

THE CONFORMATION OF AN LD TRIPEPTIDE *N*-ACETYL-*L*-PROLYL-*D*-ALANYL-METHYLAMIDE FROM PROTON AND CARBON-13 NMR

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ABSTRACT

The solution conformation of a tripeptide molecule with both *L* and *D* amino acid residues, namely *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide has been studied using both proton and carbon-13 NMR techniques. The results indicate the presence of two conformers with their percentage fraction varying in different solvents. The major fraction of the conformers have *trans* acetyl-pro bond and assume a compact β -bend conformation with *L*-pro and *D*-ala at the corners. The minor fraction of the conformers have a *cis* acetyl-pro bond and probably assume an extended structure.

The carbon-13 spin-lattice relaxation (T_1) data indicate an overall isotropic motion for the major conformers and a large mobility for the proline ring carbons which follow the order $\gamma > \beta > \delta$.

INTRODUCTION

THE conformation of peptides consisting of alternating *L* and *D* amino acid residues are of interest because of the occurrence of β -bends, as for example *LD* bends in cyclic peptides and peptide antibiotics¹⁻⁵. Detailed theoretical energy calculations^{6,7} have shown interesting possibilities of folded conformations and chain reversals for peptides with alternating *L* and *D* amino acid residues.

During the past few years, both proton and carbon-13 NMR studies have been extensively used to understand the conformation of peptides and related systems^{8,9}. Large amount of data are available on the possible *cis-trans* conformers in a number of proline containing peptides¹⁰. In addition, Carbon-13 relaxation time measurements are useful to understand the dynamics of the molecule^{10,11}, for example, in determining the mobility of the proline ring carbons in peptides containing proline residue¹⁰. In this paper the results of the ¹H and ¹³C NMR studies and also the ¹³C spin lattice relaxation time measurements on a model tripeptide, namely, *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide are reported and compared with theoretical energy calculations.

MATERIALS AND METHODS

The proton and the Carbon-13 NMR spectra were recorded using Bruker WH-270 FT spectrometer equipped with variable temperature accessory. The Carbon-13 spin-lattice relaxation time (T_1) measurements were made using a (180- τ -90- T_∞) pulse sequence with T_∞ at least five times the largest T_1 to be measured. The sample of *N*-acetyl-*L*-prolyl-*D*-alanyl-

methylamide was synthesised in our laboratory by Dr. K. S. N. Iyer and made available to us. The deuterated samples used were obtained from Stohler Isotopes Inc. The degassed solutions of the sample were used for T_1 measurements.

RESULTS

(i) ¹H NMR Studies

The 270 MHz proton NMR spectra of *N*-acetyl-*L*-Prolyl-*D*-alanyl-methylamide were recorded in CDCl₃, CD₃CN, DMSO (*d*₆) and D₂O. The spectra in CDCl₃ and DMSO (*d*₆) are shown in Fig. 1a and 1b respectively. Assignments of the different resonances have been made in comparison with the spectra of similar compounds and using double resonance methods. The data on chemical shifts (δ 's) and the spin-spin couplings (J 's) of various protons are given in Table I. In all the spectra in addition to the major resonance peaks, additional lines of smaller intensity were also observed. The intensities of these minor resonances with respect to the corresponding major lines varies as one goes from non-polar solvents like CDCl₃ to a polar solvent DMSO (*d*₆). As can be seen from Table I, the resonance due to NH of methylamide is shifted by 0.6 ppm low field whereas that of alanyl amide proton shifts by 1.9 ppm as the solvent is changed from CDCl₃ to DMSO (*d*₆). This is indicative of the fact that alanyl NH proton is perturbed to a large extent by the protonated solvent compared to the NH of methylamide. The ³ $J_{\text{NH-C}\alpha\text{-H}}$ constant for the alanyl residue in both the solvents remain the same within experimental errors.

The temperature dependence of the chemical shift for the two amide peaks in DMSO (*d*₆) is shown in Fig. 2. The temperature coefficient of chemical shift ($d\delta/dT$) for the major peaks of the alanyl NH is

