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PMR studies on N–methyl formamide oriented in a liquid crystalline nematic phase†

C L KHETRAPAL*, A C KUNWAR* and
K R K EASWARAN**
*Raman Research Institute, Bangalore 560006, India
**Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560012, India

Abstract. The PMR spectrum of N–methyl formamide dissolved in the nematic phase of 4–methoxybenzylidene–4–amino–α–methyl cinnamic acid–*n*–propyl ester is reported. The four interproton dipolar couplings are derived. Using reasonable values for the bond lengths and the bond angles, it is found that the results are consistent with the molecule having a plane of symmetry containing the fragment H.CO.NH.C. Assuming the H–CO–N fragment to be rigidly 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 are less than 10°.

Information on the mode of rotation of the methyl group about the N–C bond is derived.

The results establish that N–methyl formamide contains predominantly the '*trans*' species (NH and the C=O groups in the trans position). In addition, some weak lines, which arise from the '*cis*' species (NH and the C=O group in the cis position) present to a much smaller extent are detected.

1. Introduction

During recent years, there has been considerable interest in theoretical calculations related to the conformation of peptides and proteins[1–6]. In most of these studies a completely planar peptide unit has been used, whereas, in some cases, it has been pointed out that the peptide unit is non-planar[6, 7]. In view of the importance of such studies, it is essential to establish experimentally whether the peptide unit is planar or not; NMR studies in a nematic solvent provide an easy method for this purpose[8]. Since monosubstituted amides are the simplest structures which contain the peptide bond, it is convenient to investigate them first in order to obtain information on more complicated sytsems. In the present

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paper, which is the first in the series, results obtained from the PMR study of N-methyl formamide (figure 1) dissolved in a nematic solvent are reported.

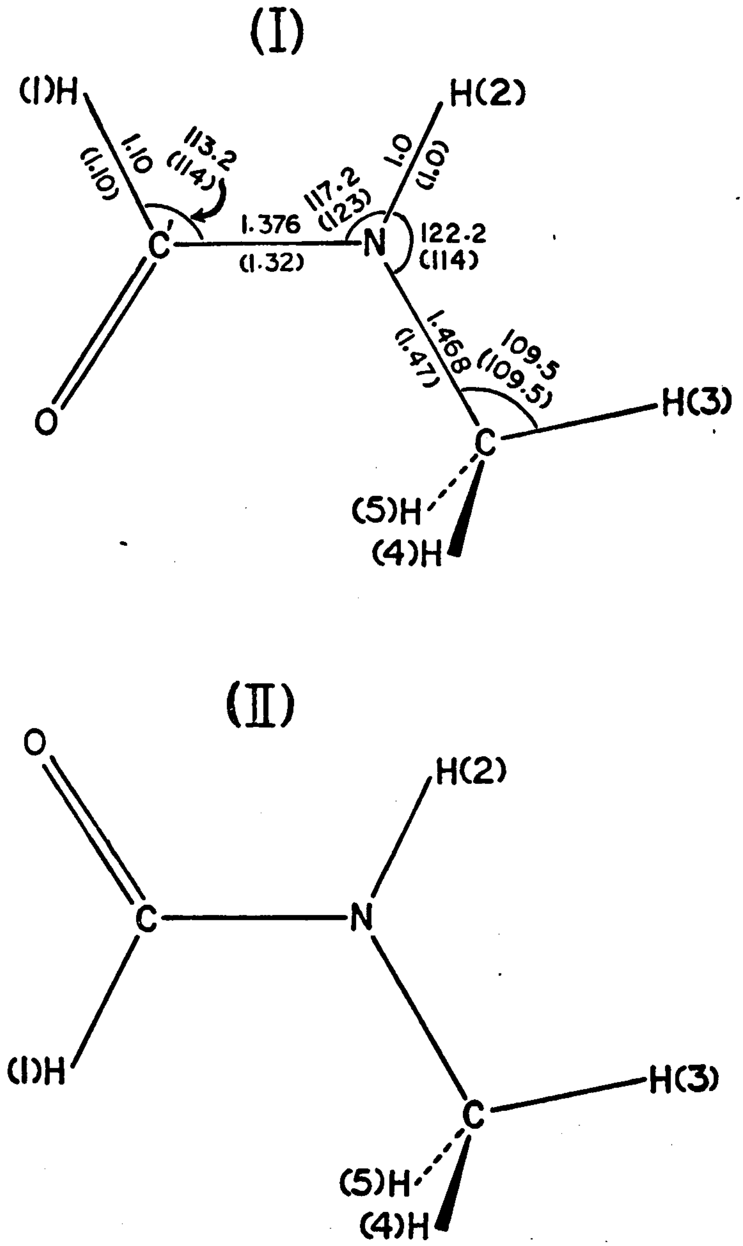

Figure 1 Structure of N-methyl formamide. Structure I is referred to as the '*trans* structure' and structure II as the '*cis* structure'. The bond lengths are given in units of 10^{-10} m and the bond angles in degrees. The two different structural data given are discussed in the text.

