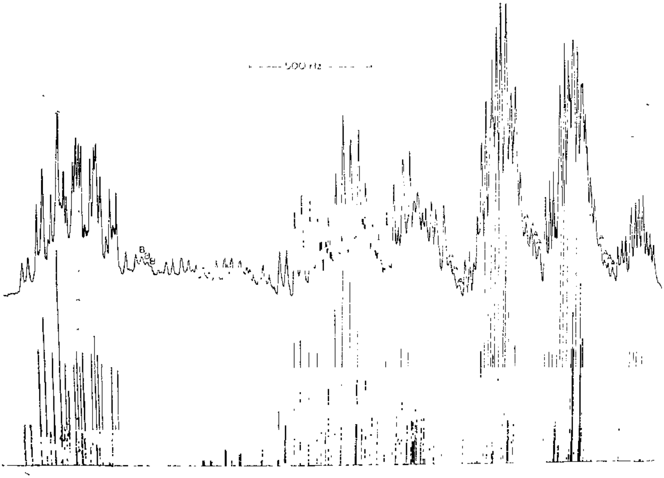


FIG. 9. Observed and calculated PMR spectra of ^{13}C- and ^{15}N-isotopically enriched N-methyl formamide oriented in the nematic phase of 4-methoxybenzylidene-4-amino-α-methyl cinnamic acid-*n*-propyl ester. The calculated spectrum is due to *trans* species of ^{15}N-methyl (^{13}C) formamide (^{13}C). The lines marked A do not belong to these species.

Solute concentration: 15 mole per cent.
Temperature: 30° C.
Spectrometer frequency: 270 MHz.

the influence of molecular vibrations and using the standard equations [8], these values are determined as 108·5° and 30·7°.

In this case, only a preliminary interpretation of the various direct dipolar couplings has yet been made. Only the 'free rotation' of the methyl group has been considered. The results indicate significant non-planar distortions around the nitrogen atom provided influences of molecular vibrations are neglected. The dihedral angles $\Delta\omega$ and θ_N describing the non-planar distortions around the nitrogen atom in this case have been found to be of opposite signs and their magnitudes were determined as $11 \pm 5°$ and $20 \pm 5°$ respectively.

It may be concluded that the NMR results in all the cases, namely, formamide, N-methyl formamide, N-methyl acetamide are consistent among themselves as also with theoretical studies. They indicate significant non-planar distortions around the nitrogen atom in a peptide unit. It may be emphasized that the NMR results neglect all influences of molecular vibrations and assume certain bond lengths.

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