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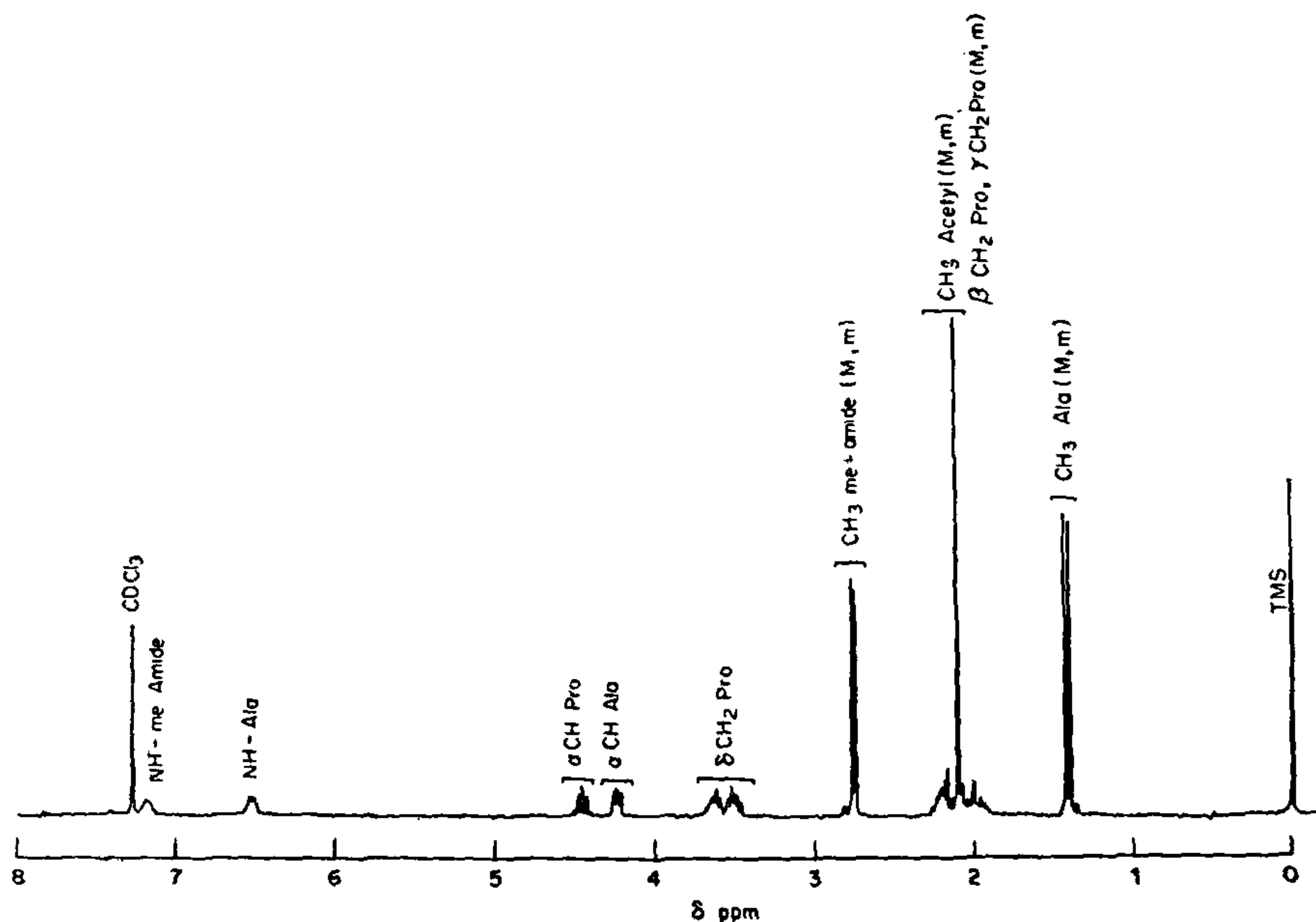


FIG. 1a. The 270 MHz ^1H NMR spectrum of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in CDCl_3 .