The PMR spectrum of N-methyl formamide has been studied earlier in the isotropic phase[9-11]. It has been found that both the '*trans*' (structure I) and the '*cis*' (structure II) species are present at room temperature. The presence of both the rotational isomers indicates a relatively high barrier to internal rotation. An estimated value of 20-28 Kcal./mole for the barrier to internal rotation[12] supports this point of view. One of the isomers is found to be present to the extent of about 92% and the other about 8%[9]. Further, from studies of a large number of substituted amides including ^{15}N enriched compounds and the effects of solvents like benzene on the methyl proton resonances in N-methyl formamide, it has been shown that it is the '*trans*' configuration (structure I) which is present predominantly ($\simeq$ 92%). NMR spectra of molecules dissolved in a liquid crystalline nematic phase which contain information about the molecular geometries provide direct knowledge of whether the '*cis*' or the '*trans*' species is more abundant. Such results are also reported in the present paper.

In the isotropic phase spectrum, the resonance of the N-H proton has been found to be considerably broadened due to the quadrupolar relaxation of ^{14}N nuclei. Analyses of the single and the double resonance PMR spectra of ^{15}N substituted N-methyl formamide[10, 11] provide the signs and the magnitudes of the inter-proton indirect spin-spin coupling constants. They have been used in the analysis of the spectrum in the nematic phase described in the paper.

2. Experimental

A 16.7 mole per cent solution of commercially available N-methyl formamide was prepared in 4-methoxybenzylidene-4-amino-α-methyl cinnamic acid *n*-propyl ester (a). The spectrum (figure 2) of the solution was recorded at probe temperature ($\simeq$30° C) on a varian HA-100 spectrometer in the field sweep mode without 'lock'.

The average line-width was 7 Hz. Several spectra were recorded and the mean line positions were measured. The statistical error in the measurement of line positions was found to be 1.0 Hz with no line deviating by more than 2.0 Hz from the mean value.

3. Results and discussion

3.1. *Analysis of the spectrum*

Analysis of the spectrum was carried out with the help of the LAOCOONOR[13] programme adapted to the IBM-360/44 computer. During the analysis, the direct dipolar couplings between nuclei i and j (D_{ij}'s as defined in the LAOCOONOR programme) and the chemical shifts ($\nu_i - \nu_j$)