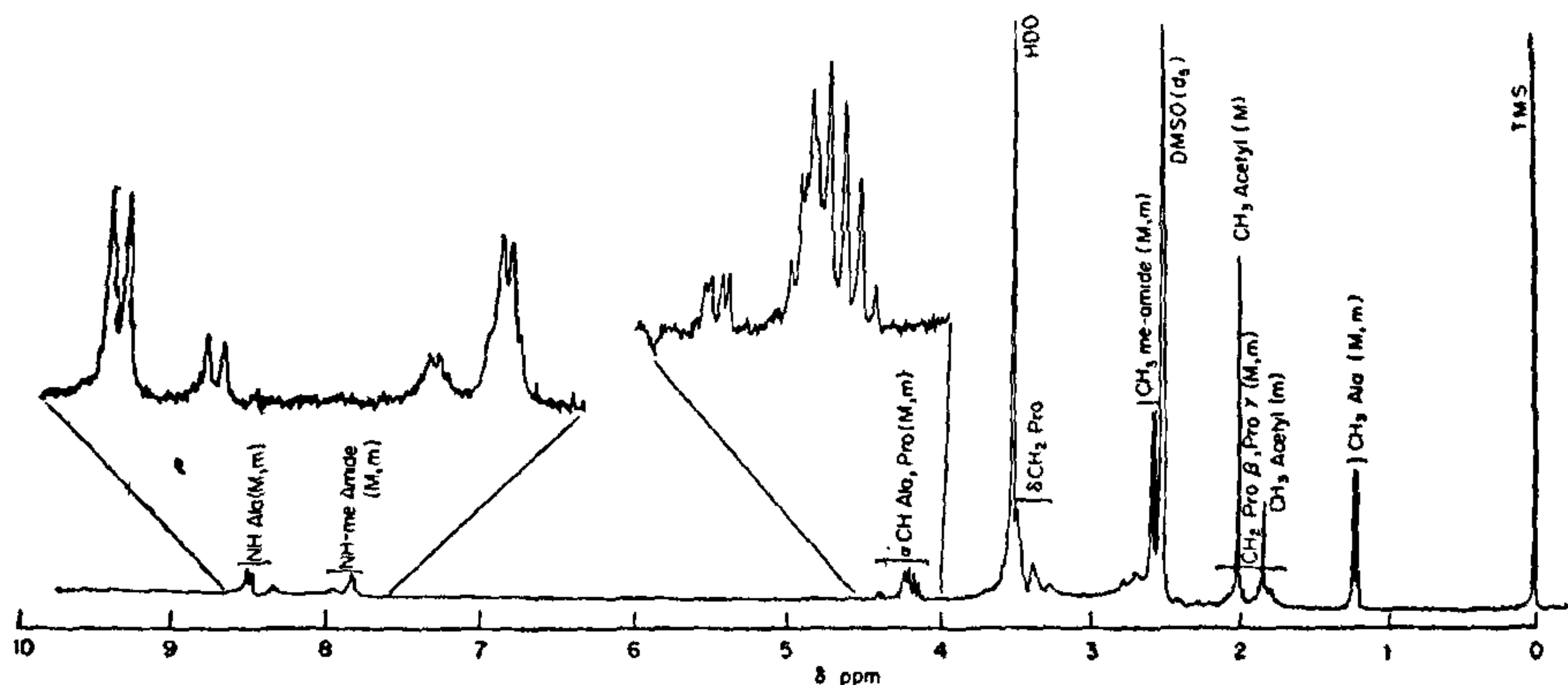


FIG. 1b. The 270 MHz ^1H NMR spectrum of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in $\text{DMSO}(d_6)$. The *a* and the amide region expansion is given as inset.

6.5×10^{-3} ppm/deg as compared to a value of 2.5×10^{-3} ppm/deg for the NH of methylamide. It is also seen that the temperature coefficient of chemical shift for the minor resonances of both the amide protons is about 6.5×10^{-3} ppm/deg.

(ii) Carbon-13 Chemical Shift

The 67.89 MHz ^{13}C NMR spectra of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in CDCl_3 and $\text{DMSO}(d_6)$ is shown in Fig. 3. The spectra were also recorded

TABLE I
The proton chemical shifts (δ)^a and the spin-spin coupling constants (J) for *N*-acetyl-L-prolyl-D-alanyl-methylamide

	Chemical shifts, (ppm)							Percentage of <i>cis</i> Conformer ^c
	Ala NH	methyl-amide NH	Pro -CH	Ala -CH	Pro -CH ₂	Pro & CH ₂	methyl-amide CH ₃	
DMSO (<i>d</i> ₆)	8.59 (8.25)	7.75 (7.87)	4.20 (4.35)	4.15 (4.32)	3.47	1.70-2.13	2.56 (2.55)	24.0
D ₂ O	4.35	4.25	3.65	1.83-2.37	2.73 (2.72)	25.0
CD ₃ CN	7.17	7.33	4.17	4.12	3.52	1.78-2.18	2.62 (2.63)	12.8
CDCl ₃	6.52	7.18	4.46	4.23	3.58	1.90-2.30	2.12 (2.00)	3.8
							1.42 (1.27)	7.7
							1.29 (1.35)	<i>b</i>
							1.37 (1.20)	7.4
							1.99 (1.82)	

^a All the chemical shifts are measured relative to TMS as internal reference.

The values given in the brackets correspond to those of *cis* conformer (M).

All other values are for the *trans* conformer (M).

^b The value could not be measured due to broadening of the peak.

^c The percentage of the *cis* conformers estimated as the average value from ¹H peak intensities of major and minor resonances.