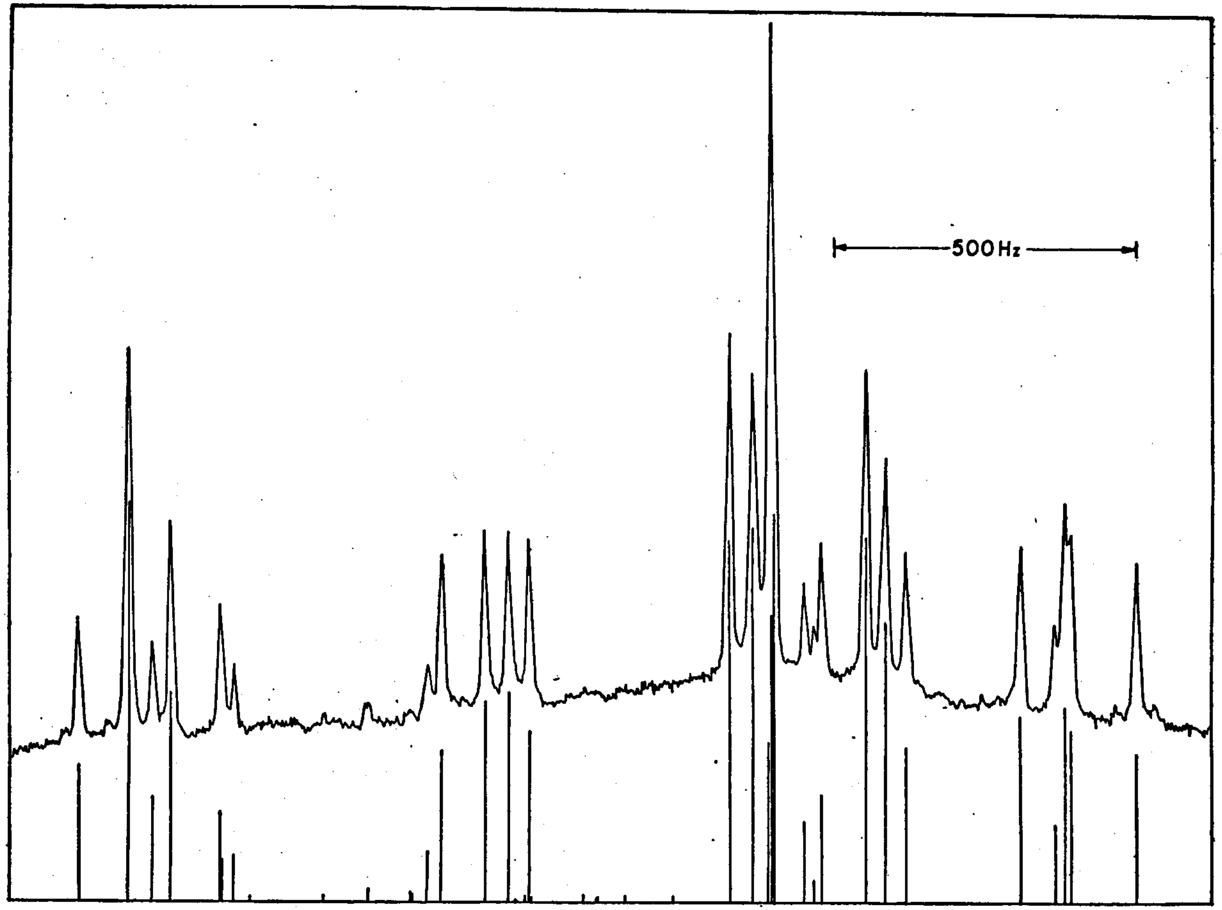

Figure 2 Observed and calculated PMR spectra of N-methyl formamide dissolved in the nematic phase of (a). Solute concentration = 16.7 mole per cent; Probe temperature = 30°C; Spectrometer frequency = 100 MHz. The calculated spectrum corresponds to the '*trans*'-species which are present predominantly. Some of the small lines in the observed spectrum which do not appear in the calculated one arise from the '*cis*' species.

were iterated upon. Values of the isotropic couplings were taken from the literature[11] and were not iterated upon. An r.m.s. error of 0·7 Hz was obtained between the observed and the calculated line positions with no line deviating by more than 3·0 Hz even when the overlapping lines were included in the analysis. The calculated spectrum is shown below the observed one in figure 2. Values of the parameters obtained are given in table 1. A comparison of the observed and the calculated spectra in figure 2 shows that some of the weak lines in the observed spectrum do not appear in the calculated one. They may arise from the '*cis*' species present in much lower abundance. This portion of the spectrum has now been studied by accumulating sufficient number of spectra with the help of a suitable 'time averaging' computer; the results will be published elsewhere[22].

Table 1. Spectral parameters for N–methyl formamide dissolved in the nematic phase of (a). Numbering of protons refers to that given in structure (I).

Nuclear pair	Coupling constants (Hz)		$(\nu_j - \nu_i)$ Hz
(i, j)	Indirect (J_{ij})	Direct dipolar (D_{ij})	
(1, 2)	1·9	−376·96 ± 0·8	123·3 ± 3·4
(1, 3) (1, 4), (1, 5)	−0·8	− 44·39 ± 0·6	533·1 ± 3·4
(2, 3) (2, 4), (2, 5)	4·9	− 36·35 ± 0·7	
(3, 4) (3, 5), (4, 5)	does not affect	163·20 ± 0·2	

Errors of the parameters given in the table 1 were determined as described in the literature[13].

3.2. Conformational information

The proton spectrum of one conformation of N–methyl formamide provides four interproton direct dipolar coupling constants. If the molecule does not possess a plane of symmetry, the four inter-proton dipolar couplings do not even provide the order matrix which has 5 independent elements.