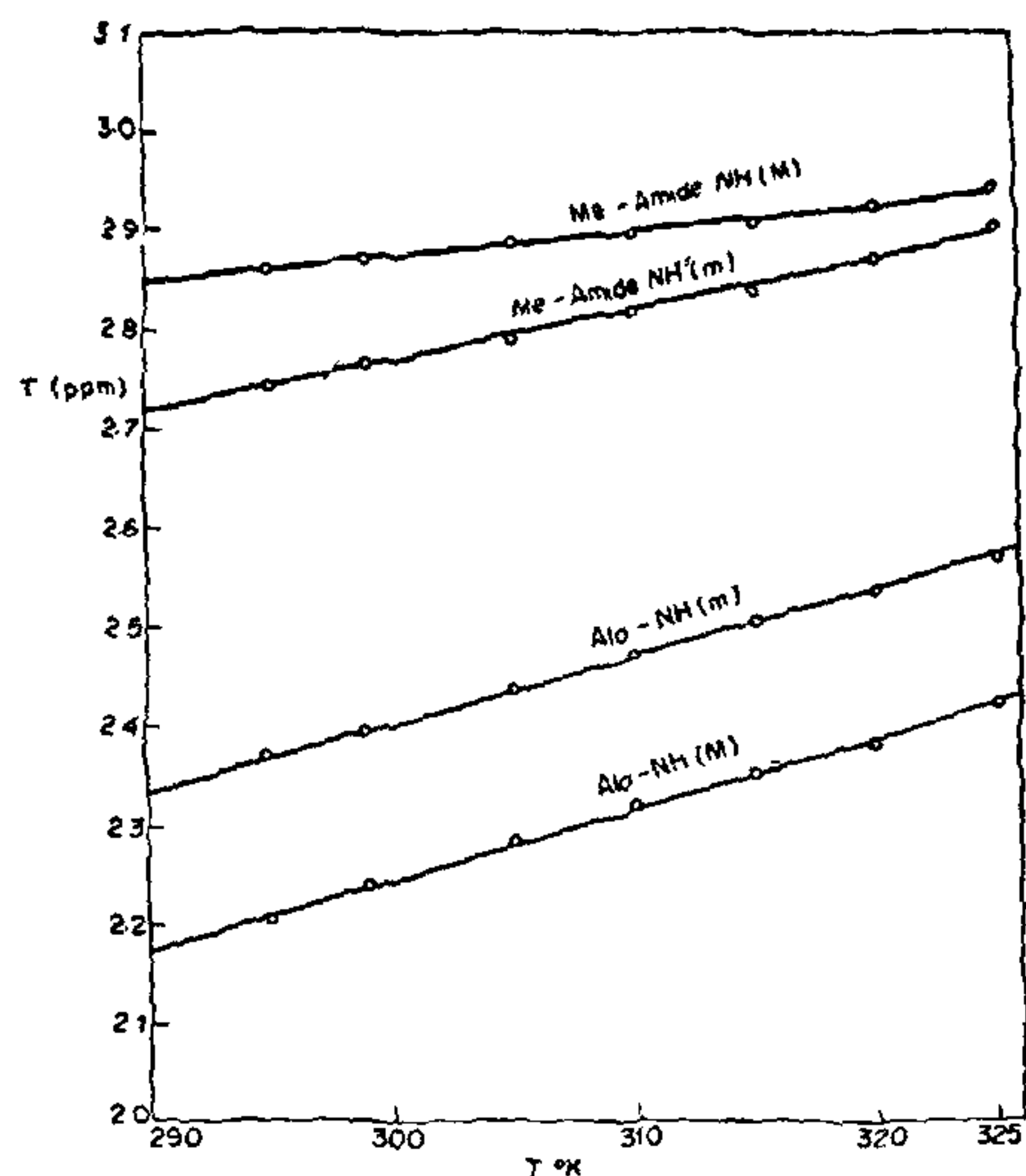


FIG. 2. The temperature dependence of chemical shift of the alanyl and methylamide NH's for the major and minor conformers of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in DMSO (d_6).

in D_2O . All assignments are made in comparison with the spectra of similar compounds^{12,13}. The chemical shift data of individual resonances in different solvents are given in Table II (data on the low field carbonyl region is not included). In addition to the major peaks another set of peaks of smaller intensity were also observed and this may be seen in Fig. 3. The ratio of the minor to major resonances varied from one solvent to the other similar to what was observed in 1H NMR studies.