In order to obtain any structural information in such a case, it is essential either to study the isotopically enriched (^{13}C and ^{15}N) species or to accumulate sufficient number of spectra with the help of a suitable computer for obtaining the direct couplings with nuclei which are present in low natural abundance. If on the other hand the molecule possesses a plane of symmetry, only three direct couplings are needed to describe the molecular order. If, therefore, a reasonable geometry for the molecule is used to compute iteratively the three elements of the order matrix[16] (the S–values) from the four direct dipolar coupling constants, an acceptable fit (*i.e.*, within experimental errors of the dipolar coupling constants) between the observed and the 'best fit' computed dipolar coupling constants indicates that either H·CO·NH·C– lie on one plane, or a rapid inversion about the nitrogen atom introduces an 'effective' plane of symmetry as discussed below.

To the best of our knowledge, the structural data on N–methyl formamide does not seem to have been reported. However, the microwave data on formamide are available[14, 15]. Of these, the more recent data[15] together with the HCH bond angle equal to 109·5° and C′–H and the N–C bond lengths equal to 1·10 Å and 1·468 Å respectively given in structure I (hereafter referred to as data I) have been used in the present calculations. A survey of the literature revealed that the data I differ from those conventionally used for the structure of the peptide unit[6]. These data (referred to as data II hereafter) given within parentheses in structure I were also used for the calculations in order to derive more reliable conclusions. Such a procedure gives an estimate of the errors arising out of the uncertainties in the structural data used.

In the present work, we discuss the possibility of the molecule possessing a plane of symmetry. We consider the following two cases:

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

Case 2. Two energetically equivalent rapidly inter-converting 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 as in the toluenes[17, 20] and the π–methylcyclopentadienyl manganese tricarbonyl complex[18]. The equations relating the dipolar couplings, D_{13} and D_{23}, with the molecular geometry and the S–values are similar to those reported earlier[18, 19] and were introduced into the 'SHAPE' programme[17] for the 'best fit' calculations, together with the equations for D_{12} and D_{34}[19]. The 'best fit' calculations for the S–values were thus made using the fixed geometry (data I and data II). 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 proton (referred to as 'staggered' hereafter) or (2) it is towards the N–H proton (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. Further, in the calculations, the trans-configuration of N-methyl formamide was assumed. Values of the 'best fit' parameters obtained using the 'SHAPE' programme are given in table 2. Errors of the S-values were estimated to be 0·0003 in each case using the errors of the observed D-values given in table 1. The 'r.m.s. error' given in table 2 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 2.

Table 2 shows that the coupling constant D_{13} is the one which is most significantly influenced by the mode of rotation of the methyl group, for both the data sets used. It is also seen from the table that the magnitude of this coupling constant is larger than the observed value (table 1) 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 2. The r.m.s. error of about 0.2 Hz for the '6-fold' or the 'free rotation' cases using 'data I' seems to support the assumption of the plane of symmetry in the molecules. Table 2 also shows that the data are strongly dependent upon the mode of rotation of the methyl group and the geometrical data used. The r.m.s. errors of 1.09, 1.41 and 2.05 Hz obtained for the 'eclipsed' and the 'staggered' cases using 'data I' and 'staggered' case using 'data II' are certainly outside the experimental errors. Although the r.m.s. errors of 0.65 and 0.75 Hz obtained for 'data II' are of about the same order of magnitude as the experimental errors of the observed coupling contants (table 1), the deviations in D_{13} are much larger and are outside the experimental errors. Consequently, in order to obtain a more direct and

Table 2. '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 I. The data are for the rigid planar conformation described in 'case I' in the text.

Parameter	Using 'data I' given in structure I			Using 'data II' given within parentheses in structure I		
	'eclipsed'	'staggered'	'6–fold' or 'free rotation'	'eclipsed'	'staggered'	'6–fold' or 'free rotation'
D_{12}	–376·90 Hz	–377·01 Hz	–376·97 Hz	–376·92 Hz	–377·03 Hz	–376·99 Hz
$D_{13}=D_{14}=D_{15}$	–46·42 Hz	–41·75 Hz	–44·00 Hz	–45·63 Hz	–40·50 Hz	–42·96 Hz
$D_{23}=D_{24}=D_{25}$	–36·91 Hz	–35.67 Hz	–36·25 Hz	–36·60 Hz	–35·62 Hz	–36·08 Hz
$D_{34}=D_{35}=D_{45}$	162·63 Hz	163·90 Hz	163·31 Hz	162·88 Hz	164·15 Hz	163·53 Hz
S_{xx}*	·0153	·0154	·0153	·0153	·0154	·0154
S_{yy}*	·0177	·0283	·0229	·0188	·0334	·0258
S_{zz}*	– ·0330	– ·0437	– ·0382	– ·0341	– ·0488	– ·0412
S_{xy}*	·0214	·0134	·0175	·0224	·0128	·0178
r.m.s. error	1·09 Hz	1·41 Hz	0·21 Hz	0·65 Hz	2·04 Hz	0·75 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 x 10^{-10}m.