(iii) ^{13}C spin-lattice Relaxation Times

The Carbon-13 spin-lattice relaxation times for the individual carbon resonances measured for both the major and minor peaks, in DMSO (d_6), are given in Table III. It is interesting to note that the prolyl and alanyl α -carbons have nearly the same NT_1 values indicating an isotropic overall motion for the backbone. The NT_1 values for the ring carbons of the prolyl residue are larger than the ones corresponding to α -carbons. The correlation time (τ_{eff}) of protonated carbons are calculated from the observed T_1 values assuming the overall motion to be isotropic and the mechanism of relaxation to be predominantly dipolar in nature and using the following equation

$$1/T_1 = N\hbar^2\gamma_c^2\gamma_H^2r_{CH}^{-6}\tau_{eff} \quad (1)$$

where N is the number of attached protons, γ_c and γ_H are the gyromagnetic ratios for carbon and proton

nuclei, r_{CH} is the internuclear separation between the carbon and the attached proton and \hbar is the planck's constant divided by 2π . In the case of pyrrolidine ring carbons, the τ_{eff} may contain a contribution from internal motion of the ring carbons (τ_{ring}) in addition to overall molecular reorientation. The internal mobility of the proline ring carbons are estimated using the expression¹⁴ namely,

$$\frac{1}{\tau_{ring}^2} = \frac{1}{\tau_{eff}^2} - \frac{1}{\tau_0^2} \quad (2)$$

where τ_{eff}^i is the overall correlation time of pyrrolidine ring carbons i ($i = \beta, \gamma$ or δ).

DISCUSSION

(i) Backbone Conformation

The ^{13}C chemical shift data (Table II) for the major resonances of C^β and C^γ carbons of the pyrrolidine ring are indicative of a *trans* acetyl-pro bond and that of the minor resonances correspond to a *cis* acetyl-pro bond¹⁵⁻¹⁷. The proton and Carbon-13 NMR spectra in different solvents indicate that the ratio of *cis* to *trans* isomers of the tripeptide varies with the solvents used. The average value of the percentage of *cis* conformer as observed from the 1H NMR data is

TABLE II

^{13}C Chemical shifts for *N*-acetyl-*L*-Protyl-*D*-alanyl-methylamide

(Resonances from carbonyl carbons not included)^a

Carbon	DMSO(d_6)	D_2O	$CDCl_3$
Pro- α (m)	61.33	61.41	61.97
Pro- α (M)	60.51	60.27	60.58
Ala- α (m)	49.16	50.20	48.83
Ala- α (M)	^b	49.86	49.26
Pro- δ (m)	47.18	47.29	46.84
Pro- δ (M)	48.85	48.94	48.55
Pro- β (m)	32.73	31.92	32.27
Pro- β (M)	30.16	29.89	29.08
N- CH_3	26.62	26.07	26.32
Pro- γ (M)	25.51	24.51	25.24
Pro- γ (m)	23.46	22.71	^b
CH_3CO (m)	23.21	21.48	23.09
CH_3CO (M)	23.08	^b	22.48
Ala- CH_3 (m)	19.09	^b	18.63
Ala- CH_3 (M)	18.41	16.59	17.70

^a Chemical shifts measured in ppm with TMS as internal standard.

^b Could not be measured due to overlap.

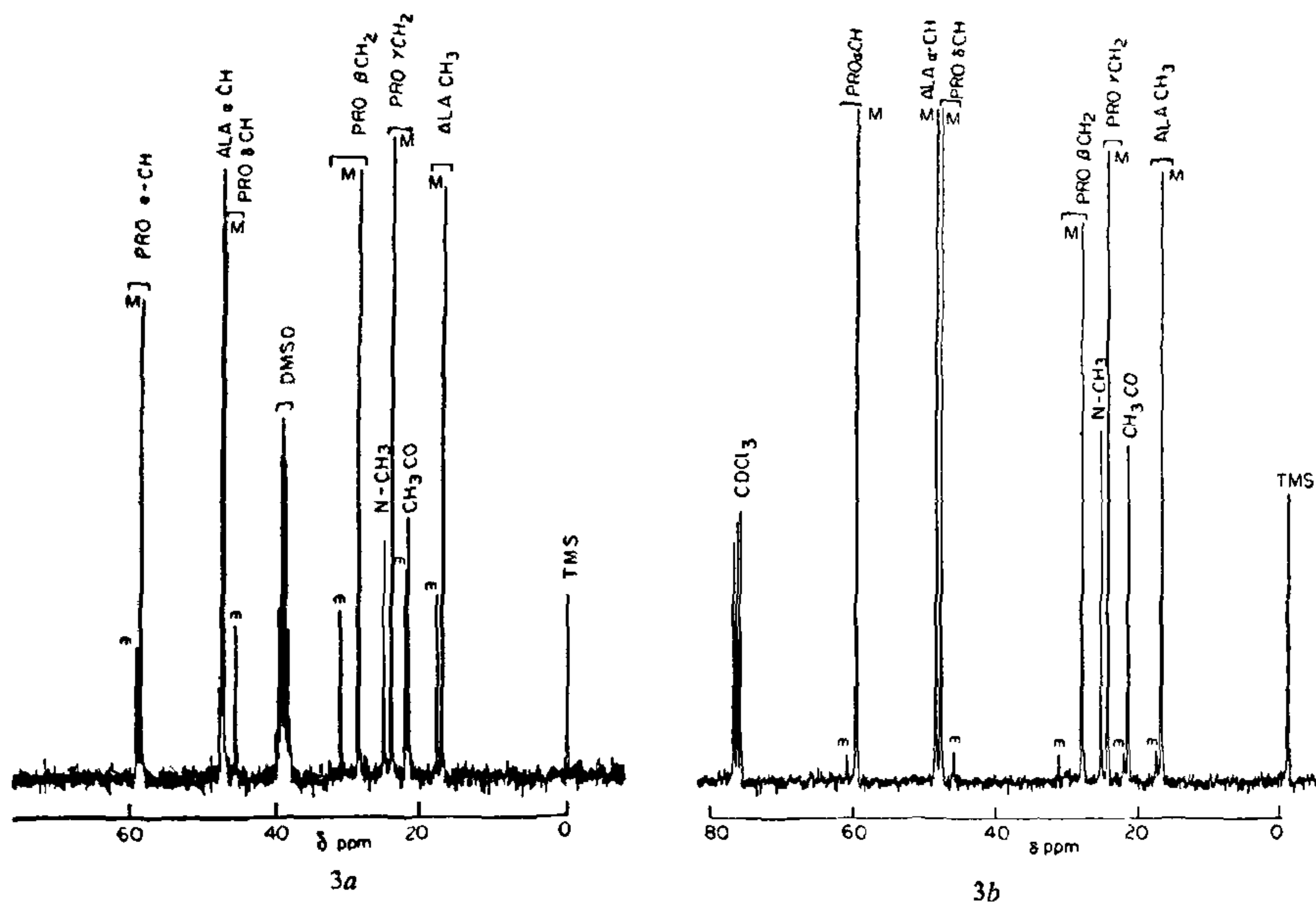


FIG. 3. The 67.89 MHz ^{13}C NMR spectrum of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in (a) CDCl_3 , (b) $\text{DMSO}(d_6)$.

TABLE III

The NT_1 values and correlation times (τ_{eff} and τ_{ring}) for ala α and pyrrolidine ring carbons of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide in $\text{DMSO}(d_6)$.

Carbon	NT ₁ in m sec.	$\tau_{\text{eff}} \times 10^{-11} \text{ sec}^{-1}$		$\tau_{\text{ring}} \times 10^{-11} \text{ sec}^{-1}$
		α -Carbons	Pyrrolidine carbons	
(a) <i>Trans</i> Conformer				
Ala- α	680 \pm 9	6.8
Pro- α	756 \pm 11	6.1
Pro- β	1212 \pm 6	..	3.80	9.25
Pro- γ	1626 \pm 12	..	2.93	5.27
Pro- δ	1002 \pm 10	..	4.60	16.04
(b) <i>cis</i> Conformer				
Ala- α
Pro- α	675 \pm 19	6.9
Pro- β	880 \pm 19	..	5.30	..
Pro- γ	1296 \pm 20	..	3.60	..
Pro- δ	720 \pm 34	..	6.45	..

given in the last column of Table I. The percentage of *cis* conformer increases as one goes from a non-polar to a polar solvent. The data indicate that while the predominant conformation of the tripeptide molecule is the one with *trans* acetyl-pro bond, the conformation with *cis* acetyl-pro bond also exists.