reliable information on the $(\mathrm{C{-}N}{<}^{\mathrm{H}}_{\mathrm{C}})$ fragment, more reliable geometrical data on N–methyl formamide should be available and the isotopically enriched ^{13}C and ^{15}N species should be studied. In the latter case besides, getting more direct coupling contants (which make it possible to obtain some of the geometrical data), it would be possible to derive the results independent of the mode of rotation of the methyl group. Work on these lines is in progress.

The above calculations for the 'free rotation' case were also made assuming the '*cis*' configuration (structure II) (obtained by a 180° rotation of the H(1)C′O plane about C′–N bond) of the molecule. It was found that the r.m.s. error between the observed and the 'best–fit' calculated *D*–values is 49.03 Hz for 'data I' and 54.72 Hz for 'data II'. The results, therefore, directly establish that the '*trans*' species are predominant in N–methyl formamide and the calculated spectrum corresponds to these.

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 inter–conversion, also needs three *S*–values to describe the molecular order. Let us consider the two bent conformations with the N-H and N-C bonds which bend on the same side of the effective plane of symmetry and make an angle $\pm\,\theta$ with it; the positive sign applies to one conformation and the negative to the other. In such a case, three *S*–values and the angle θ 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 rotation 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 the calculated dipolar couplings was obtained for the 'planar' structure (*i.e.*, for $\theta = 0$). The programme 'SHAPE' was suitably modified and adapted to IBM 360/44 computer to carry out these calculations. The resulting *S*–values and the angles (θ's) are given in table 3 for the various possibilities considered except for the 'eclipsed' case for which the values given in table 2 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 3.

Table 3 shows that, for the two rapidly inter–converting bent conformations of N–methyl formamide, the NMR results are in conformity provided (θ) is less than 10°. The value of θ in fact varies from 3 to 10° depending upon the assumed structural data and the mode of rotation of the methyl group. An error of about 5° is estimated for the value of θ given in table 2.

Table 3. 'Best fit' parameters for N-methyl formamide considering two rapidly inter-converting configurations as described in 'case 2' in the text.

Parameter	Using 'data I' given in structure I		Using 'data II' given within parentheses in structure I	
	'staggered'	'6-fold' or 'free rotation'	'staggered'	'6-fold' or 'free rotation'
$S_{xx}{}^{a}$	.0166	.0155	.0173	.0160
$S_{yy}{}^{a}$	.0265	.0225	.0304	.0245
$S_{zz}{}^{a}$	−.0431	−.0380	−.0477	−.0405
$S_{xy}{}^{a}$	.0147	.0177	.0145	.0185
θ (in degrees)	8.3	3.2	10.2	6.2

[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 x 10^{-10}m between the protons of the methyl group.

As pointed out earlier, for the 'eclipsed' form, an acceptable fit between the observed and the calculated dipolar couplings is obtained neither for the rigidly 'planar conformation' nor for the two 'rapidly inter-converting bent conformations' when reasonable bond lengths and bond angles are used. These results indicate that the 'eclipsed' form alone may not be present in N-methyl formamide if the bond length and the bond angle data used are correct.

As mentioned earlier, 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: the work on these lines is in progress.

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[*] Recently, Hirota *et al*[21] have re-determined the structure of formamide with the help of microwave spectroscopy. These data give a minimum r.m.s. error of 0.09 Hz between the observed and the 'best-fit' calculated *D*-values for a planar structure of the fragment H.CO.NH.C in N-methyl formamide; this 'minimum' is obtained for the 'free-rotation' of the methyl group about the N-C bond and using the HCH bond angle, the C-H and the N-C bond lengths equal to those for data I described in the text.

DISCUSSION

Sharma : Have you verified results for formamide ?

Khetrapal : No. The molecule formamide can be studied for obtaining such an information only if the coupling of at least one of the hetero-nuclei are observed. Such a study can be done either by taking the samples enriched in ^{13}C and/or ^{15}N or by the use of F.T.NMR. In absence of such facilities, this work has not been undertaken. However, Reeves and his group have studied the ^{15}N–enriched formamide dissolved in a lyotropic mesophase and they find a non-planar structure (Mol. Phy. 1973).