The large temperature coefficient of chemical shift ($\approx 6 \times 10^{-3}$ ppm/deg) for the NH of alanyl residue compared to small temperature coefficient (2×10^{-3} ppm/deg) for the NH of methylamide, for the *trans* case, indicates that in this conformation the NH of methylamide is possibly involved in an intramolecular hydrogen bond whereas NH of alanyl residue is exposed to the solvent. The large temperature coefficient of chemical shift for both the amide protons in the case of *cis* conformer indicates that probably both the amide protons are exposed to the solvent. These results are also supported by the relatively large low field shift of 1.9 ppm of the alanyl NH as compared to the shift of 0.6 ppm of the methylamide NH, in the *trans* case in going from CDCl_3 to $\text{DMSO}(d_6)$. All these data indicate that the tripeptide exists predominantly in the β -bond conformation with an intramolecular hydrogen bond between the NH of methylamide and CO of acetyl group such that *L*-prolyl and *D*-alanyl residues are at the corners of the bend. This conformation is very similar to that obtained from an X-ray crystal structure analysis of the compound¹⁸. The fraction of the tripeptide conformers with the *cis* acetyl-pro bond probably assume a linear conformation with both the amide protons not being involved in any intramolecular hydrogen bond.

The approximate dihedral angle $\theta(\text{H-N-C}^\alpha\text{-H})$ deduced from the average coupling constant, $^3J_{\text{NH-C}^\alpha\text{H}}$ of 7.5 Hz for alanyl NH in the *trans* conformer in all the solvents has the two possible values namely, 145° and 40° on using the Karplus-like relation between $J_{\text{NH-C}^\alpha\text{H}}$ and $\theta^{19,20}$. These θ values correspond to a ϕ value of 85° or -20° . The value of -20° for ϕ however is not energetically favourable for *D*-alanyl residue. The minimum energy conformation in the β -bond region obtained from theoretical studies gives a value of around 90° for the dihedral angle ϕ . The value of ϕ for the proline residue in proline containing peptides is close to -60° . Hence the dihedral angles ϕ_2 and ϕ_3 for this compound consistent with the NMR data for the *L*-prolyl and *D*-alanyl residues are -60° and 90° respectively. Model building with these values of ϕ and taking into account the fact that methylamide NH is hydrogen bonded to the acetyl CO group, the set of values for the dihedral angles (ϕ_2, ϕ_3) and (ϕ_2, ϕ_3) consistent with the energy calculations are $(-70^\circ, 90^\circ)$ and $(90^\circ, 30^\circ)$. The proposed conformation of the molecule with the *trans* acetyl-pro bond is shown in Fig. 4.

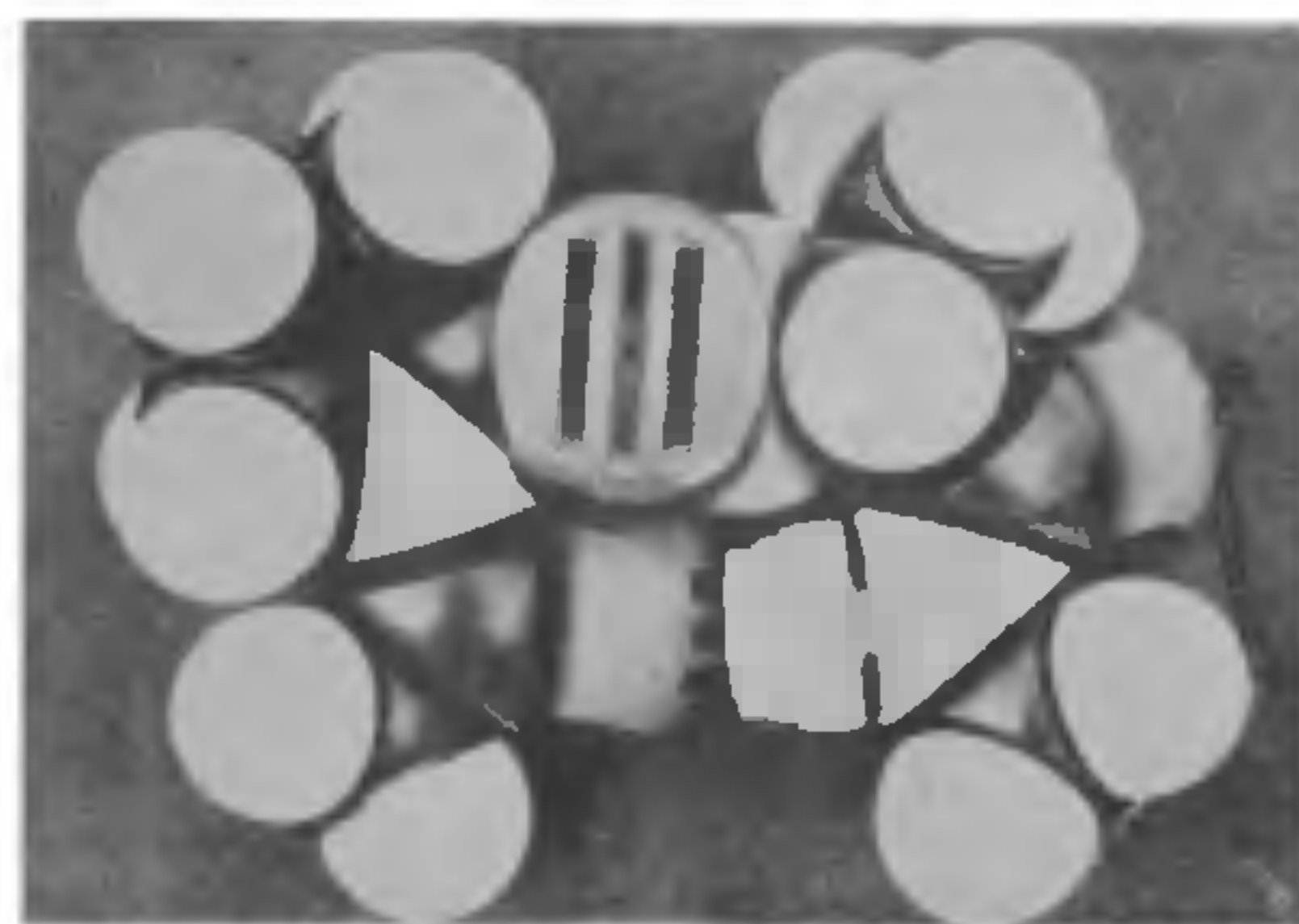


FIG. 4. The CPK model of the proposed folded conformation of *N*-acetyl-*L*-prolyl-*D*-alanyl-methylamide with the *trans* acetyl-pro bond.

(ii) Pyrrolidine Ring Mobility

The nearly same NT_1 values observed for the prolyl and alanyl α carbons given in Table II indicate an overall isotropic motion for the backbone of the molecule. The larger NT_1 values for the C^β , C^γ and C^δ carbons show the possibility of additional motion for the ring carbons. The estimated correlation times for the β , γ and δ ring carbons indicate that the motion of the δ carbons is restricted while the β and γ carbons have a large motion, the largest being for the γ carbon. The order of mobilities predicted from Carbon-13 relaxation time measurements for this molecule is $\gamma > \beta > \delta$ and hence in the *trans* conformation of the molecule the pyrrolidine ring is such that it is the γ carbon which has a large mobility compared to the rest of the atoms.

ACKNOWLEDGEMENTS

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TOPOLOGY AND PHYSICAL PROPERTIES OF *n*-ALKANES

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ABSTRACT

A relationship between the mean value of the Wiener Number and the physical properties of *n*-alkanes is presented. The root mean square value of the Wiener Number is defined. A good correlation between the topological index and physical properties has been obtained.

INTRODUCTION

MOLECULAR topology is a topic of current interest. This discipline of modern mathematics finds its various applications in chemistry. The term topology¹, in chemical literature, is generally used to represent the molecular graph², though both the terms are not synonymous. The molecular graph is a set of edges and vertices. The vertices are usually sp^3 -C atoms and edges are C-C bonds, the bond lengths of which are topologically invariant. The molecular graph is therefore a topology with defined metric³.

Considering the π -networks of conjugated hydrocarbons^{4,5} and their C-skeleton molecular graphs, topological studies have been made both on simple⁶ and heterocyclic systems^{7,8}. The problems of aromaticity and resonance energies⁹ based on the Hückel Molecular Orbital Theory, are now easy to solve with the help of topology.

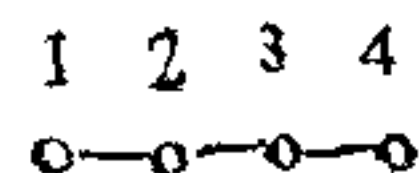
Topological indices¹⁰ have been introduced by various workers (e.g., Rouvray's index¹¹, Randic connecti-

vity index¹², Platt index¹³, and Hosoya index¹⁴). The Wiener number¹⁵ is an important topological index, which is applied by Trinajstić and Bonchev^{16,18} and Sondheimer¹⁷ to monocyclic structures and the changes in the values are related to the structural features and the physical and chemical properties¹⁷.

In this paper, it is shown that the topology can be equally applied to σ -bonded systems of sp^3 -C skeleton graph, and that the mean value of the Wiener Number is directly related to the physical properties of *n*-alkanes.

THEORY

The molecular graph (G) is defined in terms of edges, the C-C bonds and vertices, the C atoms. A labelled graph of *n*-butane may be represented by



The topological distances¹⁰ are defined as the elements of a real $N \times N$ matrix $D(G)$, which is known as the